Investigating pathogens of the gastrointestinal tract in Sudden and Unexpected Death in Infancy cases at the Tygerberg Medico-legal Mortuary, compared to an age-matched healthy control group

by
Danielle Tiffany Cupido

Dissertation presented for the degree of
Doctor of Philosophy (Medical Virology) in the Faculty of
Medicine and Health Sciences at Stellenbosch University

Supervisor: Prof. Corena de Beer
Co-supervisor: Prof. Andrew C. Whitelaw, Prof. Johan Dempers†

December 2023

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2023

Abstract

Background: Sudden and unexpected deaths in infancy (SUDI) includes infants under the age of one year that die suddenly and without apparent cause. Childhood diarrhoea is one of the leading causes of death for children under five, with around 1.7 billion cases worldwide each year and is often reported prior to death in SUDI cases. Poor socioeconomic conditions and inadequate water supplies in developing countries contribute to diarrhoea, and diarrhoeagenic *Escherichia coli* (DEC) were detected in 30-40% of these cases, while acute viral gastroenteritis causes ± 70% of all episodes. The microbiome influences host immunity, infectious susceptibility, and health, disease, and death outcomes. Limited information is available on the gastrointestinal tract (GIT) pathology, as well as the GIT microbiome as contributory factors to SUDI in South Africa. This study aims to investigate the bacterial and viral pathogens and colonisation of the GIT in SUDI cases admitted to the Tygerberg Medico-Legal Mortuary in the Western Cape in the process of determining the cause of death. Finally, the SUDI microbiome was compared to age-matched, apparently healthy infants.

Methods: Swabs of the GIT and stool samples were collected from SUDI cases at Tygerberg Medico-legal Mortuary between June 2017 and May 2018. To serve as controls, stool samples were collected from the nappies of 45 healthy and age-matched infants. In stool and swab samples positive for *Escherichia coli*, DEC were detected using the Allplex^{TM} GI-Bacteria (II) Assay and gastrointestinal viruses were detected in stool samples using the Allplex™ GI-Viral Assay. Positive rotavirus samples were genotyped and the intestinal microbiome was characterised by full-length 16S rRNA sequencing, on the PacBio Sequel IIe System platform.

Results: This study included 186 SUDI cases (107 males and 79 females) and 45 controls (24 males and 21 females). Several known demographic factors increase the risk for SUDI, including age between 2-4 months, male sex, cold season, bedsharing, prone and side sleeping positions, as well as informal housing. Enteroaggregative *Escherichia coli* (EAEC)) were detected in 87.3% of cases and enteropathogenic *Escherichia coli* (EPEC) were detected in 78.2% of cases. Co-infections between DEC pathotypes were observed in 85.2% of cases. Rotavirus was detected in 38.6%, of cases followed by norovirus GI and GII (30.0%), whereas norovirus GII was more prevalent in the controls (36.7%). Forty-eight cases had enteric virus co-detections. The association between most viruses and seasons was highly significant. Among the rotavirus genotypes, combinations of the G type and the P type, G1P[8] had the highest prevalence (40%), followed by G2P[4] (30%), while G9P[8] (20%) and G8P[4] (10%) genotypes had the lowest prevalence. *Firmicutes*, *Bacteroidota*, *Proteobacteria*, and *Actinobacteria* were found to be the most common organisms in the GIT. Significant differences were observed in alpha diversity and beta diversity between cases and controls, as well as the different final diagnoses.

Conclusion: This study demonstrated that autopsy sampling procedures should include other sampling sites, e.g., GIT, as these pathogens may contribute to death, particularly with virus and bacterium co-infections. Determining the cause of death based on GIT pathogens, may decrease the number of Sudden Infant Death Syndrome (SIDS) cases reported in the future. (513 words)

Opsomming

Agtergrond: Skielike en onverwagte sterftes in babas jonger as een jaar wat skielik en sonder duidelike oorsaak plaasvind word algemeen geag as onverwagte babadood. Diarree is een van die hoofoorsake van dood vir kinders jonger as vyf jaar, met ongeveer 1.7 miljard gevalle wêreldwyd elke jaar en dit word dikwels aangemeld in gevalle van onverwagte babadood. Swak sosio-ekonomiese toestande en onvoldoende watervoorsiening in ontwikkelende lande dra by tot diarree, en diarrogeniese *Escherichia coli* (DEC) is verantwoordelik vir 30-40% van hierdie gevalle, terwyl akute virale gastro-enteritis ± 70% van alle episodes veroorsaak. Die mikrobioom beïnvloed gasheerimmuniteit, aansteeklike vatbaarheid en gesondheid, siekte en sterftes. Beperkte inligting is beskikbaar oor die spysverteringskanaal patologie, sowel as die spysverteringskanaal-mikrobioom as bydraende faktore tot onverwagte babadood in Suid-Afrika. Hierdie studie het ten doel om die bakteriële en virale patogene en kolonisasie van die spysverteringskanaal in gevalle van onverwagte babadood te ondersoek wat in die Tygerberg Medies-geregtelike Lykshuis in die Wes-Kaap opgeneem is in die proses om die oorsaak van dood te bepaal. Laastens is die mikrobioom vergelyk tussen gevalle van onverwagte babadood en 'n groep gesonde babas van dieselfde ouderdom.

Metodes: Deppers van die spysverteringskanaal en stoelgangmonsters is van gevalle van onverwagte babadood by Tygerberg Medies-geregtelike Lykshuis ingesamel tussen Junie 2017 en Mei 2018. Om as kontroles te dien, is stoelgangmonsters van die doeke van 45 gesonde en ouderdom-ooreenstemmende babas versamel. In stoelgang- en deppermonsters positief vir *Escherichia coli*, is DEC opgespoor met behulp van die AllplexTM GI-Bacteria (II) Assay en gastroïntestinale virusse is opgespoor in stoelmonsters met behulp van die Allplex™ GI-Virale Assay. Positiewe rotavirus monsters is genotipeer en die intestinale mikrobioom is gekenmerk deur vollengte 16S rRNA volgordebepaling, op die PacBio Sequel IIe System platform.

Resultate: Hierdie studie het 186 gevalle van onverwagte babadood (107 manlik en 79 vroulik) en 45 kontroles (24 manlik en 21 vroulik) ingesluit. Verskeie bekende demografiese faktore verhoog die risiko vir onverwagte babadood, onder andere ouderdom tussen 2-4 maande, manlike geslag, koue seisoen, deel van beddens, buik- en syslaapposisies, sowel as informele behuising. Enteroaggregatiewe *Escherichia coli* (EAEC) was verantwoordelik vir 87.3% van gevalle en enteropatogene *Escherichia coli* (EPEC) was verantwoordelik vir 78.2% van gevalle. Ko-infeksie is in 85.2% van die gevalle waargeneem. Rotavirus is opgespoor in 38.6% van gevalle, gevolg deur norovirus GI en GII (30.0%), terwyl norovirus GII meer algemeen in die kontroles (36.7%) voorgekom het. Meer as een enteriese virusse is in 48 gevalle bevestig. Die verband tussen die meeste virusse en seisoene was hoogs betekenisvol. Kombinasies van G-tipe en P-tipe, G1P[8] was die algemeenste (40%), gevolg deur G2P[4] (30%), terwyl G9P[8] (20%) en G8P[4] (10%) genotipes het die laagste voorkoms getoon. *Firmicutes*, *Bacteroidota*, *Proteobacteria* en *Actinobacteria* was die algemeenste organismes in die spysverteringskanaal. Beduidende verskille is waargeneem in alfa-diversiteit en beta-diversiteit tussen gevalle en kontroles, sowel as die verskillende finale diagnoses.

Gevolgtrekking: Hierdie studie het getoon dat lykskouingsprosedures ander areas moet insluit, bv. spysverteringskanaal, aangesien hierdie patogene tot die dood kan bydra, veral met virus- en bakterie-gepaardgaande infeksies. Die bepaling van die oorsaak van dood gebaseer op spysverteringskaal-patogene kan die aantal wiegiedood gevalle wat in die toekoms aangemeld word, verminder. (514 woorde)

Acknowledgements

First and foremost, I want to thank God for giving me the strength, knowledge, ability, and opportunity to persevere and complete this research.

In recognition of my supervisor, Professor Corena de Beer, I wish to express my deepest appreciation. Your mentorship has been invaluable to me. You have been patient, supportive, and encouraging throughout my research.

I would like to express my gratitude to Professor Andrew Whitelaw, co-supervisor to this doctoral dissertation, for his guidance, support and intellectual contributions.

It is with profound appreciation that I acknowledge the contributions and guidance of the late Professor Johan Dempers.

I am grateful to all the Forensic Pathology Officers who assisted me during sample collection at the mortuary. I would also like to thank Mr Marshall Hartzenberg and Mr John Segole. You have become friends and family to me as a result.

Ms Prudence Flaendorp, Mr Wayne Claassen, and Mr Rameez Sulaiman for informing me about SUDI cases, and the Forensic Pathologists for their assistance in collecting samples. Thanks to Mrs Riley, Ms Lilly Jansen, and the rest of the staff at the Division of Forensic Medicine for their willingness to assist me at all times.

My thanks go to Ms Mathilda Claassen, Dr Kristien Nel van Zyl, Ms Bianca Leigh Hamman, Mr Hameer Vanmali, Ms Shahieda Issacs, Ms Robyn Conrad as well as the staff and students of the Division of Medical Virology for their assistance and guidance throughout this project.

Thank you to Prof Martin Kidd for his assistance during statistical analysis.

Thanks to my “unbiological” sister, Dr Bronwyn Kleinhans, for all her patience and always being willing to help me, regardless of the distance. Thank you is beyond words.

My appreciation goes out to my parents and brother for their sacrifices, guidance, and motivation in giving me this opportunity. I also wish to thank my entire family for their love and support.

I would like to thank Derrick Potgieter, my love, for being with me on this journey. Thanks for the unconditional love, support, and motivation you have shown me, and for giving me our precious daughter. It was your encouragement that pushed me through when I was ready to give up.

My beautiful daughter, Scarlett, you inspire me to achieve greatness. You have strengthened me, made me better, and made me more fulfilled than I could have imagined.

Thank you to the Harry Crossley Foundation, Poliomyelitis Research Foundation (PRF), National Research Foundation (NRF) and National Health Laboratory Service (NHLS) Research Trust for funding this study.

Dedication

This dissertation is dedicated to the late Professor Johan Dempers and all those little angels who earned their wings during the course of this project. The thesis also pays tribute to all my loved ones who were promoted to glory before I had the chance to complete it.

"There is a time for everything and a season for every activity under the heaven".

Ecclesiastes 3:1

Table of contents Page

List of Figures

List of Tables

List of Abbreviations

16S rRNA	16S ribosomal ribonucleic acid
A/E	Attaching and effacing
AAF	Aggregative adhesion fimbriae
AdV	Adenovirus
aEPEC	Atypical enteropathogenic *Escherichia coli*
AHR	Aryl hydrocarbon receptors
AIDS	Acquired immunodeficiency syndrome
AstV	Astrovirus
BBB	Blood-brain barrier
BFP	Bundle forming pilus
BLAST	Basic Local Alignment Search Tool
BoNT	Botulinum neurotoxin
C. botulinum	*Clostridium botulinum*
C. difficile	*Clostridioides difficile*
C. innocuum	*Clostridium innocuum*
C. perfringes	*Clostridium perfringes*
CCNA	Cell cytotoxicity neutralisation assays
CCS	Circular Consensus Sequences
CDC	Centres for Disease Control
cDNA	Complementary DNA
CI	Confidence interval
CMV	Cytomegalovirus
CNS	Central nervous system
CPE	Cytopathogenic effect
CSF	Cerebrospinal fluid
CV	Coxsackievirus
DAEC	Diffusely adhering *Escherichia coli*
DEC	Diarrhoeagenic *Escherichia coli*
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded deoxyribonucleic acid
DSI	Death scene investigation
dsRNA	Double-stranded ribonucleic acid
E. coli	*Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EBV	Epstein-Barr virus
EHEC	Enterohaemorrhagic *Escherichia coli*
EIA	Enzyme immunoassay
EIEC	Enteroinvasive *Escherichia coli*
ELISA	Enzyme-Linked Immunosorbent Assay
EM	Electron microscopy

EPEC	Enteropathogenic *Escherichia coli*
EspB	*Escherichia coli*-secreted protein B
ETEC	Enterotoxigenic *Escherichia coli*
GBA	Gut-Brain Axis
GDH	Glutamate dehydrogenase
GIT	Gastrointestinal tract
H & E	Haematoxyllin and eosin
HBGA	Histo-blood group antigen
HCV	Human cardiovirus
HHV	Human herpes virus
HIV	Human Immunodeficiency Virus
HPeV	Human parechovirus
HUS	Haemolytic uraemic syndrome
IC	Internal control
ICTV	International Committee on Taxonomy of Viruses
Ig	Immunoglobulin
IL	Interleukin
LB	Luria-Bertani
IMR	Infant mortality rate
IQR	Interquartile range
Lodox®	Low-dose X-ray
LT	Heat-labile enterotoxin
MAL-ED	Malnutrition and Enteric Disease Study
NAAT	Nucleic acid amplification test
NGS	Next-generation sequencing
NHLS	National Health Laboratory Service
NICHD	National Institute of Child Health and Human Development
NoV	Norovirus
NSP	Nonstructural protein
ONT	Oxford Nanopore Technology
OR	Odds ratio
ORF	Open reading frame
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
pEAF	Enteropathogenic *Escherichia coli* adherence factor plasmid
PMI	Post-mortem interval
PMBT	Post-mortem bacterial translocation
PRR	Pattern recognition receptor
PVB19	Parvovirus B19
RdRp	Ribonucleic acid-dependent ribonucleic acid polymerase
RNA	Ribonucleic acid

RT-PCR	Reverse transcription polymerase chain reaction
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
RV	Rotavirus
RV-A	Rotavirus group A
S. aureus	*Staphylococcus aureus*
SA-EPI	South African Expanded Programme of Immunisation
SAGE	Strategic Advisory Group of Experts on Immunisation
SAPS	South African Police Service
SaV	Sapovirus
SB	Sodium boric acid
SCFA	Short-chain fatty acid
SIDS	Sudden infant death syndrome
sIgA	Secretory Immunoglobulin A
SMAC	Sorbitol MacConkey Agar
SMRT	Single-molecule real-time
ST	Heat-stable enterotoxin
STEC	Shiga-toxin producing *Escherichia. coli*
SUDI	Sudden unexpected death in infancy
TBA	Tryptone blue agar
TC	Toxicogenic culture
tEPEC	Typical Enteropathogenic *Escherichia coli*
UK	United Kingdom
USA	United States of America
VLP	Virus-like particles
VT	Verocytotoxin
VTEC	Verocytotoxigenic *Escherichia coli*
VZV	Varicella zoster virus
WGS	Whole-genome sequencing
WHO	World Health Organisation

Chapter 1: Introduction

As a highly sensitive and useful measure of population health, infant mortality rate (IMR), defined as the number deaths in infants under the age of 1 per 1 000 live births in a particular year, has been used for decades (Blaxter, 1981). Accordingly, the IMR is also dependent on other factors that could influence the health status of entire populations, such as economic growth, general quality of life, social conditions, disease frequencies, and environmental factors (Reidpath & Allotey, 2003). Over the past five years, the IMR in South Africa has steadily declined to 36 per 1 000 live births in 2021 (Geoba.se: Gazetter - The World at Your fingertips, 2021). South Africa still faces enormous challenges in an effort to further reduce the IMR, despite the recent decline. Several factors complicate investigations into unexpected deaths in children, including different population densities across the country, a lack of standardised national death investigation protocols, cultural and language barriers, and a lack of funding and resources for qualified forensic pathologists, particularly in rural areas (Dempers et al., 2018).

The literature uses different terms interchangeably to describe the sudden and unexpected and / or unexplained deaths in infants younger than one year with no apparent cause. It refers to the circumstances under which the infant died, rather than a specific diagnosis (Weber & Sebire, 2009) and is commonly referred to by the general public as cot death.

Sudden unexpected death in infancy (SUDI) is a collective term to include all infant deaths where the cause of death is not immediately apparent *before* any investigation has been done to find a cause of death. On the other hand, Sudden infant death syndrome (SIDS) is defined as the unexpected death of an infant under the age of one, which appears to occur during sleep, where no explanation can be found *after* a thorough investigation has been conducted. This includes an autopsy and an examination of the circumstances of death and medical history (Krous et al., 2004).

Infection is a leading contender in terms of causation of SUDI, based on epidemiological evidence. The infection model is consistent with factors such as male sex predominance, winter seasonality, low socioeconomic status, vulnerability of the host (prematurity, low birth weight, genetic predisposition due to important polymorphisms in inflammatory, innate and adaptive immune responses) and smoke exposure. There is a correlation between an increased risk of infection and the use of the prone sleeping position (Goldwater & Bettelheim, 2013), as well as the use of contaminated surfaces as a bed for infants (Gilbert et al., 1992). As with SUDI, cases are often colonised by coliform bacteria in the respiratory tract (Blackwell et al., 2002, Blood-Siegfried et al., 2008, Weber et al., 2008) and by toxigenic organisms in the gastrointestinal tract (GIT) such as *Staphylococcus aureus (S. aureus)* (Highet & Goldwater, 2009).

Cases of unnatural deaths, including SUDI, are investigated under the auspices of the Inquests Act (Act 58 of 1959). In accordance with this Act, the South African Police Service (SAPS) is responsible for investigating the circumstances and cause of death. Moreover, the Act states that the body of a person who died as a result of other than natural causes, including SUDI, must be examined by a district surgeon or any other medical practitioner who may assess it in order to establish the cause of death with greater certainty. There is no national guideline for sudden infant death investigation in South Africa, resulting in national institutions all following different guidelines to investigate such cases (du Toit-Prinsloo et al., 2011; 2013). SUDI cases are investigated at Tygerberg Medico-legal Mortuary in accordance with standard facility procedures and may include a death scene investigation (DSI), a medical history review, as well as autopsy, and laboratory investigations including virology and microbiology to determine potential causes of death.

One of the infections that may be associated with SUDI is acute gastroenteritis. Millions of deaths in young children are caused by acute gastroenteritis (Elliott 2007). Clinically, the disease is characterised by inflammation of the mucous membrane of the GIT and increasing frequency of bowel movements with or without vomiting, fever, and abdominal pain. Three or more watery or loose bowel movements in 24 hours or at least 200 grams of stool per day are considered increasing bowel movement frequency. The disease is among the leading causes of illness worldwide and contributes to 1.5 million to 2.5 million deaths each year. Globally, diarrhoeal diseases affect 2.5 billion children per year and are the second major cause of death in children under 5 years of age (Sattar & Singh, 2021). A variety of pathogens may cause acute gastroenteritis, including bacteria, viruses, parasites, and fungi (Ciccarelli et al., 2013). While viruses are the most common cause of acute infectious diarrhoea (noro-, rota-, adeno-, and other viruses), bacterial causes are more likely to result in severe disease than other infectious causes (Sattar & Singh, 2021). Risk factors for infectious diarrhoea include a variety of factors including geography, co-morbidities, and the immune status of the host.

The human microbiome is made up of bacteria, archaea, viruses and eukaryotic microbes which exist in and on the body. The physiology of the human both in health and in disease are impacted by these microbes. They contribute metabolic functions, defend against pathogens, and educate the immune system, in doing so directly or indirectly affect most of the physiological functions (Shreiner et al., 2015). It is vital for the infants' health to establish normal GIT function for the duration of early development (Goldwater, 2015). This could influence the infants' vulnerability to infection, induction of GIT inflammation, and adverse infection outcomes (Highet et al., 2014). Factors known to influence bacterial colonisation of the colon of infants are gestational age, diet, environment,

antibiotic use (Stark & Lee, 1982; Copperstock & Zedd, 1983; Doré et al., 1998; Macfarlane & McBain, 1999; Harmsen et al., 2000) and mode of delivery (Grönlund et al., 1999).

Despite the majority of the studies related to SUDI research in Africa being conducted in South Africa (Osei-Poku et al., 2021), no studies have been done on the role of GIT pathology in determining the cause of death in SUDI as described in South Africa.

This study therefore aimed to investigate pathogens in the GIT in order to assess the role of GIT colonisation in the process of determining the cause of death in cases of SUDI, as well as profile in microbiome in these cases compared to an age-matched healthy control group.

Chapter 2: Literature Review

2.1 History of sudden unexpected infant death

Since ancient times, the sudden and unexpected death of seemingly healthy infant has been recognised (Fleming et al., 2015). In the Old Testament of the Bible reference is made to such a case. “And this woman’s son died, in the night, because she lay on him” (1 Kings 3:19). It was not until the second half of the 20th century that such deaths became the focus of medical attention. Due to the significant decrease of IMRs in England and Wales in the 20th century, from 95 per 1 000 live births in 1912, 11 in 1982 and 4 per 1 000 live births in 2012, further consideration was given to deaths for which insufficient cause of death could be found (Fleming et al., 2015).

These cases became the focus of pathologists in the 1950s and 1960s, as paediatricians would rarely see cases where no suggestive ailment requiring medical attention resulted in the demise of these infants (Mitchell & Krous, 2015). Numerous studies in the 1970s and 1980s showed an increasing number of such deaths. In Europe and New Zealand, epidemiological studies indicated an association with infants placed in a prone position while sleeping (de Jonge et al., 1989; Fleming et al., 1990).

2.2 The definitions of SUDI and SIDS

SUDI is not a clinical or pathological diagnosis and does not correspond to SIDS; rather, it signifies the presentation of death and includes all deaths in infants < 1 year of age (often restricted to 7-365 days), that present more or less suddenly and unexpectedly (Weber & Sebire, 2009), or at least without an initial clearly identifiable cause. According to the 2004 San Diego definition, SIDS is defined as the unexpected death of an infant under the age of one, which appears to occur during sleep, where no explanation can be found after a thorough investigation has been conducted. This includes an autopsy and an examination of the circumstances of death and medical history (Krous et al., 2004). The term “Borderline SIDS” is used in SUDI cases where pathological changes are clearly present, however they are not sufficient to unequivocally confirm a particular cause of death (Bajanowski et al., 2007) (Figure 2.1).

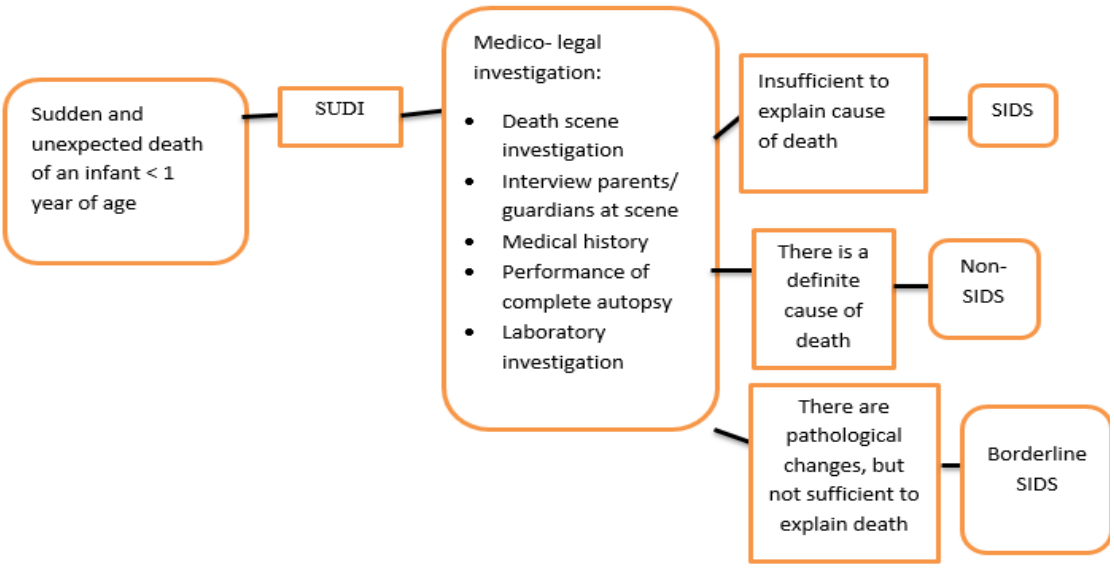


Figure 2.1: The link between SUDI and SIDS

2.3 Epidemiology of SUDI

The foremost cause of infant death outside of the neonatal period is SUDI and more specifically, SIDS (National Centre for Chronic Disease Prevention and Health Promotion, 2017). In 2015, SIDS accounted for 9% of under-5 deaths globally, resulting in approximately 19 200 deaths. This is a decrease of 8% from 20 800 deaths in 2005 (GBD 2015 Mortality and Causes of Death Collaborators, 2016). The number of SIDS globally decreased by 17% between 2007 and 2017 (GBD 2017 Causes of Death Collaborators, 2018). SIDS accounted for 27 700 deaths globally in 2019 (Institute for Health Metrics and Evaluation, 2020). In South Africa, the IMR for 2017 was 39 per 1 000 live births, compared to 38 in 2018 and 2019 and 37 in 2020. The overall IMR in 2021 was 36 per 1 000 live births, with the male IMR at 39 compared to 33 for females (Geoba.se: Gazetter - The World at Your fingertips, 2021).

There are a wide range of factors that contribute to the risk of SUDI. Risk factors include demographic factors, such as low socioeconomic status (Beckwith, 2003), male infants being more susceptible than females, infants in the age group of 2 to 4 months being particularly susceptible and there is a seasonal trend with more cases being reported during the colder months of the year (du Toit-Prinsloo et al., 2013; Moscovis et al., 2014; Fleming et el., 2015).

The literature reports specific racial and ethnic differences, despite the overall global decrease in SIDS. There are significant differences in the rates among African Americans, Native Americans, and Alaskan Native infants regardless of their socioeconomic status (Hoyert et al., 2001; Mathews et al., 2004). There is also a greater risk of SUDI among Aboriginal Australians (Beal, 2000; Panaretto et al., 2002; Freemantle et al., 2006) and Maoris in New Zealand (Mitchell et al., 1997). Moreover,

Maoris have a 6 times greater likelihood of dying of SUDI than New Zealanders of non-Maori descent (Blakely et al., 2004). Behavioural factors as well as biological differences influences the disparity.

It is estimated that African Americans place their infants in the prone position to sleep twice as often as other ethnic groups. This is despite being familiar with the *Back to Sleep* and other safe-sleeping campaigns, since many mothers do not believe the supine position is safest for their infant. Other mothers use prone positions to provide comfort for their infants or to prolong sleep time for themselves (Oden et al., 2010). In addition, African Americans are more likely to share a bed with their infant than other groups (Willinger et al., 2003).

Birth-related risk factors include prematurity (Vennemann et al., 2005) and low birth weight (Malloy and Freeman, 2000). The position the infant is placed to sleep, especially the prone and side sleeping positions, also increase the risk of SUDI. As infants between 4 and 7 months of age begin to roll over, it is easier for them to roll from the side position into the prone position than rolling from the back, as the side sleeping position is very unstable and as such confers an increased risk of SUDI. (Li et al., 2003; American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome, 2005, 2011; Hauck et al., 2010; Task Force on Sudden Infant Death Syndrome & Moon RY, 2011). Other risk factors include the use of soft bedding or pillows near the sleeping infant (Task Force on Sudden Infant Death Syndrome & Moon RY, 2011).

The room temperature, quantity of clothing on the infant, and season of the year may also contribute to SUDI (Gelfer & Tatum, 2014). Several layers of clothing or blankets on the infant and warmer room temperatures have been associated with an increase in SUDI (Moon et al., 2022). Infants sleeping in the prone position have an increased risk of overheating (Fulmer et al., 2020), when the infants are placed in the supine position this risk is unclear. Room sharing without sharing beds was linked with a decrease in SUDI, however the risk is higher when there is co-sleeping with numerous people and when the bed-sharer is intoxicated or fatigued (American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome, 2005).

A significant link between maternal smoking during pregnancy and SUDI has been confirmed by most epidemiological studies (Schoendorf & Kiely, 1992; Haglund, 1993; MacDorman et al., 1997). Smoking during pregnancy results in reduced lung volume and compliance in the foetus (Difranza et al., 2004), as well as reduced heart rate variability when stressed (Søvik et al., 2001). In addition to its neurological teratogenic effects, nicotine results in altered autonomic pathways, which contribute to a diminished response to hypoxia and other stimuli (Lewis & Bosque, 1995; Franco et al., 1999; Frøen et al., 2000; Chang et al., 2003; Horne et al., 2004). The impact of postnatal exposure to smoke

has emerged in a few studies as a distinct risk factor (Schoendorf & Kiely, 1992; Mitchell & Milerad, 2006).

Prematurity and low birth weight increases the risk of SUDI up to four-fold compared to infants born at full term (Hoffman & Hillman, 1992; Malloy & Hoffman, 1995; Blair et al., 2005). Preterm infants are frequently placed in the prone position during hospitalisation to improve respiratory function (Shepherd et al., 2020). Even though respiratory parameters are similar whether the infant is supine or prone in preterm infants nearing discharge (Levy et al., 2006), both the infant and caregiver may have become accustomed to the prone position. This can make a change in position more difficult. Other inherent risk factors include young maternal age, low maternal education, prenatal exposure to drugs, and poor, delayed or no prenatal care (Hoffman et al., 1988; Kattwinkel et al., 2000; American Academy of Pediatrics et al., 2006; Harper, 2006; Shah et al., 2006; Athanasakis et al., 2011).

Nutrition plays an important role in a child's development, both during pregnancy and after birth. Although breastfeeding may reduce the risk of SIDS (Alm et al., 2016), there is no direct evidence that maternal diet plays a role in SUDI incidence. However, a poor or unbalanced diet may result in foetal compromise that increases the risk of SUDI, such as intrauterine growth restriction (Hakeem et al., 2015). Malnutrition, however, has not been extensively discussed in the literature as a risk factor.

2.4 Aetiology of SUDI

A variety of risk factors are involved in the aetiology of SUDI, as there are many probable causes, but thus far no conclusive, acceptable pathological or genetic explanation has been established (Carroll & Wood, 2012).

Froggatt et al. (1971) was first to propose that the age range of 1-6 months represents a period of enhanced physiological vulnerability in which some critical combination of extrinsic (e.g., infection and sleep) and intrinsic (not yet unequivocally identified) factors can prove lethal. Wedgwood (1972) subsequently proposed three types of risk factors: vulnerability, age-specific risk factors, and a precipitating factor.

In 1993, Rognum and Saugstad proposed the fatal triangle as the interplay of 3 factors: (i) a vulnerable phase in the development of the central nervous system and the immune system in the first months after birth; (ii) predisposing factors, such as genetic make-up, and (iii) a trigger event such as overstimulation of the immune system.

Filiano and Kinney (1994) subsequently proposed a *Triple Risk Model* which hypothesised SUDI to result from 3 overlapping factors intersecting: (i) a vulnerable infant, (ii) a critical development period in homeostatic control; and (iii) an exogenous stressor.

SUDI may also be explained by an underlying abnormality in the brain stem neural networks that facilitate protective responses to asphyxia (Kinney et al., 2009). Serotonin and γ-aminobutyric acid deficiencies have been reported in infants who succumbed to SUDI. It has been argued that SUDI only affects infants with an underlying condition, not healthy infants (Kinney and Thach, 2009).

2.5 Interventions for risk reduction and their impact

There have been many campaigns aimed at reducing SUDI, beginning in the Netherlands in 1987, the United Kingdom (UK), New Zealand, and Australia in 1991, and Scandinavian countries in 1990-1992, as well as the United States of America (USA) in 1994 (Hauck, 2001). Reducing prone sleeping was a major focus of these risk campaigns. Although side and supine sleeping positions were initially recommended, the risk of side sleeping was found to be greater than the risk of back sleeping, thus sleeping on the back became the only recommended position (American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome, 2005). Interventions on other behaviour and practices to reduce the risk of SUDI were also included in some campaigns, such as the reduction of tobacco use during pregnancy and the promotion of breastfeeding. However, no significant changes in these behaviour patterns have been seen and avoidance of the prone sleeping position has largely attributed to reduced SUDI rates (Mitchell et al., 1994; Dwyer et al., 1995; Markestad et al., 1995).

In Hong Kong, SUDI was rare, as placing infants to sleep in the supine position was a common Chinese habit (Davies, 1985). In 1987, a campaign recommending that parents place neonates to sleep in the supine rather than prone positions was introduced in the Netherlands (Högberg & Bergström, 2000; Rusen et al., 2004). After the introduction of the *Back to Sleep* campaign (Kattwinkel et al.,1992), the SUDI rate in the USA decreased by over 50%. An analysis of SUDI in San Diego from 1991 to 2008 by Trachtenberg et al. (2012) indicated that the number of infants placed to sleep in the prone position decreased from 85% to 30%, whereas those placed to sleep in the supine position increased from 2% to 42%, and deceased infants found in the prone position decreased from 84% to 49%.

Throughout the past two decades, numerous studies have demonstrated an association between the use of pacifiers and a reduced risk of SUDI (Mitchell et al., 1993; Arnestad et al., 1997; Fleming et al., 1999; L'Hoir et al., 1999; Hauck et al., 2003; Moon et al., 2012). Meta-analysis has revealed that being placed to sleep with a pacifier has a significant protective effect against SUDI (Hauck et al.,

2005). The reduction in risk was independent of other risk factors. Researchers found that pacifier users have a 90% reduction in risk, along with a reduction in other risk factors associated with SUDI, specifically poor sleeping conditions (Li et al., 2006). Pacifiers have been suggested to exhibit protective effects in a variety of ways, including by reducing arousal thresholds, improving mouth breathing capabilities, and rendering the tongue forward in the mouth (Franco et al., 2000; Hauck et al., 2005).

2.6 Medico-legal investigation into SUDI

Under South African law, in the event that an infant has an underlying medical condition or illness and has been treated, death may be considered due to natural causes under the Births and Deaths Registration Act (Act 51 of 1992). In such circumstances, a death certificate form may be issued by the treating medical personnel, and the death will be registered with the Department of Home Affairs.

For any other deaths, such as sudden, unexplained deaths, inquest proceedings are conducted according to the Inquests Act (Act 58 of 1959), and the bodies are admitted to a medico-legal mortuary for further examination. Often the admission or not of the body is dependent on the opinion of the clinician as to whether the cause of death was regarded to be natural or unnatural.

For centuries, autopsies have been performed, in one form or another, to study the anatomy and physiology of the human body and determine the cause of death of an individual (Mark, 2002). Autopsies may be conducted in academic or anatomical pathology settings to determine the nature and degree of underlying pathology, as well as permit academics to teach students and conduct research. Medico-legal autopsies are however routinely carried out under statutory provisions requiring examinations of deceased bodies, where the cause of death is unnatural, unforeseeable or unexplained. Further legal proceedings and decisions are facilitated by this action.

The Human Tissue Act (Act 65 of 1983) governs the conduct of autopsies for academic purposes as well as anatomical pathology, in South Africa. During the post-mortem examination, the attending physician/pathologist should establish if the deceased died from natural causes.

Medico-legal post-mortem examinations are performed in cases of death that may have resulted by an unnatural cause, mainly under the Inquests Act. The Act serves to provide for the holding of inquests in cases of deaths or alleged deaths apparently occurring from other than natural causes and for matters incidental thereto, and to repeal the fire Inquests Act, 1883 (Cape of Good Hope) and the fire Inquests Law, 1884 (Natal).

The Regulations Regarding the Provision of Forensic Pathology Services (Government Gazette, R341(15 April 2005 No. 27464) regulate the provision of forensic pathology services under the National Health Act (Act 61 of 2003). Investigation of deaths that are unexpected or unexplained should be assisted by SAPS, but the performance of an autopsy may not necessarily be included in the investigation. An autopsy is further defined by these regulations as a post-mortem dissection of a corpse and post-mortem examination is defined as an examination of a human body or the remains thereof, including an autopsy with the purpose of establishing the cause of death and factors associated with the death (National Health Act (Act 61 of 2003).

Dempers et al. (2016), conducted a study to institute a standard investigation protocol for sudden infant death in the Eastern Metropole, Cape Town, South Africa. The project comprised of 18 autopsied infants. The DSIs were conducted using a standardised DSI form published by the Centres for Disease Control (CDC), Atlanta, USA (Hanzlick et al., 2007). The information obtained included the age of the infant, gestational age, the person who discovered the infant, co-sleeping information (yes/no, number of co-sleepers), the infant's medical history, such as the number and location of clinic visits, any medication, and any illnesses.

A standardised autopsy protocol was developed, partly based upon the California protocol for SUDI (Gianelli Castiglione et al., 1993), along with regional protocols. Detailed external examination of the body and histologic examination of major organ systems, as well as bacterial and viral cultures, Human Immunodeficiency virus (HIV) testing and toxicology screening where applicable, were all included in the autopsies. The autopsy results and microscopic slides of each death were reviewed by all the study pathologists in order to categorise each death according to the 1990 National Institute of Child Health and Human Development (NICHD) definition (Willinger et al., 1991): SIDS was defined as unexplained death and viewed as a diagnosis by exclusion. Based on autopsy and DSI, the known/explained cause of death was determined. There are situations in which the cause of death cannot be determined due to inexplicable autopsy results or circumstances at the scene of death, which represent an unclassified (undetermined) category.

The results revealed that the investigations conducted to determine the cause of death varied significantly in terms of the extent of the DSI and the interviews conducted with the mother and family, but not so much when it came to the autopsy performed. Initially, all the deaths were attributed to SUDI prior to the autopsy and DSI. A DSI was completed in all 18 cases, with photographs taken in 83%. Based on the results of a full autopsy and DSI conducted in each case, these deaths were categorised into three types: SIDS (n=7; 39%), known/explained cause of death (n=7; 39%), and

unclassified (n=4; 22%). The authors concluded that paediatric autopsies and DSI protocols can be developed in areas with high SUDI rates, as well as in other areas.

In medico-legal investigations, radiographs have been used since the invention of x-rays (Harcke, 2010). They are a permanent, but incomplete, record of the anatomy and pathology of the deceased before the forensic autopsy, primarily in identifying fractures and foreign materials such as bullet fragments (O'Donnell & Woodford, 2008). Medico-legal autopsies in South Africa are performed using low-dose X-rays (Lodox®) of the entire body for various reasons. The multiple views aid in rapid localisation of foreign bodies, it therefore benefits criminal investigations and religious practices which dictate fast burials. Trained staff can operate it without difficulty and its low radiation dose reduces the risk to staff (Knobel et al., 2006).

2.7 Bacterial, viral, and fungal pathogens in SUDI

2.7.1 Toxigenic bacteria and SUDI

According to epidemiological and pathological findings, *S. aureus* superantigenic enterotoxins may be responsible for SUDI (Lee et al., 1987; Malam et al., 1992; Murrell et al., 1993; Blackwell et al., 1999; Morris, 1999; Zorgani et al., 1999). An analysis of staphylococcal enterotoxins in the intestinal tract was conducted by Highet and Goldwater, 2009, in support of the staphylococcal toxic shock hypothesis of SIDS. A PCR analysis was performed to detect S. aureus, staphylococcal enterotoxins and staphylococcal toxic shock in the intestinal contents of 57 SIDS infants, as well as stool from 79 live comparison infants matched in age and gender. The proportion of SUDI babies who were positive for S. aureus and staphylococcal enterotoxin genes was significantly higher than that of comparison babies (68.4% versus 40.5%), suggesting a possible role for these organisms in SUDI.

Several studies investigated the association between *Escherichia coli* (*E. coli*) and SUDI. Within the first few days of an infant's life, *E. coli* colonises the bowel, and numerous studies have linked *E. coli* to SUDI (Bettelheim et al., 1990). *E. coli* serotypes found in the GIT of SUDI cases, however, are typically extraintestinal (Highet, 2008). The toxic effect of *E. coli* is attributed to several toxins, including heat-stable enterotoxins (ST), heat labile enterotoxins (LT) and verocytotoxins (VT). The effects of these toxins have been extensively studied (Highet, 2008). These toxins may be associated with other, unknown, or less studied toxins, as well as share a transmission vector such as bacteriophage (Bettelheim et al., 1990).

Additionally, research has linked SUDI with the presence of *Clostridium perfringens* (*C. perfringens*) in the GIT of these infants (Lindsay et al., 1993). It has also been reported that *Clostridium botulinum* (*C. botulinum*), which releases a highly toxic substance called botulinum neurotoxin (BoNT), is

associated with SUDI (Böhnel et al., 2001). The presence of *C. botulinum* further confirms the infectious hypothesis of SUDI, since *C. botulinum* is not a constituent of the normal flora of the human body (Highet, 2008). In an effort to understand how infants acquire these organisms, researchers have examined associations between numerous bacteria and SUDI. In light of the accumulation of protein within polyurethane used in cot mattresses, researchers in the UK investigated the possibility that mattresses may serve as viable harbours for bacteria. Polyurethane foam mattresses without waterproof covers at the head region of the infant were observed to have significantly higher protein levels (p=0000019), and (ii) protein levels positively correlated with both the density of culturable bacteria in the polyurethane foams and the extent to which *S. aureus* was present in the aqueous leachates. Other measured parameters and mattress type/use did not show significant associations (p>0.05) (Jenkins & Sherburn, 2008).

The other bacteria species responsible for diarrhoeal disease include *Salmonella, Shigella, Campylobacter* and *Yersinia enterocolitica*. In 2017 and 2018, *Shigella, Campylobacter,* and *Salmonella* caused deaths among children under five, in 11%, 2%, and 1% of cases respectively (Cohen et al., 2022). In the USA, *Yersinia enterocolitica* was responsible for approximately 5% of all bacterial infections in children younger than five (Scallan et al., 2013). Although *Salmonella virchow* was isolated from the myocardium of an infant who died suddenly at one month of age (Neuwirth et al., 1999), *Shigella, Camphylobacter,* and *Yersinia enterocolitica* have not been reported in literature to be associated with SUDI.

2.7.2 SUDI and viral infections

It is evident from several epidemiological characteristics of SUDI that viruses may play an important role leading to SUDI by either increasing the lethality of bacterial toxins or by synergistically influencing virulence factors or immunoregulatory polymorphisms of the bacteria. It is possible for sub-lethal doses of bacterial toxins to become lethal when viruses are present (Jakeman et al., 1991; Blackwell et al., 2005). There is no clear understanding of how this occurs, but a cytokine storm is considered to be the most likely cause (Doughty et al., 2006).

As the more likely cause of SUDI, viral-induced respiratory infections fit in well with the infectious hypothesis (Highet, 2008). Nearly 80% of reported SUDI cases present with a viral infection of the upper respiratory tract before death (Cutz et al., 2001; Highet, 2008). There has also been evidence that SUDI cases have higher rates of respiratory viruses in comparison to controls; however, no single respiratory pathogen has yet been linked to SUDI (Álvarez-Lafuente et al., 2008). Despite different study conclusions, epidemiological and pathological evidence strongly supports the association between viral infections and SUDI (Highet, 2008).

Certain enteroviral and Cytomegalovirus (CMV) infections may lead to acute myocarditis in infants, which may result in sudden death (Dettmeyer et al., 2001, 2002; Fernández-Rodriguez et al., 2006; Dettmeyer et al., 2008). Other viral infections associated with SUDI include influenza virus A, Epstein-Barr virus (EBV), and Human herpes virus (HHV) type 6 (Fernández-Rodriguez et al., 2006). It is also possible that the fairly benign Coxsackie virus (CV) A16 can contribute to SUDI (Astrup et al., 2016). Co-infection of CMV with Varicella zoster virus (VZV) resulted in the sudden death of a 2-month-old infant in 2013 (Desmons et al., 2013).

The presence of Human cardiovirus (HCV) has been detected in clinical samples from children who had diarrhoea and respiratory illness (Drexler et al., 2008). An infant whose cause of death was initially attributed to SUDI was found to have HCV in the cerebrospinal fluid (CSF) in 2011. It was the first time HCV was reported in a body compartment other than the respiratory tract or the GIT (Drexler et al., 2011).

Burger et al. (2014) found 29 polymerase chain reaction (PCR) positive cases for CMV and 2 for Adenovirus (AdV) in 82 SUDI cases at Tygerberg Medico-legal Mortuary. In France one case of SUDI was found to be positive for Human Parechovirus (HPeV) type 3 (Schuffenecker et al., 2012). Krous et al. (2009), detected no AdV or Enteroviruses with PCR, however only 7 out of 17 cases had symptoms of upper respiratory tract infection 48 hours before death.

As part of a German study, Dettemeyer et al. (2004), examined 62 SUDI cases and 11 controls who had died of unnatural causes. Enteroviruses were detected in 23%, Parvovirus B19 (PVB19) in 11%, EBV in 5%, AdV in 3% and HHV-6 in 1.6% of the SUDI groups, while no virus was found in the control group.

In many SUDI cases, the presence of viral pathogens is not an indicator of cause of death. It has been observed that viruses related to common childhood diseases such as herpes simplex, VZV, EBV and CMV are found in the lungs of deceased infants, but their involvement in death sometimes remains unclear (Alvarez-Lafuente et al., 2008). Infections can be latent, asymptomatic or pre-symptomatic. An infection that is latent can be described as an infection caused by an organism that lies hidden or dormant in the body and is not active. It is common for latent infections to remain static without causing symptoms (Larragoite & Spivak, 2019). Asymptomatic is often used to describe illnesses in which there are no symptoms for all or part of the time. A pre-symptomatic condition, on the other hand, is sometimes used to describe conditions that do not initially exhibit any symptoms but then develop them later (WHO, 2021). According to Weber & Sebire (2010), systemic responses to isolated pathogens instead of only relying on detection would provide a better understanding of pathogen involvement in disease progression.

2.7.3 Fungal infections and SUDI

Autopsies have demonstrated the presence of *Pneumocystis* in the lungs of SUDI cases without obvious pathological changes (Morgan et al., 2001; Vargas et al., 2007), however, in 105 of 128 SUDI cases, *Pneumocystis* deoxyribonucleic acid (DNA) was detected (Vargas et al., 2013). *Pneumocystis* might function as a cofactor that contributes to respiratory diseases in infants and young children, triggering excess mucus production via non-specific pathways (Vargas et al., 2013). Various species of Candida were also detected in SUDI autopsies, although none of them were associated with clinical manifestations of infection or septicaemia (Geertinger et al., 1982).

2.8 Introduction of GIT pathogens investigated in this study

The following sections will briefly introduce and characterise the diarrhoeagenic *E. coli* (DEC) and five gastrointestinal viruses and subtypes that were investigated during this study, namely enterohaemorrhagic *E. coli* (EHEC) (*stx1/stx2*, *E. coli* O157), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and diffusely adhering *E. coli* (DAEC) (Jesser & Levy, 2020), Norovirus (NoV) (GI, GII), Rotavirus (RV) A, AdV-F (Serotype 40/41), Astrovirus (AstV) and Sapovirus (SaV) (Genogroups G1, 2, 4).

E. coli is a gram-negative bacterium that is oxidase-negative, rod-shaped and belongs to the order Enterobacterales (Janda & Abbott, 2021), family *Enterobacteriaceae* (Croxen et al., 2013). It is typically about 2.0 µm long and 0.25–1.0 µm in diameter, with an estimated volume of 0.6–0.7 µm3 (Kubitschek, 1990; Yu et al., 2014). Within hours of birth, it colonises the GIT of neonates (Kaper et al., 2004) and can be readily isolated from faeces. Despite most strains of *E. coli* being harmless, there are those that are pathogenic. These strains can cause diseases like watery diarrhoea, bloody diarrhoea, urinary tract infection, meningitis, and sepsis, which are likely to be fatal (Nataro & Kaper, 1998; Gyles, 2007). These well-adapted strains of *E. coli* have acquired specific virulence factors that enable them to cause illness (Nataro & Kaper, 1998).

2.8.1 Diarrhoeagenic *E. coli*

Approximately 30–40% of acute diarrhoeal episodes among children younger than five years of age in developing countries are caused by DEC (Miliwebsky et al., 2016). It is responsible for both sporadic diarrhoea cases as well as diarrhoeal outbreaks worldwide (Croxen et al., 2013). Faecal-oral transmission occurs frequently (Gehlbach et al., 1973), and inadequate living conditions, such as insufficient water supply, poor sanitation, as well as inadequate education, are primarily responsible for disease (Croxen et al., 2013).

2.8.2 Enterohaemorrhagic *E. coli* (*stx1/stx2*, *E. coli* O157)

E. coli O157 is one of the enterohaemorrhagic group of *E. coli* strains (Coia, 1998). They are also known as verocytotoxigenic *E coli* (VTEC), after it was demonstrated that they could produce a toxin that would cause direct damage to Vero cells in cultured conditions in 1977 (Konowalchuk et al., 1977). After discovering that VT was closely related to Shiga toxin in 1982, it was termed Shiga-toxin producing *E. coli* (STEC) (O'Brien et al., 1982). Globally, EHEC is well-known to cause foodborne illnesses. The ability to produce cytotoxins from the Shiga toxin family is the primary virulence characteristic of this pathogroup of *E. coli* (Melton-Celsa, 2014). Two toxin families, *stx1* and *stx2*, encoded by respective forms of *stx1* and *stx2*, have been identified (Gallien, 2003). There are three subtypes of *stx1*, namely *stx1a*, *stx1c*, and *stx1d*, while the *stx2* group has seven subtypes, namely *stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2f*, and *stx2g* (Scheutz et al., 2012).

Infections caused by EHEC range from mild diarrhoea to more serious manifestations such as haemorrhagic colitis and haemolytic uraemic syndrome (HUS), which can have potentially life-threatening consequences. Among the most affected patients are infants and children. The prevalence of EHEC infections varies among regions, but in many countries, it is a significant cause of acute kidney failure among children (Guth et al., 2010; Majowicz et al., 2014). A distinctive feature EHEC epidemiology is the presence of its reservoir found in the digestive tracts of cattle and other animals; various foods can transmit the disease, with beef representing the most significant means of transmission, and a relatively low infectious dose, which contributes to high rates of infection and transmission among individuals (Nataro & Kaper, 1998). Diarrhoea caused by EHEC is treated with supportive care (Salvadori & Bertoni, 2013).

There have been numerous outbreaks associated with EHEC belonging to serogroup O157 over the course of history. There are many transmission vehicles associated with EHEC outbreaks, including meat products (Vygen-Bonnet et al., 2017; Furukawa et al., 2018; Wilson et al., 2018), dairy/milk products (Jaakkonen et al., 2017), vegetables/salads (Sharapov et al., 2006; Gardiner et al., 2018; Mikhail et al., 2018), and water (Probert et al., 2017). An outbreak of EHEC occurred in Germany in 2011, resulting in almost 3,000 cases of acute gastroenteritis, and 855 cases of HUS. As a result of the infection, 55 people have died (Robert Koch-Institut, 2011). In addition to several northern German states, visitors from 15 other countries were affected by the outbreak. This was also linked to a smaller outbreak in France (Rubino et al., 2011). A laboratory diagnosis determined that the causative pathogen was *E. coli* O104:H4, and an epidemiological investigation determined that fenugreek sprouts, grown in Germany but imported from Egypt, were the origin of the outbreak (Krause et al., 2011). In 2016, Japan experienced an outbreak of EHEC O157:H7 infection. Laboratory testing confirmed the presence of EHEC O157:H7 infection in 61 individuals, including

17 asymptomatic individuals, 24 patients hospitalised and 4 patients who developed HUS. This outbreak was associated with the consumption of uncooked minced meat cutlets sold frozen at supermarket branches (Furukawa et al., 2018).

In South Korea, there were five outbreaks between 2015 and 2019, with an average of 3.8 cases per outbreak. There were three outbreaks related to restaurants and two outbreaks at home. Among the 5 outbreaks, serotypes O165, O168, and O26 were associated, while O157 was not associated (Lee et al., 2021). A cluster of four HUS cases was identified in South Africa in 2017 associated with EHEC O26:H11. Patients were all females aged 5 and under. There was no evidence of an epidemiological link between the cases of HUS. It was suspected that dried meat products were the main vehicle of transmission in these cases, as three of the case-patients reported having consumed them. A test of dried meat products, however, was not able to confirm this (Smith et al., 2019).

Cytotoxicity of bacteria culture supernatants to eukaryotic cells is the gold standard for detecting *stx* (Konowalchuk et al., 1977; Karmali et al., 1983). Multiplex PCR using the *stx* gene and other virulence factors may be suitable for detecting STEC/EHEC from bacteria in confluent growth zones or from colonies on Sorbitol MacConkey Agar (SMAC) that are fermenting or non-fermenting (Leotta et al., 2005). Several assays are available to diagnose STEC based on the detection of *stx1* or *stx2*, which are important virulence factors found in this type of *E. coli* (Gould, 2009). Depending on the test format and the manufacturer, the sensitivity and specificity may differ (Donohue-Rolfe et al., 1986; Kongmuang et al., 1987; Beutin et al., 1996; Mackenzie et al., 1998; Novicki et al., 2000; Beutin et al., 2002, 2007). The cost of using these commercially available tests is too high for developing countries. The detection of STEC/EHEC can be accomplished using numerous immunoassay formats, including indirect Enzyme-Linked Immunosorbent Assay (ELISA) utilising rabbit anti-*Stx1* and anti-*Stx2* sera or capture ELISAs utilising polyclonal and monoclonal antibodies (Rocha & Piazza, 2007; Mendes-Ledesma et al., 2008; Rocha et al., 2012).

2.8.3 Enteropathogenic *E. coli*

In 1955, Neter et al., first introduced the term EPEC, to refer to a group of enteropathogenic *E. coli* strains epidemiologically associated with several infantile diarrhoea outbreaks during the 1940s and 1950s (Bray, 1945; Robins-Browne, 1987). Initially, they were classified by their serogroup, but have since been classified by the characteristic pattern of localised adhesion in tissue cultured cells. Currently, they are distinguished primarily based on the presence of distinct virulence genes. Attaching and effacing (A/E) lesions are a distinct phenotype of EPEC (Nataro & Kaper, 1998). Over the years, advances in techniques have improved the understanding of how EPEC strains differ in their genomes and virulence mechanisms. This has resulted in the subcategorisation of EPEC into

typical EPEC (tEPEC) and atypical EPEC (aEPEC) (Trabulsi et al., 2002; Kaper et al., 2004). Typical EPEC strains can cause human infectious diarrhoea and carry a large virulence plasmid, that is referred to as the EPEC adherence factor plasmid (pEAF), encoding the type IV fimbriae known as the bundle forming pilus (BFP), whereas aEPEC does not bear this plasmid (Nataro & Kaper, 1998; Trabulsi et al., 2002).

Over the span of decades, studies conducted throughout the world have strongly linked tEPEC serotypes with children <1 year of age, particularly among children in urban areas (Nataro & Kaper, 1998; Trabulsi et al., 2002; Gomes & González-Pedrajo, 2010). tEPEC is transmitted faecal-orally through contaminated surfaces, weaning fluid and direct contact with humans (Levine & Edelman, 1984). Only humans are known to harbour tEPEC and the most likely carriers are symptomatic and asymptomatic children, and asymptomatic adults (Nataro & Kaper, 1998). The pathogenic potential of aEPEC is the subject of long-standing controversy, but epidemiological studies have determined that aEPEC plays a significant role in diarrhoeal endemics and outbreaks among children (Hedberg et al., 1997; Scaletsky et al., 1999; Vieira et al., 2001; Yatsuyanagi et al., 2002; Dulguer et al., 2003). Despite the lack of direct evidence of aEPEC strains being transmissible from animals to humans, some strains may represent potential zoonotic pathogens, since diverse animal species are known reservoirs of these pathogens (Gomes et al., 2016). Additionally, foods such as raw meat, pasteurised milk, vegetables, as well as water, have been implicated in aEPEC transmission to humans (Hu & Torres, 2015; Gomes et al., 2016).

Traditional EPEC testing is still performed routinely in microbiology laboratories for children under two years of age. In order to screen *E. coli* colonies from primary isolation, antisera against the classical EPEC serogroups are used in slide agglutination. While it is widely available in most laboratories, this test has a number of significant limitations, including its inability to differentiate typical *E. coli* from aEPEC strains, cross-reactions due to serogroup diversity, and false negative results caused by strains of EPEC that belong to different serogroups than the classical strains. Molecular methods, such as PCR, are recommended for EPEC diagnosis, which can differentiate between typical and atypical infections based on the presence or absence of specific virulence factors (Mare et al., 2021). It is currently possible to identify EPEC strains by the characteristics of their virulence, however cell culture methods are labour-intensive and not available in most laboratories (Hernandes et al., 2009; Gomes et al., 2016). Available methods include immunoblotting and immunofluorescence that utilise polyclonal or monoclonal antibodies to detect BFP, as well as rapid tests, including detecting *E. coli*-secreted protein B (EspB) and a secreted protein (Girón et al., 1995; Gismero-Ordoñez et al., 2002).

2.8.4 Enteroaggregative *E. coli*

In both developed and developing countries, a pathogenic strain of *E. coli* is present which causes acute and chronic diarrhoea (Nataro et al., 2006; Hebbelstrup Jensen et al., 2014). The first EAEC strain was described by Nataro et al. in 1987 as part of their investigation of the pattern of adherence of different strains of *E. coli* to Hep-2 cells in culture. Strains were isolated from children in Chile that were suffering from diarrhoea, and they displayed a characteristic "stacked-brick" morphology, which continues to distinguish EAEC. In recent years, the EAEC has emerged as an enteric pathogen. EAEC is particularly reported as the second major cause of travelers' diarrhoea and most frequent cause of diarrhoea among children as well (Adachi et al., 2001; Huang et al., 2006).

EAEC is transmitted via the faecal-oral route through food or contaminated water (Itoh et al., 1997; Pai et al, 1997; Scavia et al., 2008). Milk samples from feeding bottles handled by mothers of low socioeconomic status were found to contain EAEC (Morais et al., 1997). EAEC is thought to progress through three distinct stages of pathogenesis. Stage 1 consists of the attachment of the EAEC to the intestinal mucosa by means of aggregative adhesion fimbriae (AAF) and other adhering projections. During stage 2, mucus accumulates on the surface of the enterocytes covering the EAEC and during the third stage, an inflammatory response is triggered, mucosal toxicity occurs, gastrointestinal secretion, as well as the release of toxins (Jenkins, 2018).

A gold standard for the detection of EAEC remains Hep-2 cell culture (Nataro et al., 1987; Jenkins et al., 2007; Bangar & Mamatha., 2008; Tokuda et al., 2010), which is performed by reference laboratories only, requires cell culture facilities, and takes a considerable amount of time (Vial et al., 1990). For detecting both typical and atypical EAEC strains, a multiplex PCR involving two plasmid-encoded genes and two chromosome-borne genes is recommended. Genes *aggR* and *aatA* (Cerna et al., 2003; Jenkins et al., 2006), as well as genes *aaiA* and *aaiG* (Dudley et al., 2006) that are included in the assay for detection of *aaiA*, *aaiG*, *aggR* and *aatA*, are quite sensitive and specific, and the assay is effective in detecting both groups of EAEC with *E. coli* isolated from stool cultures (Andrade et al., 2014).

2.8.5 Enterotoxigenic *E. coli*

ETEC was first identified by De and colleagues in Calcutta in 1956 when they administered live *E. coli* isolates obtained from children and adults suffering from cholera-like illnesses into rabbit ileal loops and found that significant amounts of fluid accumulated, as was found in the case of *Vibrio cholera* (De et al., 1956). The filtrates from these cultures were however not tested to determine whether an enterotoxin was present. These findings were not followed up until 1968, when Sack

reported that both adults and children in Calcutta with cholera-like illnesses showed almost pure *E. coli* growth in both stool and small intestine samples (Sack, 1968).

ETEC is characterised by the production of heat labile and -stable (LT and ST) enterotoxins. In endemic areas, the majority of ETEC illnesses occur within the first two years of life (Qadri et al., 2007), as well as among travellers and military personnel deployed in endemic areas (Riddle et al., 2006; Shah et al., 2009; Steffen et al., 2015; Hameed et al., 2016). Infections are usually acquired through the consumption of contaminated food or water (Dalton et al., 1999; Gilligan, 1999; Beatty et al., 2004). As a result of insufficient sanitation and sewage facilities, contaminated water is the principal source of ETEC infection (Qadri et al., 2005). The importation of food from endemic areas has recently been linked to rare sporadic cases and outbreaks in developed countries (Roussel et al., 2017).

In order to detect ETEC, the two enterotoxins must be identified. ST enterotoxins can be detected using various immunoassays, including radioimmunoassays and ELISAs, whereas LT enterotoxins of ETEC can be detected using two commercial agglutination tests. DNA probes have been used successfully to detect the LT- and ST-coding genes in stool and environment specimens. When applied to clinical samples or isolated bacteria, a variety of PCR methods have been demonstrated to be highly sensitive and specific for detecting ETEC (Qadri et al., 2005).

2.8.6 Enteroinvasive *E. coli*

It was not until 1947 that the first report of an EIEC strain was published (Ewing & Gravatti, 1947). In the late 1950s and early 1960s, it was discovered that strains of *E. coli* isolated from patients with dysentery could also cause experimental keratoconjunctivitis in guinea pigs (Séreny, 1963; Sakazaki et al., 1967). In the late 1950s and early 1960s, it was discovered that strains of *E. coli* isolated from patients with dysentery could also cause experimental keratoconjunctivitis in guinea pigs. In addition to *Shigella*, *Shigella manolovi*, *Shigella sofia*, *Shigella* strain 13, *Shigella metadysenteriae*, these *E. coli* strains have been called numerous names (Manolov, 1959; Rowe et al., 1977). Later, they were all renamed enteroinvasive *E. coli* (EIEC), which has become universally accepted (Edwards & Ewing, 1986). It is often challenging to distinguish between EIEC and Shigella spp due to the similarity of phenotypic and genotypic characteristics, especially when serogroups are shared. (Silva et al., 1980; Toledo & Trabulsi, 1983; Bando et al., 1998; Lan & Reeves, 2002; Pavlovic et al., 2011, van den Beld & Reubsaet, 2012).

Since no animal reservoir has been identified, EIEC infections in humans are likely to be transmitted through oral-faecal contact. The spread of EIEC infections occurs worldwide, but they are particularly

common in low-income countries where poor general hygiene is conducive to their spread (Chatterjee & Sanyal, 1984; Beutin et al., 1997; Kaper et al., 2004; Vieira et al., 2007). Additionally, water and cheese were described as possible sources of transmission (Borian et al., 1959; Marrier et al., 1973; Tulloch et al., 1973; Valentini et al., 1992).

A major outbreak of diarrhoea was reported in the USA in the 1970s, which affected 387 patients. The transmission vehicle was imported cheese contaminated with the O124 serogroup (Marrier et al., 1973). In Europe, outbreaks involving EIEC have been reported, including one in Italy in 2012 involving 109 cases (Escher et al., 2014; Pettengill et al., 2015). In 2014, two connected outbreaks of gastroenteritis led to more than 100 cases in the UK (Newitt et al., 2016), and in 2013 traveler's diarrhoea was reported in Spain with the same EIEC serotype (Michelacci et al., 2016). In Africa, EIEC has been reported sporadically and infrequently (Rappelli et al., 2005; Bonkoungou et al., 2013).

A simple stool test based on apyrase activity was described for EIEC detection. This enzyme is essential for the pathogen's intracellular and inter-cellular spread and can be measured by a colorimetric reaction. In laboratories with limited resources, the method can be applied for routine use in laboratories with robust equipment and affordable reagents.

2.8.7 Diffusely adherent *E. coli*

In 2006, DAEC were reported to also belong to the diarrheagenic group of *E. coli* (Blanco et al., 2006). Diffusely adherent *E. coli* strains are distinguished by their diffuse adherence pattern to cultured HeLa or HEp-2a cells, in which the bacteria are uniformly distributed across the surface of each cell (Scaletsky et al., 2002a). There are two groups of DAEC strains that can be identified based on the expression of adhesins, Afa/Dr DAEC and AIDA-I DAEC. The adherence phenotype in DAEC strains may be caused by the Afa/Dr family of adhesions (Scaletsky et al., 2002b).

In children, especially those 6 months of age and older, Afa/Dr DAEC strains have been associated with acute diarrhoea as well as persistent diarrhoea. Consequently, patients with the DAEC pathogroup can experience diarrhoea caused by genes encoding the Afa/Dr adhesin (Lozer et al., 2013). In addition to fimbrial and afimbrial adhesins, the Afa/Dr family includes the afimbrial adhesins Afa-I-VIII and Dr-2 as well as the fimbrial adhesins Dr and F1845. These adhesins have been detected in *E. coli* strains isolated from human urinary tract infections or diarrhoea. However, Afa-VII has only been found in *E. coli* strains isolated from bovine faeces. The first report of F1845 adhesin was made from an *E coli* strain (C1845) isolated from a child suffering from chronic diarrhoea (Lalioui et al.,1999). In *E. coli* isolates from diarrhoea patients, Afa-I, Afa-II, Afa-III, and Afa-V genes have been isolated (Servin, 2014).

The main route by which DAEC pathogens are transmitted is through food or water contaminated with human or animal faeces. Watery or bloody diarrhoea, abdominal pain, dehydration, and fever may be symptoms of diarrhoea caused by DAEC. However, DAEC diarrhoea has no specific clinical feature (Gunzburg et al., 1993). In stool specimens from children with diarrhoea, DAEC is less prevalent than other DEC pathotypes. Studies have linked DAEC strains to diarrhea in infants, children, and adults (Daigle et al., 1994). It has been reported that DAEC may be present in children without clinical symptoms, while other studies indicate a relationship between DAEC infection and clinical symptoms (Meraz et al., 2007; Spano et al., 2008). Diffusely adherent E. coli can be identified using HEp2 cell adherence assays based on diffuse patterns (Cabrera-Sosa & Ochoa, 2020).

2.8.8 Norovirus (GI, GII)

Known formerly as Norwalk virus, human NoV was initially identified in stool samples obtained in Norwalk, Ohio, USA, during an outbreak of gastroenteritis and was the first to be associated with gastroenteritis (Kapikian et al., 1972). Acute illness due to this virus was first named “winter vomiting disease” in 1929 because of the seasonality of the illness and the prevalence of vomiting among patients (Zahorsky, 1929).

NoVs are small, nonenveloped, positive-stranded ribonucleic acid (RNA) viruses belonging to the Caliciviridae family (Green, 2013). This genome consists of a linear, positive-sense RNA that measures ~7.6 kb in length (Jiang et al., 1993). There is a covalent linkage at the 5’ end of the genome to the viral protein genome and a polyadenylation at the 3’ end (Thorne & Goodfellow, 2014). At least seven genogroups of NoV have been described (GI, GII, GIII, GIV, GV, GVI, and GVII), further divided into different genetic clusters or genotypes (Atmar et al., 2019). Genogroups GI and GII are the most common NoV that cause human illness (Vinjé et al., 2000). Genogroup II, genotype 4 NoV (also referred to as GII.4) causes the majority of adult gastroenteritis outbreaks around the world (Noel et al., 1999).

An elementary school in Norwalk experienced an outbreak in 1968 that resulted in the identification of the virus and approximately half of the students experienced nausea, vomiting, diarrhoea, and low-grade fever (Adler & Zickl, 1969). The virus is typically transmitted through the faecal-oral route via contaminated water or food, or by direct contact between people. In addition to contaminated surfaces or vomit from infected individuals, other risk factors include unhygienic food preparation and sharing close quarters with others (Brunette, 2017). In addition to accounting for nearly 20% of acute diarrhoeal cases worldwide, it is resulting in a reported 685 million episodes and 212 000 deaths each year (Ahmed et al., 2014; GBD Diarrhoeal Diseases Collaborators, 2017; Farahmand et al., 2022). Children younger than five years of age are generally the most vulnerable to endemic NoV

gastroenteritis (Kabue et al., 2016; Cannon et al., 2019; Farahmand et al, 2022). Among infants less than one year of age, the NoV infection rate was highest, according to meta-analysis conducted recently (Farahmand et al, 2022).

NoV detection is best performed by real-time reverse transcription-PCR (RT-PCR). It is extremely sensitive and can be applied to clinical and environmental samples, as well as to quantify viral loads using quantitative reverse transcription PCR (RT-qPCR). For the surveillance of NoV outbreaks, the CDC uses RT-PCR for genotyping. In outbreaks, enzyme immunoassays (EIA) provide rapid and cost-effective monitoring, but their sensitivity is only about 50%, which requires negative samples to be confirmed using RT-PCR (Gastañaduy & Bégué, 2016).

2.8.9 Rotavirus A

RVs are members of the family Sedoreoviridae (Matthijnssens et al., 2022) and possess a genome consisting of 11 segments of double-stranded (ds) RNA enclosed in three concentric layers of protein (Estes & Cohen, 1989; Estes, 2001). Viral particles can measure up to 76.5 nm in diameter (Prasad & Chiu, 1994). One gene product code for each of the 11 genes, with six proteins found in virus particles, VP1-VP7, and five nonstructural proteins (NSP), NSP1-NSP5 (Ciarlet & Estes, 2001; Lundgren & Svensson, 2001). RVs are classified into nine genetic groups (A-J), with K and L proposed as new species (Johne et al., 2019). In humans, RV-A is responsible for more than 90% of RV infections (Leung et al., 2005).

In 1943, researchers showed that an agent that caused infectious diarrhoea in children also caused diarrhoea among cattle (Light & Hodes, 1943). More than 30 years after the discovery of the agent, preserved samples were characterised as RV (Mebus et al., 1976). A related group of viruses was found in children with gastroenteritis by Ruth Bishop and colleagues in 1973 (Bishop, 2009). Hospitalised infants, children in day-care centres, and the elderly living in nursing homes are commonly affected by RV-A diarrhoea outbreaks (Anderson & Weber, 2004; Sassi et al., 2015). The largest recorded epidemic of diarrhoea occurred in Central America during 2005. A mutation in the RV-A genome may have caused such an unusually large and severe outbreak, enabling the virus to escape immunity in the population (Bucardo et al., 2007). In 1977, Brazil experienced a similar large outbreak (Linhares et al., 1981). Mild to severe symptoms of the illness include nausea, vomiting, diarrhoea and a low-grade fever. Infected children usually show symptoms within two days of exposure (Hochwald & Kivela, 1999). Infections caused by rotavirus are more likely to cause dehydration than infections caused by most bacteria. This is the most common cause of death associated with rotavirus infection. (Maldonado & Yolken, 1990).

A monovalent 2-dose vaccine with an attenuated human strain of RV G1P[8] (Rotarix; GlaxoSmithKline), as well as a pentavalent bovine RV-based vaccine with capsid proteins bearing human serotypes G1, G2, G3, G4, and P[8] (RotaTeq; Merck) were licensed in the USA in 2006 (Ruiz-Palacios et al., 2006; Vesikari et al., 2006). Globally, the World Health Organisation (WHO) Strategic Advisory Group of Experts on Immunisation (SAGE) has recommended that RV vaccines be included in all childhood immunisation programs in 2009 (WHO, 2009). As a result of vaccination, hospital admissions and emergency room visits have declined by 67% globally (Burnett et al., 2017).

RV-A infections are diagnosed by direct analysis of faecal samples, which can contain as many as 1 x 10^{10} viral particles per gram of stool. In laboratories engaged in clinical and public health, EIAs are preferred due to their high specificity and sensitivity (>90%). In addition to lateral flow immunoassays, other antigen detection methods, such as latex agglutination, are also available for point-of-care testing (Esona & Gautam, 2015). The presence of RV antigen correlates more strongly with illness than does the presence of viral nucleic acids. By detecting viral nucleic acids, subclinical infections can also be detected. The new generation of molecular detection methods, which can identify multiple gastroenteritis pathogens simultaneously and require expensive laboratory equipment, are highly sensitive and specific (>90%) for identifying RV (Reddington et al., 2014; Buss et al., 2015; Deng et al., 2015).

2.8.10 Adenovirus-F (Serotype 40/41)

AdVs are members of the Adenoviridae family and are nonenveloped viruses that contain a double-stranded (ds) deoxyribonucleic acid (DNA) genome enclosed in an icosahedral nucleocapsid. They are medium size ranging from 90-100 nm. AdVs are the largest non-enveloped viruses structurally and their non-segmented dsDNA genomes range between 26 and 45 kbp, which is larger than other dsDNA viruses (Anon, 2021). At present, there are 88 human AdVs, which are categorised into seven species (AdV A to G) (Dhingra et al., 2019). Paediatric gastroenteritis, accompanied by fever, vomiting, and diarrhoea, is a common complication of type 40/41 (species F) (Uhnoo et al., 1984; Kotloff et al., 1989; Grimwood et al., 1995).

The gastroenteritis caused by AdV 40/41 is a common cause of hospitalisation as well as serious illness (Afrad et al., 2018; Iturriza-Gómara et al., 2019; Praharaj et al., 2019). In children younger than two years of age, especially in infants younger than 12 months, the disease burden is highest (Banerjee et al., 2017; Platts-Mills et al., 2018; Bray et al., 2019). The prevalence of type 41 is higher than that of type 40 (Afrad et al., 2018; Primo et al., 2018; Qiu et al., 2018; Hassan et al., 2019; Kumthip et al., 2019). Diarrhoea has also been associated with AdV species A, B, C, and D. Types

12, 18, and 31 of species A are classified as enteric AdV, along with AdV 40 and 41 (Magwalivha et al., 2010; Ghebremedhin, 2014; La Rosa et al., 2015; Qiu et al., 2018).

AdV in humans can be spread through inhalation, contact with small droplets, or the faecal-oral route. As a result of AdV contaminating food and water, outbreaks have been reported in schools, nurseries, and military camps (Fong & Lipp, 2005; Filho et al., 2007; Huh et al., 2009; Lazić et al., 2015).

Direct or indirect immunofluorescence, conventional cell culture methods or shell vial cultures, as well as PCR can be used to detect AdV (Lee et al., 2010). While conventional viral culture is the gold standard for RV detection, it may not be sensitive enough for some samples and the cytopathic effect (CPE) may not be apparent for up to 21 days (Ison, 2006; Lee et al., 2010). Since EIAs are 98% more sensitive and specific than electron microscopy (EM) in detecting viral antigens in stool, it is the most convenient method of detection. Real-time RT-PCR has demonstrated superior performance over EIAs and EM (Logan et al., 2006). Serotypes can be determined by PCR amplification. The use of serological tests is relatively uncommon; however, neutralisation or haemagglutination inhibition tests are used to detect these antibodies. The growth of enteric AdVs in routine cell lines has been difficult, but isolation techniques have improved (Arcangeletti et al., 2014).

2.8.11 Astrovirus

The human AstV genome consists of a non-segmented positive sense ssRNA that is encapsulated within a non-enveloped icosahedral capsid (Matsui et al., 2001). EM was first used to detect the presence of AstV in infants hospitalised with diarrhoea (Madeley & Cosgrove, 1975). Based on virion morphology, human AstV was the earliest member of the Astroviridae family to be detected (Monroe et al., 1995). Two genera were later established according to their hosts, the Mamastrovirus, which affects mammals, and the Avastrovirus, which affects birds (Mayo, 2002). AstV has a genome size of about 6.8 (6.2 to 7.8) kb, excluding the polyadenylated tail at the 3′ end (Bosch et al., 2011). Open reading frames (ORF) 1a,1b, and 2 appear in the genome from the 5′ to 3′ ends. NSPs are encoded by ORF1a and ORF1b, both of which are involved in the transcription and replication of RNA, whereas structural proteins are encoded by ORF2, which are expressed from a sub-genomic RNA (Monroe et al., 1993; Willcocks & Carter, 1993).

Human AstV have eight known serotypes (1 to 8), with serotype 1 being the most dominant worldwide (Matsui & Greenberg, 2001). In addition to RV and caliciviruses such as norovirus and sapovirus, AstV is one of the leading causes of acute gastroenteritis among infants (Glass et al., 1996). In particular, children and immunocompromised adults are particularly susceptible to gastroenteritis (Cortez et al., 2017), with approximately 2–8% of all cases of non-bacterial acute gastroenteritis in

children being caused by AstV. Duodenal epithelial cells usually contain viral particles (Maclachlan et al., 2017). An infection typically causes mild symptoms and is linked to outbreaks of gastroenteritis in indoor locations, such as day-care centres and schools (Pankovics et al., 2011; Tan et al., 2017).

Faecal-oral transmission is primarily facilitated by direct contact with faeces or by consuming contaminated food or water (Roach & Langlois, 2021). Due to lower temperatures which enhance virus stability, infection is more common during the winter months in temperate climates (Abad et al., 2001), whereas infections in tropical climates are more prevalent during the rainy season (Cruz et al., 1992; Maldonado et al., 1998). It is also possible for AstV to be transmitted from animals to humans, with abattoir employees having three times the likelihood of having antibodies against turkey AstV (Meliopoulos et al., 2014).

There has been some research reporting use of real-time RT-PCR to be effective in detecting all AstV genotypes (Royuela et al., 2006) and there are a few RT-qPCR techniques that can be used to diagnose AstV simultaneously with other enteric viruses that cause gastroenteritis (Liu et al., 2012). In addition, microarrays are used to distinguish between the eight distinct types of AstV (Brown et al., 2008). Recent developments have enabled multiplex RT-PCR panels to diagnose AstV and other GIT pathogens more rapidly, efficiently, and at a reasonable cost. In the early attempts to detect AstV, NoV, AdV, SaV, and enteroviruses in stool samples, either end-point (Rohayem et al., 2004; Yan et al., 2004) or qPCR (Beuret, 2004) were as effective as single-plex PCR, while a melting curve analysis allowed detection of dual-infection through the formation of dual peaks, although the sensitivity was approximately 10 times greater than end-point PCR (Beuret, 2004).

2.8.12 Sapovirus (Genogroups G1, 2, 4)

SaV comprises a genus of genetically diverse non-enveloped, positive-sense ssRNA viruses in the family *Caliciviridae* (Vinjé et al., 2019). The genome contains two ORFs and is approximately 7.5-8.5 kb in length. ORF1 encodes a large polyprotein that contains NSPs (such as RNA-dependent RNA polymerase [RdRp]) followed by the major capsid protein, VP1, while ORF2 encodes for the minor structural protein, VP2 (Desselberger, 2019). VP1 sequences are widely used in SaV classification as the VP1 region has greater genetic diversity than the RdRp region (Oka et al., 2015). Human and animal SaVs are classified into 19 genogroups, of which viruses from GI, GII, GIV, and GV are associated with human gastroenteritis infection and human SaV can further be subdivided into at least 18 genotypes (Vinjé et al., 2019). As of recent years, the SaV GI and GII genogroups have been detected more frequently globally (Diez-Valcarce et al., 2018; Varela et al., 2019; Makhaola et al, 2020), whereas the GIV genogroup is relatively uncommon, but can occasionally be found in South

Africa, Spain, Canada, and Guatemala since it is the third most prevalent genogroup (Pang et al., 2009; Murray et al., 2016; Diez-Valcarce et al., 2019; Varela et al., 2019).

The prevalence of Caliciviruses, such as NoV and SaV, has increased as RV vaccines have been widely used (Hemming et al., 2013; Koo et al., 2013; Payne et al., 2013; Doll et al., 2016). SaV infections are less detrimental to health than NoV or RV infections (Sakai et al., 2001). However, evidence suggests that infections can lead to severe dehydration and hospitalisation (Romani et al., 2012; Bucardo et al., 2014). There have been outbreaks as well as sporadic cases of acute gastroenteritis associated with SaV (Liu et al., 2015; 2016), with a higher prevalence in children than in adults (Liu et al., 2016; Pongsuwanna et al., 2017). The faecal–oral route allows viruses to be transmitted from one individual to another by consuming contaminated food and drinking water, and handling faeces that contains or have been contaminated by SaV (Iizuka et al., 2010; Räsänen et al., 2010; Kitajima et al., 2011; Kobayashi et al., 2012; Lee et al., 2012).

SaVs are typically detected by RT-PCR. It is normally not diagnosed due to costs and limited accessibility to PCR in many parts of the world and generally, diagnosis does not affect treatment. Several multiplex GIT pathogen panel tests now include SaV (Freeman et al., 2017). Epidemiological studies are frequently conducted using SaV genotyping (Sánchez et al., 2018).

2.9 Overview of the GIT and microbiota

There are several distinctly defined anatomical regions of the GIT, extending from the lips to the anus. Microbiology of the GIT can be accessed from the oral cavity to the stomach, small intestine, and large intestine as well as faeces. Most microbiome research on the GIT focuses on faeces, as it is more readily accessible (Mackie et al., 1999). The GIT is inhabited by a microbial community that is characterised by its high population density, extensive diversity, and intricacy of interactions. GIT microbes include all major groups, with bacteria being the most researched. However, a diversity of protozoa is commonly observed in the GIT as well (Clarke, 1977; Dehority, 1997; Hespell et al., 1997).

The human microbiome is a microbial community defined as the sum of all microbial life living in or on the human body (Fricke, 2014). It has expansive metabolic, nutritional, and immunological effects on the host. Throughout a person's lifetime, the microbiome evolves within the host, constantly adjusting to maintain homeostasis with the host's immune system (Gritz & Bhandari, 2015). Shortly after birth, the GIT is colonised with commensal bacteria, which compromise approximately 1 000 species that form significant components of the microbiota ecosystem (Bischoff & Kramer, 2007; Honda & Takeda, 2009).

The virome, or viral microbiome (Oh et al., 2014), is comprised of eukaryotic RNA, DNA viruses and bacteriophages. The virome is relatively unknown, yet there are indications that it plays a role in human health. In addition to being an indicator of paediatric febrile illness and Acquired Immunodeficiency Syndrome (AIDS), anellovirus, a DNA virus originating in eukaryotic cells, is directly associated with host immunosuppression and organ transplant outcome (McElvania Tekippe et al., 2012; De Vlaminck et al., 2013; Li et al., 2013; Béland et al., 2014). As chronic virus infection can confer increased resistance to pathogens (Barton et al., 2007), the ongoing characterisation and annotation of the virome will provide the opportunity to identify novel pathogens, species that are mutualistic and symbiotic, and genetic elements that can be found on host chromosomes derived from viruses (Virgin, 2014).

The mycobiome refers to the commensal fungi and archaea in the human microbiome (Gillevet et al., 2009). Commensal fungi that normally inhabit the human body have been much less studied, as the minority of overall commensal organisms in the human body is formed by fungi. A large number of fungi is also unculturable (Huffnagle & Noverr, 2013). Despite constituting as little as 0.1% of the total microbiome, the fungi found in the microbiome may be crucial to maintaining the structure, function, and immune priming of the microbial community (Qin et al., 2010).

2.9.1 Bacterial diversity in the GIT

The human GIT mucosa is made up of epithelial cells, lamina propria and the muscularis mucosae, which is populated by approximately 10^{14} microbes (Clark & Coopersmith, 2007). As a result of a low pH level and rapid flow rates of the stomach and small intestine, there are relatively few microbes in these regions (10^{3}-10^{5} bacteria/g or mL content). The upper small intestine is predominated by acid-tolerant *lactobacilli* and *streptococci*. As a transition region preceding the large intestine, the distal small intestine (ileum) maintains a more varied microbiota and has a higher number of bacteria (10^{8}/g or mL content) than the upper bowel. Due to its slow turnover, the large intestine (colon) is the primary location of microbial colonisation. It is characterised by high bacteria numbers (10^{10}-10^{11}/g or mL content), low redox potential, and high short-chain fatty acid (SCFA) contents. Additionally, there are distinct spatial patterns of organism distribution within each compartment of the GIT, as well as an increasing gradient of indigenous microbes from the stomach to the colon. A total of four microhabitats are known, including the intestinal lumen, the unstirred mucus layer or gel that covers the entire epithelium of the intestinal tract, the deep mucus layer in the intestinal crypts, and the surface of mucosal epithelial cells (Lee, 1984; Berg, 1996).

2.9.2 Development of the infant GIT microbiome

The in-utero environment was previously regarded as being largely sterile, and that the foetus was only colonised with bacteria at the time of birth (Gritz & Bhandari, 2015). The presence of microbes has been confirmed by both culture-based and culture independent studies in the placenta (Satokari et al., 2009; Aagaard et al., 2014), amniotic fluid (DiGiulio et al., 2008; Oh et al., 2010), foetal membrane (Steel et al., 2005), umbilical cord blood (Jiménez et al., 2005) and meconium (Jiménez et al., 2008; Hu et al., 2013).

Infants born via vaginal delivery initially have skin, GIT, oral, and nasopharyngeal cavities enriched with *Lactobacillus spp*, which is very similar to the mother's vaginal microbiome (Dominguez-Bello et al., 2010). In the case of a caesarean delivery, the infant is colonised by skin and environmental bacteria, such as *Staphylococcus*, *Streptococcus* and *Propionibacteria* (Bäckhed et al., 2015; Dominguez-Bello et al., 2016). In contrast to emergency caesarean delivery, infant GIT microbiome development is different following elective caesarean deliveries. An emergency caesarean delivery is usually laboured, whereas an elective caesarean delivery is not laboured (Chu et al., 2017). The rupture of membranes during labour often results in the neonate being exposed to vaginal microbes, which may explain the difference in the infant's GIT colonisation following the two types of caesarean deliveries (Stinson et al., 2018). Over time, the microbiota changes and adapt to the physiochemical and biochemical composition of each organ in the body, as well as to the availability of certain nutrients (Koenig et al., 2011; Yatsunenko et al., 2012; Lim et al., 2015).

Infants receive their nutrients primarily from either breastmilk or formula feed during their first few months of life. Many benefits to the infant are associated with breastfeeding, including an enhanced resistance to infection (WHO, 2000; Sadeharja et al., 2007), lower risk of obesity (Harder et al., 2005; Weng et al., 2012) or decreased risk of allergies (Greer et al., 2008). Breastmilk is believed to be beneficial due in part to factors secreted in the milk, such as immunoglobulin (Ig) A, lactoferrin, and defensins (Lönnerdal, 2003; Brandtzaeg, 2010). In comparison with formula-fed infants, breastfed infants are enriched with *Bifidobacterium* and *Lactobacillus*, resulting in an increased intestinal acidity as well as a greater quantity of SCFA (Yoshioka et al., 1983). In addition to reducing GIT pH, it also serves to protect against common pathogenic organisms. SCFAs are produced by bacteria in neonates through the fermentation of human milk oligosaccharide, which do not directly enter the host's digestive system, but rather provide a food source for colonic bacteria. Up to 10^7 bacteria can be found in 800 mL of breastmilk, including *Staphylococcus*, *Bifidobacterium, Lactobacillus* and *Streptococcus*, all of which act as inoculants for the neonate (Heikkilä & Saris, 2003; Martin et al., 2003). There has been no definitive determination of the source of these maternal bacteria (Arrieta et al., 2014).

An increase in the variety of dietary substrates consumed by growing infants after a period of contraction of bacterial diversity (Pantoja- Feliciano et al., 2013; Dominguez-Bello et al., 2016), is associated with a change in bacterial composition. This change may also result in an increase in the ability of these bacteria to perform carbohydrate utilisation, amino acid synthesis, and produce vitamins (Bäckhed et al., 2015). By the age of 3, a child's microbiome should be comparable to that of adults in their population (Yatsunenko et al., 2012).

The GIT bacterial microbiome also varies with gestational age. A longitudinal study of preterm infants showed that the pace of the GIT bacterial microbiome development was strongly determined by postconceptional age (La Rosa et al., 2014; Gibson et al., 2016). Preterm infants have a very different, 'sparse' microbiome compared to full-term infants. This developmentally challenged microbiota is dominated by enterococci, *Staphylococcus* coagulase-negative, yeasts and *Enterobacteriaceae*, as very few anaerobes are present (Adlerberth, 2009). Furthermore, a number of pre- and postnatal insults can also adversely affect the GIT microbiome of a preterm infant. Since these infants are delivered rapidly, they are less likely to be exposed to maternal vaginal and enteric bacteria. Mothers at risk of preterm delivery are usually hospitalised for an extended period and receive antibiotic treatment. This may alter the microbiome of the mother and her foetus. Compared with full-term infants, preterm infants are susceptible to several inflammatory factors during pregnancy (prenatal maternal illness, infection, smoking, and physiological stress) and postnatally (formula feeding, antibiotics, and medications that affect pH levels in the gastric area). As a result, they are also exposed to different microbial sources. Additionally, the hospital environment is likely to contain microbes and microbial resistance that differ from those in a home environment. Consequently, many preterm infants develop "neonatal intensive care unit" flora as they require prolonged hospitalisation (Brooks et al., 2014). In addition, preterm infants are commonly fed formula such as cow's milk or fortified human milk, causing their GIT microbiota to be completely different (Poroyko et al., 2011).

The GIT of preterm babies are often underdeveloped, fragile, and vulnerable to complications from prolonged fasting and altered gastrointestinal motility and perfusion. This can lead to difficulties in gastrointestinal colonising and exacerbation of inflammation. The successional development of the microbiota will be influenced by the composition of the gastrointestinal microbiota of infants with very low birth weight, and significant niches will be occupied later in life by bacteria that was established early after birth. Compared to full-term infants, there is usually a delay in developing adult microbiota. The alterations and delays in commensal colonisation early in life may pre-empt gastrointestinal diseases and immune balances may shift. This may result in neurodevelopmental effects and atopic allergies during childhood and later life (Weng & Walker, 2013).

The development of the bacterial microbiome is also influenced by environmental factors, such as geographical location, family interactions and dust. More similarities are found in the bacterial microbiomes of individuals residing in the same household compared to those that do not (Yatsunenko et al., 2012). Firstborn children tend to have a less rich and diverse bacterial microbiome than children with a higher birth order, suggesting a transfer of bacteria from interactions with older siblings and / or modified sanitation practices of the parents as more children are born in the family (Laursen et al., 2015). There is evidence that the practice of sucking a baby's pacifier in order to clean it results in altered oral microbiota in infants, as well as a lower risk of allergies (Hesselmar et al., 2013). Moreover, early exposure to animals and pets can have a protective effect on infants, since exposure to microbes stimulates the development of immune tolerance (Tamburini et al., 2016), and also decrease the occurrence of allergies and asthma (Ownby et al., 2002), although the mechanisms for this are not yet well understood. The decrease in *Lactobacillus*, *Bifidobacterium adolecentis*, and *Clostridioides difficile (C. difficile)* during the first 2 months of life, as well as the proliferation of *Bifidobacterium* in infants, is attributed to larger household sizes and increased exposure to dust, which is linked to the development of allergies (Sjögren et al., 2009).

Only recently has the interaction between microbiota and host genetics been recognised to play a major role in human disease. When a diverse gastrointestinal microbiota composition is associated with metabolic disorders that a have genetic component (Herbert et al., 2006; Frayling et al., 2007; Turnbaugh et al., 2009; Qin et al., 2012; Karlsson et al., 2013), it is suggested that the host impaired bacterial regulation is a likely mechanism of pathogenesis. It is very difficult to interpret the interaction between host genetics and microbiota due to the large number of interactions between numerous bacterial species and numerous genetic polymorphisms (Knights et al., 2014).

2.9.3 Effect of antibiotic use on the microbiome

Antibiotic treatment targets not only pathogenic microorganisms but also the host-associated microbial communities in the GIT. Antibiotics are considered effective in treating a wide range of health conditions since most antibiotics possess a broad spectrum activity, but in addition to destroying pathogenic bacteria, other microbes are also affected. This results in a lasting negative impact on the microbiota of the GIT (Jernberg et al., 2007).

Exposure to antibiotics and its influence on the human microbiome can begin in utero and continue through critical stages of foetal growth and development (Yang et al., 2019). The developing infant bacterial microbiome and accompanying enteric pathogen colonisation can be significantly altered by antibiotic usage and may fail to recover fully (Dethlefsen et al., 2008; Fouhy et al., 2012). Antibiotic use by pregnant women is linked to altered microbial compositions, depending on the type

of antibiotic administered (Azad et al., 2016; Coker et al., 2020). Additionally, it has been associated with developmental and cognitive impairments (Kenyon et al., 2008), diabetes development and immunological changes (Tormo Badia et al., 2014) and obesity (Mueller et al., 2015). Antibiotic use during infancy and childhood has been linked to altered metabolic functions and a change in the microbial composition (Korpela et al., 2016), as well as an increased risk for the development of asthma and allergies (Ni et al., 2019; Yamamoto-Hanada et al., 2017), and obesity (Bailey et al., 2014) later in life.

The adult bacterial microbiome, however, seems to be more resilient to disruption by antibiotic usage (Lozupone et al, 2012), and will eventually recover despite sudden changes. A mouse model has been used to demonstrate that the microbial balance amongst bacteria, viruses and fungi can also be altered by antibiotic treatment. It can cause gastrointestinal fungal cells (*Candida albicans*) to multiply, causing a host of lung disorders facilitated by mast cell activation, interleukin (IL)-5, IL-13, and various inflammation-related mediators (Noverr et al., 2005). This may also be the reason for impaired immune responses to viral infections in humans (Gonzales-Perez et al., 2016).

2.10 Impact of infant microbiota on health

The composition of the neonatal gastrointestinal microbiota plays an important role in the development of the immune system, central nervous system (CNS), and metabolic processing of nutrients (Yao et al., 2021).

2.10.1 Immune system development

The mucosal surfaces in neonates and adults differ considerably. Among respiratory and digestive epithelia, permeability is much higher, and the neonatal epithelium does not produce antimicrobial peptides and enzymes. In addition, the pH in the infant stomach is higher, and the composition of the secreted mucus layer, as well as its glycosylation, differ from that of adults. Infants have fewer innate and acquired immune cells, including granulocytes, neutrophils, eosinophils, lymphocytes, and regulatory T cells, which are less able to produce antibodies, cytokines, and chemokines. It is particularly apparent in the body's largest immune tissue, the GIT (Martin et al., 2010). Upon pathogen invasion, the neonate's immune system preferentially develops tolerance, which is also why the immune system of a neonate is different from that of an adult (Forsthuber et al., 1996; Ridge et al., 1996; Sarzotti et al., 1996).

As gastrointestinal microbiota digest exogenous, and ferment endogenous compounds, metabolites are produced, which can act directly on mucosal epithelial cells or enter the bloodstream. In order to modulate immunity, these metabolites may act through the following mechanisms:

(i) microbes in the GIT digest fibre in food, producing SCFAs, which have a variety of immune-modulating properties;

(ii) specific host receptors bind to GIT microbes, such as aryl hydrocarbon receptors (AHR), G protein-coupled receptors 41 and 43, Toll-like receptors, and Pregnane X receptors;

(iii) polyamines, which include spermine, putrescine, and spermidine, play an important role in gene transcription and translation and are found in the majority of cells.

Pattern recognition receptors (PRR) are critical elements of innate immunity that detect, recognise, and coordinate self- and non-self-antigens (Rooks & Garrett, 2016). It is possible for these receptors to respond to peptidoglycans, lipopolysaccharides, formyl peptides, nucleic acids, and flagellins. A signal cascade linked to PRRs triggers the release of chemokines, apoptotic factors, and cytokines, possibly contributing to the development of disease (Yuan et al., 2021).

Secretory IgA (sIgA) serves a vital regulatory function in this priming and immune maturation process. An important component of maintaining the diversity within the bacterial communities inhabiting the GIT mucosa is the presence of sIgA which targets a significant number of GIT bacteria and modulate their growth. Through the selective modulatory activity of sIgA, maternal antibodies must work together to support the development of an infant's immune system from the earliest stages of life (Levi Mortera et al., 2016).

2.10.2 Gut-brain axis

During early development, the infant brain gradually develops axons and dendrites, forms synapses, expands neurogliocytes, and becomes myelinated. Therefore, the first two years of life are also critical for establishing the GIT microbiota, as the infant's brain develops optimally when its GIT microbiota has been established (Diaz, 2016). The CNS is considered to be immune-privileged. Throughout the brain, blood vessels are formed by endothelial cells and tight junctions that form the blood-brain barrier (BBB). The BBB strictly controls movement of molecules, ions, and cells between the periphery and the brain (Engelhardt & Liebner, 2014). The BBB further protects the brain, neurons and their connections from pathogens and unwanted immune responses (Daneman & Prat, 2015). The CNS, intestine, and microbiota communicate through a complex bidirectional communication network called the Gut-Brain Axis (GBA), which mediates communication between intestines and the CNS (Sudo et al., 2004; Skonieczna-Żydecka et al., 2018). A range of pathways are involved in this axis, including the autonomic and enteric nervous systems, the endocrine system, the hypothalamic-pituitary-adrenal axis, the immune system, as well as the microbiota and its metabolites (Duvallet et al., 2017; Blacher et al., 2019; Burberry et al., 2020).

A number of neurotransmitters and metabolic products, such as essential vitamins, secondary bile acids, amino acids, and SCFAs, modulate several immune pathways, which affect behaviour, memory, learning, locomotion, and neurodegenerative diseases (Peng et al., 2009; Yano et al., 2015; Ellwardt et al., 2016; Engelhardt et al., 2016; Jenkins et al., 2016; Kipnis, 2016; O'Keefe, 2016; Kennedy et al., 2017; Mertens et al., 2017; Mittal et al., 2017; Skonieczna-Żydecka et al., 2018; Baj et al., 2019; Dalile et al., 2019; Feng et al., 2021).

2.11 An overview of methods for studying gastrointestinal bacteria

A reductionist approach was used in the past to study the human microbiome, using culture media and microscopes to identify and characterise single bacteria strains. Only culturable bacteria were initially identified and classified phylogenetically. Over 40% of GIT bacteria cannot survive outside their natural habitat and the application of culture-dependent as well as culture-independent analytical methods has therefore improved the understanding of gastrointestinal microbiota. The recent development of next-generation sequencing (NGS) in biological science has been a significant advancement. It uses metagenomic techniques based on 16S ribosomal RNA (16S rRNA) gene amplification by PCR and whole-genome sequencing (WGS) to study and define the human gastrointestinal microbiota (Singh, 2021).

2.11.1 Culture-based methods

For the identification of new species, culture-based methods are still widely regarded as the gold standard protocol. These are reliable and inexpensive methods for identifying bacteria, however, their effectiveness cannot be guaranteed against anaerobic and non-amenable bacteria (Singh, 2021). Furthermore, the value in detecting changes in the microbiota profiles is limited, due to a major underestimation of the diversity of bacteria present in the intestinal luminal contents. Consequently, these methods are not best suited to study gastrointestinal microbiota profiles and are only used for the analysis of individual culturable bacterial groups in specific clinical settings. In the late 20th century, several molecular approaches have been developed to overcome the limitations of culture technology and the study of bacterial genomic material and identification is now based on 16S rRNA sequences of the bacteria (Sarangi et al., 2019).

2.11.2 Culture-independent methods

The metagenomic technique was the first method of phylogenetically identifying 80% of uncultured microbes. The development of this culture-independent technique for the identification of microorganisms has revolutionised research into human microbiotas. 16S Amplicon sequencing protocols involve amplifying species-specific 1500-bp-long whole 16S rRNA genes by PCR using

nucleic acid extracted from the sample (Olsen et al., 1986). Additionally, it includes hypervariable regions, specifically, the V4-V5 region as a subset of the nine short hypervariable regions, from V1-V9. DNA fragments are physically separated from each other using gel electrophoresis following PCR-based amplification with universal and specific primers (Lane et al., 1985). In contrast, shotgun DNA sequencing enables the analysis of metagenomics by sequencing the entire genome rather than amplifying specific gene regions (Quince et al., 2017). This application allows identification of a microbial community at the species/strain level by identifying all organisms present within it. Using this technique, it is possible to determine the biological functions of the microbiota present in the sample. Furthermore, the PCR does not amplify specific regions of the genome, thereby avoiding any bias arising from the amplification step itself or during primer selection (Hodkinson and Grice, 2015).

While amplicon sequencing can be used to characterize and compare communities, the use of specific primers and multiple amplification cycles introduce inherent biases (Hodkinson and Grice, 2015). The cost of shotgun DNA sequencing is higher than 16S profiling, as it generates massive amounts of sequence data. Data analysis requires highly trained experts as well as expensive equipment and facilities to deal with the vast amounts of data generated. Furthermore, a large amount of information has been accumulated by several studies with regard to 16S profiling; however, due to the absence of a database on uncultured microorganisms and their functions, it is difficult to obtain the complete biological information by comparing the sequences obtained by experiments to those deposited in the database. Due to this, numerous attempts are being made to gather information regarding functional genes and to construct a database profile of the entire microbial genome. In addition, metagenomic analyses, which are based on DNA, do not reflect viable microbial communities, as the DNA used is extracted from microbial organisms that may or may not be alive at the time of analysis (Ercolini, 2013).

With the advent of DNA NGS, metagenomic and WGS methods have become more sophisticated and rapid. Recent methods such as 454 pyrosequencing, Illumina, SOLiD, Ion Torrent, and single-molecule real-time (SMRT) circular consensus sequencing equipment from Pacific Biosciences (Lu et al., 2009) and Oxford Nanopore Technology (ONT) have provided a deeper understanding of the gastrointestinal microbiome through improved speed and analytic power (Nicholls et al., 2019). Using MinION™ nanopore sequencing technology, ONT has demonstrated that it can overcome PCR limitations when sampling gastrointestinal microbes, this includes temperature variations, cloning, and long and deep sequencing. (Singh, 2021). In addition to phylogenetic analysis, NGS analysis can also be used for the functional analysis of microbial communities. It provides a framework for omics-based methods, such as metatranscriptomics, metaproteomics, and metabolomics, which have

enabled increased understanding of the function of metagenomes comprising whole microbial communities (Petriz & Franco, 2017).

2.12 Post-mortem bacteriology

2.12.1 Post-mortem samples: source of bacteria

In addition to causing disease, bacteria can also travel through the blood without affecting health. At room temperature a deceased body will be putrefied due to post-mortem bacterial growth and tissue invasion, and contamination may occur during or after death (Weinbaum et al., 1997; Weinstein, 2003). Furthermore, in the course of death, the mucosal surfaces become ischaemic, and the immune system becomes compromised, favouring bacterial invasion (Morris et al., 2006).

Bacteria can be isolated from post-mortem mucosal and epithelial surfaces, including the GIT, oral cavity, and respiratory tract. The presence of the bacteria may be explained by either genuine positivity, agonal spreading, post-mortem translocation, or contamination (Morris et al., 2006). In the case of genuine positivity, bacteria are found in the body throughout life, and are still detectable after death. These may not necessarily result in disease; however, if an infection occurs prior to death and during the period of death, it cannot be ruled out as a contributing cause. In the case of agonal spread, bacteria are introduced during death or artificial resuscitation. Therefore, mixed bacterial growth may result from decreased mucosal integrity, leading to bacterial invasion. Post-mortem translocation refers to the movement of bacteria from the mucosal surface to blood and tissues following death and cessation of circulation (Morris et al., 2006). Consequently, this leads to polymicrobial growth in cultures and reduces the sterility of samples (Wilson et al., 1993). Lastly, contamination during sample collection by external sources may also result in mixed growths of microbes (Morris et al., 2006).

2.12.2 Effect of post-mortem interval

It is challenging to determine the contribution of a detected microorganism to death following post-mortem translocation, because the presence of a potentially pathogenic microorganism may result from an ante-mortem bacterial infection or a post-mortem event (Balzan et al., 2007; Yatsunenko et al., 2012). An increased post-mortem interval (PMI) is commonly associated with more positive cultures and an increased number of microbes isolated per culture (Wilson et al., 1993). Post-mortem translocation is less likely to occur if the body is stored at low temperatures or retrieved relatively soon after death (Morris et al., 2006). Weber et al. (2010) reported that a prolonged PMI does not result in a higher frequency of positive cultures or mixed-growth episodes as had been hypothesised with post-mortem translocations. Extended PMIs may therefore cause the death of microbes. While

interpreting positive microbiological culture results in SUDI post-mortem samples, a prolonged PMI of several days has not been shown to be associated with an increased risk of translocation. It is therefore recommended that routine microbiological sampling be conducted in all autopsies of SUDI cases, regardless of the PMI as more research is required into bacterial behaviour after death.

2.12.3 Post-mortem interpretation of microbiology

In addition to providing valuable information, microbiological cultures can sometimes raise some doubts. The most common challenge is determining whether the organism colonises or infects. Bacteria are sometimes isolated in the absence of signs and symptoms of disease, while in other instances, microbial cultures are negative in the presence of acute symptoms of an infectious disease. Interpretation of post-mortem microbiological tests is even more challenging, because their quality depends on a number of factors, namely the post-mortem interval, antibiotic treatment, and methods used to collect samples. Contamination may occur when specimens are not obtained in a sterile manner. Microbiological results can also be affected by the manner in which the body was handled after death as well as storage conditions. Consequently, the results could be compromised by microorganisms that colonize one part of the body but not another, which would result in a reduction in the quality of the results and a more complicated final assessment (Fujita et al., 2002; Morris et al., 2007; Pryce et al., 2011; Christoffersen, 2015; Saegeman et al., 2017; Fernandes-Rodrigues et al., 2019).

In SUDI, viral detection depends on a number of factors, including the methods used, the method of sample collection, which viruses are tested, and whether the results are meaningful or not. A prolonged post-mortem interval may affect virus viability and nucleic acid integrity, resulting in an inability to detect viruses during molecular testing and culture (Weber et al., 2010).

2.12.4 The interpretation of a true pathogen in a post-mortem setting

Different diets, lifestyles, and geographical locations significantly affect the microbial ecology of a dead human body (Finley et al., 2015; Hauther et al., 2015). Microbiology results from post-mortem examinations may reflect an actual infection, but they may also occur as a result of contamination, commensal bacteria, and/or post-mortem bacterial translocation (PMBT) (Saegeman et al., 2021).

Post-mortem bacterial translocation results in the proliferation and migration of endogenous commensal gut bacteria, such as Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Enterococcus, and Clostridium species. Putrefactive decomposition is primarily caused by PMBT, which occurs approximately four minutes after death and is not primarily determined by the postmortem interval (Christoffersen, 2015; Finley et al., 2015; Bucheli & Lynne, 2016; Burcham et

al.,2016). Putrefaction begins with aerobic bacteria but is rapidly overtaken by anaerobes as time progresses. In most cases, peripheral blood samples are contaminated by coagulase-negative staphylococci that are introduced into the sample from the skin (Palmiere et al., 2016). The presence of mixed bacterial growth generally indicates contamination of the sample, whereas the presence of a single isolate is typically interpreted as a true positive (Morris et al., 2007; Lobmaier et al., 2009; Weber & Sebire, 2009). The presence of a single microorganism from a number of different sites may still indicate contamination rather than infection, even when samples are taken according to protocol (Palmiere et al., 2015; Palmiere et al.,2016). However, the identification of a pathogenic organism from multiple locations at autopsy indicates true ante-mortem bacteremia (Palmiere et al., 2015). Post-mortem microbiology yields are typically reduced with prolonged PMI and putrefaction (Saegeman et al., 2017).

2.13 The microbiome and SUDI

In forensic and paediatric medicine, it is essential to determine the cause of SUDI (García et al., 2020). This is the most frequent cause of infant death (Carlin & Moon, 2017; Oliveira & Amorim, 2018; Young & Shipstone, 2018; Horne, 2019) and accounts for 40–50% of all infant deaths in developed countries. The incidence is highest in the first few months of life and during the first year (Wu, 2010). The immunity and physiological processes of the host are influenced by microbiota and the relationship between changes in the GIT microbiota during neonatal development and paediatric disorders (Rautava et al., 2012) has profound implications for human health (Clemente et al., 2012). Weber et al. published multiple studies of unexplained SUDI, non-infectious explained sudden infant deaths, and bacterial-caused SUDI (Weber et al., 2008, 2010, 2011). An increase in bacterial isolates of *Streptococcus pneumoniae*, *S. aureus*, *Neisseria meningitidis*, beta-haemolytic *Streptococcus* type A and B, and *E. coli* was found in infants whose deaths were explained by non-infectious causes (Weber et al., 2008). No significant difference was found in the detection of viruses between sudden unexplained deaths and sudden deaths due to non-infectious causes, according to the results of virological testing (Weber et al., 2010).

Highet et al. (2014) examined the intestinal contents of 52 SUDI cases and 102 samples from faeces of age- and sex-matched control infants. In SUDI babies, alterations in the GIT microbiome caused by increasing age were evident. Compared to the controls, SUDI samples showed significant increases in *Bacteroides thetaiotaomicron*, *Clostridium innocuum* (*C. innocuum*), and *C. difficile*. The number of cases with dual colonisation was significantly greater in SUDI than in healthy infant samples. The incidence of triple colonisation with *C. difficile*, *C. perfringens*, and *C. innocuum* was also significantly higher. SUDI babies placed to sleep in the prone position were more likely to contract *S. aureus* than those positioned on their sides or in the supine position. Additionally, more

than half of the organisms were isolated from sterile environments. In summary, it is still unclear whether the microbiomes of SUDI and healthy babies differ in a way that makes infants more prone to infection and, consequently, sudden death, and should be elucidated with larger and longer studies (Leong et al., 2017).

2.14 Research aims and objectives

The overall aim of this study was to investigate the bacterial as well as viral pathogens of the GIT in the medico-legal environment at the Tygerberg Medico-Legal Mortuary in the Western Cape, in order to assess the role of GIT colonisation in the process of determining the cause of death in cases of SUDI over a one-year period (June 2017-May 2018). Demographic data was also collected and analysed to assess potential risk factors for SUDI in this specific infant population (Chapter 3).

2.14.1 Aim I

Profile the bacterial and viral pathogens present in the GIT of SUDI cases admitted to the Tygerberg Medico-legal Mortuary, Cape Town.

Objectives

- To sub-type *E. coli* positive stool and swabs from different sections of the GIT in SUDI cases (Chapter 4).
- To detect gastrointestinal viruses in the stool collected from the SUDI cases (Chapter 5).
- To characterise the human group A RV genotypes in SUDI cases (Chapter 6).

2.14.2 Aim II

Profile the microbiome in stool samples collected from SUDI cases and age-matched, healthy infants with comparable sociodemographic status as the SUDI cases (Chapter 7).

Objectives

- To use full-length 16S rRNA gene amplicon sequencing to profile the microbiome
- To describe alpha and beta diversity in the different infant groups.

Chapter 3: Characterisation of sociodemographic and risk factors in Sudden Unexpected Death in Infancy cases at the Tygerberg Medico-legal Mortuary, Cape Town

Abstract

Background: Literature suggests that sudden unexpected death in infancy (SUDI) results from the combination of three individual risk factors, namely a susceptible infant, a critical developmental stage for homeostasis, and an external stressor. Investigation of risk factors is crucial for identifying the cause and manner of death in each case of SUDI. The aim of this study was to assess if there was any correlation between sociodemographic data and possible risk factors for SUDI in cases admitted to the Tygerberg Medico-legal Mortuary over a 1-year period.

Materials and methods: A total of 186 SUDI cases were included in the study, and demographic and epidemiological data were collected. Statistical analysis was conducted to determine if sociodemographic factors were associated with the causes of death.

Results: Male infants constituted 58% of the cases for this particular study period. At the time of death, the median age of the infants was 8.4 weeks, and most of the deaths occurred during the colder months of the year (66%). Among the sleep environment-related risk factors, bed-sharing accounted for 96%, while based on birth-related risk factors, 43% of babies were born premature. Age in weeks and the cause of death ($p=0.007$) showed a highly significant association.

Conclusion: Sociodemographic and risk factors derived from this study appear to be in good agreement with the risk factors published in the literature. A valuable preventive measure is education and awareness of modifiable risks in the community.

3.1 Introduction

The *Triple Risk Model* described in 1994 indicates that SUDI occurs when infants with latent vulnerabilities, e.g., brain stem or genetic abnormalities, are exposed to a trigger or extrinsic risk factor (prone sleeping, obstructions of the airway) during a critical developmental phase (Filiano & Kinney, 1994). The breathing, autonomic, and cardiac processes develop between the ages of two and four months, and a combination of intrinsic and extrinsic factors may lead to a life-threatening condition while the infant is sleeping. There is a significantly increased likelihood of SUDI occurring if protective mechanisms are ineffective during these episodes. However, if one of these factors is eliminated, SUDI will be less likely to occur (Moon et al., 2007; Goldstein et al., 2016).

Extrinsic risk factors refer to physiological stressors that were experienced by the infant at the time of death, often caused by the infant's environment. Among these factors are sleeping in prone position, bedsharing, overheating, using soft bedding, sleeping on inappropriate surfaces, and covering the face of an infant while sleeping. Infants are more vulnerable to the influences of extrinsic risks due to intrinsic risk factors (Duncan & Byard, 2018). Several intrinsic factors can contribute to the risk of SUDI, including male sex (Lewak et al., 1979), prematurity (Horne, 2006), maternal alcohol consumption, and tobacco use (Schoendorf & Kiely, 1992; Strandberg-Larsen, 2009).

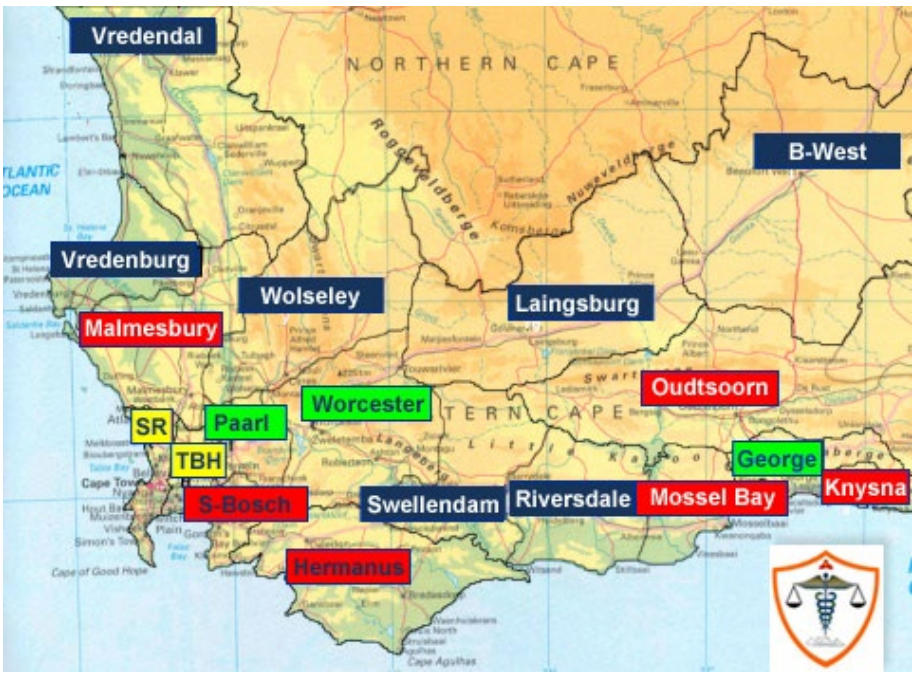
A significant advancement has been the identification of factors that may be modified, particularly those that relate to the care of infants (Mitchell et al., 1992). As a result of the *Back to Sleep* campaigns that were implemented in the 1990s and early 2000s, SUDI rates have declined substantially (Hauck & Tanabe, 2008; 2010), however, it remains a global burden, despite a reduction in absolute numbers (Heron et al., 2009).

It is crucial to investigate risk factors and circumstances for each case of SUDI in order to diagnose the cause and manner of death. Additionally, an analysis of the aggregate results of these investigations might assist in preventing or limiting the occurrence of SUDI in the future (Hunt & Hauck, 2006). The study investigated potential associations between sociodemographic data and possible risk factors, and SUDI.

3.2 Materials and methods

3.2.1 Study setting

The Tygerberg Medico-legal Mortuary is an academic forensic pathology mortuary in Cape Town, a city of approximately 4.8 million people. This facility serves the Eastern Metropole of the City of Cape Town referral areas, including Khayelitsha, Tygerberg and the Eastern and Northern Sub Districts (Figures 3.1 and 3.2).



(SR=Salt River Mortuary; TBH=Tygerberg Hospital and Medico-legal Mortuary)

Figure 3.1: Forensic Pathology centres in the Western Cape (Western Cape Government, 2014)

Figure 3.2: The referral areas for the Tygerberg Forensic Pathology Medico-legal Mortuary depicted by the red outline (Western Cape Provisional Government, 2020)

The prevalence of SUDI in Cape Town was recently reported to be 3.7 per 1 000 live births (Elliot et al., 2020). In 2019, approximately 46% of the families residing in the City of Cape Town lived below the poverty line with a monthly income of less than R2 000, while a 2016 community survey showed that 18% of the population lived in informal dwellings, less than 1% had no access to sanitation and 1.3% had no access to electricity (City of Cape Town Profile and Analysis District Development Model, 2020). The Tygerberg Medico-legal Mortuary has an average case load of approximately 3 000-4 000 cases per annum of which infant deaths comprise 5-8% (personal communication with Dr Zandré Smith, Specialist Forensic Pathologist, 24/03/2023).

3.2.2 Ethics

The study was approved by the Stellenbosch University Health Research Ethics Committee (S16/10/214) and a waiver of consent was granted for the SUDI cases (Appendix A). Consent was obtained pursuant to section 3(a) of the Inquests Act (58 of 1959) and the Criminal Procedure Act (1977). The Western Cape Forensic Pathology Service, SUDI Questionnaire FPS006(b) is routinely completed at the time the infant is admitted to the Tygerberg Medico-legal Mortuary (Appendix B).

As for the control group, informed consent was obtained from the parents or caregivers to non-invasively collect stool samples from soiled nappies and a questionnaire was subsequently completed by all consentees to collect information similar to the SUDI cases (Appendix C).

3.2.3 Sample collection

Between June 2017 and May 2018, swabs were collected during autopsies from the ileum, colon, and rectum of 186 SUDI cases admitted to the Tygerberg Medico-legal Mortuary in Cape Town, along with stool samples taken through an incision in the large intestine. The swabs were placed in a Cary-Blair transport medium and the stool samples were transported in leak-proof containers to the National Laboratory Health Service (NHLS) Medical Microbiology laboratory at Tygerberg Hospital within 3 hours of collection. For each case, demographic information was collected from the Forensic Pathology case files. These samples were collected and tested with the intention of reporting the results to the Division of Forensic Pathology in order to assist in the process of determining the cause of death.

Baby sanctuaries and day-care centres provided stool samples from 45 apparently healthy and age-matched infants to serve as controls. Samples were submitted to the NHLS Medical Microbiology laboratory at Tygerberg Hospital within 24 hours of collection.

3.2.4 Cause of death determination

The South African Inquests Act (No. 58 of 1959) stipulates that all unnatural deaths, including SUDI, are required to undergo a medico-legal investigation. A comprehensive investigation into the cause of the infant's death includes external, internal, and X-ray examinations, along with measuring the infant's body to determine how he or she developed (Bajanowski et al., 2007). It is necessary to conduct three key investigations in SUDI, i.e., a review of the clinical history, an investigation of the death scene, and a post-mortem examination, which includes microbiology and virology testing. Death scene investigations in unexpected deaths are vital to determine risk factors and, possibly, indicate the cause of death. In most cases, macroscopic autopsies cannot reveal morphological changes related to the cause of death (Vorontsov & Kelmanson, 1990). In addition, all autopsies include histological examinations of major organ systems, and toxicology testing, as indicated (Dempers et al., 2016). If no cause of death can be ascertained through the medico-legal investigation in SUDI cases, the case is classified as sudden infant death syndrome (SIDS), which represents a diagnosis by exclusion (Willinger et al., 1991).

3.2.5 Reviewing of case files

A Microsoft Office® Excel spreadsheet was used to capture demographic and epidemiological data from all SUDI cases included in this study. Information collected included PMI, season, sex, area in which they reside (by sub district), age in weeks, prematurity, birth weight, feeding method , amount of people in the household, type of housing, medical history prior to death and cause of death the pathologists assigned to the cases.

3.2.6 Statistical analysis

Statistical analysis was performed using R software version 4.2.2. Associations were calculated using the Fisher Exact test for categorical data and Kruskal-Wallis rank test for numerical data. Statistical significance was observed at $p<0.05$ at a 95% confidence interval, with a strong significance observed at $p<0.01$. The categorical data included sex, prematurity, low birth weight, bedsharing, type of housing, season, the position the infant was placed to sleep, and cause of death, while the numerical data included age and birth weight.

3.3 Results

3.3.1 Sociodemographic profiles of the SUDI cases

Due to incomplete questionnaires, information regarding the infant's death was not captured in every case.

The male to female ratio was 1.4:1, with 107 (58%) male cases. The median age of the infants at the time of death was 8.4 weeks (interquartile range [IQR]: 5.8–16.8) and the mean post-mortem interval (PMI) was 7±3.5 days. The majority of the cases (65.6%) presented during the colder months of the year (mid-March to mid-September) (Figure 3.3).

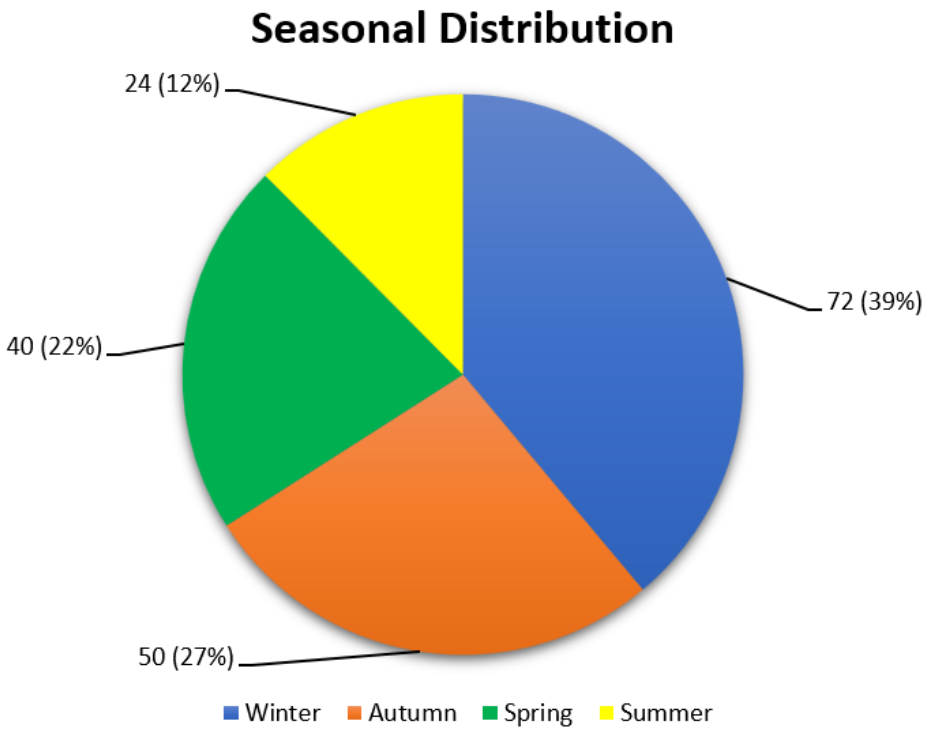


Figure 3.3: The seasonal distribution of the SUDI cases from June 2017 to May 2018, showing an increase of cases during autumn and winter

3.3.2 Risk factors identified in the SUDI cases

Risk factors associated with the sleep environment of the infant included bed-sharing in 96% of cases (154/161) and side sleeping position in 61% (91/150). Fifty-two percent (77/149) cases were reported to live in informal housing (Figure 3.4). Birth-related risk factors comprised 43% (74/172) of the babies being born prematurely and 47% (78/165) had birth weights of less than 2 500 g, representing low birth weight infants.

3.3.3 Sociodemographic profile of the control group

Some sociodemographic information was not available for all controls. This may be due to the fact that most of them live in baby sanctuaries and the caregiver was unaware of this information at the time of sample collection.

The control group consisted of 24 male and 21 female infants, with a median age of 10.5 weeks (IQR: 5.3–13). Bed-sharing was reported in 12% (4/34) of the controls, with side sleeping position in 66% (19/29). Thirty-three percent (8/24) of the infants were born prematurely.

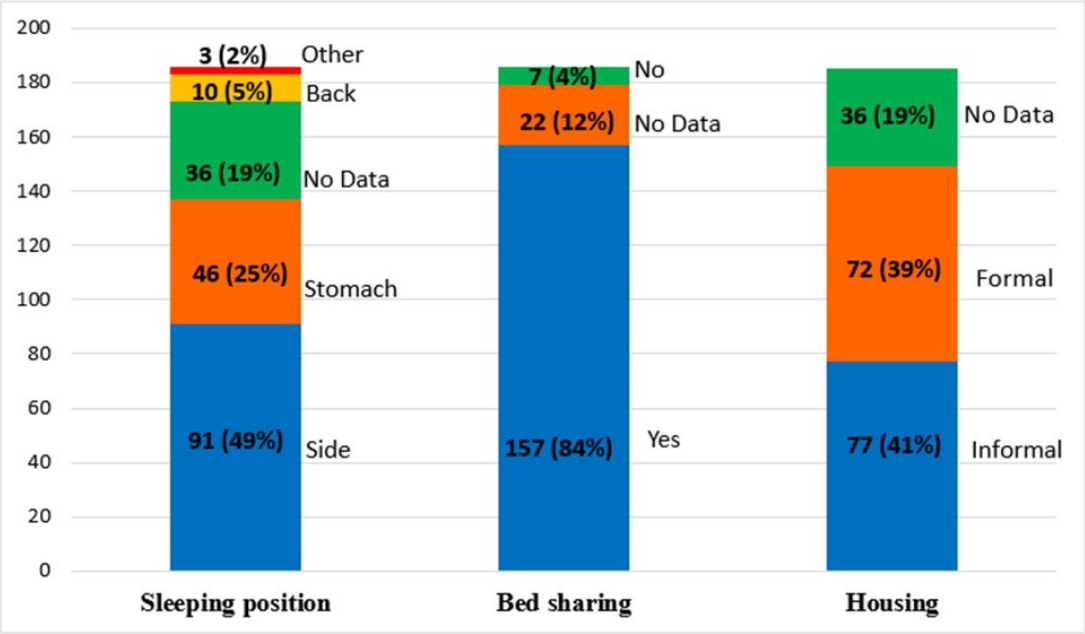


Figure 3.4: Distribution of the position the infant was placed to sleep, bed-sharing and housing reported in the SUDI group (n=186)

3.3.4 Final cause of death classification in the SUDI cases

At the date of submitting this thesis, the final cause of death had not been assigned for 1 of the 186 SUDI cases. The most common cause of death in the remainder of cases was infection in 58% (107/185) of cases, followed by SIDS in 41% (76/185), while 1% (2/185) died of other causes (bronchopneumonia secondary to heart failure and cardiomyopathy and complications of prematurity in another).

3.4 Statistical analysis

3.4.1 Sociodemographic data and potential risk factors for SUDI

This study compared sociodemographic risk factors with the final cause of death, i.e., SIDS, infection, and other.

Table 3.1 summarises the sociodemographic factors for the three different final cause of death classifications. No significant association could be demonstrated between cause of death classification and categorical sociodemographic factors such as sex, prematurity, type of housing, seasonality, low birth weight, bedsharing, and the position the infant was placed to sleep. The actual birth weight in gram and final cause of death did not show any significant association (p=0.73) either.

The only highly significant association was observed between age in weeks and final cause of death (p=0.009) where infants under the age of 13 weeks were more often assigned SIDS as final cause of death 6 weeks (interquartile range [IQR]: 7.16–12.44) compared to Infection 10 weeks (interquartile

range [IQR]: 11.89–12.44)) (Table 3.1, Figure 3.5). The ages of the two infants that died of other causes were 10.7 and 41.1 weeks respectively.

Table 3.1: Associations between sociodemographic information and the different cause of death classification groups (n=185)

	Final cause of death			
	SIDS	**Infection**	**Other**	
Number of cases	**76**	**107**	**2**	
Age in weeks (Median, (IQR))	6 (7.16–12.44)	10 (11.89–12.44)		**0.009**
Risk factors*	**n (%)****	**n (%)****	**n (%)****	**p-value**
Sex *(n=127)*: Male Sex	18 (14.2%)	37 (29.1%)	1 (0.8%)	**0.43**
Gestation *(n=172)*: Prematurity	27 (15.7%)	43 (25.0%)	2 (1.2%)	**0.20**
Birth weight *(n=165)*: Low birth weight	32 (19.4%)	45 (27.3%)	1 (0.6%)	**0.97**
Bedsharing *(n=161)*: Yes	64 (39.8%)	87 (54.0%)	2 (1.2%)	**0.31**
Position placed to sleep *(n=185)*				**0.22**
Side	45 (24.3%)	26 (14.1%)	0 (0.0%)	
Prone	91 (49.2%)	49 (26.5%)	2 (1.1%)	
Supine	10 (5.4%)	7 (3.8%)	0 (0.0%)	
Housing *(n=149)*: Informal	30 (20.1%)	46 (30.1%)	1 (0.7%)	**0.99**
Seasons *(n=185)*				**0.08**
Summer	6 (3.2%)	18 (9.7%)	0 (0.0%)	
Autumn	16 (8.7%)	34 (19.4%)	0 (0.0%)	
Winter	33 (17.8%)	36 (19.5%)	2 (1.0%)	
Spring	21 (11.4%)	19 (10.3%)	0 (0.0%)	

* Number in parenthesis represents the total number of observations available per risk factor
** Percentages were calculated out of the total number of observations available per risk factor

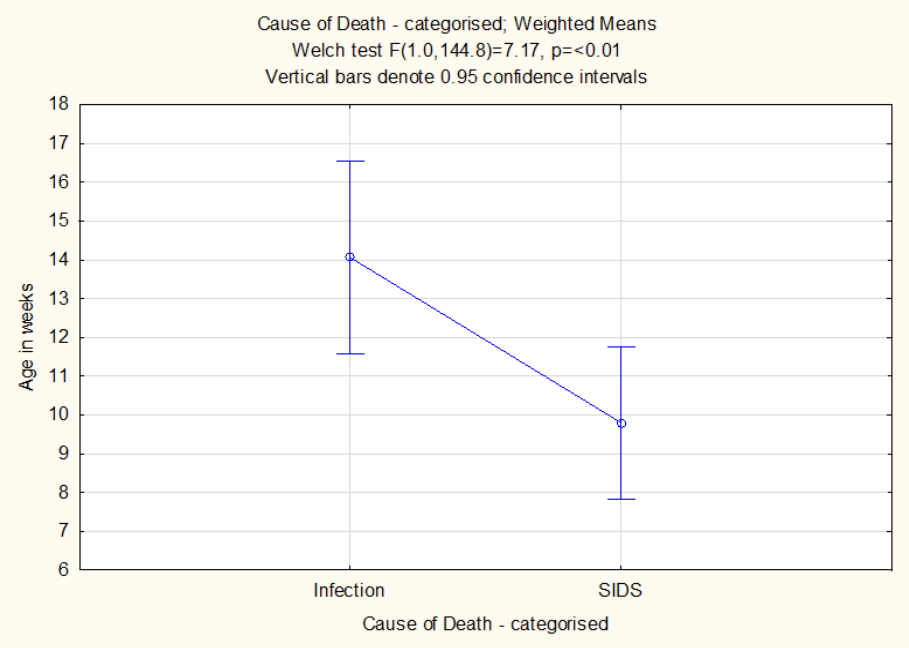


Figure 3.5: Highly significant association between the Age in weeks and Cause of death ($p < 0.01$)

3.5 Discussion

In infants, sudden death has long been proposed as a multifactorial phenomenon, involving the interaction of a variety of factors, each of which on its own might not be sufficient to cause death, but can be a contributing element to sudden death when combined with other factors (Bergman, 1970). As part of this study, the socio-demographic profile of the study population were characterised, and the risk factors were compared across the different cause of death categories.

In the literature, male infants account for approximately 60% of SUDI cases (Jørgensen et al., 1979; Fleming et al., 2015). This was also the case in the current study, where 58% of the SUDI cases were males, while 21% of the cases assigned a final cause of death of SIDS were males, compared to 31% males in the Infection group. There are several theories postulating why male infants are most affected by SUDI. Generally, females have higher Ig levels, suggesting better antigen response and humoral immunity to infection (Verthelyi, 2001; Whitacre, 2001; Fish, 2008). The cytokine response of males has also been found to be altered, which is associated with sudden unexpected deaths, SUDI, sepsis mortality, and trauma-related deaths, probably due to the influence of oestrogen and testosterone (Choudhry et al., 2007). The medullae of male infants are more likely to exhibit increased apoptotic neuronal cell death than females, altering arousal pathways and subsequently increasing the risk of SUDI (Paterson et al, 2006; Machaalani & Waters, 2008). Moscovis et al (2014) evaluated the effects of cigarette smoke, virus infection, and male sex on inflammatory responses in neonates at risk of SUDI and found that male infants had lower pro-inflammatory cytokine responses than females, suggests that a reduced pro-inflammatory response may limit damage when pathogenic stimuli are present, but may also make them more susceptible to initial pathogen invasion.

The peak incidence of SUDI is between 1 and 4 months of age (Shapiro-Mendoza et al., 2006); Fleming et al., 2015), with 90% of cases occurring before the age of 6 months (Moon & Task Force on Sudden Infant Death Syndrome, 2016).

There was reasonable agreement between the average age of the SUDI cases included in this study and what has been reported in the literature. During this period, a number of factors may increase susceptibility to infection, in particular, the loss of maternal antibodies. From 1 to 5 months of age, males develop circadian rhythms, changes in their night-time cortisol levels, and testosterone surges (Blackwell et al.,2015). In addition to this, the infant brain undergoes significant changes as it develops, particularly in its systems that control homeostasis (Fleming et al., 2015). This could also explain the highly significant association between infection and age in weeks.

SUDI occurs more often during the colder compared to the warmer months (Chang et al. 2008). This study also found a higher prevalence of SUDI cases in the colder months (65.6%). Research suggests that excessive clothing and overwrapping of infants may increase the risk of SUDI during colder months (Moon et al., 2007). Colder months are also associated with an increase in respiratory infections, and it has been found that respiratory infections are spread more easily to infants (Guntheroth et al., 1992).

Sharing a bed with an infant is a common practice in many cultures in order to facilitate breastfeeding (Blair et al., 2010; Huang et al., 2013) and enhance the relationship between parent and child (Mosko et al., 1997). Despite the prevalence of bedsharing (96%) in most SUDI cases in the current study, the association between bedsharing and the causes of death was not significant. Several factors may contribute to the increased risk of SUDI associated with bedsharing, such as overheating and overlays (Carpenter et al., 2004; Tappin et al., 2005). Furthermore, the risk may also be increased when the person sharing the bed is intoxicated or overtired (Blair et al., 1999; Carpenter et al., 2004). Among infants whose mothers smoke, bedsharing is an additional risk factor, as the infant may suffer hypoxia as a result of rebreathing expired air from the mother (Scragg et al., 1993). The majority of the control infants did not share a bed, possibly because they lived in baby sanctuaries and slept in cots or cribs.

Although SUDI is most commonly associated with prone sleeping (Groswasser et al., 2001), being placed to sleep on the side was the most prevalent position in the SUDI cases and controls in this study. Prone sleeping prone is a significant risk factor for SUDI, since it puts a significant physical strain on the heart and lungs (Mitchell et al., 2012), decreasing the protective reflexes to hypoxia and hypercapnia during sleep phases. Blood pressure, cerebral oxygenation, and cerebral blood flow are also reduced (Yiallourou et al., 2008; Wong et al., 2011). When term infants reach an age of 2 to 4 months, decreased arousal and depressed baroreflex responses have been shown (Horne et al., 2001;

Yiallourou et al., 2008). The majority of these characteristics are also evident in premature babies when they are in a prone position (Witcombe et al., 2008; Fyfe et al., 2014). Due to the *Back to Sleep* campaign, the number of infants placed to sleep in the prone position has decreased significantly. Therefore, the side sleep position has now been recognised to be as significant as the prone position (Fleming et al., 1996; Mitchell et al., 1997; Oyen et al., 1997; Hauck et al., 2002; Li et al., 2003). In several SUDI cases, the infants were found in the prone position at the time of death, having been placed to sleep on their sides. The side sleeping position is quite unstable and allows infants to easily roll into the prone position (Li et al., 2003).

Informal housing accounted for 52% of the cases in this study. Heathfield et al (2020) hypothesised that infants living in informal conditions are immunocompromised and that maternal IgG is more rapidly reduced than infants living in formal housing or settlements. Thus, infants in overcrowded living conditions cannot cope with the extent of microorganism exposure and the burden of disease and succumb to infections sooner (Webb et al., 1994; le Roux et al., 2015). Compared to babies born at term, premature or low birth weight babies have up to four times the risk of SUDI. As gestational age or birth weight decreases, this risk increases (Hoffman & Hillman, 1992; Malloy & Hoffman, 1995; Blair et al., 2006). The immaturity of these infants' autonomic systems may contribute to their increased vulnerability (Duncan & Byard, 2018). Among the cases and controls in the present study, less than half were premature or had low birth weights and it was not possible to assess any associations with prematurity and low birth weight.

In the present study, infection was the most common cause of death. This is consistent with the literature, which found that infection is the most common cause of explained SUDI (Weber et al., 2008; du Toit-Prinsloo et al., 2013, Heathfield et al., 2020; Winterbach et al., 2021; Fitzgerald et al., 2022). Indirect or direct interactions between bacterial and viral agents have been suggested as possible contributors to the pathogenesis of SUDI (Highet, 2008).

3.6 Conclusion

The sociodemographic information and risk factors derived from this study are generally in good agreement with risk factors published in the literature. In the study, the only highly significant association found was between age in weeks and infection. Infant mortality is at its highest between the ages of 2 and 4 months, probably due to the steady decline of maternal IgG levels following birth (Blackwell et al., 2005). In the wake of the successful *Back to Sleep* campaign, one of the most effective preventive measures is to educate and inform the public about other modifiable risks, especially for those who are responsible for the care of infants.

Chapter 4: Subtyping *Escherichia coli* in gastrointestinal samples from Sudden Unexpected Death in Infancy cases at the Tygerberg Medico-legal Mortuary, Cape Town

Abstract

Background: Diarrhoea is the fifth leading cause of overall mortality in children under the age of five years. Most countries do not regularly screen for diarrhoeagenic *Escherichia coli* (DEC), leading to limited data on its presence in children. Various studies have suggested that bacterial infections, particularly strains of *Escherichia coli (E. coli)*, are associated with sudden and unexpected death in infancy (SUDI). This study investigated the different *E. coli* strains in culture-positive post-mortem gastrointestinal tract (GIT) samples collected from SUDI cases at the Tygerberg Medico-legal Mortuary, Cape Town, over a one-year period.

Materials and methods: GIT swabs and stool samples were collected from 156 SUDI cases between 2017 and 2018. Routine bacterial culture was performed on all the samples. Real-time polymerase chain reaction (PCR) was performed on *E. coli* isolates for identification of DEC strains.

Results: Only 14 SUDI cases were culture-negative and real-time PCR analysis was performed on 434 isolates from 142 cases. Most cases tested positive for enteroaggregative *E. coli* (EAEC) (87.3%) and enteropathogenic *E. coli* (EPEC) (78.2%), followed by enterotoxigenic *E. coli* (ETEC) (50.7%), while the O157 strain was found to be the lowest among the cases (11.9%).

Conclusion: The results from this study confirmed the presence of EPEC, EAEC and ETEC in SUDI cases. There is still a need to investigate the association between specific *E. coli* strains and SUDI to elucidate the potential role of *E. coli* in the death of these infants.

Manuscript submitted to and is under review by: ***Forensic Science International: Synergy***

Authors: DT Cupido (DTC), AC Whitelaw (ACW), C de Beer (CDB)

Author contributions: DTC collected the samples at the Tygerberg Medico-legal Mortuary and carried out the laboratory investigations, acquired all data and drafted the manuscript. ACW and CDB conceptualised the study and reviewed the manuscript.

4.1 Introduction

Almost 1.7 billion cases of childhood diarrhoeal disease are reported per year globally (WHO, 2017). In 2016, diarrhoea was the eighth foremost cause of mortality among children, accounting for an estimated 1.6 million deaths, and the fifth foremost cause of death in children under the age of five years (GBD 2016 Diarrhoeal Disease Collaborators et al., 2018). Developing countries in Africa, Asia and Latin America are most frequently affected, with diarrhoeal diseases largely due to low socio-economic conditions, such as insufficient water supply and poor hygiene and sanitation (Croxen et al., 2013).

In developing countries, one of the most common causes of bacterial diarrhoea is *E. coli* (Saeed et al., 2015; Shrivastava et al., 2017). As a commensal bacterium, *E. coli* can be found in the intestinal microflora of several animals, including humans. However, some strains are harmful to human health and can cause debilitating, and even fatal, diseases (Bélanger et al., 2011). DEC is the foremost cause of bacterial paediatric diarrhoea (Canizalez-Roman et al., 2016) and is further divided according to specific virulence factors into EPEC, EAEC, ETEC, enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC) and diffusely adherent *E. coli* (DAEC) (Bettelheim & Goldwater, 2015). The EPEC strain can further be divided into two subtypes based on the existence of bundle-forming pili. Typical EPEC (tEPEC) has fimbrial adhesins as a virulence factor, which are absent in atypical EPEC (aEPEC) (Tennant et al., 2009; Gomes et al., 2016). The specific adhesins carried on the diarrhoeagenic strains increase their capability to colonise the GIT. The strains differ in their disease patterns and intensity once they are established in the GIT (Nataro & Kaper, 1998).

In developing countries, DEC strains account for 30 to 40% of diarrhoea cases in children, most notably EAEC, EPEC and ETEC (Cabrera-Sosa & Ochoa, 2020). In 2013, Kotloff et al. published results from the Global Enteric Multicenter Study indicating that aEPEC was the fifth most frequently detected pathogen in children under 12 months of age who died of acute gastroenteritis. Most countries, however, do not routinely screen for DEC in clinical samples and there is a lack of data relating to DEC strains isolated from children (Yu et al., 2015). As in many developing and sub-Saharan African countries, South Africa has a high prevalence of diarrhoea among infants and immunocompromised adults (Tau et al., 2012). Due to the absence or poor quality of waste disposal and sanitation facilities, low-income individuals are disproportionately affected by such diseases (Baker et al., 2016). Studies investigating DEC in South Africa include site specific studies from clinical specimens (Msolo et al., 2020) and food and environmental sources (Adefisoye & Okoh, 2016; Aijuka et al., 2018; Abdalla et al., 2021; Abdalla et al., 2022). There are, however, several gaps in the understanding of the prevalence of DEC pathotype(s) in cases of SUDI.

Various studies have suggested that bacterial infections, particularly strains of *E. coli*, are associated with SUDI where infants younger than one year die suddenly and without apparent cause (Bettelheim et al., 1989, 1990; Bettiol et al., 1994; Blackwell et al., 1999; Goldwater & Bettelheim, 2002; Pearce et al., 2004; Highet, 2008; Weber & Sebire, 2009). It has often been noted that these infants had mild clinical symptoms during the days leading up to death, with diarrhoea often being reported (Hoffman et al., 1988). In 2010, Pearce et al. investigated the diversity of *E. coli* serotypes found in the GIT contents of Australian infants who died of SUDI, compared with faecal material from healthy infants. They found that the seven frequent serotypes belonging to those linked with extra-intestinal infections in humans as listed above were more commonly isolated from SUDI cases. Significantly higher proportions were found among infants who died from other causes (13%, $p < 0.05$) or sudden infant death syndrome (SIDS) (18.7%, $p=0.0002$) than healthy infants (6%). They suggested a possible link between SUDI and specific patterns of *E. coli* strains.

While *E. coli* is an integral part of the intestinal microbiome, it has been detected in infants who died suddenly and unexpectedly (Gilbert et al., 1992; Weber et al., 2008; Goldwater, 2009). Therefore, this study aimed to identify the different *E. coli* strains in culture-positive post-mortem GIT swabs collected from SUDI cases at the Tygerberg Medico-legal Mortuary, Cape Town.

4.2 Materials and methods

4.2.1 Study population

A total of 156 SUDI cases have been collected between June 2017 and May 2018 and analysed in this chapter, as well as 10 apparently healthy and age-matched infants who served as controls.

4.2.2 Isolation of *E. coli*

All samples were sent to the National Health Laboratory Services (NHLS), Tygerberg Hospital, microbiology laboratory for routine identification of organisms. *Salmonella* and *Shigella* were detected using Xylose lysine deoxycholate agar (XLD agar) and MacConkey agar (MCC). The blood agar was used for detecting *Vibrio cholera*, while the charcoal cefoperazone deoxycholate (CCD) agar was used for detecting *Campylobacter*. The presence of *E. coli* was confirmed by inoculating the samples onto MacConkey agar plates and incubating the plates at 35°C for 24 hours in an aerobic incubator. Lactose-fermenting organisms, such as *E. coli*, produce acidic by-products that lower the pH, causing the pH indicator to turn pink (Jung & Hoilat, 2022). *E. coli* colonies were transferred to Microbank™ microbeads (Pro-lab Diagnostics, Canada) and stored at -80°C until further analysis was performed. On the day of analysis, the vials were removed from storage and one bead was aseptically inoculated directly onto a Tryptone Bile Agar (TBA) plate and incubated aerobically at

35°C overnight. Subsequently, a single *E. coli* colony was picked from the TBA plate, inoculated into Luria-Bertani (LB) broth, and incubated overnight aerobically on a shaker at 35°C.

4.2.3 Nucleic acid extraction

Deoxyribonucleic acid (DNA) was extracted from the *E. coli* contained in the LB broth using a Zymo Quick-DNA™ Miniprep Plus Kit (Zymo Research, USA). After centrifuging the *E. coli* cells for 2 minutes at 5000 x g, the supernatant was removed and the pellets were resuspended in 200 µL DNA Elution Buffer, along with 10 µL GI-BP internal control (Seegene Inc, Korea), in preparation for Real-Time PCR. Thereafter, 200 µL BioFluid and Cell buffer as well as 20 µL Proteinase K were added. The tube was vortexed (S0200, Labnet, New Jersey, USA) for 10-15 seconds and incubated at 65°C for 10 minutes. Genomic binding buffer totalling 420 µL was added to the tube and vortexed for an additional 10-15 seconds. After adding the lysate stepwise to the Zymo-Spin™ IIC-XL column, the sample was centrifuged for 1 minute at 12 000 x g. Upon completion of centrifugation, the flow-through was discarded and the column was placed in a new collection tube. The column was then washed with 400 µL DNA Pre-wash buffer and centrifuged at 12 000 x g for 1 minute. After discarding the flow-through again, the second wash step was conducted by adding 700 µL g-DNA wash buffer to the column and centrifuging it at 12 000 x g for 1 minute. Once more, the flow-through was discarded and 200 µL g-DNA wash buffer were added to the column and centrifuged for 1 minute at 12 000 x g.

The DNA elution buffer was preheated to 65°C before being added to the matrix. A total of 50 µL of DNA elution buffer was added directly to the matrix and incubated at room temperature for 5 minutes. Afterward, the eluate was loaded again and incubated for 3 minutes at room temperature before it was centrifuged a second time. As a result, the overall yield was increased. The concentration and purity of the extracted DNA were determined using a NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific, USA).

4.2.4 Qualitative real-time polymerase chain reaction

The Allplex™ GI-Bacteria (II) Assay (Seegene Inc, Korea) was performed on a CFX96 system (Bio-Rad, USA) according to the manufacturer's instructions to detect genes for EHEC (*stx1/stx2*, *E. coli* O157), EPEC (eaeA), ETEC (lt/st) and EAEC (aggR) and internal control. The positive control consisted of 4 GI-bacteria and IC clones, while RNAse-free water was used as the negative control. A total volume of 25 µL was used in the reaction, with 20 µL of the master mix and 5 µL DNA template (Table 4.1). Real-time PCR was conducted in a 96-well skirted PCR plate sealed with adhesive Microseal® 'C' PCR Plate Sealing Film (Bio-Rad, USA). In each reaction, the positive control and negative control that were supplied were tested.

Table 4.1: Reaction mixture for real-time PCR analysis

Reagent	Volume
RNase-free Water	10 µL
5X GI-B(II) MOM	5 µL
5X Anyplex PCR Master Mix	5 µL
Total volume of PCR Mastermix	**20 µL**
PCR Mastermix	20 µL
DNA Template	5 µL
Total volume of reaction	**25 µL**

Thermal cycling was carried out on a CFX96™ Real-time PCR machine (Bio-Rad, USA) and the thermal cycling conditions for each reaction are outlined in Table 4.2 and the fluorophores used to detect the analytes in Table 4.3.

Table 4.2: Real-time PCR thermal cycling conditions

Step	No. of cycles	Temperature	Duration
1	1	50°C	20 min
2		95°C	15 min
3	45	95°C	10 sec
4*		60°C	1 min
5*		72°C	30 sec
6	**REPEAT STEP 3-5, 44 TIMES**		

***:** Plate Read at Step 4 and 5.

Table 4.3: Fluorophores used to detect analytes

Fluorophore	Analyte	
FAM	Shiga toxin (*stx1/2*)	EPEC (eaeA)
HEX	Internal Control (IC)	ETEC (lt/st)
Cal Red 610	*E. coli* O157 (O157)	EAEC (aggR)

The Seegene Export tool was used to quantitate data obtained from the Seegene Allplex™ GI-Bacteria (II) PCR performed on the CFX96™ were automatically saved in two folders (QuantStep4 and QuantStep5). The QuantStep4 data file was imported into Seegene Viewer for Real-Time PCR instruments (V3), and the test kit (Novaplex™ GI-Bacteria (II) Assay (96 plate)) was selected from the product menu for analysis of the results for each well. A Ct value of ≤ 43 indicates a positive result, whereas a Ct value of > 43 or N/A (not detected) indicates a negative result.

4.2.5 Statistical analysis

Statistica® version 14.0 statistical software (TIBCO Software, Inc.) was used for statistical analysis. Associations were calculated using the F-test for numerical data and the Fisher exact test for categorical data. Statistical significance was observed at $p < 0.05$ at a 95% confidence interval (CI), with a strong significance observed at $p < 0.01$. Logistic regression was used to analyse the associations between strains and sociodemographic risk factors. The data were adjusted to remove the effect of predictor variables when odds ratios (OR) were calculated for the main variables. Adjusted ORs ratios were reported.

4.3 Results

4.3.1 Epidemiological data

The male to female ratio was 1.3:1. The median age of the infants at the time of death was 9 weeks (interquartile range [IQR]: 5.0–16.6) and the mean post-mortem interval (PMI) was 7±3.5 days. The control group consisted of seven male and three female infants, with a median age of 15.2 weeks (IQR: 9.8–23.9).

4.3.2 Laboratory results

Campylobacter was found in one case while *Shigella* was found in another case. *Salmonella* was detected in three cases. Only 14 (9%) of the 156 cases were culture-negative for *E. coli*. As there were so many cases positive for *E coli*, it was subtyped to determine if it was a true occurrence or contamination. The qualitative PCR results of *E. coli* isolates from the 142 culture-positive cases are summarised in Tables 4.4 and 4.5. One case was negative for all *E. coli* subtypes, while 14.1% had evidence of only one DEC pathotype. Mixed infections were common, with 121 (85.2%) having evidence of at least two different pathotypes. Although routine microbial culture confirmed the presence of *E. coli* in all 10 control samples, no pathogenic *E. coli* could be identified in any of them.

Table 4.4: Enteric pathogens detected in gastrointestinal samples from 142 SUDI cases

Enteric pathogens	n	%
EAEC (aggR)	124	87.3%
EPEC (eaeA)	111	78.2%
ETEC (lt/st)	72	50.7%
O157	17	11.9%
Negative	1	0.7%

Table 4.5: Prevalence of single and multiple DEC pathotypes detected in GIT samples from SUDI cases (n=142)

Infection combinations	n	%
Single pathotype	20	14.1%
Mixed pathotypes	121	85.2%
Two pathotypes	*64*	*45.1%*
Three or four pathotypes	*57*	*40.1%*

4.3.3 Statistical analysis

EPEC was 4 times less likely to be present in premature than full-term infants (OR= 0.24; 95% Cl, 0.07-0.78; p=0.02). However, in further statistical analyses confirmed it was observed that EPEC was significantly more prevalent in full-term infants ($p < 0.01$). Male infants were 3 times more likely to be positive for ETEC than female infants (OR= 3.19, 95% Cl, 1.29-7.89, p=0.01) (Table 4.6), further statistical analyses supported these results and confirmed a significant association between ETEC and sex (p=0.04) (Table 4.7). Male infants were more likely to have ETEC, and ETEC, EAEC and EPEC were more often confirmed in the colder months ($p < 0.01$). ETEC was significantly associated with breastfeeding (p=0.04) and the association between ETEC and low birth weight showed a trend toward significance (p=0.09).

There was no significant association between the cause of death and the pathogenic strains of *E. coli* (Table 4.8).

Positive correlations were found between the number of people in the household and ETEC (Figure 4.1), as well as PMI and O157 (Figure 4.2).

Table 4.6: Adjusted Odds Ratio for sociodemographic risk factors categorised by *E. coli* pathogenic strains (n= 141)

Strain	Sociodemographic risk factor	Adjusted Odds Ratio (95% CI)	p value
EPEC (n=110)			
	Male Sex	1.14 (0.34-3.77)	0.83
	Prematurity	0.24 (0.07-0.78)	**0.02**
	Age (weeks)	0.98 (0.93-1.04)	0.55
	Number of people in the household	0.93 (0.65-1.31)	0.66
ETEC (n=72)			
	Male Sex	3.19 (1.29-7.89)	**0.01**
	Prematurity	0.96 (0.39-2.37)	0.92
	Age (weeks)	1 (0.96-1.04)	0.96
	Number of people in the household	1.23 (0.94-1.62)	0.13
EAEC (n=124)			
	Male Sex	3.47 (0.9-13.41)	0.07
	Prematurity	1.2 (0.28-5.14)	0.81
	Age (weeks)	1.03 (0.96-1.12)	0.39
	Number of people in the household	1.51 (0.9-2.55)	0.12
O157 (n=18)			
	Male Sex	0.7 (0.18-2.78)	0.61
	Prematurity	0.97 (0.22-4.3)	0.96
	Age (weeks)	0.96 (0.88-1.04)	0.27
	Number of people in the household	0.63 (0.37-1.07)	0.08

Table 4.7: Sociodemographic risk factors categorised by *E. coli* pathogenic strains (n= 141)

Sociodemographic risk factor	Strains n (%)	p value
	ETEC (n=72)	
Male Sex	48 (58.5%)	**0.04**
Seasons		**<0.01**
Cold months	39 (54.2%)	
Warm months	33 (45.8%)	
Breastfed	61 (56.0%)	**0.04**
Low birth weight	41 (58.6%)	**0.09**
	EAEC (n=124)	
Seasons		**<0.01**
Cold months	82 (66.1%)	
Warm months	42 (33.9%)	
	EPEC (n=110)	
Prematurity	41 (67.2%)	**<0.01**
Seasons		**<0.01**
Cold months	87 (79.1%)	
Warm months	23 (20.9%)	
Low birth weight	45 (64.3%)	**<0.01**

Table 4.8: Cause of death categorised by *E. coli* pathogenic strains (n=133)

Cause of death	EPEC n (%)	ETEC n (%)	EAEC n (%)	O157 n (%)
SIDS (n=53)	43 (41.4%)	25 (36.7%)	47 (39.8%)	9 (56.3%)
Infection (n=80)	61 (58.7%)	45 (64.3%)	71 (60.2%)	7 (43.8%)
p value	**0.53**	**0.38**	**1.00**	**0.18**

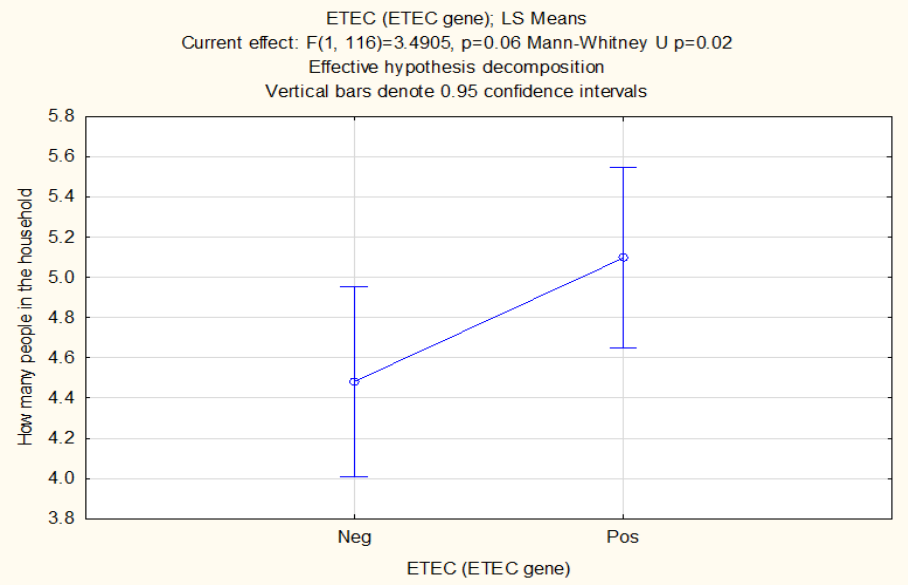


Figure 4.1: Positive correlation between the number of people in the household and ETEC

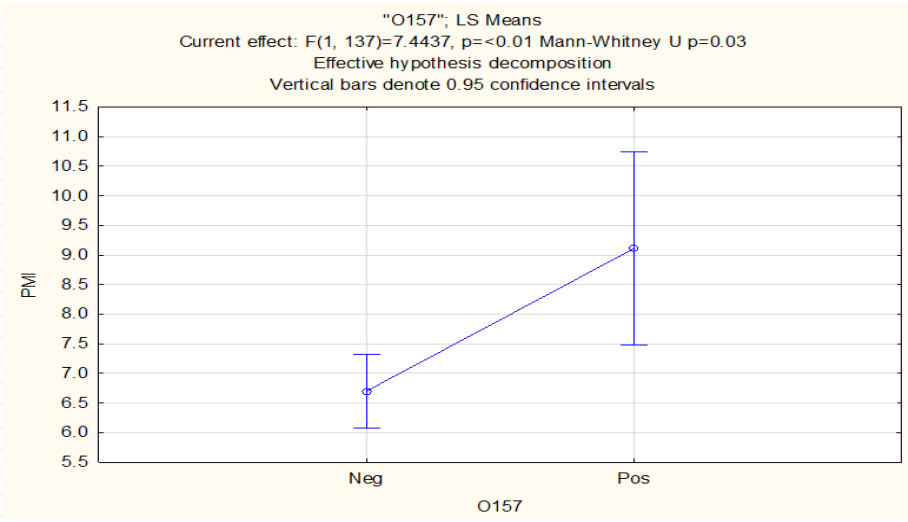


Figure 4.2: Significant positive correlation between PMI and O157 ($p < 0.03$)

4.4 Discussion

Infections associated with DEC are a major health concern among children in developing countries due to the associated morbidity and mortality in children younger than 5 years old (Gomes et al., 2016). Despite numerous post-mortem bacteriological cultures producing organisms in SUDI cases, the majority appear not to be associated with death. In cases of SUDI that are otherwise unexplained, a high rate of *E. coli* has been found, indicating a possible causal relationship (Weber et al., 2008). This study examined the occurrence of different *E. coli* strains in culture-positive post-mortem GIT samples collected from SUDI cases.

In the present study, EAEC was detected in more than 80% of cases, similar to findings from a previous study in South Africa, in which this strain was detected in just more than half of the samples from infants without and with diarrhoea (Tanih et al., 2014). Over the past few years, EAEC has been recognised as an emerging diarrhoeagenic pathogen, accounting for up to 15% of diarrhoeal cases in developed and developing countries (Aijuka et al., 2018; Lääveri et al., 2018; Fedor et al., 2019; Ellis et al., 2020). Severe diarrhoea in children is often associated with EAEC, which can cause chronic diarrhoea, especially in malnourished infants. It is also regarded as the second most frequent cause of travellers' diarrhoea (Estrada-Garcia & Navarro-Garcia, 2012). According to a recent study conducted in South Africa investigating genes coding for virulence and phylogroups among *E. coli* isolated from children hospitalised due to diarrhoea in Limpopo Province, EAEC was the second most prevalent pathotype (Alfinete et al., 2022).

EPEC is an important causative agent of infant diarrhoea in developing countries, mostly in children under 2 years old (Cabrera-Sosa & Ochoa, 2020), but it can also be detected in non-diarrhoeal samples (Chellapandi et al., 2017). For decades, global studies have shown that in developed countries, tEPEC was strongly associated with diarrhoea in infants (Nataro & Kaper, 1998; Trabulsi et al., 2002; Gomes & González-Pedrajo, 2010) and studies conducted in South Africa, Brazil, Chile, and Mexico showed that tEPEC caused 30-40% of infantile diarrhoea cases (Gomes et al., 1989; Ochoa et al., 2008; Gomes & González-Pedrajo, 2010). However, aEPEC was confirmed in a few studies on children from India (Wani et al., 2006; Nair et al., 2010; Rajeshwari et al., 2015), but most studies still report tEPEC as being more prevalent in causing diarrhoea than aEPEC (Alikhani et al., 2006). In addition, in developing countries such as Africa and Asia, tEPEC is still the major primary enteropathogen (Rajendran et al., 2010; Kotloff et al., 2013; Santona et al., 2013; Ben Salem-Ben Nejma et al., 2014; Langendorf et al., 2015; Odetoyin et al., 2016). Although EPEC was the second most common strain detected in this study, the subtypes of EPEC were not investigated.

ETEC causes hundreds of millions of diarrhoeal illness cases globally (Khalil et al., 2018), particularly affecting young children under the age of 5 who have not yet developed immunity from prior exposure to ETEC (Qadri et al., 2007). In low- to middle-income countries, ETEC remains one of the most common causes of death from diarrhoeal illness among young children (Kotloff et al., 2013) and approximately 30-60% of all cases of travellers' diarrhoea are caused by ETEC (Gascón et al., 1998; Jiang et al., 2002). In various countries, ETEC has been described as the most frequent cause of diarrhoea amid all *E. coli* strains (Presterl et al., 2003; Shaheen et al., 2004; Jafari et al., 2008; Moriel et al., 2012; Gomez-Duarte et al., 2013). In the current study, however, ETEC was found to be less prevalent than EAEC and EPEC. According to data on isolation rates by age, 10-

20% of children under 12 months were infected with ETEC (Nguyen et al., 2005), this could explain the lower prevalence rate found in this study.

A previous study from South Africa reported an O157 prevalence of only 3.8% (Tanih et al., 2014), which was considerably lower than the frequency detected in the current study (11.9%). This strain, also known as EHEC, is a subtype of Shiga toxin-producing *E. coli* (STEC) (Nataro & Kaper, 1998), which is mostly associated with outbreaks of diseases, such as haemorrhagic colitis and haemolytic uraemic syndrome (Parsons et al., 2016). Water sources contaminated by human and/or animal waste were likely to have played a role in the prevalence found in the current study (Mabika et al., 2021).

Mixed DEC infections were described in studies conducted in Kenya (Iijima et al., 2017; Nyanga et al., 2017). The occurrence of mixed DEC infections is common in communities with limited access to water supplies and sanitation, where transmission takes place through food, water, and the environment (Nimri et al., 2004). *E. coli* are among the first bacteria to colonise neonatal GIT after birth (Mueller et al., 2015) and are typically found in the lower intestines of humans (Tenaillon et al., 2010). This could explain the *E. coli* detected in the controls in this study.

ETEC is predominantly transmitted from person to person via the faecal–oral route and from the environment through contaminated water, food, or soil (Madigan & Martinko, 2006). Similarly, close space sharing and host contacts may also be significant in the transfer of ETEC, as well as the other *E. coli* pathotypes (Blyton et al., 2014). UN-Habitat, 2021 defines overcrowding as the presence of more than three people in the same habitable room within a dwelling unit. With urbanisation increasing rapidly in many developing countries, such as South Africa, there is a shortage of housing, resulting in overcrowding. Various health outcomes are associated with household overcrowding, including respiratory infections and diarrhoeal illnesses (Nkosi et al., 2019), which supports the positive correlation found between the number of people in the household and the presence of ETEC in this study.

The PMI showed a positive correlation with O157. It is unclear what role O157 plays during the PMI, and further research is needed to clarify this. Hurtado et al. (2018), however, concluded that *Enterobacteriaceae* infections are likely to be overestimated as contributors to death, especially if autopsies are conducted more than 24 hours after death. In such cases, it is not possible to confirm these bacteria to be the actual cause of death. It has also been demonstrated that *E. coli* can survive at temperatures of 4°C for extended periods of time, although prolonged storage at low temperatures may result in a loss of viability (Tuttle et al., 2022).

The significant association between male infants and ETEC found in this study was similar to a study in Egypt that also identified being male as one of the risk factors associated with ETEC-related infections (Mansour et al., 2014). In an Indian study, ETEC was also detected more often in stool samples of male than female children younger than 5 years (Yadav et al., 2020). Poor homeostatic mechanisms, caused by factors such as low birth weight, can increase an infant's vulnerability to diarrhoeal infections caused by DEC and enteric viruses (Bobak & Guerrant, 2015), and this could possibly contribute to the presence of ETEC.

Despite low birth weight and prematurity increasing the risk of infection in infants, EPEC is a major source of infection in children under 2 years of age in developing countries (Cabrera-Sosa & Ochoa, 2020) and this may explain the high prevalence of EPEC in full-term infants of normal birth weight found in this study.

Environmental and climate factors contribute to an infant's susceptibility to infection and disease and could partly explain the associations between seasons and the prevalence of EAEC found in this study. Results from Bolivia showed a peak in EAEC infections during the colder months (April–September). However, during the warmer months (October–March), a higher proportion of DEC-positive samples was found (Gonzales et al., 2013). Awotiwon et al. (2016) also described a higher incidence of diarrhoea cases due to bacterial enteropathogens during the summer months in South Africa.

França et al. (2011) determined that when opsonised by human colostrum supernatants, ETEC is destroyed by the phagocytes. The dominant antibody contained in human milk is secretory immunoglobin A. These antibodies provide protection against ETEC infection in fully breastfed infants (Hanson et al., 1994), but the results from the current study did not support this and found an association between ETEC and breastfed infants. Similarly, studies from Bangladesh and Egypt did not demonstrate protection against ETEC. These results suggest that the promotion of breastfeeding alone will not lead to an effective reduction of ETEC in children in underdeveloped countries (Abu-Elyazeed et al., 1999).

4.5 Conclusion

In the present study, stool samples collected from SUDI cases showed a high prevalence of EAEC, EPEC and ETEC. These results agree with literature that found that children were commonly affected by these strains. The association between certain *E. coli* strains and death requires further investigation, as the exact mechanisms and roles need to be systematically elucidated.

The main strengths of this study was the relatively large sample size, and the fact that sampling was done from multiple areas of GIT, thus increasing chance of isolation of organisms. Some of the challenges encountered included the small control group, the variable PMI which may affect culture yield and the fact that characterisation of *E. coli* was done on cultured isolates only. Future research should investigate the value of performing PCR directly on stool samples.

Chapter 5: Screening for viral pathogens in the gastrointestinal tract from Sudden Unexpected Death in Infancy cases at the Tygerberg Medico-legal Mortuary, Cape Town

Abstract

Background: In 2013, diarrhoeal disease was estimated to be one of the leading causes of death in sub-Saharan Africa, in addition to HIV/AIDS, malaria and lower respiratory tract infections. Most paediatric gastroenteritis cases are caused by acute viral gastroenteritis, yet studies on viral enteropathogens in sudden unexpected death in infancy (SUDI) cases have not been conducted. The aim of this study was to describe specific viral pathogens in stool samples collected from SUDI cases and age-matched, apparently healthy infants in Cape Town, South Africa.

Materials and methods: Stool samples were collected from 176 SUDI cases between June 2017 and May 2018. In addition, stool samples were collected from the nappies of 30 age-matched, apparently healthy infants as a control group. Real-time polymerase chain reaction (PCR) was performed on the stool samples for identification of the viruses.

Results: A total of 111 SUDI cases were positive for viruses, while no viruses were identified in the remaining 65 cases. Most cases were positive for rotavirus (RV) (38.6%), followed by norovirus GI and GII (30.0%). However, in the control samples, norovirus (NoV) GII (36.7%) was more prevalent, followed by RV (33.3%).

Conclusion: This study revealed that RV and NoV were the most prevalent viruses in infants, highlighting the importance of RV-A vaccinations. It is also necessary to emphasise the significance of norovirus infection in children.

Manuscript submitted and it is under review by: ***Virology Journal, Springer Nature***

Authors: DT Cupido (DTC), C de Beer (CDB)

Author contributions: DTC collected the samples at the Tygerberg Medico-legal Mortuary and carried out the laboratory investigations, acquired all data and drafted the manuscript. CDB conceived the research, read, and reviewed the manuscript, and approved the final version for publication.

5.1 Introduction

The sudden and unexpected death of seemingly healthy infants has been recognised since ancient times (Fleming et al., 2015). SUDI is not a clinical or pathological diagnosis and is different from sudden infant death syndrome (SIDS). SUDI includes all deaths in infants younger than 1 year (often restricted to 7-365 days) that present suddenly and unexpectedly (Weber & Sebire, 2009), or at least without a clearly identifiable cause at the time of death and *before* any investigations have been performed. SIDS is defined as the unexpected death of an infant under the age of one, which appears to occur during sleep, where no explanation can be found after a thorough investigation has been conducted. This includes an autopsy and an examination of the circumstances of death and medical history (Krous et al., 2004).

Viruses can either directly or indirectly, through synergistic interactions with bacterial virulence features or immunoregulatory polymorphisms, enhance the noxiousness of bacterial toxins in SUDI (Jakeman et al., 1991; Blackwell et al., 2005). Viruses in the respiratory tract of an infant can cause sublethal bacterial toxin levels to become lethal (Highet, 2008). The mechanism behind this, however, is not yet entirely understood (Doughty et al., 2006). Virus-induced diarrhoea is caused by viral pathogens colonising different environments of the small intestine, disrupting the natural fluid balance of the GIT. Enteric viruses are stable at low pH and resistant to digestive enzymes, characteristics that enhance their ability to infect (Michelangeli & Ruiz, 2003).

Despite a global decrease in deaths caused by diarrhoeal disease from 2.6 million to 1.3 million in 2013, diarrhoeal disease remains a significant health concern, especially in Africa. During this time, diarrhoeal disease was estimated to be one of the leading causes of death in sub-Saharan Africa, in addition to human immunodeficiency virus (HIV) infection and associated acquired immunodeficiency syndrome (AIDS), malaria and lower respiratory tract infections (GBD 2013 Mortality and Causes of Death Collaborators, 2015). Globally, acute gastroenteritis still accounts for up to 10% of hospitalisations and 19% of deaths in children under the age of five years (Adadey & Quaye, 2017). In developing countries, where inadequate supply of clean drinking water and suboptimal sanitation are major contributing factors (Chakravarty et al., 2017; Squire & Ryan, 2017), diarrhoea is the second major cause of infant death (Platts-Mills et al., 2015; Kotloff, 2017). Annually, 12.5% one in eight deaths amongst children under the age of five in Africa, South America and Asia is the result of diarrhoea (Keddy et al., 2016; Kotloff, 2017). Acute gastroenteritis can be caused by a variety of viral, bacterial, parasitic, and fungal infections (Ciccarelli et al., 2013), with acute viral gastroenteritis comprising almost 70% of all incidents in children (Webb & Starr, 2005). More than 20 different viruses have been confirmed as aetiological agents of gastroenteritis (Wilhelmi et al., 2003). Cases present with mild fever, watery diarrhoea without blood and damage to the intestinal

enterocytes. Dehydration, which may lead to electrolyte disturbance and metabolic acidosis, is the most common and dangerous complication. The risk of complications is increased in children with poor nourishment (Elliott, 2007).

Globally, RV is the most frequently identified viral enteropathogen in infants and young children (Kotloff et al., 2013), particularly in acute diarrhoea. Each year, RV infections result in the hospitalisation of two million children under 5 years of age and approximately 527 000 deaths (Enweronu-Laryea et al., 2012). It has been estimated that over 200 000 children under 5 years of age have died from RV infection in developing countries in sub-Saharan Africa and Southeast Asia in 2013. RV group A (RV-A) is responsible for the majority of human infections (Desselberger, 2017) and since the introduction of the RV vaccine, there has been a marked reduction in RV-specific diarrhoea and a 38% reduction in all-cause diarrhoea (Burnett et al., 2017). Four live-attenuated RV vaccines are currently prequalified by the World Health Organisation (WHO) (Rotarix, RotaTeq, Rotavac and RotaSiil) (Mphahlela et al., 2021). The South African Expanded Programme on Immunisation (SA-EPI) introduced the RV vaccines in 2009 (National Department of Health, 2012), leading to a 33-57% national decline in all-cause diarrhoea (Msimang et al., 2013; Groome et al., 2014, 2016).

Since the RV vaccine significantly reduced RV-specific diarrhoea, NoV has been recognised as the primary cause of severe viral gastroenteritis (Hemming et al., 2013; Payne et al., 2013). Genetic variations in the expression of Histo-blood group antigen (HBGA) in the mucosa play a significant role in norovirus infection risk, and rotavirus infections may follow a similar pattern across populations (Payne et al., 2015). Most human infections are caused by NoV GII strains; specifically, genotype GII.4 is accountable for approximately 55-85% of cases globally (Ramani et al., 2014). In children, NoV disease is mostly moderate. Acute infections require hospitalisation in between 7.2 and 16 per 10 000 children under 5 years of age, and diarrhoea is involved in approximately 10-12% of deaths annually (Hall et al., 2013; Lopman et al., 2015). In 2017, NoV was detected in 15% of hospitalised South African children under 5 years of age with diarrhoea with the majority being under the age of two years (Page et al., 2017). NoV vaccine development has been identified as a high priority, but due to genetic and antigenic diversity and the presence of multiple co-circulated variants of different genotypes, it remains challenging (Chhabra et al., 2019). Clinical trials are currently underway for recombinant VP1-based virus-like particles (VLPs), which mimic major antigens from NoVs and are safe, immunogenic, and protective, suggesting the potential for developing an effective NoV vaccine (Tan, 2021).

In countries where RV vaccination coverage is high, sapovirus (SaV) has emerged as the second major viral enteropathogen following NoV (Bucardo et al., 2014). SaV is associated with both sporadic and epidemic cases of acute gastroenteritis, but children are more often affected than adults (Liu et al., 2015, 2016; Pongsuwanna et al., 2017). Unlike NoV, SaV research is not progressive (Oka et al., 2015). Prevalence rates in children under 5 years of age have been reported in a few sub-Saharan African countries, ranging from approximately 6% in Tanzania (Liu et al., 2011; Elfving et al., 2014), to 8% in Malawi (Dove et al., 2005), 10% in Gabon (Lekana-Douki et al., 2015) and 18% in Burkina Faso (Matussek et al., 2015). A five-year study on children under the 5 years of age who were hospitalised for diarrhoea in a South African setting with a high HIV prevalence, found an 8% prevalence for SaV with many children being infected during their second year of life. SaV was confirmed to be the sole cause of death in two patients and was also present as coinfection in 11% of the fatal cases (Page et al., 2016b).

The majority of diarrhoeal diseases caused by astrovirus (AstV) have been described in children under 5 years of age, the elderly (Bosch et al., 2014; Jarchow-Macdonald et al., 2015) and immunosuppressed individuals of all ages (Grohmann et al., 1993; Liste et al., 2000; Vu et al., 2017). Numerous African countries have detected AstV in children with diarrhoea, such as Mali where up to 3% of confirmed AstV cases were found in children under the age of five (Liu et al., 2016). Other countries have also reported positive cases in children, with prevalence rates varying from a low of 2% in Burkina Faso (Phan et al., 2014), to 3% in Ghana (Silva et al., 2008), 4% in Tunisia (Monastiri et al., 2016), 6% in Egypt (Ahmed et al., 2011) and Kenya (Kiulia et al., 2007), 10% in The Gambia (Meyer et al., 2015) and a high of 40% in Nigeria (Ayolabi et al., 2012). In a 5-year South African epidemiological study among hospitalised children under 5 years of age, AstV was detected in 7% of cases, of which 9% were infants between the ages of 7 and 12 months (Nadan et al., 2019).

Globally, human adenovirus (AdV) was responsible for the deaths of about 13% of children under 5 years of age in 2016 (GBD 2016 Lower Respiratory Infections Collaborators, 2018). Gastroenteritis associated with AdV is commonly caused by types 40 and 41 (Ghebremedhin, 2014; Ouedraogo et al., 2016; Reis et al., 2016; Khanal et al., 2018). A meta-analysis investigating the aetiology of enteric pathogens associated with gastroenteritis in children under 5 years of age in sub-Saharan Africa reported that in a pooled sample, AdV was the least detected virus in only about 1% of cases (Oppong et al., 2020).

To date, no studies have investigated the role of viral enteropathogens in SUDI cases. This study therefore aimed to describe specific viral pathogens present in stool samples collected from SUDI cases and age-matched, clinically healthy infants in Cape Town, South Africa.

5.2 Materials and methods

5.2.1 Sample collection

Stool samples were collected from 176 SUDI cases admitted to Tygerberg Medico-legal Mortuary in Cape Town between June 2017 and May 2018. Moreover, stool samples were obtained non-invasively from the nappies of 30 age-matched, apparently healthy infants as controls.

5.2.2 Nucleic acid extraction

Stool samples were collected in sterile leakproof containers and stored in DNA/RNA Shield™ (Zymo Research, California, USA) at -80°C until analysis. Approximately 60 mg of stool was dissolved in 600 µL of phosphate-buffered saline (PBS) (Gibco®, California, USA) containing five metal beads. The stool was disrupted with the Qiagen TissueLyser LT (QIAGEN, Hilden, Germany), followed by centrifugation for 2 minutes at 11 000 x g (5424 Microcentrifuge, Eppendorf, Hamburg, Germany). Viral DNA and RNA were extracted using NucleoSpin Virus Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. In the 1.5 mL tube provided, 200 µL of supernatant was added along with 10 µL of GI-V internal control, 5 µL of proteinase K liquid (Zymo Research, California, USA) and 200 µL lysis Buffer VL added after mixing by pipetting. The tube was vortexed (S0200, Labnet, New Jersey, USA) for 10-15 seconds prior to the addition of 5.6 µL of the supplied carrier RNA. After vortexing the mixture again, it was incubated at room temperature for 3 minutes. Following incubation, 200 µL molecular biology grade ethanol (Sigma-Aldrich, USA) was added and briefly vortexed before the tube was incubated for five minutes. To remove drops from the lid, the tube was centrifuged for 1 second at 2000 x g.

The lysate was added stepwise to the column and centrifuged for 3 minutes at 4 000 x g. After centrifugation was completed, the flow-through was discarded and the column was placed in a new 2 mL collection tube. The column was then washed with 400 µL wash buffer VW1 and centrifuged at 11 000 x g for 30 seconds. After discarding the flow-through again, the column was placed in a new collection tube. A second wash step was performed by adding 400 µL wash buffer VW2 to the column and centrifuging it for 11 000 x g for 30 seconds. Once again, the flow-through was discarded and a new collection tube was placed in the column. As a final step, 200 µL VW2 were added to the column and centrifuged for 5 minutes at full speed. After placing the column in the clean 1.5 mL Elution tube provided, it was incubated at 56°C for 5 minutes with the lid open. As a final step, 30 µL of RNase-Free H2O heated to 70°C was added to the column in order to elute viral RNA and DNA. The concentration and purity of the extracted DNA/RNA were determined using the NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific, USA).

5.2.3 Qualitative real-time polymerase chain reaction

Multiplex PCR using the Allplex ™ GI-Viral Assay (Seegene Inc, Korea) was performed following the manufacturer's instructions for detection of NoV GI, NoV GII, RV-A, AdV-F (Serotype 40/41), AstV and SaV (Genogroups G1, 2, 4) and an internal control. The positive control consisted of 6 GI-virus and internal control (IC) clones, while RNAse-free water was used as the negative control. A total volume of 25 µL was used in the reaction, with 20 µL of the master mix and 5 µL DNA/RNA template (Table 5.1). Real-time PCR was conducted in white, low profile 0.2 mL 8-Tube PCR Strips without caps and sealed with clear, flat optical 0.2 mL 8-strip PCR caps (Bio-Rad, USA). In each reaction, the positive control and negative control that were supplied were tested.

Table 5.1: Reaction mixture for real-time PCR analysis

Reagent	Volume
RNase-free Water	8 µL
5X GI-V MOM	5 µL
5X Real-time One-step Buffer	5 µL
Real-time One-step Enzyme	2 µL
Total volume of one-step RT-PCR Mastermix	**20 µL**
One-step RT-PCR Mastermix	20 µL
DNA/RNA Template	5 µL
Total volume of reaction	**25 µL**

The thermal cycling conditions for each reaction carried out on a CFX96TM Real-Time PCR System (Bio-Rad, USA) are outlined in Table 5.2 and the fluorophores used to detect the analytes in Table 5.3.

Table 5.2: Real-time PCR thermal cycling conditions

Step	No. of cycles	Temperature	Duration
1	1	50°C	20 min
2		95°C	15 min
3	45	95°C	10 sec
4*		60°C	1 min
5*		72°C	30 sec
6	**REPEAT STEP 3-5, 44 TIMES**		

***:** Plate Read at Step 4 and 5.

Table 5.3: Fluorophores used to detect analytes

Fluorophore	Analyte	
FAM	AstV	NoV GII
HEX	IC	AdV-F
Cal Red 610	SaV	NoV GI
Quasar 670	RV-A	

The Seegene Export tool was used to obtain the quantitation data from the Seegene Allplex ™ GI-Viral PCR performed on the CFX was automatically saved in two folders (QuantStep4 and QuantStep5). The QuantStep4 data file was imported into Seegene Viewer for Real-Time PCR instruments (V3), and the test kit (Allplex ™ GI-Virus Assay (8 strip)) was chosen from the product menu for analysis of the results for each well. A Ct value of ≤40 indicates a positive result, whereas a Ct value of > 40 or N/A (not detected) indicates a negative result.

5.2.4 Histology

Tissue samples from the gastrointestinal tract (GIT) (duodenum, small and large intestine) were collected for histological analysis and placed in Tissue-Tek Uni-Cassettes and fixed in 10% formalin for up to 24 hours to harden and preserve the tissue. Following preservation, the preserved tissue was processed using the Tissue-Tek® VIPTM 5 Vacuum Infiltrator Processor (Sakura® Finetek, Europe). The fixation process involved 12-24 hours in formalin, followed by the dehydration of the tissue and xylene submersion. The processed tissue samples were then embedded in paraffin wax blocks. The embedded tissue was cut into 3-5 µm thick sections using an Accu-Cut® SRMTM microtome (Sakura® Finetek, Europe) and then placed in the AWB 210 Water Bath (Amos Scientific, Australia) at 60°C to remove folds. To facilitate the attachment of tissue to the microscope slide, sections were mounted on glass microscope slides (Starfrost®, UK) and incubated (Scientific Series 9000) for 30 minutes at 77°C. The tissue was stained with haematoxylin and eosin (H&E) (Sigma-Aldrich, South Africa) for visualisation under light microscope (Titford, 2005) The H&E staining technique allowed identification of the morphological changes within GIT tissue. An Olympus® BX41 light microscope was used to analyse the stained microscope slides at magnifications of 40x, 100x and 200x. The histology slides were analysed to identify features such as lymphocytic and neutrophil infiltrates, oedema, and inflammation.

5.2.5 Statistical analysis

Statistica® version 14.0 Statistical Software (TIBCO Software, Inc.) was used to perform analyses. Associations were calculated using the Mann-Whitney U test for numerical data and the Fisher exact

test for categorical data. Statistical significance was observed at $p < 0.05$ at a 95% confidence interval, with a strong significance observed at $p < 0.01$. In the case of non-normally distributed data, the Mann-Whitney U and Fisher exact tests were used.

5.3 Results

5.3.1 Epidemiological data

A total of 176 cases (57.4% males and 42.6% females) were included in the study group. The median age of the infants at the time of death was 8.3 weeks (interquartile range [IQR]: 4.8-16.6), and the mean post-mortem interval (PMI) was 6.6±3.5 days. The control group consisted of 12 (40%) males and 18 (60%) females, with a median age of 24 weeks (IQR: 9-38).

5.3.2 Laboratory results

Viruses were detected in 111 of the 176 (63%) SUDI cases. A single virus was detected in 63 SUDI cases and 17 controls. NoV GII was detected in 11 samples, while 10 were negative for all viruses (Table 5.4). Among the enteric viruses detected, 36 cases had co-detections of 2 viruses, while 3 controls had co-detections of 2 viruses (Table 5.5). Viral and bacterial co- infections were not detected in 93 (53%) cases while co-infections were detected in 83 (47%) cases. The most prevalent co-infections were one virus and two or more bacteria (Table 5.6). The most prevalent virus and bacteria co-infections patterns were NoV (GI/GII) and DEC 21(25.3%) as well as RV-A and DEC 21(25.3%).

Seasonal variations in the occurrence of enteric viruses were evident (Figure 5.1). RV-A, NoV GI, NoV GII, AdV-F and AstV were more frequently detected in the colder months (49.5%, 33.3%, 22.5% and 8.1% respectively) compared to the warmer months, with SaV the least frequently detected (0.9%).

Table 5.4: Frequency (n, %) of GIT viruses detected in 176 SUDI cases and 30 control samples

GIT viruses	SUDI Cases	Controls
RV-A	68 (38.6%)	10 (33.3%)
NoV	53 (30.0%)	11 (36.7%)
AdV-F	28 (15.9%)	0 (0.0%)
AstV	17 (9.7%)	0 (0.0%)
SaV	1 (0.6%)	2 (6.7%)
Negative	65 (36.9%)	10 (33.3%)

Table 5.5: Prevalence of virus and virus co-infections detected in the cases and control group

No. of viruses	Virus combinations	SUDI Cases (n=48)	Controls (n=3)
Two	NoV GII + RV-A	8	3
	AdV-F + RV-A	8	0
	NoV GII + AdV-F	3	0
	ASV + RV-A	13	0
	NoV GI + RV-A	1	0
	ASV + SaV	1	0
	NoV GII + NoV GI	2	0
	Double infections in total (%)	36 (75.0%)	3 (100.0%)
Three	NoV GII + AdV-F + RV-A	10	0
	NoV GII + AdV-F + NoV GI	1	0
	Triple infections in total (%)	11 (22.9%)	0 (0.0%)
Four	ASV + NoV GII + NoV GI + RV-A	1	0
	Quadruple infections in total (%)	1 (2.1%)	0 (0.0%)

Table 5.6: Prevalence of virus and bacteria co-infections detected in the cases (n=176)

Virus and bacteria co-infection	Virus and bacteria combinations	SUDI cases
One virus + two or more bacteria	NoV (GI/GII) +DEC	21
	RV-A+DEC	21
	ADV-F+DEC	4
	Total co-infections (%)	46 (26.1%)
Two viruses + two or more bacteria	ASV+RV-A+DEC	7
	ADV-F+RV-A+DEC	5
	NoV (GI/GII)+RV-A+DEC	5
	NoV (GI/GII)+ADV-F+DEC	2
	Total co-infections (%)	19 (10.8%)
Three viruses + two or more bacteria	NoV (GI/GII)+ADV-F+RV-A+DEC	6
	Total co-infections (%)	6 (3.4%)
One virus + one bacterium	NoV (GI/GII) +DEC	3
	RV-A+DEC	1
	ADV-F+DEC	1
	Total co-infections (%)	5 (2.8.7%)
Three viruses + one bacterium	NoV (GI/GII)+ADV-F+RV-A+DEC	3
	Total co-infections (%)	3 (1.7%)
Two viruses + one bacterium	NoV (GI/GII) +DEC	2
	ADV-F+DEC	1
	Total co-infections (%)	3 (1.7%)

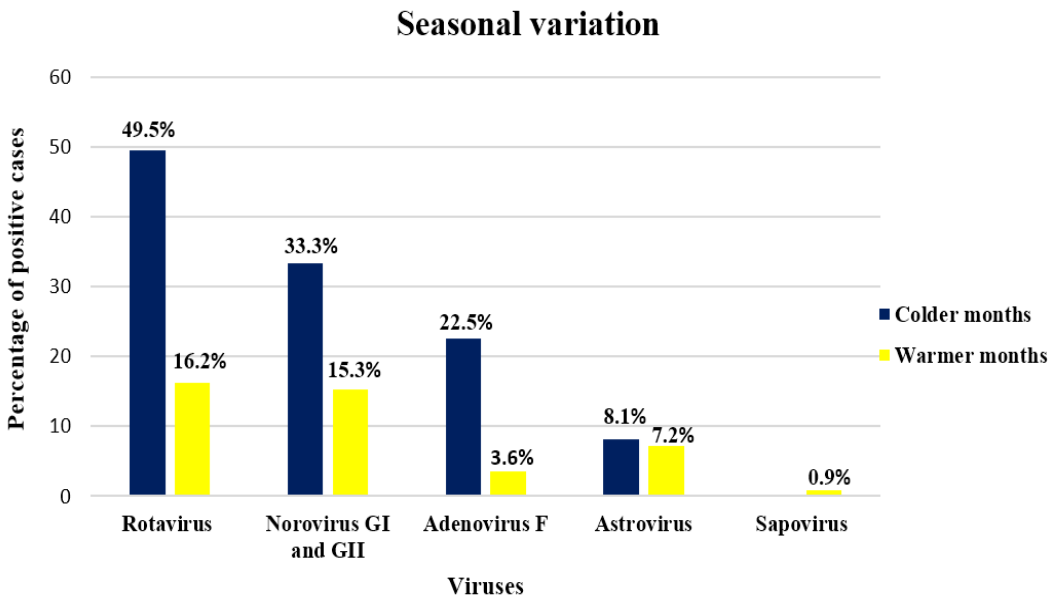


Figure 5.1: Seasonal variation of viruses detected in the SUDI cases from June 2017 to May 2018

5.3.3 Histology results

Histopathological analysis did not provide any significant results. Autolysis was prevalent on most slides, and it was thus not possible to determine whether autolysis have obscured any morphological changes.

5.3.4 Statistical analysis

In the SUDI group, a highly significant association was observed between RV-A, NoV GII, AdV-F, AstV and season (Fisher exact test, $p < 0.01$). NoV GI and SaV displayed no association with season. None of the viruses were associated with sex, prematurity, bed-sharing, PMI, or number of people in the household. AdV-F showed significant associations with both age in weeks and birth weight (Mann-Whitney U test, $p=0.01$), while NoV GI had a significant association with the position the infant was placed to sleep (Fisher exact test, $p=0.03$) and medical history (Fisher exact test, $p=0.04$).

5.4 Discussion

Globally, enteric viruses have been recognised as the most common cause of gastroenteritis (Bányai et al., 2018). In the current study, enteric viruses were detected in more than 60% of both SUDI cases and controls. A similar prevalence was found in Gabon (Lekana-Douki et al., 2015) but it was considerably lower than Burkina Faso (Ouédraogo et al., 2016). In contrast, lower prevalence rates between 30% and 54% were found in Cameroon (Ayukekbong et al., 2011), India (Chitambar et al., 2012), Nigeria (Arowolo et al., 2019), Europe (Flahault & Hanslik, 2010) and Italy (Biscaro et al., 2018). It is therefore apparent that the viral gastroenteritis burden varies among locations and

countries, but it is clear that low- and middle-income countries are affected more severely (Arowolo et al., 2019).

RV-A was the most prominent pathogen detected in the SUDI cases. Previous South African studies described the distribution and diversity of enteric viruses in stool samples collected from children younger than five years (Rossouw et al., 2021) as well as the possible mixed diarrhoeal aetiology in stool samples collected from children younger than four years (Chukwu et al., 2019). RV-A was the predominant enteric virus in children (22% and 82%, respectively). In developing countries such as South Africa, RV is still the primary cause of viral gastroenteritis hospitalisations, despite routine vaccination programmes (Operario et al., 2017).

NoV, predominantly NoV GII, was the second most frequently detected virus in the SUDI cases and the most frequent virus in the control group. Similarly, genogroup NoV GII has been found in clinical cases and disseminated in communities globally (Patel et al., 2008; Siebenga et al., 2009; Hoa Tran et al., 2013). Generally, children living in areas with poor sanitation and hygiene practices are at increased risk of exposure to enteropathogens, which, similar to other developing countries such as Mexico, Brazil, Bolivia and China, could be associated with high reported NoV GII prevalence rates (García et al., 2006; Ferreira et al., 2010; Zou et al., 2015; McAtee et al., 2016).

The low prevalence of AdV-F found in this study is marginally higher than in previous South African studies that found AdV in 7-12% of cases (Govender et al., 2017; Rossouw et al., 2020; 2021). The marginally higher prevalence rate is likely to result from the virus being more endemic in the sampling region in comparison to the other study regions. In contrast, a study from Ethiopia describing the prevalence and genetic diversity of human AdV and human AstV in stool samples collected from infants and children with diarrhoea found a much higher AdV prevalence (32%) (Gelaw et al., 2019). The lower prevalence in the current study may be the result of the use of a less sensitive assay than that used in the study in Ethiopia, which was more sensitive and able to detect all known types of human AdV.

AstV usually causes milder infections not requiring hospitalisation (Bosch et al., 2014). A study from Kenya and Gambia investigated the prevalence and diversity of both classic and novel AstV in children under five and confirmed the presence of AstV in 10% of cases (Meyer et al., 2015). However, a Nigerian study examining the prevalence, seasonality, and risk factors of enteric viruses in stool samples of children with acute gastroenteritis found AstV in 20% of these cases (Arowolo et al., 2019). In contrast to the current study, a retrospective South African study (Nadan et al., 2019) screened diarrhoeagenic stool samples from children under the age of five for viruses, bacteria and parasites and found AstV in only about 6% of infants who died during the course of the study. Infants

in the current study died suddenly and unexpectedly before hospitalisation could possibly occur, whereas those in the retrospective study were hospitalised for an average of four days before passing away. This may explain the higher prevalence observed. Furthermore, it is likely that the higher prevalence rate may be due to the fact that AstV infection is frequently associated with the lack of a source of indoor water, and contaminated water sources such as boreholes, cisterns, communal taps, and rivers (Hwang et al., 2015).

Although SaV was the least prevalent virus in the cases in this study, found in less than 1%, the prevalence found in the control group was comparable to the 8% found in hospitalised children under five years old with acute diarrhoea between 2009 and 2013 (Page et al., 2016b). In comparison to RV or NoV, SaV can present with a rather mild clinical presentation (Pang et al., 2000; Sakai et al., 2001), and although mortality associated with SaV is rare, outbreaks have been reported in elderly long-term care facilities (Oka et al., 2015). This may explain the higher prevalence in the control group than in the cases. The Malnutrition and Enteric Disease Study (MAL-ED) reported a 23% SaV detection rate globally, including South Africa (Liu et al., 2016). Different prevalence rates amid distinct studies are common and may possibly be linked to different study designs, study settings, sample sizes, sampling seasons, socioeconomic status of the population and viral detection procedures used during the investigations (Kotloff et al., 2019).

Almost half of the current cases had coinfections, which was about 10% more than a previous study of South African children under five years of age hospitalised for gastroenteritis (Rossouw et al., 2020, 2021). Similarly, a study in Burkina Faso reported enteric viruses in 35% of children under five years of age (Ouédraogo et al., 2016). The most commonly reported coinfection in this study was between AstV and RV-A, which is in agreement with previous findings from South Africa (Rossouw et al., 2020, 2021). The prevalence rate was, however, much lower than reports from Nigeria (59%) (Arowolo et al., 2019). This study also found coinfections between NoV GII, RV-A and AdV more frequently than results from Kuwait (21% versus 8%) (Mohammad et al., 2020). There are biological and epidemiological implications of mixed viral infections whereby viruses interact either synergistically or antagonistically, changing the concentration of either or both viruses and impacting the outcome of the infection (Mohammad et al., 2020).

Co-infections with bacteria and viruses were detected in 47% of cases. Similarly, a study conducted in South Africa to determine the prevalence of individual and multiple diarrhoea-causing pathogen combinations among children suffering from diarrhoea in rural and peri urban communities reported a 47% prevalence of bacterial and viral co-infection (Potgieter et al., 2023). The prevalence of co-infection between NoV (GI/GII) and DEC as well as RV-A and DEC in this study was higher than

that in Southwest China (25.3% versus 1.1% and 25.3% versus 2.3%, respectively) (Zhang et al., 2016). The effects of co-infections on the intestinal flora have been shown to alter its composition, reduce its diversity, and increase the frequency of intestinal flora disorders (Li et al., 2020; Sabey et al., 2021). Inflammatory processes may be triggered by microbiota that directly interact with epithelial cells (Mathew et al., 2019).

In the GIT, autolysis is more rapid due to the presence of self-digesting enzymes (What is Autolysis? - Definition & Histology, 2021), possibly explaining the frequency of autolysis found in the histology slides. Compared with histology slides of other organs, such as the lung and heart, which are routinely examined, it was difficult to analyse the GIT slides in this study due to PMI and autolysis. As a longer PMI has the effect of deteriorating the quality of the tissues and consequently, the results obtained (Heimesaat et al., 2012)

Colder months have repeatedly been associated with a spike in infant deaths compared to warmer months (Chang et al. 2008). Different mechanisms may be at play. Cold weather increases the likelihood of close contact among people who may have been exposed to fomites contaminated with viruses, thereby increasing the risk of transmission from person to person (Arowolo et al., 2019). These conditions may contribute to the organism's spreading, transmitting, and maintaining itself (D'Souza et al., 2008), which was supported by the incidence of viral peaks during the colder months in this study.

The highest incidence of SUDI has repeatedly been shown to occur in early infancy (2-4 months) when immune responses are still immature, and levels of maternal antibodies are declining (Raza & Blackwell, 1999). Birth weight below 2 500 grams is also associated with a higher risk of infection (Read et al., 1994) as well as impaired homeostasis, which can lead to infection (Bobak & Guerrant, 2015). This supports the associations observed between age and AdV-F as well as birth weight. As opposed to other GIT viruses, AdV infections can be contracted throughout the year, which may explain the statistical associations observed, especially among infants with compromised immune systems.

An association was observed between NoV GI and GII and the position the infant was placed to sleep as well as the medical history of the infant. It has been reported in a study conducted in Tasmania that SIDS can occur in the prone position if accompanying symptoms, such as cough, fever, nasal congestion, vomiting, or diarrhoea, are present on the day of death or the day prior. Among infants in the prone position, the risk of SIDS was higher for those who were ill than for those who were healthy (Ponsonby et al. 1993). This could explain the association observed in the current study.

5.5 Conclusion

Based on the results of this study, RV-A and NoV were identified as the most common enteric viruses in infants under 12 months of age. It is imperative to highlight both the importance of RV-A vaccinations and the significance of NoV infection in children following RV vaccination. It is particularly critical in a developing country such as South Africa where infections caused by RV-A remain the leading cause of viral gastroenteritis despite routine vaccinations. It has been demonstrated that co-infection with multiple microbes can increase both morbidity and mortality. The identification of pathogens in infants with diarrhoea is therefore imperative.

Chapter 6: Characterisation of human rotavirus group A genotypes in Sudden Unexpected Death in Infancy cases at the Tygerberg Medico-legal Mortuary, Cape Town

Abstract

Background: Rotavirus (RV) affects neonates and children under the age of five in both in developed and developing countries each year. Among young children, RV-A is the most common cause of seasonal endemic diarrhoea. RV particles have outer capsid proteins, VP7 and VP4, that can independently elicit an immune response and were used during the development of vaccines. The distribution of RV strains in cases of sudden and unexpected deaths in infancy (SUDI) is unknown; therefore, the aim of this study was to characterise RV strains in SUDI cases and controls.

Materials and methods: Thirteen previously extracted and confirmed RV positive samples from the main SUDI study were included in this sub-study. Genotyping was conducted using standard methods.

Results: G1P[8] was the most frequent combination of G type and P type (4/10, 40%). This was followed by G2P[4] (3/10, 30%) while G9P[8] was present in only two cases (20%) and G8P[4] was the least prevalent in a single case only (10%).

Conclusion: In this study, G1P[8] was the most prevalent genotype combination, followed by G2P[4]. This finding agrees with the literature; however, further research with a larger sample size will enable a better understanding of the genotypes prevalent in this specific study population.

6.1 Introduction

In South Africa, diarrhoea accounts for 7.2% of infant mortality, making it the third leading cause of infant death (Statistics South Africa, 2018a). Malnutrition is a major risk factor in the development of diarrhoeal disease in children, as well as inadequate access to clean water, and unsanitary conditions. These risk factors are responsible for 84% of all childhood deaths due to diarrhoeal diseases. RV affects neonates and children under five years in both developed and developing countries, causing around 258 million cases of morbidity and 128 000 diarrhoeal deaths every year al, 2018). Approximately 80% of these deaths occur in Sub-Saharan African countries (Troeger et al, 2018; Steele & Groome, 2020).

As a result of its segmented genome, RV can adopt remarkable genetic diversity (Zeller et al., 2015). There are a number of new strains arising every year as a result of recombination of genes, genomic re-assortment, point mutation accumulation, and inter-species transmission mechanisms (Kirkwood, 2010; Seheri et al., 2018). Humans are susceptible to RV groups A-H, with RV-A being the most common cause of seasonal endemic diarrhoea among young children. In addition to infecting mammals, RV-A is also capable of infecting birds (ICTV, 2021).

A binary classification system was devised based on the antigenic reactivity of VP4 and VP7, which induce neutralising antibodies independently (Estes & Kapikian, 2007; Matthijnssens et al., 2011). VP7 and VP4 are two outer capsid proteins that are genetically distinct, and this allows the virus to be classified into two genotypes, G (glycoprotein) and P (protease-sensitive), respectively (Estes & Kapikian, 2007). Through the use of reverse transcription-polymerase chain reaction (RT-PCR) and sequencing techniques, 36 different G genotypes and 51 different P genotypes have been described so far (Steger et al., 2019).

Wa-like and DS-1-like genotype constellations are two major genotype constellations found in humans (Matthijnssens et al., 2009). P[8] genotypes are frequently associated with a Wa-like genotype constellation, whereas P[4] genotypes are usually associated with a DS-1-like genotype constellation. The Wa-like genotype constellation is the most important genotype in humans and it is responsible for more than 90% of all infections caused by RV (Bányai et al., 2012; Matthijnssens & Van Ranst, 2012). The dominant genotypes of VP7 RV on the African continent are G1, G2, G3, G4, G8, G9 and G12; G4 strains predominated in the 1980s and 1990s, but have since declined greatly (Steele et al., 2003). In terms of VP4 genotypes, the most frequently circulating ones are P[8], P[6], and P[4] (Mwenda et al., 2010; Seheri et al., 2014; Nyaga et al., 2018). Prior to the introduction of RV vaccines in Africa, genotype G1P [8] strains were prevalent (Seheri et al., 2018).

Currently, four RV vaccines have been prequalified for routine use by the WHO: RotaTeq (Merck and Co., West Point, PA. USA), Rotarix (GlaxoSmithKline Biologicals, Rixenstart, Belgium) in 2008 and 2009, respectively and two vaccines manufactured in India, Rotavac (Bharat Biotech, Hyderabad, India) and RotaSil (Serum Institute of India) in 2018 (Global Alliance for Vaccines and Immunizations Detailed Product Profiles/Gavi, the Vaccine Alliance). Based on a sub-analysis of high-mortality countries in Africa and Asia, the 4 vaccines were similar in their efficacy against severe rotavirus gastroenteritis at 1 year of follow-up, with vaccine efficiencies ranging from 48% to 57% (WHO, 2021). According to an analysis of data from 69 countries participating in the Global Rotavirus Surveillance Network (GRSN), rotavirus prevalence decreased by 40% following the introduction of the rotavirus vaccine (Aliabadi et al., 2019). Vaccination has also been shown to reduce hospitalisations associated with rotavirus infections, all cause acute gastroenteritis hospitalisations, and gastroenteritis mortality in several countries (WHO, 2021). As of August 2009, the RV vaccine was introduced into the South African Expanded Programme of Immunisation (SA-EPI) (Hemming et al., 2013). Despite improved vaccination coverage, studies have documented alarming rates of RV-associated diarrhoeal morbidity and hospitalisation in certain regions in South Africa over the past decade (Iyaloo et al., 2013, Asowata et al., 2018).

Efficacy trials on Rotarix and RotaTeq showed that they were more effective in developed countries with low diarrhoeal mortality than in developing countries with high diarrheal mortality (Bergman et al., 2021). The lower efficacy of rotavirus vaccination in developing countries is likely to be a multifactorial phenomenon due to differences in rotavirus epidemiology with high levels of infection, deficiencies in essential vitamins and minerals, exposure to certain pathogens prior to vaccination, and chronic diseases such as malaria and human immunodeficiency virus (HIV). Consequently, the poor GIT response to the live vaccine has a negative impact on the efficacy thereof (Parker et al., 2018). There is currently no research that characterises the distribution of RV in SUDI cases; therefore, this study aimed to characterise the RV strains in SUDI cases and control samples.

6.2 Materials and methods

6.2.1 Samples

A total of 13 RV positive PCR products were successfully sequenced in the study. These PCR products were obtained from previously extracted RV positive samples. It included 7 cases and 3 controls, of whom vaccination statuses were unknown for 6 infants, while 2 did not receive vaccinations, and 2 only received the first vaccination dose. Among the seven cases, two amplified both VP7 and VP4 genes, while the remaining five amplified either VP4 or VP7 genes. This resulted in nine successfully sequenced PCR products. Among the three controls, one control amplified both

the VP7 and VP4 genes, while the remaining two controls amplified either the VP4 or VP7 genes. This resulted in four successfully sequenced PCR products.

6.2.2 cDNA synthesis and conventional PCR

The complimentary deoxyribonucleic acid (cDNA) was synthesised on the extracted ribonucleic acid (RNA) template using the LunaScript RT Supermix Kit (New England Biolab, USA). A total of 8 µL RNA template was added to 2 µL LunaScript RT Supermix at the following cycling parameters: 25°C for 2 minutes, 55°C for 10 minutes, 95°C for 1 minute, and 4°C hold. Following the addition of the reaction mixture to the SimpliAmp™ thermal cycler (Life Technologies, Applied Biosystems® by ThermoFisher Scientific™, Randburg), the cDNA synthesis was then initiated.

The GoTaq® Flexi DNA Polymerase Kit (Promega, USA) was used to conduct conventional PCR on both the VP7 and the VP4 genes. The components and volumes for the PCR master mix are listed in Table 6.1 and the primer sequences are shown in Table 6.2. Geneious Prime® 2021.1.1 was used to determine primer annealing temperature and target specificity. PCR mixtures were prepared in sterile 1.5 mL Eppendorf tubes and 47 µL was aliquoted into sterile 0.2 mL PCR strips with strip caps (STARLAB International, Hamburg). Following the addition of the template to the PCR tubes, the mixture was centrifuged to eliminate any bubbles that were present. A total volume of 50 µL was used for the PCR assay. The PCR runs included a positive control, a negative control, and a non-template control in order to avoid false-positive results. As a positive control, a cultured RV positive sample was used.

For the PCR, the reaction mixture was placed into a SimpliAmp™ thermal cycler (Life Technologies, Applied Biosystems® by ThermoFisher Scientific™, Randburg) according to the conditions outlined in Tables 6.3 and 6.4.

Table 6.1: PCR master mix volume of each reagent used per reaction s

Reagent	Volume per run (µL)
GoTaq Flexi buffer	10
Primer (forward)	5
Primer (reverse)	5
dNTP mix	1
GoTaq DNA polymerase	0.25
Template	3
Nuclease-free water	23.75
Total volume	**50 µL**

Table 6.2: The consensus primers used in this study

Primer	Primer sequence 5'-3	Reference	Product size
VP7-F VP7-R	5′ ATG TAT GGT ATT GAA TAT ACC AC 3′ 5′ AAC TTG CCA CCA TTT TTT CC 3′	Iturriza-Gómara et al., 2004	842bp
VP4-F VP4-R	5′ TAT GCT CCA GTN AAT TGG 3′ 5′ ATT GCA TTT CTT TCC ATA ATG 3′	Simmonds et al., 2008	624bp

Table 6.3: VP4 PCR thermocycling conditions

Step	Temperature (°C)	Duration	Cycle
1	95°C	2 min	1
2	94°C	45 sec	35
3	45°C	45 sec	
4	72°C	1 min	
5	72°C	5 min	1
6	4°C	∞	

Table 6.4: VP7 PCR thermocycling conditions

Step	Temperature (°C)	Duration	Cycle
1	95°C	2 min	1
2	94°C	45 sec	25
3	50°C	45 sec	
4	72°C	51 sec	
5	72°C	5 min	1
6	4°C	∞	

6.2.3 PCR product visualisation

Visualisation of the PCR products was carried out by gel electrophoresis, using sodium borate (SB) buffer, which has a lower conductivity, operates at a faster speed, and produces brighter bands than standard buffers (Brody & Kern, 2004). To prepare a 20X SB buffer stock solution, 8 grams of sodium hydroxide and 45 grams of boric acid were dissolved in 1 litre of distilled water from Milli-Q® (Merck Millipore, Germany). A 1X SB buffer working solution was subsequently prepared by diluting 5 mL of SB buffer stock solution in 995 mL of MilliQ water.

A 2% agarose gel was prepared by dissolving 2 grams of Lonza® LE agarose (Lonza BioWhittaker, Verviers, Belgium) in 1X SB buffer to make a 100 mL mixture. It was then heated in a microwave oven for 3 minutes or until the solution was clear. After the gel mixture had cooled down sufficiently, 5 µL of Condasafe gel dye was added to it (Laboratorios CONDA, Madrid, Spain) and the gel mixture was poured into an electrophoresis tray with a 1 mm gel comb. This was left at room temperature for approximately 30 minutes before the comb was removed. The gel was placed in the Enduro™ Electrophoresis System (Labnet, USA) and 1X SB buffer was added to completely cover the gel to allow for effective electricity conduction. A 3 µL ready-to-use GeneRuler™ 100 bp DNA ladder (ThermoScientific, USA) was loaded in the first and last wells of the gel to provide a size reference. To load the rest of the wells, 5 µL of the PCR product was mixed with 1 µL of 6X orange loading dye (ThermoScientific, USA). After electrophoresis at 100V for 40 minutes, the gels were visualised using ChemiDoc™ Imaging Systems (Bio-Rad, USA).

6.2.4 PCR product purification

The unpurified PCR products were frozen at -20°C and delivered on ice to Inqaba Biotechnical Industries (Pty) for the purification of PCR products and sequencing. Purification of PCR products was carried out using the Rapid PCR Cleanup Enzyme Set (New England Biolabs, USA). By adding 50 µL Exonuclease I and 200 µL Shrimp Alkaline Phosphatase, a master mix of Exo/SAP was prepared. To 10 µL amplified PCR product, 2.5 µL Exo/SAP master mix was added. After mixing thoroughly, the mixture was incubated for 15 minutes at 37°C. Following this, it was heat inactivated at 80°C for 15 minutes.

6.2.5 Sequencing PCR

Sanger sequencing was performed using the Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1 (NimaGen, The Netherlands). In order to sequence the specific region of interest, a master mix was prepared for each primer used. In accordance with the manufacturer's instructions, 1 µL of purified PCR product was added to a reaction mix containing the reagents shown in Table 6.5. Based on the cycling parameters described below, the reaction was performed on either a GeneAmp® 9700 thermal cycler or a Veriti® (Applied Biosystems, USA). The following parameters were used: 96°C for 45 seconds, 25 cycles at 96°C for 10 seconds, 50° C for 5 seconds, and 60°C for 4 minutes.

Table 6.5: The volume of each reagent per reaction used in the sequencing PCR assays.

Reagent	Volume per run (µL)
BrilliantDye v3.1 rr Premix	1
5x Sequencing Buffer	1.5
Primer	1
Template	1
nuclease-free water	5.5
Total volume	**10 µL**

6.2.6 Sequencing clean-up

The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Zymo Research, USA) by adding 240 µL of Sequencing Binding Buffer to 5-10 µL sequencing reaction. The mixture was transferred to the Zymo-Spin™ IB-96 plate mounted on a collection plate provided. Following centrifugation at 3 000 x g for 2 minutes, 300 µL of Sequencing Wash Buffer was added to each well of the collection plate. It was then centrifuged at ≥ 3 000 x g for 5 minutes. Water (15-20 µL) was added directly to the column matrix filter plate. The Zymo-Spin™ IB-96 plate was placed on top of the 96-Well PCR plate and the assembly was mounted onto the collection plate. The plate was centrifuged for 2 minutes at 3 000 x g to elute the DNA. The cleaned-up products were injected into the Applied Biosystems ABI 3500XL Genetic Analyser or Applied Biosystems ABI 3730XL Genetic Analyser (ThermoFisher Scientific, USA) with a 50 cm array, using POP7.

6.2.7 Phylogenetic Analysis

For each sample, sequence data files were uploaded to Geneious Prime® 2023.1 (Biomatters Inc., USA) and consensus sequences were assembled with the De Novo Assemble tool and primers were trimmed accordingly. The National Center for Biotechnology Information's (NCBI) online Basic Local Alignment Search Tool (BLAST) was used to verify that the obtained sequences were human RV-A. GenBank® was used to download the related sequences. Appendix D includes a list of GenBank® sequences downloaded.

Multiple sequence alignment of the nucleotide sequences was performed using Multiple Alignment using Fast Fourier Transformation software version 7 (https://mafft.cbrc.jp/alignment/server/). To determine the best model test, maximum likelihood tree, and bootstrapping support, Randomised Axelerated Maximum Likelihood (RAxMLGUI 2.0) was used. Bootstrap analysis of 1 000 replicates were performed and only clusters with 70% or more support were considered significant. The HKY+G4 model (Hasegawa-Kishino-Yano with gamma distributed rates) was found to best fit the

sequence data for the VP4 and VP7 genes, respectively (Hasegawa et al., 1985). FigTree version 1.4.4 was used to edit the trees (Rambaut, 2018).

6.3 Results

6.3.1 Phylogenetic analyses based on the VP7 and VP4 genes

During the current study, six sequences were obtained using conventional PCR targeting a fragment of 842 bp of the VP7 gene. Additionally, seven sequences were obtained using conventional PCR targeting a fragment of 624 bp of the VP4 gene.

In Figure 6.1, at position one, the sequence derived from sample 1174_2 clusters with other RV strains from South Africa (Genbank IDs MW552597, MW552146, MW552685, unpublished study by Nyaga et al., 2021) and Kenya (Genbank IDs MZ096975, unpublished study by Lambisia et al., 2021, and MN194385, unpublished study by Mwanga et al., 2020). This sample was 100% identical to a strain genotyped as G2P[4]. At position two, the sequence from 897_VP7 does not appear to cluster directly with other RV sequences. Instead, it is positioned close to clusters of RV strains from Kenya (Genbank IDs MK434785, MK434784 and MK434780, unpublished study by Mwanga et al., 2019; Genbank ID MH402590, unpublished study by Owor et al., 2018; Genbank ID MZ093894, unpublished study by Lambisia et al., 2021). In addition, this sample was 99% identical to an isolate and strain that was genotyped G8P[4].

At position three, sequence H16_2 clusters with RV strains (Genbank ID MN787037, unpublished study by Kuča and Mans, 2021) from South Africa and Zimbabwe (Genbank IDs KJ752797, KJ753710, KJ753539 direct submission by Wentworth et al., 2015 and Genbank ID KP753098, direct submission by Das et al., 2015). There was 99% similarity between this sample and an isolate and strains genotyped as G9P[8]. At position four, the sample sequences from 1606_2, H12_2 and 0124_2 are seen to cluster with RV strains from Belguim (Genbank IDs ON855111 and ON855125, published study by Simsek et al., 2022), South Korea (Genbank IDs MG922993 and MG922997, unpublished study by Tran & Kim, 2018), Brazil (Genbank IDs MG590362 and MG590364, unpublished study by Pankov et al., 2018; Genbank ID MT633136 published study by Silva-Sales et al., 2021), Japan (Genbank ID KY616899, published study by Kaneko et al., 2018) and Venezuela (Genbank ID MG571803, unpublished study by Siqueira et al., 2018). These samples were between 99-100% identical to isolates and strains genotyped as G1P[8]. Additionally, sequences from H12_2 and 0124_2 also showed strong phylogenetic relatedness to a Rotarix vaccine strain from Brazil (Genbank ID MH884610, unpublished study by Gelaw et al., 2019).

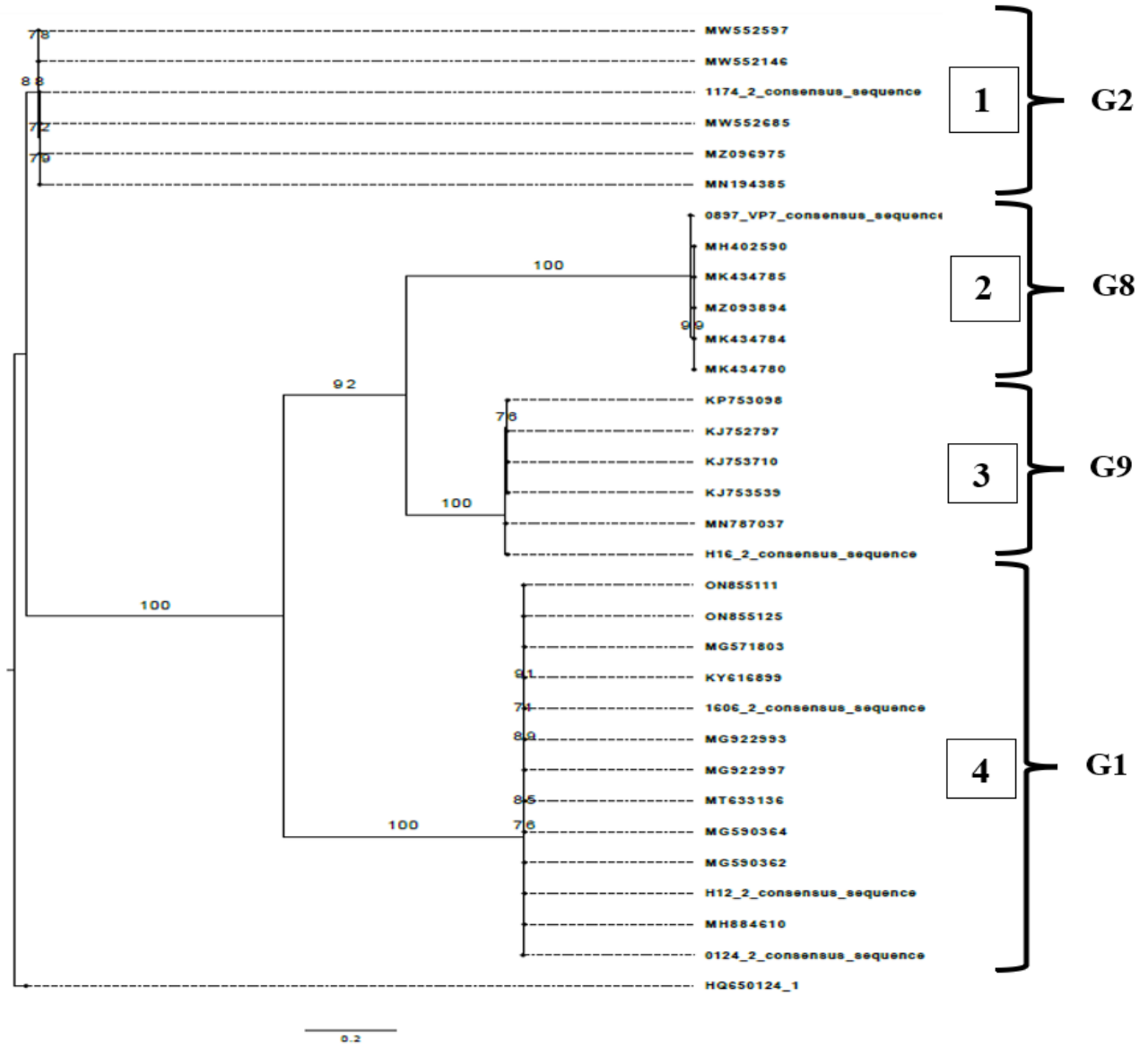


Figure 6.1: Phylogenetic analysis of the Rotavirus VP7 gene (842 bp)

Phylogenetic inference was performed by the Maximum Likelihood method with 1 000 bootstrap replicates in RAxMLGUI 2.0. The Hasegawa-Kishino-Yano model with gamma distributed rates was used (HKY+G4). The tree was rooted with RV-A/Human-tc/USA/DS-1/1976/G2P[4] (HQ650124_1). The scale bar indicates the number of nucleotide substitutions per site.

As shown in Figure 6.2, at position one, the sequence derived from sample 0897_VP4 clusters with that of other RV strains from South Africa (Genbank IDs MW392029, MW392039, MW392041, MW392006, unpublished study by Rossouw et al., 2021), as well as Kenya (Genbank IDs MZ094320, unpublished study by Lambisia et al., 2021). In addition, this sample was 100% identical to an isolate that was genotyped G8P[4]. At position two, the sequence from samples 1366_VP4, 1525_2 and 1174_2 appears to cluster with other RV strains from South Africa (Genbank IDs MW552576, MW552642 and MW552554, unpublished study by Nyaga et al., 2021; Genbank ID MW392003,

unpublished study by Rossouw et al., 2021). These sequences further clustered with a RV strain from Kenya (Genbank ID MZ096973, unpublished study by Lambisia et al., 2021) and 100% identical to isolates and strains genotyped as G2P[4].

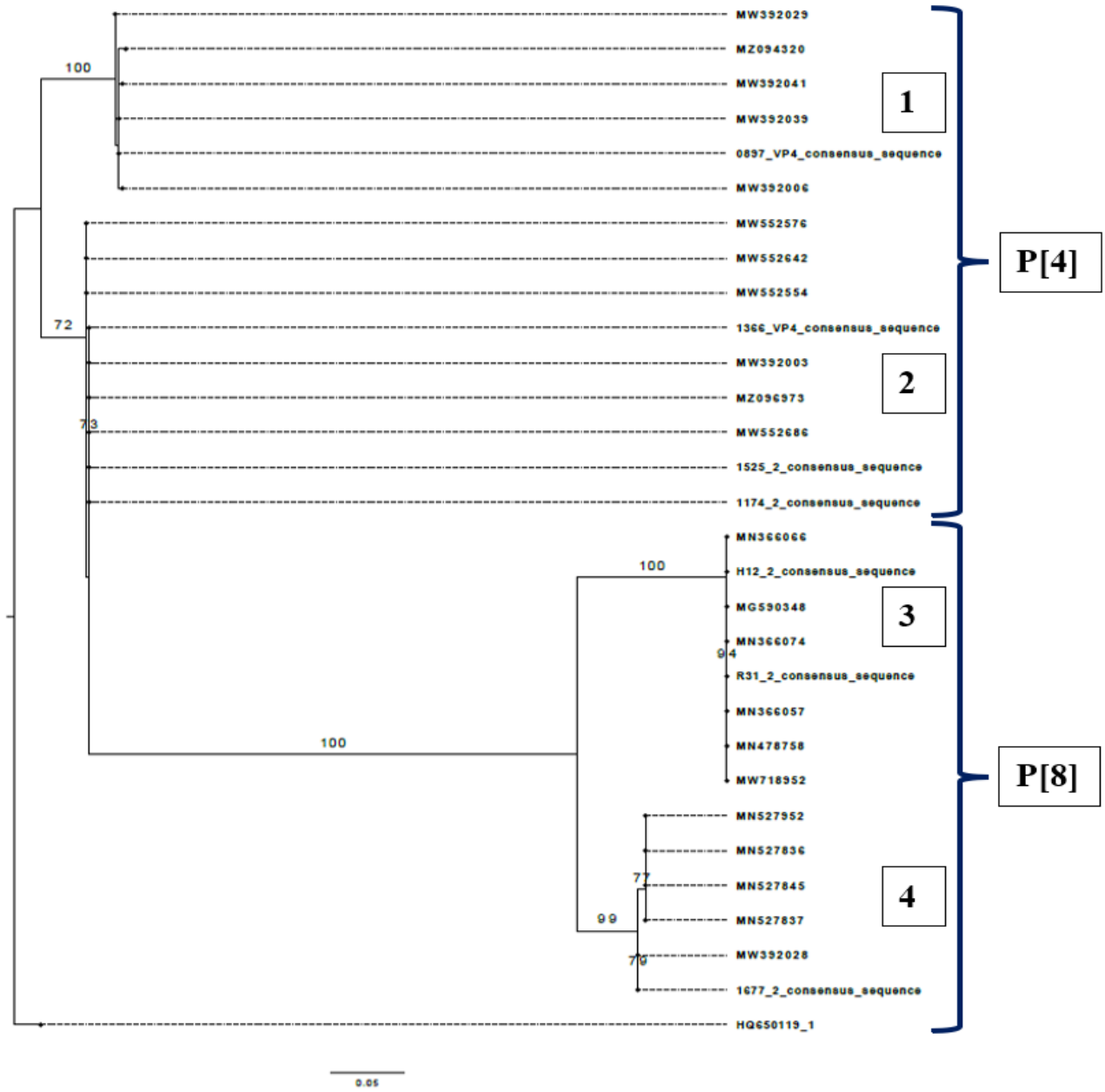


Figure 6.2: Phylogenetic analysis of the Rotavirus VP4 gene (624 bp)

At position three, H12_2 and R31_2 sample sequences were clustered with RV strains from Brazil (Genbank IDs MN366066, MN366074 and MN366057, unpublished study by Cantelli et al., 2020; Genbank ID MG590348, unpublished study by Pankov et al., 2018), the USA (Genbank ID MN478758, unpublished study by Esona et al., 2021) and Tanzania (Genbank ID MW718952, direct submission by Malakalinga et al., 2022). The two control samples were also 100% similar to an isolate and strains genotyped as G1P[8].

Moreover, at position four, the sequence from 1677_2 is clustered with RV strains from South Africa (Genbank ID MW392028, published study by Rossouw et al., 2021) and China (Genbank IDs MN527952, MN527836, MN527845 and MN527837, unpublished study by Gao et al., 2022). There was a 100% similarity between this sample and an isolate genotyped as G9P[8].

Phylogenetic inference was performed by the Maximum Likelihood method with 1 000 bootstrap replicates in RAxMLGUI 2.0. The Hasegawa-Kishino-Yano model with gamma distributed rates was used (HKY+G4). The tree was rooted with RV-A/Human-tc/USA/DS-1/1976/G2P[4] (HQ650119.1). The scale bar indicates the number of nucleotide substitutions per site. The prevalence of the genotypes confirmed in this study is summarised in Table 6.6.

Table 6.6: Prevalence of genotypes in the study (n=10)

Genotype	Prevalence (n, %)
G1P[8]	4 (40%)
G2P[4]	3 (30%)
G9P[8]	2 (20%)
G8P[4]	1 (10%)

6.4 Discussion

Among the most common G and P genotype combinations worldwide are G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8], with G1P[8] being the most common (Santos & Hoshino, 2005; Rahman et al., 2007; Matthijnssens et al., 2009). During the past few years, the epidemiological relevance of unusual RV genotypes, such as G1P[4], G2P[8], G9P[4], G12P[4], G8P[6], G8P[8], and G12P[6], has increased on the African continent (Moure et al., 2018; Seheri et al., 2018). It is estimated that over 90% of cases of RV gastroenteritis in young children are caused by strains G1P[8], G2P[4], G3P[8], and G9P[8] (Linhares et al., 2011; Patel et al., 2012).

Phylogenetic analysis in the current study has shown that the genotype combination G1P[8] clusters closely with other genotypes G1P[8] described throughout the world. This includes Brazil, the USA, Tanzania, Belguim, Venezuela, Japan, and South Korea. This genotype may reflect the impact of human movements across the borders of South Africa. Globally, G1P[8] is one of the most prevalent and clinically important strains of RV-A (Dóró et al., 2014). VP4 and VP7 variants persist despite antigenic and genetic heterogeneity among G1P[8] strains, and their epidemiological fitness may account for their global prevalence (Santos et al., 2019).

In this study, the most prevalent combination of G type and P type was G1P[8] (4/10, 40%), which contrasts with South African studies by Rossouw et al. (2021) who found G3P[4] to be most prevalent, and Page et al. (2016a) who found G3P[8] and G9P[8]. There was, however, a similar finding in a review article which explained genetic diversity of RV, potential changes in strain types, and changes in the burden of RV after the introduction of RV vaccination in children under five years old in Tanzania, where G1P[8] was also found to be the most prevalent genotype (Malakalinga et al., 2019). As shown by Magagula et al. (2015), all G1P[8] strains displayed Wa-like genetic constellations and shared a moderate degree of nucleotide identity with Rotarix and RotaTeq G1P[8]. There is evidence that vaccine-derived virus is shed in faeces during the first 28 days following the initial dose and 15 days following the second dose, with the G1P[8] RV1 virus detected at higher levels following the first dose (Phua et al., 2005; Anderson, 2008; do Carmo et al., 2011; Atchison et al., 2016; Roczo-Farkas et al., 2018; Bruun et al., 2021). Other countries have reported a higher incidence of acute gastroenteritis due to vaccine shedding or horizontal transmission of the vaccine strain (Payne et al., 2010; Kaneko et al., 2017; Bennett et al., 2019). This study detected that samples sequences H12 and 0124 were phylogenetically related to a Rotarix vaccine strain. This is further supported by the similarity to genotype G1P[8]. However, the vaccination status of the infants in these two samples is unknown. To determine whether the observed vaccine strain is the result of vaccine shedding or horizontal transmission, additional research will be required.

It has been reported that after the introduction of Rotarix® in South Africa, the number of non-G1P[8] strains such as G2P[4], which are not included in the monovalent G1P[8] vaccine, has significantly increased (Page et al., 2018). G2P[4] tends to possess the DS-1-like genogroup and its dominance has been documented in certain geographic areas (Doan et al., 2011; Vizzi et al., 2017; Khandoker et al., 2018; Thanh et al., 2018). During 1984, 1990, and 1993, the South African G2 strains predominated alongside the G1 strains. A ten-year cyclic pattern was observed between 1987 and 1997 with major epidemics in both years (Page & Steele, 2004). In the phylogenetic analysis, genotype G2P[4] clustered closely with other genotype G2P[4] described in South Africa between 2013 and 2017. It was reported in South Africa that the frequency of G2P[4] increased between 2012 and 2014 but decreased between 2015 and 2016 (GERMS-SA Annual Report, 2017). The presence of G2P[4] in these samples may suggest that it was still circulating in South Africa at the time they were collected. Kenya is one of the top ten countries in terms of the number of tourists visiting South Africa from other African countries (Statistics South Africa, 2018b), and it is possible that the circulating genotypes of Kenya could be transmitted into the country through tourists.

G2P[4] (3/10, 30%) was the second most prevalent genotype in this study. A diarrhoeal surveillance in South Africa (GERMS-SA Annual Report, 2017) as well as studies in Tanzania (Malakalinga et

al., 2019) and Benin (Agbla et al., 2020) also revealed this genotype to be the second most prevalent. In Latin America, Belgium, Botswana, and Australia, despite reports of widespread vaccination with Rotarix, diarrhoeal episodes were consistently associated with the heterotypic G2P[4] RV genotype (Zeller et al., 2010; Gastañaduy et al., 2016; Santos et al., 2017; Roczo-Farkas et al., 2018). A higher prevalence of G2P[4] genotypes appear to be associated with older individuals (European Centre for Disease Prevention and Control, 2017), and some studies have suggested that older people not eligible for vaccination may serve as a permanent reservoir for G2P[4] infection in children (Markkula et al., 2017). This may account for the prevalence found in the current study.

RV genotype G9 was first detected in 1983-1984 in the USA, causing diarrhoea in infants. Subsequently, G9 associated with diarrhoea was reported in several countries during the 1990s (Santos & Hoshino, 2005). G9P[8] is one of the six most common genotypes worldwide (along with G1P[8], G2P[4], G3P[8], G4P[8], and G12P[8]), causing 90% of severe RV cases that require hospitalisation (Santos & Hoshino, 2005; Dhita et al., 2017). Study sequences from G9P[8] grouped with other genotype G9P[8] from South Africa, Zimbabwe, and China based on phylogenetic analysis. In China, G9P[8] RV-A has been the dominant genotype since 2012 (Zhou et al., 2020), while G9P[8] was the third dominant genotype in Zimbabwe (Seheri et al., 2018). Due to its proximity to Zimbabwe on the north and the influx of Chinese tourists, the presence of this genotype in South Africa may be attributed to human movement across national borders.

A low prevalence of G9P[8] was observed in this study (2/10, 20%). Similar findings were previously reported from Zimbabwe and Zambia, where G9P[8] accounted for 20% of the circulating genotypes (Seheri et al., 2014). In South Africa, G9P[8] was the most prevalent genotype in stool samples collected between 2014 and 2015 (Asowata et al., 2018), but in 2017 this genotype was the least prevalent genotype (GERMS-SA Annual Report, 2017).

While RV-A genotypes such as G8P[4] are substantially prevalent in Africa, they are relatively uncommon throughout the rest of the world (Mwenda et al., 2010; Bányai et al., 2011; Dóró et al., 2014; Seheri et al., 2018). There is evidence that G8 genotype is more common in Africa as a result of RV transmission between humans and cattle (Cunliffe et al., 2000; Esona et al., 2009; Jere et al., 2012). This supports the phylogenetic analysis, as G8P[4] was grouped with genotypes from South Africa and Kenya. Among the genotype combinations in the current study, G8P[4] was the least frequently detected. This genotype was predominant in South Africa in 2017, however, in 2018 it was less prevalent, which is also in line with the results of this study (GERMS-SA Annual Report, 2017, 2018). It may represent a hybrid RV between humans and animals. As a result, it is likely that human and bovine RVs have dynamic interactions and are able to transmit across species, which could

provide a mechanism for generating more genetic diversity through reassortment of genomes (Omatola et al., 2021).

There were only a limited number of samples included in this part of the study, which may be a result of low viral loads (Ct > 32) or mutations in primer binding sites which inhibit specific primers, preventing many samples from being amplified (Rossouw et al., 2020).

6.5 Conclusion

The most prevalent genotype combination in this study was G1P[8], followed by G2P[4]. Although this is similar to what has been described in the literature, further research with a larger sample size will enable a better understanding of the genotypes prevalent in this specific study population. It will be necessary to conduct additional analyses in order to determine whether the observed vaccine strain was caused by vaccine shedding or horizontal transmission. Additionally, whole genome sequencing should be considered since it will provide a more comprehensive view of RV epidemiology by providing insight into the specific strains detected, their origins, and their reassortments.

Chapter 7: Profiling the human gastrointestinal microbiome in Sudden and Unexpected Death in Infancy cases at the Tygerberg Medico-legal Mortuary, and healthy infants in Cape Town

Abstract

Background: Infancy is an extremely critical period for establishing the gastrointestinal microbiome, a long-term process that affects health and disease risk. Various studies have investigated possible associations between the infant's intestinal microbiome and sudden unexpected death in infancy (SUDI). Research on the microbiome and its specific role in SUDI have been largely unexplored, particularly in South Africa. For Aim II of this study, the microbiomes in stool samples collected from SUDI cases were compared with those collected from age-matched, apparently healthy infants.

Materials and methods: Stool samples were collected from 34 SUDI cases, as well as 11 age-matched, apparently healthy infants between June 2017 and May 2018. Full-length 16S rRNA gene amplicon sequencing was performed on the PacBio Sequel IIe System platform to profile the microbiome.

Results: The predominant taxonomic phylum in control samples was *Bacteroidota* (90.9%, n=10/11), while *Proteobacteria* were more prevalent in cases with gastroenteritis (50.0%, n=2/4), *Firmicutes* were more prevalent in cases with respiratory tract infections (42.9%, n=6/14) and *Actinobacteriota* were more prevalent in sudden infant death syndrome (SIDS) cases (37.5%).

Conclusion: The findings from this study suggested that the gastrointestinal composition of infants who died from infection and SIDS was different than that of the control group. Although the current results show promise, it is not yet possible to show temporality between the composition of the microbiome and ultimate occurrence of SUDI and will be particularly beneficial.

7.1 Introduction

The microbiome is described as collections of organisms or microbial genomes that inhabit an ecological niche (Bäckhed et al., 2005; Ley et al., 2006; Turnbaugh et al., 2007). Each individual has microflora consisting of 10 to 100 trillion mutualistic cells (Turnbaugh et al., 2007). There is a variety of diverse microbial communities present in various areas of the human body, including the oro-nasopharyngeal sphere, the skin, the vagina, and the gastrointestinal tract (GIT). The human GIT microflora consists mostly of bacteria, a few archaea, eukaryotes, and viruses. Health and disease are influenced by the interaction between these microbial communities and the host (Rajilić-Stojanović & de Vos, 2014).

During pregnancy, the mother's microbiota impacts foetal development, particularly the brain. In addition, maternal microbiota disorders can adversely affect pregnancy outcomes and pose a serious health risk to the foetus. Both environmental and genetic factors affect the healthy growth of the infant's microbiota following birth (Yao et al., 2021).

A woman's oral cavity, GIT, and vaginal microbiota are significantly altered during pregnancy. A number of factors contribute to these changes, including dietary factors such as weight and nutrition, infections, antibiotic use, stress, and the genetic makeup of the individual (Baker et al., 2004; Goodrich et al., 2016; Jašarević et al., 2017; Kim et al., 2017; Codagnone et al., 2019; Zhou et al., 2020).

The maternal GIT microbiota may affect the well-being of the infant and nutrition may account for at least part of this regulation (Morrison & Regnault, 2016). Due to the abundant use of processed foods, dietary fat, and sugars in the typical Western diet, it promotes excessive weight gain, dysbiosis of the GIT, and is associated with adverse effects on both maternal and child health (Luoto et al., 2013; Dunlop et al, 2015; Morrison & Regnault, 2016). During pregnancy, mothers who adhere to recommended dietary allowance for fat and fibre may experience beneficial changes in their GIT microbiota composition, such as high GIT microbiota diversity (Röytiö et al., 2017). As early as in utero and during delivery, the changes in the mother's microbiota can affect the infant's microbiota. A difference in bacterial microbiota patterns is observed among infants born to obese mothers compared to those born to lean mothers. The differences in intestinal microbiota that result from maternal obesity last for at least one year, demonstrating the long-term impact of maternal obesity on the microbiota of their offspring (Collado et al., 2010; Galley et al., 2014; Zheng et al., 2015; Garcia-Mantrana & Collado, 2016).

Antibiotic use during pregnancy also affects the GIT microbiome of the infant (Zou et al., 2018; Zhang et al., 2019; Coker et al., 2020), and it may lead to increased hospitalisation of the infant due to infections (Miller et al., 2018). In comparison to mothers who used oral antibiotics prior to delivery, infants born to mothers who received pre-delivery antibiotics showed microbial changes in the GIT for as long as 12 months after delivery (Azad et al., 2016).

It has been demonstrated that supplementing the GIT with probiotics using established bacterial strains facilitates the colonisation of the GIT flora. Probiotics have been shown to reduce the incidence and mortality of necrotising enterocolitis and septicaemia in very low birth weight and / or preterm infants (Thomas et al., 2017). It is furthermore possible to minimise the adverse effects of antibiotics by co-administering probiotics (Arboleya et al., 2016).

Neonates delivered by caesarean section are not exposed to their mother's vaginal fluid and microbiota during birth. Vaginal seeding is often performed by inoculating a cotton gauze or a cotton swab with vaginal fluids to transfer the vaginal flora to the mouth, nose, or skin of the neonate immediately after delivery. The purpose this procedure is to transfer maternal vaginal bacteria to the neonate, which theoretically contributes to the diversification of GIT microbiota in treated infants to counteract the decreased microbial diversity seen in caesarean sections. A study conducted in 2016 confirmed that infants delivered by caesarean section and that received vaginal seeding had a similar microbiota as their vaginally delivered counterparts. While the study was relatively small, women who carried possible vaginal pathogens were excluded, and the clinical outcome of the infants was not addressed (Dominguez-Bello et al., 2016). As a result of the lack of studies and information regarding the safety and lasting effects of vaginal seeding, this practice is still widely disparaged (Cunnington et al., 2016).

The faecal microbiota transplantation procedure could also be an excellent alternative for introducing neonates to their mother's GIT microbiota when vaginal delivery is not possible. A proof-of-concept study conducted by Korpela et al. (2020) in Finland suggests that this approach may be feasible. Although no concrete evidence exists yet, vaginal seeding combined with faecal microbiota transplantation may be able to establish an environment resembling that of a vaginally delivered infant.

During the perinatal period, the mode of delivery and gestational age at birth have a significant impact on the infant GIT microbiome. Mode of feeding, maternal nutrition, host genetics and environmental factors influence infant GIT microbiota development in early life (Yao et al., 2021). Infancy represents an extremely crucial period for the establishment of the GIT microbiome, an intricate process that has long-term implications for health and disease. The colonisation of the GIT by

microorganisms is fundamentally related to metabolic programming, immunological maturation, and proper development of the GIT (Collado et al., 2012; Bäckhed et al., 2015). At birth, a variety of beneficial and pathogenic microbes colonise the infant's intestines, making them crucial to their health (Rodríguez et al., 2015), especially the microbes from the mother (Korpela & de Vos, 2018).

Both environmental and genetic factors affect the infant microbiota after birth, which is necessary for healthy growth (Yao et al., 2021). Disturbances in the infant GIT microbiome characterised by the overrepresentation of potentially harmful taxa have been linked to persistent disease, including immunological disorders later in life and acute chronic inflammation (Prescott, 2013; Cox et al., 2014; Stiemsma & Turvey, 2017; Olin et al., 2018). It has also been demonstrated that factors related to the establishment of the GIT microbiome, such as delivery mode and feeding type (Schwartz et al., 2012; Jakobsson et al., 2014; Bäckhed et al., 2015; Madan et al., 2016; Reyman et al., 2019), are important factors in the development of infections (Laubereau et al., 2004; Duijts et al., 2010; Ladomenou et al., 2010; Dieterich et al., 2013; Bosch et al., 2016; Reyman et al., 2019).

Numerous theories have been proposed to explain sudden unexpected infant death (SUDI), including microbiological and immunological factors (Gleeson et al., 2004). In 2017, Leong et al. investigated whether the infant's intestinal microbiome, such as carriage of toxigenic bacteria, could be associated with SUDI. Stool samples from 44 cases and 44 age-matched controls were collected. Both bacterial alpha diversity and unconstrained ordination were used to study the microbiota composition and compare the results between the two groups. The intestinal carriage of *Staphylococcus aureus (S. aureus)*, *Clostridioides difficile* and pathogenic *Escherichia coli* were detected using quantitative polymerase chain reaction (PCR) assays. The microbiota diversity in SUDI cases was not significantly different from the controls. Species richness and age were positively associated. Only a few South African studies have examined the microbiome of the human GIT. These are usually birth cohort studies involving infants under one year of age (Claassen-Weitz et al., 2018; Wood et al., 2018; Brown & Jaspan, 2020; Naudé et al., 2020). There has been very little research on the GIT microbiome and its role in SUDI, especially in South Africa. This section aims to profile the microbiome in stool collected from SUDI cases compared to stool collected from age-matched, apparently healthy infants.

7.2 Materials and methods

7.2.1 Sample collection

Stool samples from 34 SUDI cases were included in this study. In accordance with the Forensic Pathology case record files, 16 deaths were attributed to SIDS, 4 to gastroenteritis, and 14 to

respiratory tract infections. As a control group, stool samples were collected from 11 age-matched, apparently healthy infants.

7.2.2 DNA Extraction

Stool samples were collected in sterile leak-proof containers and stored in DNA/RNA Shield™ (Zymo Research, California, USA) at -80°C until analysis. DNA was extracted using QIAamp® PowerFecal® Pro DNA Kit (QIAGEN®, Cape Town) according to the manufacturer's instructions. A mixture of approximately 250 mg of stool and 800 µL of Solution CD1 was added to the PowerBead Pro tube. Brief vortexing was performed to ensure that the contents were thoroughly mixed. After disrupting the stool with the Qiagen TissueLyser LT (QIAGEN, Hilden, Germany) for 5 minutes at 25 Hz, it was centrifuged for 1 minute at 15 000 x g.

The supernatant was transferred to a clean 2 mL microcentrifuge tube provided, and 200 µL Solution CD2 was added and vortexed for 5 seconds. After centrifugation for 1 minute at 15 000 x g, 700 µL supernatant was transferred again to a clean 2 mL microcentrifuge tube. A total of 600 µL of Solution CD3 was then added and vortexed for 5 minutes and 650 µL of the lysate was loaded onto the MB Spin Column and centrifuged for 1 minute at 15 000 x g. The flow-through was discarded and the rest of the lysate was passed through the MB Spin Column. The column was placed in a 2 mL collection tube, washed with 500 µL of Solution EA and centrifuged at 15 000 x g for 1 minute. Once again, the flow-through was discarded and the column washed a second time with 500 µL Solution C5 and centrifuged at 15 000 x g for 1 minute once again. Once more, the flow-through was discarded, and the column was transferred to a new 2 mL tube and centrifuged at 16 000 x g for 2 minutes. After the column was placed in a new 1.5 mL elution tube, 50 µL of Solution C6 was added to the matrix. This was centrifuged at 15 000 x g for 0 minute.

The Qubit® dsDNA HS Assay Kit (Life Technologies™, Johannesburg) was used according to the manufacturer's instructions, Briefly, the reactions were set up with two standards for each sample. The Qubit® working solution was prepared in a 1:200 dilution with Qubit® DNA in Qubit® DNA Buffer (Table 7.1).

Table 7.1: Reagents used for DNA quantification

Reagent	Volumes for Standards	Volumes for Samples
Working solution	190 μL	199 μL
Standard	10 μL	-
Sample	-	1 μL
Total volume	**200 μL**	**200 μL**

7.2.3 PCR assay

The genomic DNA was diluted to 5 ng/µL, frozen at -20°C and delivered on ice to Inqaba Biotechnical Industries (Pty) for PCR amplification. The PacBio Sequel IIe System platform was used to sequence the full-length 16S rRNA gene amplicon. The PCR was performed using the Q5® High-Fidelity 2X Master Mix (New England Biolab, USA), the reaction volume was 25 µL, consisting of 12.5 µL Q5 High-Fidelity 2X Master Mix, 1.25 µL each of amplicon PCR 27 Forward and 1492 Reverse primers (10 µM each) (Table 7.2), 1 µL template DNA and 9 µL nuclease-free water. Table 7.3 illustrates the cycling parameters.

Table 7.2: Modified (5'amino-PB M13 adaptor) universal full length 16S primers

Primer name	Primer sequence 5'-3'	Reference
27f	5AmMC6/gtaaaacgacggccagt AGRGTTYGATYMTGGCTCAG	Lane, 1991
1492r	5AmMC6/caggaaacagctatgac RGYTACCTTGTTACGACTT	Lane, 1991

Table 7.3: PCR thermocycling conditions

Step	Temperature (°C)	Duration	Cycle
Initial denaturation	95°C	3 min	1
Denaturation	98°C	20 sec	1
Annealing	57°C	15 sec	2
Extension	72°C	60 sec	20
	98°C	20 sec	
	65°C	15 sec	
	72°C	60 sec	
Final extension	72°C	5 min	1
	4°C	5 min	1
Hold	**10°C**	∞	

7.2.4 PacBio full-length 16S rRNA sequencing

The Unsupported Full-Length 16S Amplification, SMRTbellTM Library Preparation and Sequencing Protocol (Pacific Biosciences of California, Inc) was followed to prepare amplicons for PacBio SMRT sequencing. PacBio M13 barcodes were used to label the full-length 16S rRNA amplicons from the first PCR for multiplexing in a second round PCR. The PCR was performed using the Kapa HiFi HotStart ready reaction mix (Kapa Biosystems, Roche, Basel, Switzerland).

The reaction volume was 25 µL, consisting of 12.5 µL KAPA HiFi Hotstart ReadyMix; 2.5 µL each of M13 forward Barcoded Primer and M13 reverse Barcoded Primer (0.3 µM each), 1 µL Round 1 PCR products and 6.5 µL HPLC Water. PCR cycling conditions are outlined in Table 7.4. Following PCR amplification, the second-round products were visualised on an agarose gel. AMPure PB beads were used to purify amplicons (PacBio, USA). The PCR reaction volume of 25 µL was filled with 25 µL of nuclease-free water, followed by 30 µL of bead solution. Following purification, Qubit dsDNA HS was used to quantify the amplicons and normalised to 10 nM for pooling.

Table 7.4: Cycling conditions for the 2nd round PCR

Step	Temperature (°C)	Duration
1	95°C	3 min
2	98°C	20 sec
3	60°C	15 sec
4	72°C	60 sec
5	Repeat steps 2 to 4	
6	98°C	20 sec
7	65°C	15 sec
8	72°C	60 sec
9	Repeat steps 6 to 8 (20 cycles total)	
10	72°C	5 min
11	4°C	∞

SMRTbell Express Template Prep Kit 2.0 (PacBio, USA) was used for the construction of the SMRTBell library on the pooled, barcoded amplicon samples. As part of the library construction process, DNA damage repair, DNA end repair/A-tailing and adapter ligation were performed prior to purification with AMPure PB beads. To remove damaged SMRTbell templates, each library sample was nuclease-treated, followed by a second purification step with AMPure PB beads. The PacBio Sequel II system was used for sequencing the pooled amplicon mixes.

7.2.5 Sequence Data Analysis

Raw subreads were processed through the SMRTlink (v9.0). Based on the Circular Consensus Sequences (CCS) algorithm, highly accurate reads (> QV20) were generated. To process these highly accurate reads, vsearch (https://github.com/torognes/vsearch) was used to perform trimming, dereplication, chimeral removal and OTU clustering (at 97%). On the Stellenbosch University high performance computing cluster 2, demultiplexed single-end FASTQ sequences were imported and

analysed utilising the Quantitative Insights Into Microbial Ecology (QIIME2 2022.8) bioinformatics platform (Bolyen et al., 2019).

Sequence QC was performed using DADA2 plugin and taxonomic analysis was performed on the SILVA 138 99% OTU V4 region database (https://docs.qiime2.org/2022.8/data-resources/). Phylogenetic diversity analysis was performed using mafft-fasttree plugin (Appendix E).

At a sampling depth of 8 062 bp, alpha diversity (diversity within samples) was performed using the observed features diversity metrics (richness of a community as measured qualitatively) (Faith, 1992). As part of the beta diversity (diversity between samples), the unweighted Uni-Frac (dissimilarities between communities are assessed qualitatively using phylogenetic relationships between the features) (Lozupone & Knight, 2005) and weighted Uni-Frac (dissimilarities between communities are assessed quantitatively using phylogenetic relationships between the features) (Lozupone et al., 2007) dissimilarity metrics were used.

To determine statistical significance for alpha diversity, Kruskal–Wallis pairwise tests and Benjamini–Hochberg False Discovery Rate corrections (Benjamini and Hochberg 1995) were used when necessary. For beta diversity, PERMANOVA with BH-FDR adjustment was used to determine the significance of differences. A p value of ≤ 0.05 was considered statistically significant for both alpha and beta diversity.

7.3. Results

7.3.1 Epidemiological data

A total of 45 stool samples were analysed, including 34 SUDI cases (61.8% males and 38.2% females) with a median age of 10 weeks (Interquartile Range [IQR]: 6–20) and 11 samples (45.4% males and 54.6% females) from apparently healthy infants with a median age of 22 weeks (IQR: 10.5–36.25) (Table 7.5).

7.3.2 Taxonomic profile of controls and various causes of death

The various causes of death were categorised as gastroenteritis, respiratory tract infections and SIDS. It was observed that *Bacteroidota* was the most phylum in control samples (10/11, 90.9%), while *Proteobacteria* were more abundant in gastroenteritis cases (2/4, 50%), *Firmicutes* were more abundant in respiratory tract infections (6/14, 42.9%) and Actinobacteriota in SIDS cases (6/16, 37.5%) (Figure 7.1). Control group is indicated by the red block.

Table 7.5: The sociodemographic profiles of the SUDI and control groups

Sociodemographic factors		SUDI Cases (n, %)	Controls (n, %)
Age in weeks (Median, (IQR))		10 (6.0-20.0)	22 (10.5-36.3)
Sex	Male	21 (61.8)	5 (45.4)
	Female	13 (38.2)	6 (54.6)
Mode of Delivery	Normal Vaginal Delivery	16 (47.1)	6 (54.6)
	Caesarean Section	16 (47.1)	5 (45.4)
	Unknown	2 (5.8)	0 (0.0)
Feeding method	Breastfed	14 (41.2)	1 (9.1)
	Bottle-fed	6 (17.6)	7 (63.6)
	Mixed bottle- and breastfed	11 (32.4)	3 (27.3)
	Unknown	3 (8.8)	0 (0.0)
Premature	Yes	13 (38.2)	3 (27.3)
	No	21 (61.8)	5 (45.4)
	Unknown	0 (0.0)	3 (27.3)
Place of birth	Hospital	25 (73.5)	9 (81.8)
	Clinic	3 (8.8)	0 (0.0)
	Home	3 (8.8)	2 (18.2)
	Unknown	3 (8.8)	0 (0.0)
Position placed to sleep	Prone	9 (26.5)	1 (9.1)
	Supine	2 (5.9)	5 (45.4)
	Side	15 (44.1)	5 (45.4)
	Unknown	8 (23.5)	0 (0.0)
Cause of death	SIDS	16 (47.1)	N/A
	Respiratory infection	14 (41.2)	N/A
	Gastroenteritis	4 (11.8)	N/A

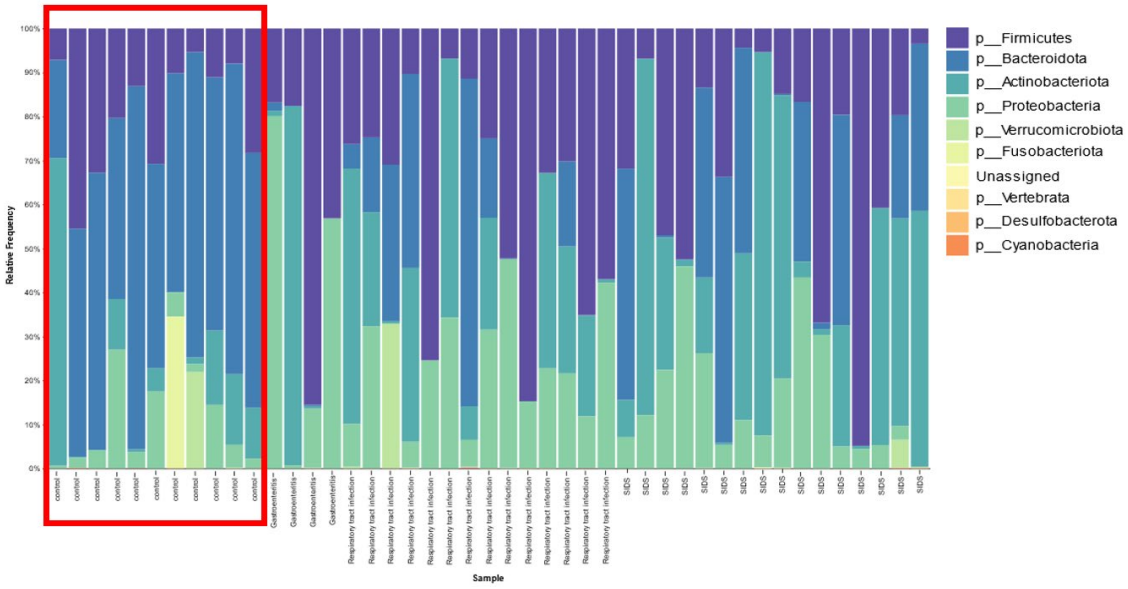


Figure 7.1: Phylum-level taxonomic profiles of the control and the various causes of death

7.3.3 Taxonomic profile according to age in weeks

Among both young (< 25 weeks of age) and older infants (> 25 weeks of age), *Bacteroidota* (18/45, 40%) and *Actinobacteriota* (11/45, 24.4%) were the most abundant phyla (Figure 7.2).

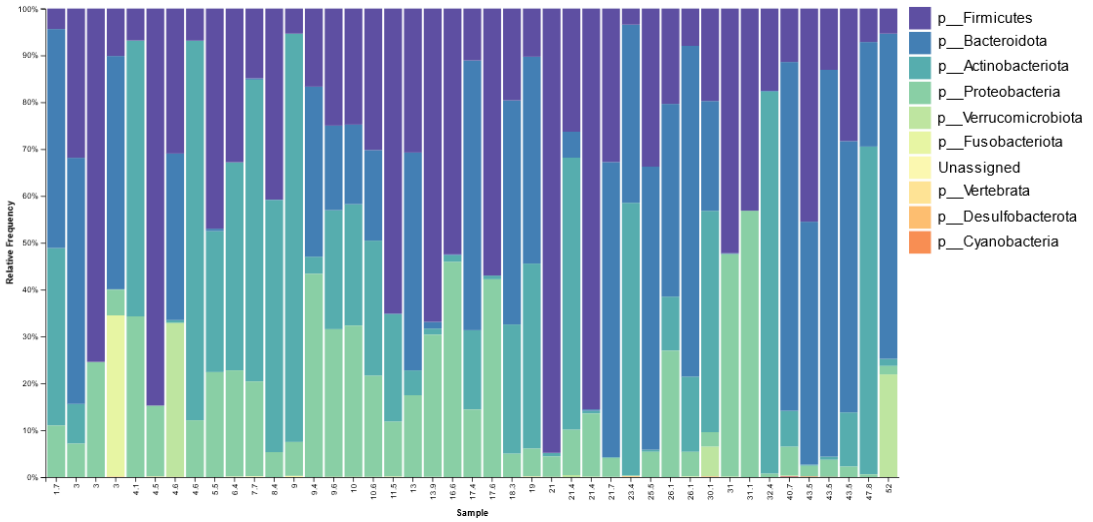


Figure 7.2: Phylum-level taxonomic profiles of samples according to age in weeks

7.3.4 Taxonomic profiles of controls and cause of death according to mode of delivery

The mode of delivery was classified as either normal vaginal delivery or caesarean section. In the control group, *Bacteroidota* was the most abundant phyla in both vaginal delivery (6/11, 54.6%) and caesarean section (4/11, 36.4%). However, *Firmicutes* (5/9, 55.5%) dominated caesarean sections while *Bacteroidota* (3/8, 37.5%) dominated vaginal deliveries in the infection cases. Among SIDS cases, both *Actinobacteriota* (3/7, 42.9%) and *Firmicutes* (3/7, 42.9%) were observed in the caesarean

sections, whereas *Bacteroidota* (4/8, 50%) were abundant in vaginal deliveries. Due to unknown delivery modes, two samples were excluded from the visualisation (Figure 7.3).

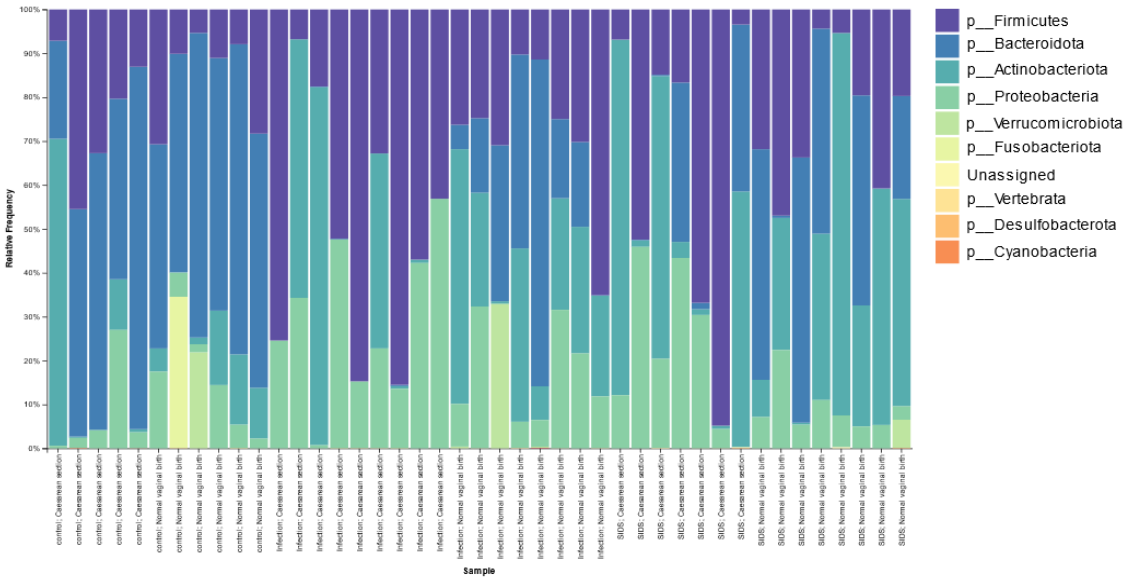


Figure 7.3: Phylum-level taxonomic profiles of controls and cause of death according to mode of delivery

7.3.5 Taxonomic profiles of controls and cause of death according to feeding method

The feeding method of the infants were classified into three categories: bottle-fed, breastfed or both bottle-fed and breastfed. In the control group, *Bacteroidota* (10/11, 90.9%) was abundant in all three categories, with most of the controls being bottle-fed (7/11, 63.6%). According to the infection cases among the bottle-fed infants, *Actinobacteriota* (1/4, 25%), *Firmicutes* (1/4, 25.0%), *Bacteroidota* (1/4, 25.0%), and *Proteobacteria* (1/4, 25.0%) were equally abundant. *Actinobacteriota* (3/8, 37.5%) and *Firmicutes* (3/8, 37.5%) were more abundant among breastfed infants, whereas *Firmicutes* (3/6, 60.0%) were more abundant among bottle and breastfed infants. In SIDS cases, *Actinobacteriota* (3/5, 60.0%) were the most abundant phyla found in bottle and breastfed infants, while *Bacteroidota* (1/2, 50%) and *Firmicutes* (1/2, 50%) were abundant in the bottle-fed infants. *Bacteroidota* (2/6, 33.3%), *Firmicutes* (2/6, 33.3%), and *Actinobacteriota* (2/6, 33.3%) were equally abundant in breastfed infants. There were three samples omitted from the visualisation due to their unknown feeding method (Figure 7.4).

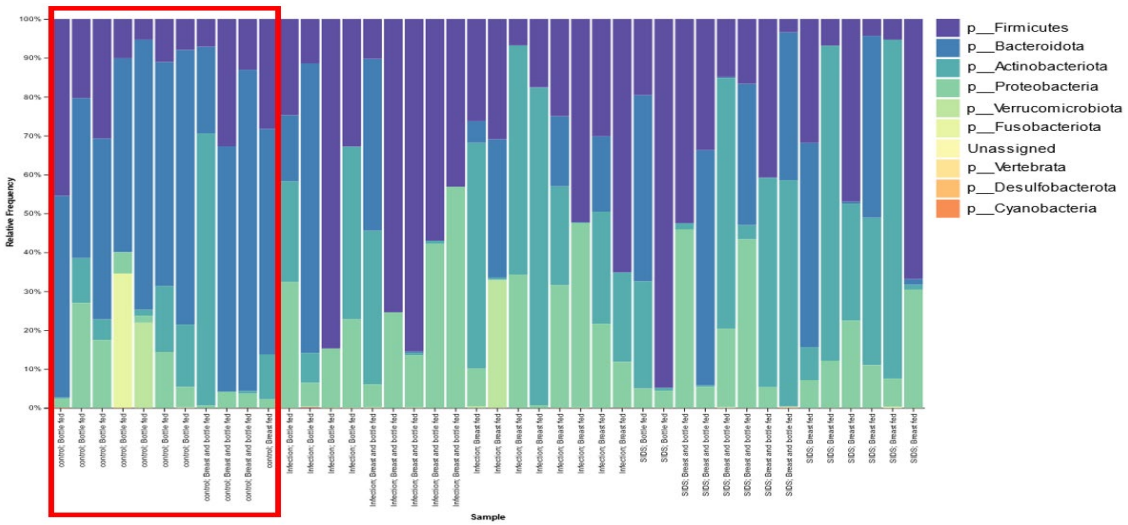


Figure 7.4: Phylum-level taxonomic profiles of controls and cause of death according to feeding method

7.3.6 Taxonomic profiles of controls and cause of death according to the position the infant was placed to sleep

The position the infants were placed to sleep is referred to as sleeping position for the purpose of this study and were classified into supine, side, and prone sleeping positions. In the control group, *Bacteroidota* were abundant in all three sleeping positions (10/11, 90.9%). Among the infection cases, *Bacteroidota* (3/6, 50%) were abundant in the supine and side sleeping positions, while *Firmicutes* (5/7, 71.4%) were abundant in the prone sleeping position. Among the SIDS cases, *Firmicutes* (4/10, 40%) were abundant in the side sleeping position, *Actinobacteriota* (10/10, 100%) in the supine sleeping position, and *Bacteroidota* (5/10, 50%) and *Proteobacteria* (5/10, 50%) in the prone sleeping position. As a result of their unknown sleeping position, eight samples were omitted from the visualisation (Figure 7.5).

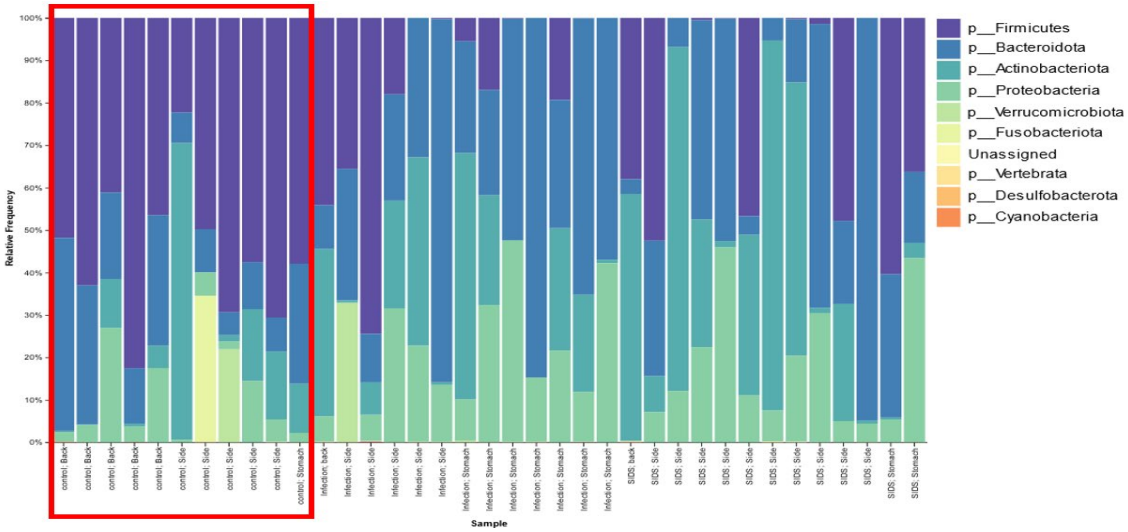


Figure 7.5: Phylum-level taxonomic profiles of controls and cause of death according to the position the infant was placed to sleep

7.3.7 Taxonomic profiles at the genus level of the control and the various causes of death

Bifidobacterium were abundant among respiratory tract infection cases and SIDS cases, *Bacteroides* were abundant among controls, and *Escherichia - Shigella* abundant in gastroenteritis cases (Figure 7.6).

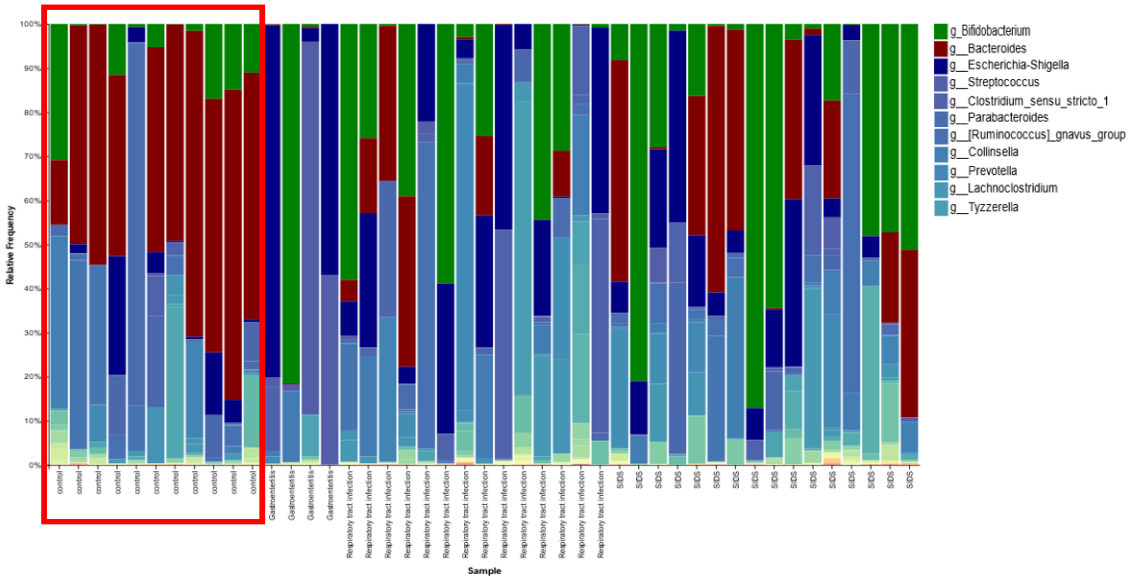


Figure 7.6: Genus-level taxonomic profiles of the control and the various causes of death

7.3.8 Alpha diversity

Alpha diversity was significantly high in the control group compared to the infection and SIDS cases (Figure 7.7(c); (p=0.03 and p=0.007) respectively. In addition, normal vaginal births were associated with significantly higher diversity (Figure 7.7(a); p=0.01), while bottle-fed infants had a significantly higher diversity than both bottle- and breastfed infants (Figure 7.7(b); p=0.006).

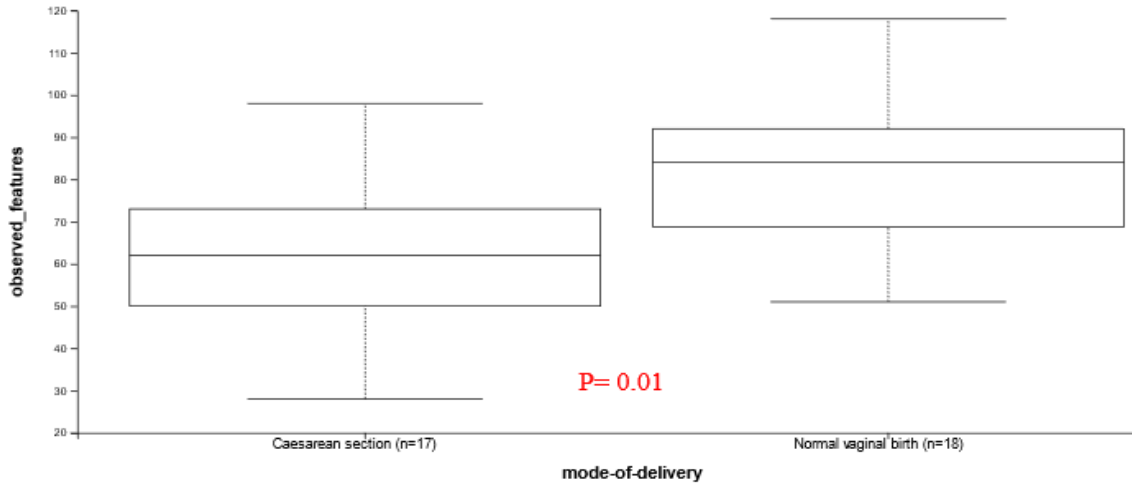


Fig 7.7 (a): Alpha diversity between mode of delivery

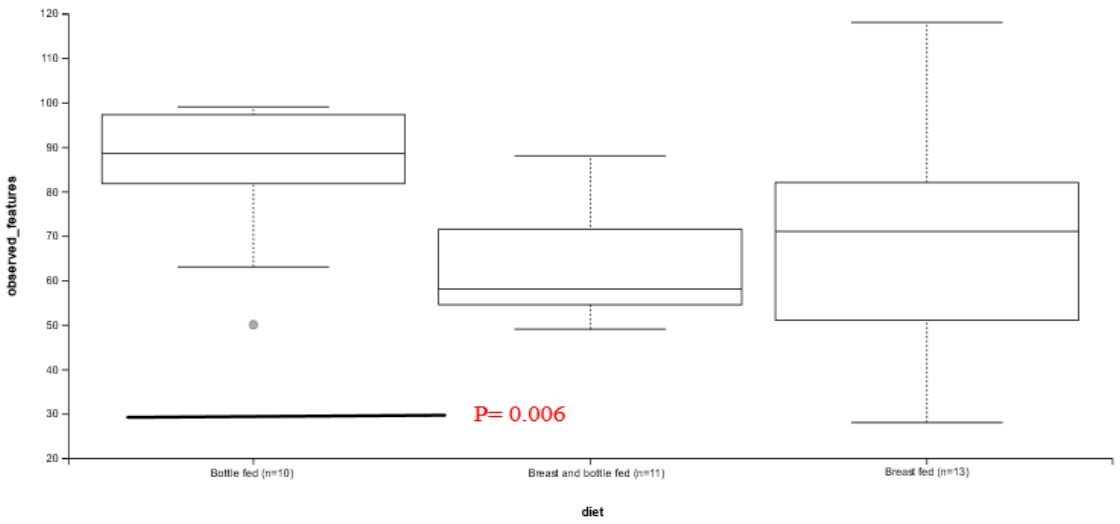


Fig 7.7(b): Alpha diversity according to infant feeding method of infants

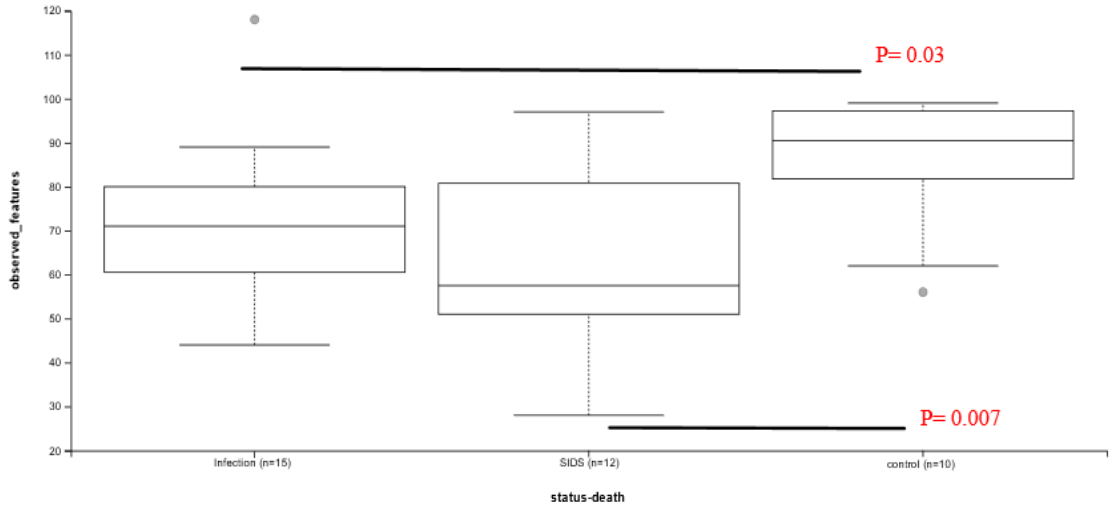


Fig 7.7(c): Alpha diversity between infection, SIDS, and the control group

Figure 7.7: Alpha diversity was measured by calculating observed features and Kruskal–Wallis pairwise test was used to perform statistical analysis

7.3.9 Beta diversity

According to the unweighted Uni-Frac dissimilarity metrics, a significant difference between infection cases and the control group (Figure 7.8(b); p=0.004). There were also significant differences between vaginal delivery and caesarean sections (Figure 7.8(a); p=0.03), Weighted Uni-frac dissimilarity metrics revealed a significant difference when the control group was compared to the infection and SIDS cases (Figure 7.8(f); p=0.001 and p=0.006) respectively.

Significant differences were observed between bottle-fed infants and those that were both bottle- and breastfed, as well as bottle-fed compared to breastfed infants (Figure 7.8(c); p=0.02 and p=0.01) respectively. In addition, significant differences were also observed between vaginal delivery and

caesarean sections (Figure 7.8(d); p=0.009) as well as between the supine and prone sleeping positions (Figure 7.8(e); p=0.04).

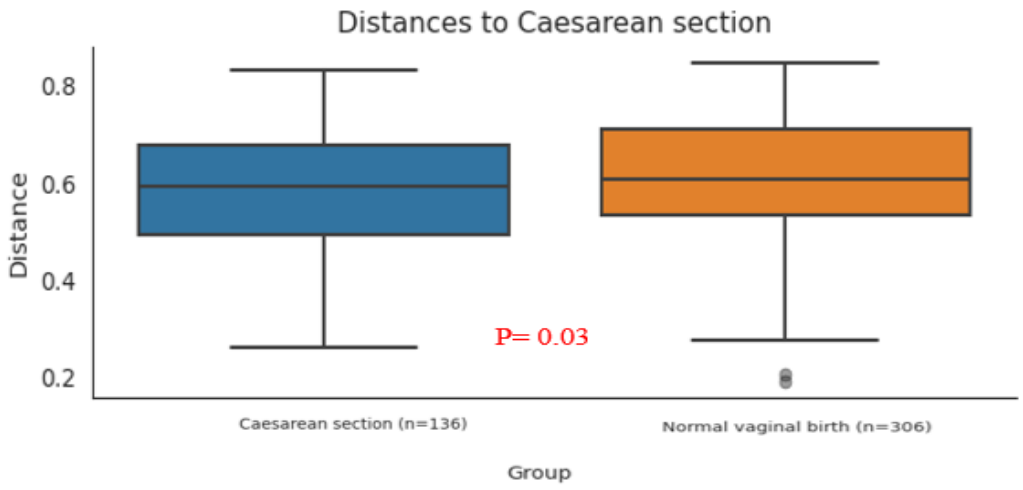


Fig 7.8(a): Unweighted Uni-Frac showing differences between mode of delivery

Fig 7.8(b): Unweighted Uni-Frac showing differences between cause of death and the control group

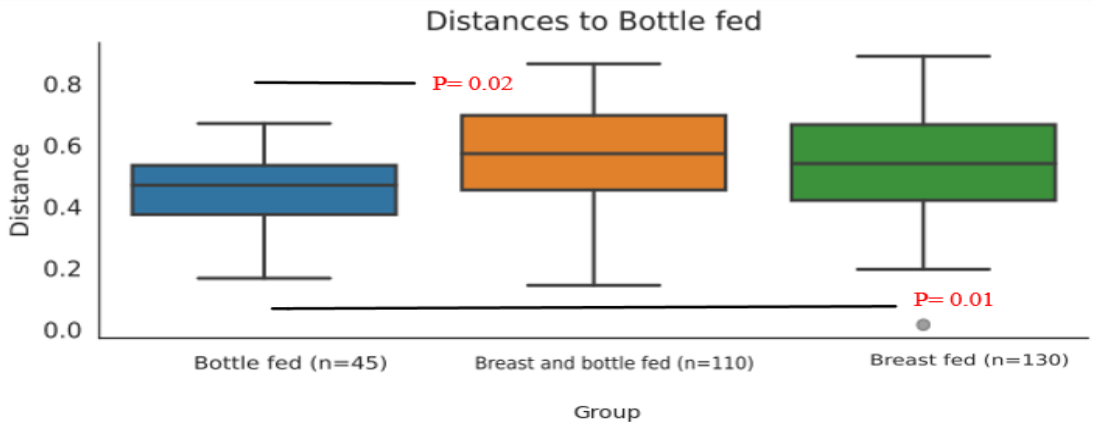


Fig 7.8(c): Weighted Uni-Frac showing differences between feeding method

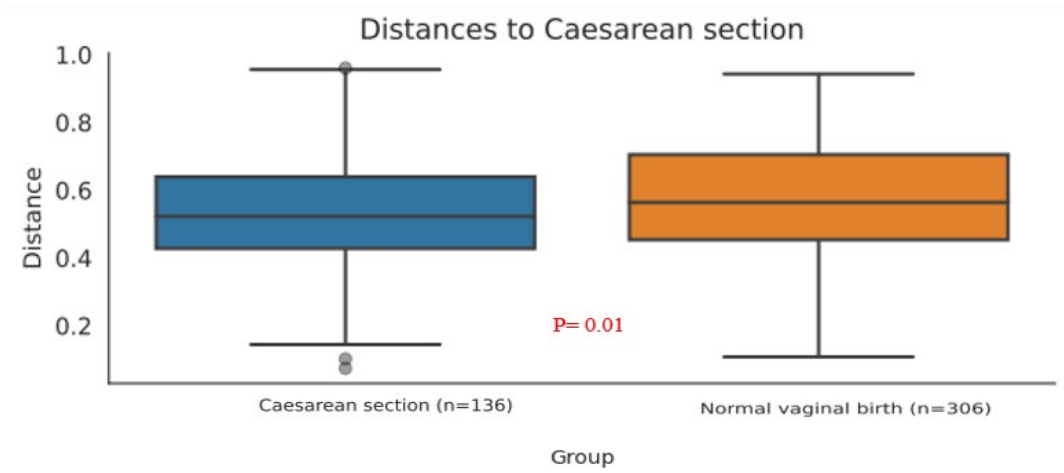


Fig 7.8(d): Differences between modes of delivery as shown by weighted Uni-Frac

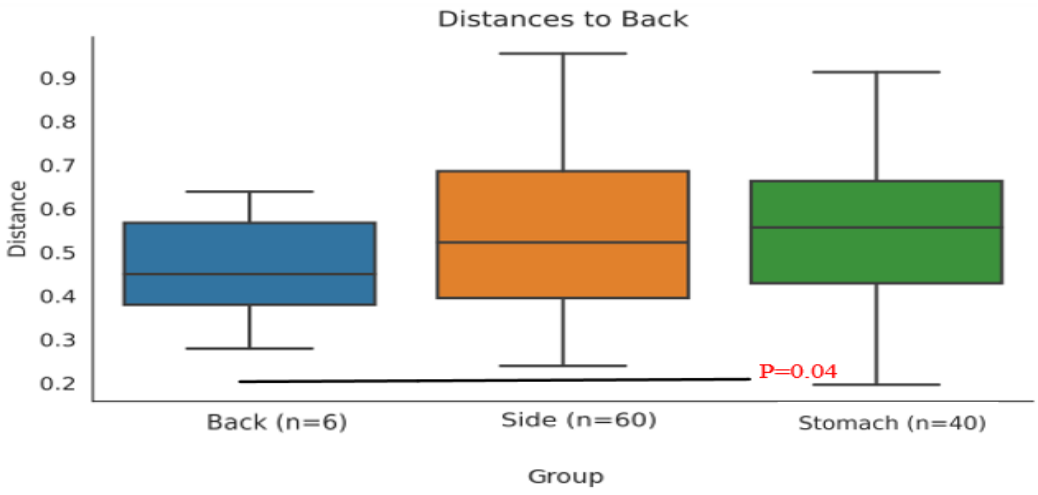


Fig 7.8(e): Differences between the position the infant was placed to sleep as shown by weighted Uni-Frac

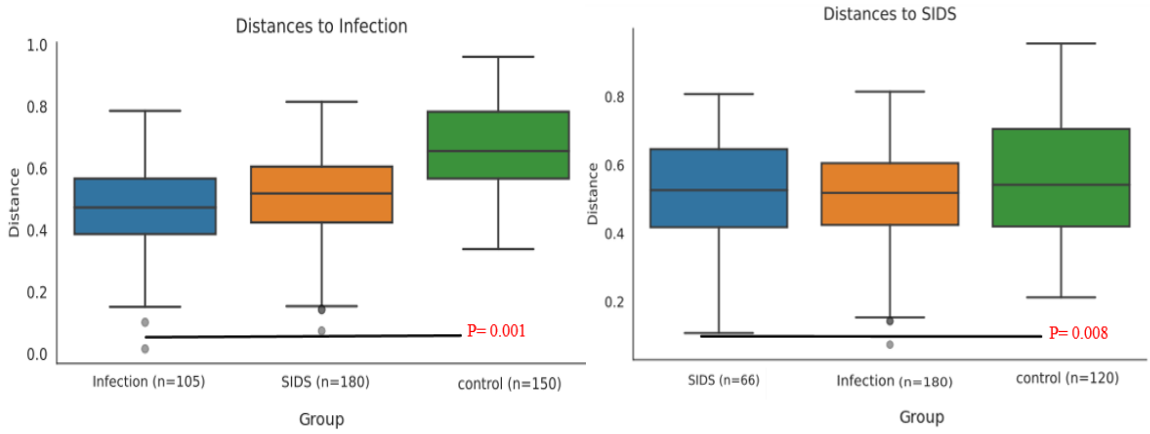


Fig 7.8(f): Weighted Uni-Frac showing differences between cause of death and the control group

Figure 7.8: Beta diversity was measured using unweighted Uni-Frac and weighted Uni-Frac dissimilarity metrics and PERMANOVA was used to perform statistical analysis

7.4. Discussion

This pilot studied aimed to describe the GIT microbiota of apparently healthy controls and SUDI cases with a final cause of death of SIDS or infection. In addition, the risk factors associated with SUDI were analysed using diversity metrics and the taxonomic composition profiled.

Among the most abundant organisms in the GIT are *Firmicutes, Bacteroidota, Proteobacteria,* and *Actinobacteria* (Brown et al., 2013; Power et al., 2014). These organisms were also confirmed in the control group as well as the SUDI cases. It has been reported in the literature that anaerobes such as *Bifidobacteria* proliferate during periods of low oxygen availability and nutrient scarcity (Gates et al., 2021), and this could possibly be the reason for the high abundance of *Bifidobacteria* in SIDS and infection cases in the current study. Similarly, Li and colleagues found an enrichment of phyla such as *Firmicutes* among paediatric patients with frequent respiratory tract infections (Li et al., 2019). Gram-negative bacteria, particularly *Proteobacteria*, can inhibit certain innate immune responses, causing acute gastroenteritis due to the production of flagellin or toxigenic lipopolysaccharides (Adler et al., 2005). *Proteobacteria* and *Escherichia - Shigella* were found to be comparatively abundant in cases with gastroenteritis.

There was an abundance of *Actinobacteria* in the younger infants, whereas *Bacteroidota* were abundant in the older infants. This is consistent with *Actinobacteria* genus *Bifidobacterium*, becoming abundant soon after birth. The *Bifidobacterium*-dominated microbiota persists until solid foods are introduced (Palmer et al., 2007; Vallès et al., 2014). As solids are introduced to older infants, there is an increase in phyla abundance, especially an increase in *Bacteroidota* (Koening et al., 2011; Vallès et al., 2014).

During vaginal delivery, infants are colonised by similar flora to that colonising the maternal vagina, while during caesarean delivery, infants are colonised by maternal skin bacteria rather than vaginal bacteria (Dominguez-Bello et al., 2010; Montoya-Williams et al., 2018). In the absence of mother-to-child transfer during vaginal delivery, the GIT is colonised primarily by microbes from the environment (Stokholm et al., 2016). Vaginally delivered infants have a high abundant of *Bacteroides* species in their GIT (Fouhy et al., 2012; Milani et al., 2017), as in the current study, *Bacteroidota* was found in all the vaginally delivered infants. *Actinobacteria* were detected in less than 50% of caesarean-born infants among the SIDS cases. This finding is similar to previous reports that caesarean-born infants harbour fewer *Bifidobacterium* and *Bacteroides* species (Adlerberth et al., 2006; Penders et al., 2006; Biasucci et al., 2008). The dominance of *Firmicutes* in caesarean-born infants in infection and SIDS cases in the current study is similar to previous reports that showed

caesarean section delivery exhibited decreased *Bacteroidetes* colonisation and increased *Firmicute* abundance (Jakobsson et al., 2014; Rutayisire et al., 2016; Yang et al., 2019).

Neonatal GIT microbiota colonisation patterns are heavily influenced by nutrition, whether breastmilk or formula (Gritz & Bhandari, 2015). In breastfed infants, *Bifidobacterium* species represent the abundant *Actinobacteria* (Harmsen et al., 2000; Jost et al., 2012) and lactic acid bacteria represent the abundant *Firmicutes* phylum (Turroni et al., 2012; Bergström et al., 2014). This is similar to the phyla found in the current study. In the breastfed SIDS cases and controls, *Bacteroidota* may be present due to non-digestible carbohydrates in breastmilk that ferment in the colon and support the growth of probiotics *Bifidobacterium* and *Bacteroides* (Jain & Walker, 2015). The GIT microbiota of formula-fed infants is dominated by *Bifidobacterium* and *Bacteroides* (Harmsen et al., 2000), in addition to *Escherichia coli*, *Clostridioides difficile*, *Prevotella*, and *Lactobacillus* (Piacentini et al., 2010; Madan et al., 2012; Jost et al., 2012; Di Mauro et al., 2013; Gomez-Llorente et al., 2013; Torrazza & Neu, 2013; Power et al., 2014; Jain & Walker, 2015).

The presence of *Bacteroidota* in the controls, who were both bottle- and breastfed, suggests that these infants had GIT microbiota similar to formula-fed infants. Similarly, it has been reported that supplementing breastfed infants with formula, even in relatively small amounts, can result in the transition in feeding pattern from breastfed to formula-fed (Mackie et al., 1999; Guaraldi & Salvatori, 2012). In 2011, Hascoët et al. published a study that demonstrated that if infants were fed a formula consisting mainly of whey protein, containing low levels of phosphate and protein, similar in composition to human milk, they developed a faecal microbiota profile resembling that of breastfed infants. It was also found that the GIT composition of breast- and bottle-fed infants in the infection and SIDS cases was comparable to that of breastfed infants.

SUDI has been most often associated with the prone sleeping position (Willinger et al., 1994). In a study conducted by Highet et al. (2014), the prevalence of *S. aureus* colonisation among SUDI cases found in the prone position was higher than among those found on their sides or in the supine position. This contrasts with the bacteria found in the current study. Currently, there is no other literature describing the composition of the infant's GIT in relation to various sleeping positions. Novel results have thus been obtained in this study. The phyla identified in the study are however prominent in the GIT (Brown et al., 2013; Power et al., 2014).

The statistically significant differences in alpha and beta diversity (unweighted and weighted Unifrac) observed between vaginal delivery and caesarean section may be a result of complications during pregnancy, antibiotics being administered during delivery and inactivity of the mother that occurs during a caesarean section (Stinson et al., 2018). The first inoculum in caesarean section babies

is not derived directly from the mother's vaginal microbiota during delivery. Instead, it originates from other maternal sources, such as the mother's skin or mouth, or from non-maternal sources, such as the environment in which the baby was delivered (Milani et al., 2017). To restore the GIT microbiota of an infant after a caesarean section, Dominguez-Bello et al. (2016) exposed infants to vaginal fluid. In the 30 days following the delivery of the infant, the microbiota of the mouth, GIT, and skin improved significantly. In accordance with these findings, it is evident that the vertical transfer of maternal vaginal microbes following delivery can be partially reversed.

In the current study, the beta diversity metric (weighted Unifrac) indicates that the diversity of bottle-fed infant groups is lower than that of breastfed infants. A comparison of human breastmilk and formula has shown that breastmilk has superior health benefits for infants, particularly in protecting intestinal barrier integrity and mucosal defences (Stewart et al., 2018). Additionally, immunoactive factors, such as polymeric IgA, antibacterial peptides, and elements of the innate immune response, can influence health-promoting microorganisms (Walker & Iyengar, 2015). Although commercial formulas are increasingly similar in composition to breastmilk, the microbiota of breastfed and formula-fed infants differs (Baumann-Dudenhoeffer et al., 2018). Breastfed infants generally have higher levels of faecal short-chain fatty acids, which are the main products of human milk oligosaccharides fermentation (Salli et al., 2019). Moreover, supplementing infants who do not receive breastmilk exclusively with a breastmilk-like formula may allow them to develop a partially breastfed GIT microbiota (Gritz & Bhandari, 2015), explaining the significant differences observed in the alpha and beta (weighted Uni-frac) diversity metrics between bottle-fed infants and those who were both bottle- and breastfed.

Escherichia-Shigella was the predominant genus among infants placed to sleep in the prone position, whereas the genus *Bacteroides* dominated among infants placed to sleep in the supine position in the current study. This supports the difference observed in beta diversity (weighted Uni-frac) between the prone and supine sleeping positions. Other studies compared the gut microflora of 52 SIDS infants with 102 faecal samples from age-matched live comparison infants and found that *S. aureus* was significantly associated with the prone sleeping position. This suggests sleeping in the prone position could increase the risk of ingesting or inhaling bacteria that are present on the bed surface (Highet et al., 2014). Additionally, skin scales containing *S. aureus* and *E. coli* are shed onto sleeping surfaces, causing the infants to inhale or ingest these bacteria (Goldwater & Bettelheim, 2013). The prone sleeping position also increases the risk of colonisation by inducing temperature-dependent toxins, such as pyrogenic toxins of *S. aureus*, since prone sleeping significantly increases the nasal temperature of the infant (Molony et al., 1999).

The results suggest that the diversity and abundance of the GIT microbiota in infection cases significantly decreased compared to the control group. Studies have shown that gastrointestinal infections may affect aerobic bacteria since these bacteria use oxygen to obtain energy and metabolism (Swidsinski et al., 2008; Conway & Cohen, 2015; Gao et al., 2018). Moreover, respiratory tract infections can promote the emergence of potentially harmful bacteria. The presence of infection appears to inhibit bacteria growth that benefits health (Wang et al., 2014). Significant differences were observed in alpha diversity and beta diversity (weighted Uni-frac) between the cases of SIDS and the control group. In comparison to healthy controls, SUDI may be associated with an altered GIT microbiome characterised by innate lymphoid cells and pro-inflammatory microorganisms (Goldwater, 2015).

7.5 Conclusion

In SUDI cases and controls, the GIT microbiome was similar to that described previously for infants in the first year of life, the predominant bacteria belonged to the phyla *Firmicutes*, *Bacteroidota*, *Proteobacteria* and *Actinobacteria*. The study also revealed that the GIT composition of infants who died from infection and SIDS was different than the GIT composition of the control group. Other studies specifically screened for *C. innocuum*, *C. Perfringens*, *C. difficile*, *Bacteroides thetaiotaomicron* and *S. aureus*, but this was the first study to perform full-length sequencing to identify organisms in infants who died from infection and SIDS and apparently health, live infants. Further research with a larger sample size may be particularly useful in determining whether these differences contribute to the development of SUDI.

Chapter 8: Conclusion

The overall aim of this study was to investigate the presence of bacterial and viral pathogens in the gastrointestinal tract in SUDI cases admitted to Tygerberg Medico-Legal Mortuary over a 1-year period between June 2017 and May 2018, thus including all four seasons in a single year. As an exploratory pilot study, the GIT microbiome of the SUDI cases was compared to the GIT microbiome of seemingly healthy living infants who served as a control group.

Several sociodemographic risk factors were compared with the cause of death, with the only highly significant association being observed between the age in weeks and the cause of death. The results suggest that younger infants may have a higher probability to be classified as SIDS compared to infection. This is in line with common practices where older infants are usually exposed to more pathogens when attending creches or day-care centres and have a higher probability of contracting infection from the environment or other individuals.

The identification of certain sociodemographic risk factors has been the focus of research worldwide and is primarily aimed at finding positive predictive factors to guide modifiable behaviour in parents and communities to lower SUDI rates. This is an essential first step in investigating a disease with no known cause.

E. coli was detected in the majority of the SUDI cases. Qualitative analysis confirmed that the most common pathogenic *E. coli* strains (EAEC, EPEC, and ETEC) found in these cases are similar to those previously described in the literature. Coinfections between two or more strains were the most common. Literature describes similar mixed DEC infections. Although PMI and O157 showed a significant association in this study, it is unclear what role O157 plays during the PMI, and further research is necessary to clarify this.

The study further indicated that RV continued to be the most prevalent enteric virus despite the availability of the RV vaccine as part of the SA-EPI. In addition, NoV has emerged as a predominant paediatric viral enteric pathogen since the RV vaccine introduction. As Histo-blood group antigen expression in the mucosa can influence the risk of NoV infection, RV infections may also follow a similar pattern. The frequency of autolysis on the histological slides made analysis difficult. As a result of the statistical significance observed between NoV GI and GII and the position the infant was placed to sleep, it is imperative that continuous campaigns are conducted throughout the community to raise awareness of the risks associated with prone sleeping.

The study revealed that G1P[8], G2P[4], G9P[8] and G8P[4] were the most prevalent genotypes of RV, with G1P[8] and G2P[4] having the highest prevalence. The presence of these genotypes at the

time the samples were collected may suggest that they were still circulating in South Africa. An accurate conclusion, however, would require a larger sample size. According to the phylogenetic analysis, two samples from the current study were closely related to the Rotarix vaccine strain. However, it was unknown whether these infants had indeed received the vaccine. Additional studies will be necessary to determine whether observed vaccine strains were transmitted horizontally or could be a result of vaccine shedding.

Finally, this study described the GIT microbiome of apparently healthy controls and infants who died from SIDS or infection. The findings indicated that the most prevalent organisms in the GIT of the infants included in this study were *Firmicutes*, *Bacteroidota*, *Proteobacteria*, and *Actinobacteria*, in agreement with the literature, with *Bifidobacterium* being more prevalent in SIDS and infection cases. This may suggest that there is a difference in GIT microbiota between the control group and the cases. However, further research is required to determine the extent of these differences and their impact in causing death.

8.1 Limitations of the study

A major limitation of this study was the small number of controls included. A suitable control group would be infants who died from unnatural causes. However, due to several factors and ethical constraints, this was not possible, as samples of these infants were not used for determining the cause of death, and therefore are not covered by the waiver of consent. Healthy babies represent the closest control group from which samples could be collected, but the findings would have been more significant if a larger number of control samples were available for comparison purposes.

The medical history of the SUDI cases, i.e., whether they had diarrhoea before death, was not always available or subject to recall bias when the parents completed the questionnaire upon admission of the infant to the Tygerberg Medico-legal Mortuary.

A lack of resources prevented further subtypes of EPEC from being studied.

As a result of the long PMI, reviewing histological slides was generally difficult. The constant volume of cases admitted to the Tygerberg Medico-legal Mortuary, criminal cases often take precedence over infant cases, often resulted in a longer PMI. Furthermore, these infants must be identified by their parent(s) and their sociodemographic and medical history must be provided to the pathologist before an autopsy can be conducted.

It is not possible to accurately predict the viral genotypes circulating in the study population due to the small sample size and unequal distribution of samples between cases and controls. As a result of

time constraints, it was not possible to conduct further troubleshooting in order to include more samples in the study.

Based on the small sample size and convenient sampling method used for the microbiome section of this study, bias was introduced. Due to the high cost of microbiome research, the number of samples that could be used in the study was also limited. As the laboratory work for this area was outsourced, the negative controls indicated that environmental contamination might have occurred during the PCR process.

8.2 Future directions

It is recommended that future studies include larger case and control groups. As a result, a better understanding of how these two groups compare can be gained, and bias will be reduced.

Additional subtyping of EPEC would be beneficial, as aEPEC is the fifth most commonly detected pathogen in infants who die from acute gastroenteritis. An in-depth investigation of the association between certain strains of *E. coli* and death is necessary, as the exact mechanisms and roles need to be systematically understood.

Additional analysis is necessary to determine whether the observed RV vaccine strain was the result of shedding or horizontal transmission. It is also recommended to consider whole genome sequencing in order to gather a more comprehensive picture of RV epidemiology by providing insight into the strains detected, their origins, and reassortment patterns.

The extent of the differences in the GIT microbiota between the cases and the controls needs to be established to determine their impact on death. Additionally, further investigation is needed regarding the GIT microbiota when sleeping in different positions, as previous studies have only investigated prone sleeping positions. As part of further microbiome analysis, shotgun metagenomics could be used as a more sensitive method.

Future research should not only focus on the viruses that are present, but also into their characteristics and significance.

It would be useful to conduct a multi-center study in which post-mortem samples could be collected from a larger number of deceased infants and the findings compared across numerous medico-legal mortuaries.

Furthermore, it would be beneficial to investigate the markers of inflammation.

Further troubleshooting and rerunning of the denoising step were also not possible to prevent the loss of so many reads for differential abundance analysis.

There may be value in expanding the studies to also include virome profiling in such cases. It has been widely demonstrated that bacterial communities play an important role in human health and disease. It is, however, becoming increasingly apparent that the GIT virome also plays an important role in the pathogenesis of many diseases. Profiling the GIT virome will therefore provide insight not only into the viruses that are present, but also into their characteristics and significance.

References

- Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med. 2014 May 21;6(237):237ra65. doi: 10.1126/scitranslmed.3008599. PMID: 24848255; PMCID: PMC4929217.

- Abad FX, Villena C, Guix S, Caballero S, Pintó RM, Bosch A. Potential role of fomites in the vehicular transmission of human astroviruses. Appl Environ Microbiol. 2001 Sep;67(9):3904-7. doi: 10.1128/AEM.67.9.3904-3907.2001. PMID: 11525984; PMCID: PMC93108.

- Abdalla SE, Abia ALK, Amoako DG, Perrett K, Bester LA, Essack SY. Food animals as reservoirs and potential sources of multidrug-resistant diarrheagenic *E. coli* pathotypes: Focus on intensive pig farming in South Africa. Onderstepoort J Vet Res. 2022 Jan 20;89(1): e1-e13. doi: 10.4102/ojvr. v89i1.1963. PMID: 35144444; PMCID: PMC8832000.

- Abdalla SE, Abia ALK, Amoako DG, Perrett K, Bester LA, Essack SY. From Farm-to-Fork: E. Coli from an Intensive Pig Production System in South Africa Shows High Resistance to Critically Important Antibiotics for Human and Animal Use. Antibiotics (Basel). 2021 Feb 10;10(2):178. doi: 10.3390/antibiotics10020178. PMID: 33578692; PMCID: PMC7916376.

- Abu-Elyazeed R, Wierzba TF, Mourad AS, Peruski LF, Kay BA, Rao M, Churilla AM, Bourgeois AL, Mortagy AK, Kamal SM, Savarino SJ, Campbell JR, Murphy JR, Naficy A, Clemens JD. Epidemiology of enterotoxigenic Escherichia coli diarrhea in a pediatric cohort in a periurban area of lower Egypt. J Infect Dis. 1999 Feb;179(2):382-9. doi: 10.1086/314593. PMID: 9878022.

- Adachi JA, Jiang ZD, Mathewson JJ, Verenkar MP, Thompson S, Martinez-Sandoval F, Steffen R, Ericsson CD, DuPont HL. Enteroaggregative Escherichia coli as a major etiologic agent in traveler's diarrhea in 3 regions of the world. Clin Infect Dis. 2001 Jun 15;32(12):1706-9. doi: 10.1086/320756. Epub 2001 May 21. PMID: 11360211.

- Adadey SM, Quaye O. The burden of gastroenteritis in the post-Rotavirus vaccine era in Ghana: a hospital diagnoses-based study. Int J Med Res Health Sci. 2017;6(7):45-9.

- Adefisoye MA, Okoh AI. Identification and antimicrobial resistance prevalence of pathogenic Escherichia coli strains from treated wastewater effluents in Eastern Cape, South Africa. Microbiologyopen. 2016 Feb;5(1):143-51. doi: 10.1002/mbo3.319. Epub 2016 Jan 13. PMID: 26758686; PMCID: PMC4767426.

- Adler A, Wollach B, Kilman A, Gottesman G. Enteric gram-negative sepsis complicating rotavirus gastroenteritis in previously healthy infants. Clin Pediatr (Phila). 2005 May;44(4):351-4. doi: 10.1177/000992280504400411. PMID: 15864369.

- Adler JL, Zickl R. Winter vomiting disease. J Infect Dis. 1969 Jun;119(6):668-73. doi: 10.1093/infdis/119.6.668. PMID: 5795109.

- Adlerberth I, Lindberg E, Aberg N, Hesselmar B, Saalman R, Strannegård IL, Wold AE. Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: an effect of hygienic lifestyle? Pediatr Res. 2006 Jan;59(1):96-101. doi: 10.1203/01.pdr.0000191137. 12774.b2. Epub 2005 Nov 30. PMID: 16380405.

- Adlerberth I, Wold AE. Establishment of the gut microbiota in Western infants. Acta Paediatr. 2009 Feb;98(2):229-38. doi: 10.1111/j.1651-2227.2008.01060. x. PMID: 19143664.

- Afrad MH, Avzun T, Haque J, Haque W, Hossain ME, Rahman AR, Ahmed S, Faruque ASG, Rahman MZ, Rahman M. Detection of enteric- and non-enteric adenoviruses in gastroenteritis patients, Bangladesh, 2012-2015. J Med Virol. 2018 Apr;90(4):677-684. doi: 10.1002/jmv.25008. Epub 2018 Jan 11. PMID: 29244212.

- Agbla JM, Esona MD, Agbankpe AJ, Capo-Chichi A, Gautam R, Dougnon TV, Razack O, Bowen MD, Bankole HS. Molecular characteristics of rotavirus genotypes circulating in the south of Benin, 2016-2018. BMC Res Notes. 2020 Oct 19;13(1):485. doi: 10.1186/s13104-020-05332-7. PMID: 33076976; PMCID: PMC7574571.

- Ahmed SF, Sebeny PJ, Klena JD, Pimentel G, Mansour A, Naguib AM, Bruton J, Young SY, Holtz LR, Wang D. Novel astroviruses in children, Egypt. Emerg Infect Dis. 2011 Dec;17(12):2391-3. doi: 10.3201/eid1712.110909. PMID: 22172277; PMCID: PMC3311207.

- Ahmed SM, Hall AJ, Robinson AE, Verhoef L, Premkumar P, Parashar UD, Koopmans M, Lopman BA. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. Lancet Infect Dis. 2014 Aug;14(8):725-730. doi: 10.1016/S1473-3099(14)70767-4. Epub 2014 Jun 26. PMID: 24981041; PMCID: PMC8006533.

- Aijuka M, Santiago AE, Girón JA, Nataro JP, Buys EM. Enteroaggregative Escherichia coli is the predominant diarrheagenic *E. coli* pathotype among irrigation water and food sources in South Africa. Int J Food Microbiol. 2018 Aug 2; 278:44-51. doi: 10.1016/j.ijfoodmicro.2018.04.018. Epub 2018 Apr 20. PMID: 29702315.

- Alfinete NW, Bolukaoto JY, Heine L, Potgieter N, Barnard TG. Virulence and phylogenetic analysis of enteric pathogenic Escherichia coli isolated from children with diarrhoea in South Africa. Int J Infect Dis. 2022 Jan; 114:226-232. doi: 10.1016/j.ijid.2021.11.017. Epub 2021 Nov 11. PMID: 34775113.

- Aliabadi N, Antoni S, Mwenda JM, Weldegebriel G, Biey JNM, Cheikh D, Fahmy K, Teleb N, Ashmony HA, Ahmed H, Daniels DS, Videbaek D, Wasley A, Singh S, de Oliveira LH, Rey-Benito G, Sanwogou NJ, Wijesinghe PR, Liyanage JBL, Nyambat B, Grabovac V, Heffelfinger JD, Fox K, Paladin FJ, Nakamura T, Agócs M, Murray J, Cherian T, Yen C, Parashar UD, Serhan F, Tate JE, Cohen AL. Global impact of rotavirus vaccine introduction on rotavirus hospitalisations among children under 5 years of age, 2008-16: findings from the Global Rotavirus Surveillance Network. Lancet Glob Health. 2019 Jul;7(7):e893-e903. doi: 10.1016/S2214-109X(19)30207-4. PMID: 31200889; PMCID: PMC7336990.

- Alikhani MY, Mirsalehian A, Aslani MM. Detection of typical and atypical enteropathogenic Escherichia coli (EPEC) in Iranian children with and without diarrhoea. J Med Microbiol. 2006 Sep;55(Pt 9):1159-1163. doi: 10.1099/jmm.0.46539-0. PMID: 16914644.

- Alm B, Wennergren G, Möllborg P, Lagercrantz H. Breastfeeding and dummy use have a protective effect on sudden infant death syndrome. Acta Paediatr. 2016 Jan;105(1):31-8. doi: 10.1111/apa.13124. Epub 2015 Sep 2. PMID: 26175065; PMCID: PMC5049485.

- Alvarez-Lafuente R, Aguilera B, Suárez-Mier MA, Morentin B, Vallejo G, Gómez J, Fernández-Rodríguez A. Detection of human herpesvirus-6, Epstein-Barr virus, and cytomegalovirus in formalin-fixed tissues from sudden infant death: a study with quantitative real-time PCR. Forensic Sci Int. 2008 Jul 4;178(2-3):106-11. doi: 10.1016/j.forsciint.2008.02.007. PMID: 18424026.

- American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome. The changing concept of sudden infant death syndrome: diagnostic coding shifts, controversies regarding the sleeping environment, and new variables to consider in reducing risk. Pediatrics. 2005 Nov;116(5):1245-55. doi: 10.1542/peds.2005-1499. Epub 2005 Oct 10. PMID: 16216901.

- American Academy of Pediatrics; Hymel KP; Committee on Child Abuse and Neglect; National Association of Medical Examiners. Distinguishing sudden infant death syndrome from child abuse fatalities. Pediatrics. 2006 Jul;118(1):421-7. doi: 10.1542/peds.2006-1245. PMID: 16818592.

- Anderson EJ, Weber SG. Rotavirus infection in adults. Lancet Infect Dis. 2004 Feb;4(2):91-9. doi: 10.1016/S1473-3099(04)00928-4. PMID: 14871633; PMCID: PMC7106507.

- Anderson EJ. Rotavirus vaccines: viral shedding and risk of transmission. Lancet Infect Dis. 2008 Oct;8(10):642-9. doi: 10.1016/S1473-3099(08)70231-7. PMID: 18922486.

- Andrade FB, Gomes TAT, Elias WP. A sensitive and specific molecular tool for detection of both typical and atypical enteroaggregative Escherichia coli. J Microbiol Methods. 2014 Nov; 106:16-18. doi: 10.1016/j.mimet.2014.07.030. Epub 2014 Aug 6. PMID: 25108292.

- Anon. Double-Stranded DNA Viruses- Adenoviruses [Website]. 2021. Available from: https://bio.libretexts.org/@go/page/9925. [Date accessed 7 March 2022].

- Arboleya S, Sánchez B, Solís G, Fernández N, Suárez M, Hernández-Barranco AM, Milani C, Margolles A, de Los Reyes-Gavilán CG, Ventura M, Gueimonde M. Impact of Prematurity and Perinatal Antibiotics on the Developing Intestinal Microbiota: A Functional Inference Study. Int J Mol Sci. 2016 Apr 29;17(5):649. doi: 10.3390/ijms17050649. PMID: 27136545; PMCID: PMC4881475.

- Arcangeletti MC, Germini D, Martorana D, Rodighiero I, De Conto F, Medici MC, Chezzi C, Calderaro A. High frequency of cultivable human subgroup F adenoviruses in stool samples from a paediatric population admitted to hospital with acute gastroenteritis. J Med Microbiol. 2014 Jun;63(Pt 6):812-818. doi: 10.1099/jmm.0.072413-0. Epub 2014 Mar 21. PMID: 24659810.

- Arnestad M, Andersen M, Rognum TO. Is the use of dummy or carry-cot of importance for sudden infant death? Eur J Pediatr. 1997 Dec;156(12):968-70. doi: 10.1007/s004310050753. PMID: 9453383.

- Arowolo KO, Ayolabi CI, Lapinski B, Santos JS, Raboni SM. Epidemiology of enteric viruses in children with gastroenteritis in Ogun State, Nigeria. J Med Virol. 2019 Jun;91(6):1022-1029. doi: 10.1002/jmv.25399. Epub 2019 Jan 28. PMID: 30636345.

- Arrieta MC, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The intestinal microbiome in early life: health and disease. Front Immunol. 2014 Sep 5; 5:427. doi: 10.3389/fimmu.2014.00427. PMID: 25250028; PMCID: PMC4155789.

- Asowata OE, Ashiru OT, Mahomed S, Sturm AW, Moodley P. Influence of vaccination status and clinical, seasonal and sociodemographic factors on rotavirus prevalence in KwaZulu-Natal, South Africa. Southern African Journal of Infectious Diseases. 2018 Dec 1;33(5):1-7. Doi.org/10.1080/23120053.2018.1551850

- Astrup BS, Johnsen IB, Engsbro AL. The role of Coxsackievirus A16 in a case of sudden unexplained death in an infant - A SUDI case. Forensic Sci Int. 2016 Feb;259: e9-13. doi: 10.1016/j.forsciint.2015.12.017. Epub 2015 Dec 21. PMID: 26747753.

- Atchison CJ, Stowe J, Andrews N, Collins S, Allen DJ, Nawaz S, Brown D, Ramsay ME, Ladhani SN. Rapid Declines in Age Group-Specific Rotavirus Infection and Acute Gastroenteritis Among Vaccinated and Unvaccinated Individuals Within 1 Year of Rotavirus Vaccine Introduction in England and Wales. J Infect Dis. 2016 Jan 15;213(2):243-9. doi: 10.1093/infdis/jiv398. Epub 2015 Jul 30. PMID: 26232438.

- Athanasakis E, Karavasiliadou S, Styliadis I. The factors contributing to the risk of sudden infant death syndrome. Hippokratia. 2011 Apr;15(2):127-31. PMID: 22110293; PMCID: PMC3209674.

- Atmar RL, Baehner F, Cramer JP, Lloyd E, Sherwood J, Borkowski A, Mendelman PM; NOR-201 Study Group. Persistence of Antibodies to 2 Virus-Like Particle Norovirus Vaccine Candidate Formulations in Healthy Adults: 1-Year Follow-up With Memory Probe Vaccination. J Infect Dis. 2019 Jul 19;220(4):603-614. doi: 10.1093/infdis/jiz170. PMID: 31001633.

- Awotiwon OF, Pillay-van Wyk V, Dhansay A, Day C, Bradshaw D. Diarrhoea in children under five years of age in South Africa (1997-2014). Trop Med Int Health. 2016 Sep;21(9):1060-70. doi: 10.1111/tmi.12739. Epub 2016 Jul 6. PMID: 27314457.

- Ayolabi C, Ojo D, Akpan I. Astrovirus infection in children in lagos, Nigeria. Afr J Infect Dis. 2012;6(1):1-4. doi: 10.4314/ajid. v6i1.77733. PMID: 23878708; PMCID: PMC3555437.

- Ayukekbong J, Lindh M, Nenonen N, Tah F, Nkuo-Akenji T, Bergström T. Enteric viruses in healthy children in Cameroon: viral load and genotyping of norovirus strains. J Med Virol. 2011 Dec;83(12):2135-42. doi: 10.1002/jmv.22243. PMID: 22012721.

- Azad MB, Konya T, Persaud RR, Guttman DS, Chari RS, Field CJ, Sears MR, Mandhane PJ, Turvey SE, Subbarao P, Becker AB, Scott JA, Kozyrskyj AL; CHILD Study Investigators. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG. 2016 May;123(6):983-93. doi: 10.1111/1471-0528.13601. Epub 2015 Sep 28. PMID: 26412384.

- Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005 Mar 25;307(5717):1915-20. doi: 10.1126/science.1104816. PMID: 15790844.

- Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, Tremaroli V, Yin Y, Bergman S, Xu X, Madsen L, Kristiansen K, Dahlgren J, Wang J. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell Host Microbe. 2015 May 13;17(5):690-703. doi: 10.1016/j.chom.2015.04.004. Erratum in: Cell Host Microbe. 2015 Jun 10;17(6):852. Jun, Wang [corrected to Wang, Jun]. Erratum in: Cell Host Microbe. 2015 Jun 10;17(6):852. PMID: 25974306.

- Bailey LC, Forrest CB, Zhang P, Richards TM, Livshits A, DeRusso PA. Association of antibiotics in infancy with early childhood obesity. JAMA Pediatr. 2014 Nov;168(11):1063-9. doi: 10.1001/jamapediatrics.2014.1539. PMID: 25265089.

- Baj A, Moro E, Bistoletti M, Orlandi V, Crema F, Giaroni C. Glutamatergic Signaling Along The Microbiota-Gut-Brain Axis. Int J Mol Sci. 2019 Mar 25;20(6):1482. doi: 10.3390/ijms20061482. PMID: 30934533; PMCID: PMC6471396.

- Bajanowski T, Vege A, Byard RW, Krous HF, Arnestad M, Bachs L, Banner J, Blair PS, Borthne A, Dettmeyer R, Fleming P, Gaustad P, Gregersen M, Grøgaard J, Holter E, Isaksen CV, Jorgensen JV, de Lange C, Madea B, Moore I, Morland J, Opdal SH, Råsten-Almqvist P, Schlaud M, Sidebotham P, Skullerud K, Stoltenburg-Didinger G, Stray-Pedersen A, Sveum L, Rognum TO. Sudden infant death syndrome (SIDS)--standardised investigations and classification: recommendations. Forensic Sci Int. 2007 Jan 17;165(2-3):129-43. doi: 10.1016/j.forsciint.2006.05.028. Epub 2006 Jun 27. PMID: 16806765.

- Baker JL, Michaelsen KF, Rasmussen KM, Sørensen TI. Maternal prepregnant body mass index, duration of breastfeeding, and timing of complementary food introduction are associated with infant weight gain. Am J Clin Nutr. 2004 Dec;80(6):1579-88. doi: 10.1093/ajcn/80.6.1579. PMID: 15585772.

- Baker KK, Padhi B, Torondel B, Das P, Dutta A, Sahoo KC, Das B, Dreibelbis R, Caruso B, Freeman MC, Sager L, Panigrahi P. From menarche to menopause: A population-based assessment of water, sanitation, and hygiene risk factors for reproductive tract infection symptoms over life stages in rural girls and women in India. PLoS One. 2017 Dec 5;12(12): e0188234. doi: 10.1371/journal.pone.0188234. PMID: 29206842; PMCID: PMC5716553.

- Balzan S, de Almeida Quadros C, de Cleva R, Zilberstein B, Cecconello I. Bacterial translocation: overview of mechanisms and clinical impact. J Gastroenterol Hepatol. 2007 Apr;22(4):464-71. doi: 10.1111/j.1440-1746.2007.04933. x. PMID: 17376034.

- Bando SY, do Valle GR, Martinez MB, Trabulsi LR, Moreira-Filho CA. Characterization of enteroinvasive Escherichia coli and Shigella strains by RAPD analysis. FEMS Microbiol Lett. 1998 Aug 1;165(1):159-65. doi: 10.1111/j.1574-6968.1998.tb13141.x. PMID: 9711853.

- Banerjee A, De P, Manna B, Chawla-Sarkar M. Molecular characterization of enteric adenovirus genotypes 40 and 41 identified in children with acute gastroenteritis in Kolkata, India during 2013-2014. J Med Virol. 2017 Apr;89(4):606-614. doi: 10.1002/jmv.24672. Epub 2016 Sep 19. PMID: 27584661.

- Bangar R, Mamatha B. Identification of enteroaggregative Escherichia coli in infants with acute diarrhea based on biofilm production in Manipal, south India. Indian J Med Sci. 2008 Jan;62(1):8-12. PMID: 18239265.

- Bányai K, László B, Duque J, Steele AD, Nelson EA, Gentsch JR, Parashar UD. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for understanding the impact of rotavirus vaccination programs. Vaccine. 2012 Apr 27;30 Suppl 1: A122-30. doi: 10.1016/j.vaccine.2011.09.111. PMID: 22520121.

- Bányai K, Mijatovic-Rustempasic S, Hull JJ, Esona MD, Freeman MM, Frace AM, Bowen MD, Gentsch JR. Sequencing and phylogenetic analysis of the coding region of six common rotavirus strains: evidence for intragenogroup reassortment among co-circulating G1P[8] and G2P[4] strains from the United States. J Med Virol. 2011 Mar;83(3):532-9. doi: 10.1002/jmv.21977. PMID: 21264876.

- Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, Diamond MS, Miller VL, Virgin HW 4th. Herpesvirus latency confers symbiotic protection from bacterial infection. Nature. 2007 May 17;447(7142):326-9. doi: 10.1038/nature05762. PMID: 17507983.

- Baumann-Dudenhoeffer AM, D'Souza AW, Tarr PI, Warner BB, Dantas G. Infant diet and maternal gestational weight gain predict early metabolic maturation of gut microbiomes. Nat Med. 2018 Dec;24(12):1822-1829. doi: 10.1038/s41591-018-0216-2. Epub 2018 Oct 29. PMID: 30374198; PMCID: PMC6294307.

- Beal SM. Sudden infant death syndrome in South Australia 1968-97. Part I: changes over time. J Paediatr Child Health. 2000 Dec;36(6):540-7. doi: 10.1046/j.1440-1754.2000.00563.x. PMID: 11115028.

- Beatty ME, Bopp CA, Wells JG, Greene KD, Puhr ND, Mintz ED. Enterotoxin-producing Escherichia coli O169:H41, United States. Emerg Infect Dis. 2004 Mar;10(3):518-21. doi: 10.3201/eid1003.030268. PMID: 15109427; PMCID: PMC3322800.

- Beckwith JB. Defining the sudden infant death syndrome. Arch Pediatr Adolesc Med. 2003 Mar;157(3):286-90. doi: 10.1001/archpedi.157.3.286. PMID: 12622679.

- Béland K, Dore-Nguyen M, Gagné MJ, Patey N, Brassard J, Alvarez F, Halac U. Torque Teno virus in children who underwent orthotopic liver transplantation: new insights about a common pathogen. J Infect Dis. 2014 Jan 15;209(2):247-54. doi: 10.1093/infdis/jit423. Epub 2013 Aug 6. PMID: 23922368.

- Bélanger L, Garenaux A, Harel J, Boulianne M, Nadeau E, Dozois CM. Escherichia coli from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. FEMS Immunol Med Microbiol. 2011 Jun;62(1):1-10. doi: 10.1111/j.1574-695X.2011.00797. x. Epub 2011 Mar 24. PMID: 21362060.

- Ben Salem-Ben Nejma I, Hassine Zaafrane M, Hassine F, Sdiri-Loulizi K, Ben Said M, Aouni M, Mzoughi R. Etiology of Acute Diarrhea in Tunisian Children with Emphasis on Diarrheagenic Escherichia coli: Prevalence and

Identification of *E. coli* Virulence Markers. Iran J Public Health. 2014 Jul;43(7):947-60. PMID: 25909062; PMCID: PMC4401059.

- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological). 1995 Jan;57(1):289-300. doi.org/10.1111/j.2517-6161. 1995.tb02031. x.

- Bennett A, Pollock L, Jere KC, Pitzer VE, Lopman B, Parashar U, Everett D, Heyderman RS, Bar-Zeev N, Cunliffe NA, Iturriza-Gomara M. Infrequent Transmission of Monovalent Human Rotavirus Vaccine Virus to Household Contacts of Vaccinated Infants in Malawi. J Infect Dis. 2019 May 5;219(11):1730-1734. doi: 10.1093/infdis/jiz002. PMID: 30689911; PMCID: PMC6500552.

- Berg RD. The indigenous gastrointestinal microflora. Trends Microbiol. 1996 Nov;4(11):430-5. doi: 10.1016/0966-842x (96)10057-3. PMID: 8950812.

- Bergman AB. Synthesis. In: Sudden infant death syndrome. Edited by: Bergman AB, Beckwith JB, Ray CG. 1970, Seattle, WA: University of Washington Press, 210-221.

- Bergman H, Henschke N, Hungerford D, Pitan F, Ndwandwe D, Cunliffe N, Soares-Weiser K. Vaccines for preventing rotavirus diarrhoea: vaccines in use. Cochrane Database Syst Rev. 2021 Nov 17;11(11):CD008521. doi: 10.1002/14651858.CD008521.pub6. PMID: 34788488; PMCID: PMC8597890.

- Bergström A, Skov TH, Bahl MI, Roager HM, Christensen LB, Ejlerskov KT, Mølgaard C, Michaelsen KF, Licht TR. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. Appl Environ Microbiol. 2014 May;80(9):2889-900. doi: 10.1128/AEM.00342-14. Epub 2014 Feb 28. PMID: 24584251; PMCID: PMC3993305.

- Bettelheim KA, Dwyer BW, Smith DL, Goldwater PN, Bourne AJ. Toxigenic Escherichia coli associated with sudden infant death syndrome. Med J Aust. 1989 Nov 6;151(9):538. doi: 10.5694/j.1326-5377. 1989.tb128512. x. PMID: 2682151.

- Bettelheim KA, Goldwater PN, Dwyer BW, Bourne AJ, Smith DL. Toxigenic Escherichia coli associated with sudden infant death syndrome. Scand J Infect Dis. 1990;22(4):467-76. doi: 10.3109/00365549009027079. PMID: 2218409.

- Bettelheim KA, Goldwater PN. Escherichia coli and Sudden Infant Death Syndrome. Front Immunol. 2015 Jul 3; 6:343. doi: 10.3389/fimmu.2015.00343. PMID: 26191064; PMCID: PMC4490240.

- Bettiol SS, Radcliff FJ, Hunt AL, Goldsmid JM. Bacterial flora of Tasmanian SIDS infants with special reference to pathogenic strains of Escherichia coli. Epidemiol Infect. 1994 Apr;112(2):275-84. doi: 10.1017/s095026880005768x. PMID: 8150001; PMCID: PMC2271463.

- Beuret C. Simultaneous detection of enteric viruses by multiplex real-time RT-PCR. J Virol Methods. 2004 Jan;115(1):1-8. doi: 10.1016/j.jviromet.2003.09.005. PMID: 14656455.

- Beutin L, Gleier K, Kontny I, Echeverria P, Scheutz F. Origin and characteristics of enteroinvasive strains of Escherichia coli (EIEC) isolated in Germany. Epidemiol Infect. 1997 Jun;118(3):199-205. doi: 10.1017/s0950268897007413. PMID: 9207729; PMCID: PMC2808807.

- Beutin L, Steinrück H, Krause G, Steege K, Haby S, Hultsch G, Appel B. Comparative evaluation of the Ridascreen Verotoxin enzyme immunoassay for detection of Shiga-toxin producing strains of Escherichia coli (STEC) from food and other sources. J Appl Microbiol. 2007 Mar;102(3):630-9. doi: 10.1111/j.1365-2672.2006.03139. x. PMID: 17309611.

- Beutin L, Zimmermann S, Gleier K. Evaluation of the VTEC-Screen "Seiken" test for detection of different types of Shiga toxin (verotoxin)-producing Escherichia coli (STEC) in human stool samples. Diagn Microbiol Infect Dis. 2002 Jan;42(1):1-8. doi: 10.1016/s0732-8893(01)00325-x. PMID: 11821164.

- Beutin L, Zimmermann S, Gleier K. Rapid detection, and isolation of shiga-like toxin (verocytotoxin)-producing Escherichia coli by direct testing of individual enterohemolytic colonies from washed sheep blood agar plates in the VTEC-RPLA assay. J Clin Microbiol. 1996 Nov;34(11):2812-4. doi: 10.1128/jcm.34.11.2812-2814.1996. PMID: 8897187; PMCID: PMC229408.

- Biasucci G, Benenati B, Morelli L, Bessi E, Boehm G. Cesarean delivery may affect the early biodiversity of intestinal bacteria. J Nutr. 2008 Sep;138(9):1796S-1800S. doi: 10.1093/jn/138.9.1796S. PMID: 18716189.

- Biscaro V, Piccinelli G, Gargiulo F, Ianiro G, Caruso A, Caccuri F, De Francesco MA. Detection and molecular characterization of enteric viruses in children with acute gastroenteritis in Northern Italy. Infect Genet Evol. 2018 Jun; 60:35-41. doi: 10.1016/j.meegid.2018.02.011. Epub 2018 Feb 10. PMID: 29438743.

- Bischoff SC, Krämer S. Human mast cells, bacteria, and intestinal immunity. Immunol Rev. 2007 Jun; 217:329-37. doi: 10.1111/j.1600-065X.2007.00523. x. PMID: 17498069.

- Bishop R. Discovery of rotavirus: Implications for child health. J Gastroenterol Hepatol. 2009 Oct;24 Suppl 3: S81-5. doi: 10.1111/j.1440-1746.2009.06076. x. PMID: 19799704.

- Blacher E, Bashiardes S, Shapiro H, Rothschild D, Mor U, Dori-Bachash M, Kleimeyer C, Moresi C, Harnik Y, Zur M, Zabari M, Brik RB, Kviatcovsky D, Zmora N, Cohen Y, Bar N, Levi I, Amar N, Mehlman T, Brandis A, Biton I, Kuperman Y, Tsoory M, Alfahel L, Harmelin A, Schwartz M, Israelson A, Arike L, Johansson MEV, Hansson GC, Gotkine M, Segal E, Elinav E. Potential roles of gut microbiome and metabolites in modulating ALS in mice. Nature. 2019 Aug;572(7770):474-480. doi: 10.1038/s41586-019-1443-5. Epub 2019 Jul 22. PMID: 31330533.

- Blackwell C, Moscovis S, Hall S, Burns C, Scott RJ. Exploring the risk factors for sudden infant deaths and their role in inflammatory responses to infection. Front Immunol. 2015 Mar 5; 6:44. doi: 10.3389/fimmu.2015.00044. PMID: 25798137; PMCID: PMC4350416.

- Blackwell CC, Gordon AE, James VS, MacKenzie DA, Mogensen-Buchanan M, El Ahmer OR, Al Madani OM, Törö K, Csukás Z, Sótonyi P, Weir DM, Busuttil A. The role of bacterial toxins in sudden infant death syndrome (SIDS). Int J Med Microbiol. 2002 Feb;291(6-7):561-70. doi: 10.1078/1438-4221-00168. PMID: 11892683.

- Blackwell CC, MacKenzie DA, James VS, Elton RA, Zorgani AA, Weir DM, Busuttil A. Toxigenic bacteria and sudden infant death syndrome (SIDS): nasopharyngeal flora during the first year of life. FEMS Immunol Med Microbiol. 1999 Aug 1;25(1-2):51-8. doi: 10.1111/j.1574-695X.1999.tb01326. x. PMID: 10443491.

- Blackwell CC, Moscovis SM, Gordon AE, Al Madani OM, Hall ST, Gleeson M, Scott RJ, Roberts-Thomson J, Weir DM, Busuttil A. Cytokine responses and sudden infant death syndrome: genetic, developmental, and environmental risk factors. J Leukoc Biol. 2005 Dec;78(6):1242-54. doi: 10.1189/jlb.0505253. Epub 2005 Oct 4. PMID: 16204631.

- Blair PS, Fleming PJ, Smith IJ, Platt MW, Young J, Nadin P, Berry PJ, Golding J. Babies sleeping with parents: case-control study of factors influencing the risk of the sudden infant death syndrome. CESDI SUDI research group. BMJ. 1999 Dec 4;319(7223):1457-61. doi: 10.1136/bmj.319.7223.1457. PMID: 10582925; PMCID: PMC28288.

- Blair PS, Heron J, Fleming PJ. Relationship between bed sharing and breastfeeding: longitudinal, population-based analysis. Pediatrics. 2010 Nov;126(5): e1119-26. doi: 10.1542/peds.2010-1277. Epub 2010 Oct 18. PMID: 20956410.

- Blair PS, Platt MW, Smith IJ, Fleming PJ; CESDI SUDI Research Group. Sudden infant death syndrome and sleeping position in pre-term and low birth weight infants: an opportunity for targeted intervention. Arch Dis Child. 2006 Feb;91(2):101-6. doi: 10.1136/adc.2004.070391. Epub 2005 May 24. PMID: 15914498; PMCID: PMC2082697.

- Blakely T, Ajwani S, Robson B, Tobias M, Bonné M. Decades of disparity: widening ethnic mortality gaps from 1980 to 1999. N Z Med J. 2004 Aug 6;117(1199): U995. PMID: 15475978.

- Blanco M, Blanco JE, Dahbi G, Alonso MP, Mora A, Coira MA, Madrid C, Juárez A, Bernárdez MI, González EA, Blanco J. Identification of two new intimin types in atypical enteropathogenic Escherichia coli. Int Microbiol. 2006 Jun;9(2):103-10. PMID: 16835840.

- Blaxter M. The health of the children: a review of research on the place of health in cycles of disadvantage. 1981, London: Heinemann Educational, 219.

- Blood-Siegfried J, Rambaud C, Nyska A, Germolec DR. Evidence for infection, inflammation and shock in sudden infant death: parallels between a neonatal rat model of sudden death and infants who died of sudden infant death syndrome. Innate Immun. 2008 Jun;14(3):145-52. doi: 10.1177/1753425908090730. PMID: 18562573; PMCID: PMC2679510.

- Blyton MD, Banks SC, Peakall R, Lindenmayer DB, Gordon DM. Not all types of host contacts are equal when it comes to *E. coli* transmission. Ecol Lett. 2014 Aug;17(8):970-8. doi: 10.1111/ele.12300. Epub 2014 May 25. PMID: 24861219.

- Bobak DA, Guerrant RL. Nausea, Vomiting, and Noninflammatory Diarrhea. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 2015:1253–1262.e5. doi: 10.1016/B978-1-4557-4801-3.00100-4. Epub 2014 Oct 31. PMCID: PMC7173487.

- Böhnel H, Behrens S, Loch P, Lube K, Gessler F. Is there a link between infant botulism and sudden infant death? Bacteriological results obtained in central Germany. Eur J Pediatr. 2001 Oct;160(10):623-8. doi: 10.1007/s004310100807. PMID: 11686509.

- Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019 Aug;37(8):852-857. doi: 10.1038/s41587-019-0209-9. Erratum in: Nat Biotechnol. 2019 Sep;37(9):1091. PMID: 31341288; PMCID: PMC7015180.

- Bonkoungou IJ, Haukka K, Österblad M, Hakanen AJ, Traoré AS, Barro N, Siitonen A. Bacterial and viral etiology of childhood diarrhea in Ouagadougou, Burkina Faso. BMC Pediatr. 2013 Mar 19;13:36. doi: 10.1186/1471-2431-13-36. PMID: 23506294; PMCID: PMC3616825.

- Borian A, Csizmazia F, Karvaly E, Mihalffy F, Redey B. [Enterocolitis epidemic caused by water contaminated with Coli O 124 in Veszprem]. Orv Hetil. 1959 Jul 26;100:1072-4. Hungarian. PMID: 13802829.

- Bosch A, Pintó RM, Guix S. Human astroviruses. Clin Microbiol Rev. 2014 Oct;27(4):1048-74. doi: 10.1128/CMR.00013-14. PMID: 25278582; PMCID: PMC4187635.

- Bosch A., Guix S., Krishna NK., Méndez E., Monroe, S.S., Pantin-Jackwood, M. and Schultz-Cherry, S. In: Virus taxonomy: classification and nomenclature of viruses (Ninth Report of the International Committee on the Taxonomy of Viruses). Edited by: King AMQ, Lefkowitz E, Adams MJ, Carstens EB. 2011, New York: Elsevier Academic Press, 953–959.

- Bosch AATM, Levin E, van Houten MA, Hasrat R, Kalkman G, Biesbroek G, de Steenhuijsen Piters WAA, de Groot PCM, Pernet P, Keijser BJF, Sanders EAM, Bogaert D. Development of Upper Respiratory Tract Microbiota in Infancy is Affected by Mode of Delivery. EBioMedicine. 2016 Jul; 9:336-345. doi: 10.1016/j.ebiom.2016.05.031. Epub 2016 May 26. PMID: 27333043; PMCID: PMC4972531.

- Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. J Pediatr. 2010 Feb;156(2 Suppl): S8-15. doi: 10.1016/j.jpeds.2009.11.014. PMID: 20105666.

- Bray AE, Ahmed S, Das SK, Khan SH, Chisti MJ, Ahmed T, Faruque ASG, Fuchs GJ. Viral Pathogen-Specific Clinical and Demographic Characteristics of Children with Moderate-to-Severe Diarrhea in Rural Bangladesh. Am J Trop Med Hyg. 2019 Aug;101(2):304-309. doi: 10.4269/ajtmh.19-0152. PMID: 31264563; PMCID: PMC6685579.

- Bray J. Isolation of antigenically homogeneous strains of *Bacterium coli neopolitanum* from summer diarrhoea of infants. J Pathol Bacteriol. 1945;57(2):239–247. doi.org/10.1002/path.1700570210.

- Brody JR, Kern SE. History and principles of conductive media for standard DNA electrophoresis. Anal Biochem. 2004 Oct 1;333(1):1-13. doi: 10.1016/j.ab.2004.05.054. PMID: 15351274.

- Brooks B, Firek BA, Miller CS, Sharon I, Thomas BC, Baker R, Morowitz MJ, Banfield JF. Microbes in the neonatal intensive care unit resemble those found in the gut of premature infants. Microbiome. 2014 Jan 28;2(1):1. doi: 10.1186/2049-2618-2-1. PMID: 24468033; PMCID: PMC4392516.

- Brown BP, Jaspan HB. Compositional analyses reveal correlations between taxon-level gut bacterial abundance and peripheral T cell marker expression in African infants. Gut Microbes. 2020;11(2):237-244. doi: 10.1080/19490976.2019.1643673. Epub 2019 Jul 26. PMID: 31347944; PMCID: PMC7053881.

- Brown DW, Gunning KB, Henry DM, Awdeh ZL, Brinker JP, Tzipori S, Herrmann JE. A DNA oligonucleotide microarray for detecting human astrovirus serotypes. J Virol Methods. 2008 Jan;147(1):86-92. doi: 10.1016/j.jviromet.2007.07.028. Epub 2007 Oct 1. PMID: 17905448; PMCID: PMC2238180.

- Brown J, De Vos WM, DiStefano PS, Doré J, Huttenhower C, Knight R, Lawley TD, Raes J, Turnbaugh P. Translating the human microbiome. Nat Biotechnol. 2013 Apr;31(4):304-8. doi.org/10.1038/nbt.2543.

- Brunette GW. CDC Yellow Book 2018: Health Information for International Travel. 2017, Oxford University Press, 269.

- Bruun T, Salamanca BV, Bekkevold T, Døllner H, Gibory M, Gilje AM, Haarr E, Kran AB, Leegaard TM, Nakstad B, Nordbø SA, Rojahn A, Størdal K, Flem E; Norwegian Enhanced Pediatric Immunisation Surveillance Network. Impact of the Rotavirus Vaccination Program in Norway After Four Years With High Coverage. Pediatr Infect Dis J. 2021 Apr 1;40(4):368-374. doi: 10.1097/INF.0000000000003020. PMID: 33399430.

- Bucardo F, Karlsson B, Nordgren J, Paniagua M, González A, Amador JJ, Espinoza F, Svensson L. Mutated G4P [8] rotavirus associated with a nationwide outbreak of gastroenteritis in Nicaragua in 2005. J Clin Microbiol. 2007 Mar;45(3):990-7. doi: 10.1128/JCM.01992-06. Epub 2007 Jan 17. PMID: 17229854; PMCID: PMC1829148.

- Bucardo F, Reyes Y, Svensson L, Nordgren J. Predominance of norovirus and sapovirus in Nicaragua after implementation of universal rotavirus vaccination. PLoS One. 2014 May 21;9(5): e98201. doi: 10.1371/journal.pone.0098201. PMID: 24849288; PMCID: PMC4029982.

- Bucheli SR, Lynne AM. The microbiome of human decomposition. Microbe Magazine. 2016;11:165-71. DOI: 10.1128/microbe.11.165.1.

- Burberry A, Wells MF, Limone F, Couto A, Smith KS, Keaney J, Gillet G, van Gastel N, Wang JY, Pietilainen O, Qian M, Eggan P, Cantrell C, Mok J, Kadiu I, Scadden DT, Eggan K. C9orf72 suppresses systemic and neural inflammation induced by gut bacteria. Nature. 2020 Jun;582(7810):89-94. doi: 10.1038/s41586-020-2288-7. Epub 2020 May 13. PMID: 32483373; PMCID: PMC7416879.

- Burcham ZM, Hood JA, Pechal JL, Krausz KL, Bose JL, Schmidt CJ, Benbow ME, Jordan HR. Fluorescently labeled bacteria provide insight on post-mortem microbial transmigration. Forensic Sci Int. 2016 Jul;264:63-9. doi: 10.1016/j.forsciint.2016.03.019. Epub 2016 Mar 17. PMID: 27032615.

- Burger MC, Dempers JJ, de Beer C. Profiling the approach to the investigation of viral infections in cases of sudden unexpected death in infancy in the Western Cape Province, South Africa. Forensic Sci Int. 2014 Jun; 239:27-30. doi: 10.1016/j.forsciint.2014.03.007. Epub 2014 Mar 18. PMID: 24727219.

- Burnett E, Jonesteller CL, Tate JE, Yen C, Parashar UD. Global Impact of Rotavirus Vaccination on Childhood Hospitalizations and Mortality From Diarrhea. J Infect Dis. 2017 Jun 1;215(11):1666-1672. doi: 10.1093/infdis/jix186. PMID: 28430997; PMCID: PMC5543929.

- Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, Rogatcheva M, Kanack KJ, Bourzac KM. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol. 2015 Mar;53(3):915-25. doi: 10.1128/JCM.02674-14. Epub 2015 Jan 14. PMID: 25588652; PMCID: PMC4390666.

- Cabrera-Sosa L, Ochoa TJ. Escherichia coli diarrhea. Edited by: Ryan ET, Hill DR, Solomon T, Aronson NE, Endy TP. 2020, Hunter's Tropical Medicine and Emerging Infectious Diseases (Tenth Edition), Amsterdam, The Netherlands: Elsevier, 481-485. doi.org/10.1016/C2016-0-01879-X.

- Canizalez-Roman A, Flores-Villaseñor HM, Gonzalez-Nuñez E, Velazquez-Roman J, Vidal JE, Muro-Amador S, Alapizco-Castro G, Díaz-Quiñonez JA, León-Sicairos N. Surveillance of Diarrheagenic Escherichia coli Strains Isolated from Diarrhea Cases from Children, Adults and Elderly at Northwest of Mexico. Front Microbiol. 2016 Nov 30; 7:1924. doi: 10.3389/fmicb.2016.01924. PMID: 27965648; PMCID: PMC5127799.

- Cannon JL, Lopman BA, Payne DC, Vinjé J. Birth Cohort Studies Assessing Norovirus Infection and Immunity in Young Children: A Review. Clin Infect Dis. 2019 Jul 2;69(2):357-365. doi: 10.1093/cid/ciy985. Erratum in: Clin Infect Dis. 2021 Dec 16;73(12):2374. PMID: 30753367; PMCID: PMC7962893.

- Carlin RF, Moon RY. Risk Factors, Protective Factors, and Current Recommendations to Reduce Sudden Infant Death Syndrome: A Review. JAMA Pediatr. 2017 Feb 1;171(2):175-180. doi: 10.1001/jamapediatrics.2016.3345. PMID: 27918760.

- Carpenter RG, Irgens LM, Blair PS, England PD, Fleming P, Huber J, Jorch G, Schreuder P. Sudden unexplained infant death in 20 regions in Europe: case control study. Lancet. 2004 Jan 17;363(9404):185-91. doi: 10.1016/s0140-6736(03)15323-8. PMID: 14738790.

- Carroll R, Wood JN. Sudden unexpected infant death: a compassionate forensic approach to care. Clinical Pediatric Emergency Medicine. 2012 Sep 1;13(3):239-48. doi: 10.1016/j.cpem.2012.06.011.

- Cerna JF, Nataro JP, Estrada-Garcia T. Multiplex PCR for detection of three plasmid-borne genes of enteroaggregative Escherichia coli strains. J Clin Microbiol. 2003 May;41(5):2138-40. doi: 10.1128/JCM.41.5.2138-2140.2003. PMID: 12734261; PMCID: PMC154749.

- Chakravarty I, Bhattacharya A, Das SK. Water, sanitation and hygiene: The unfinished agenda in the World Health Organization South-East Asia Region. WHO South East Asia J Public Health. 2017 Sep;6(2):22-33. doi: 10.4103/2224-3151.213787. PMID: 28857059.

- Chang AB, Wilson SJ, Masters IB, Yuill M, Williams J, Williams G, Hubbard M. Altered arousal response in infants exposed to cigarette smoke. Arch Dis Child. 2003 Jan;88(1):30-3. doi: 10.1136/adc.88.1.30. PMID: 12495956; PMCID: PMC1719296.

- Chang RK, Keens TG, Rodriguez S, Chen AY. Sudden infant death syndrome: changing epidemiologic patterns in California 1989-2004. J Pediatr. 2008 Oct;153(4):498-502. doi: 10.1016/j.jpeds.2008.04.022. Epub 2008 May 27. PMID: 18534214.

- Chatterjee BD, Sanyal SN. Is it all shigellosis? Lancet. 1984 Sep 8;2(8402):574. doi: 10.1016/s0140-6736(84)90783-9. PMID: 6147618.

- Chellapandi K, Dutta TK, Sharma I, De Mandal S, Kumar NS, Ralte L. Prevalence of multi drug resistant enteropathogenic and enteroinvasive Escherichia coli isolated from children with and without diarrhea in Northeast Indian population. Ann Clin Microbiol Antimicrob. 2017 Jul 11;16(1):49. doi: 10.1186/s12941-017-0225-x. PMID: 28693504; PMCID: PMC5504610.

- Chhabra P, de Graaf M, Parra GI, Chan MC, Green K, Martella V, Wang Q, White PA, Katayama K, Vennema H, Koopmans MPG, Vinjé J. Updated classification of norovirus genogroups and genotypes. J Gen Virol. 2019 Oct;100(10):1393-1406. doi: 10.1099/jgv.0.001318. Erratum in: J Gen Virol. 2020 Aug;101(8):893. PMID: 31483239; PMCID: PMC7011714.

- Chitambar S, Gopalkrishna V, Chhabra P, Patil P, Verma H, Lahon A, Arora R, Tatte V, Ranshing S, Dhale G, Kolhapure R, Tikute S, Kulkarni J, Bhardwaj R, Akarte S, Pawar S. Diversity in the enteric viruses detected in outbreaks of gastroenteritis from Mumbai, Western India. Int J Environ Res Public Health. 2012 Mar;9(3):895-915. doi: 10.3390/ijerph9030895. Epub 2012 Mar 14. PMID: 22690171; PMCID: PMC3367285.

- Choudhry MA, Bland KI, Chaudry IH. Trauma and immune response--effect of gender differences. Injury. 2007 Dec;38(12):1382-91. doi: 10.1016/j.injury.2007.09.027. Epub 2007 Nov 28. PMID: 18048037; PMCID: PMC2692838.

- Christoffersen S. The importance of microbiological testing for establishing cause of death in 42 forensic autopsies. Forensic Sci Int. 2015 May;250:27-32. doi: 10.1016/j.forsciint.2015.02.020. Epub 2015 Feb 28. PMID: 25769131; PMCID: PMC7130849.

- Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nat Med. 2017 Mar;23(3):314-326. doi: 10.1038/nm.4272. Epub 2017 Jan 23. PMID: 28112736; PMCID: PMC5345907.

- Chukwu MO, Abia ALK, Ubomba-Jaswa E, Dewar JB, Obi CL. Mixed Aetiology of Diarrhoea in Infants Attending Clinics in the North-West Province of South Africa: Potential for Sub-Optimal Treatment. Pathogens. 2020 Mar 6;9(3):198. doi: 10.3390/pathogens9030198. PMID: 32155961; PMCID: PMC7157715.

- Ciarlet M, Estes MK. Interactions between rotavirus and gastrointestinal cells. Curr Opin Microbiol. 2001 Aug;4(4):435-41. doi: 10.1016/s1369-5274(00)00232-0. PMID: 11495808.

- Ciccarelli S, Stolfi I, Caramia G. Management strategies in the treatment of neonatal and pediatric gastroenteritis. Infect Drug Resist. 2013 Oct 29; 6:133-61. doi: 10.2147/IDR.S12718. PMID: 24194646; PMCID: PMC3815002.

- City of Cape Town Profile and Analysis District Development Model [Website]. 2020. Available from: https://www.cogta.gov.za/ddm/wp-content/uploads/2020/11/City-of-CT-September-2020.pdf. [Date accessed: 16 February 2022].

- Claassen-Weitz S, Gardner-Lubbe S, Nicol P, Botha G, Mounaud S, Shankar J, Nierman WC, Mulder N, Budree S, Zar HJ, Nicol MP, Kaba M. HIV-exposure, early life feeding practices and delivery mode impacts on faecal bacterial profiles in a South African birth cohort. Sci Rep. 2018 Mar 22;8(1):5078. doi: 10.1038/s41598-018-22244-6. PMID: 29567959; PMCID: PMC5864830.

- Clark JA, Coopersmith CM. Intestinal crosstalk: a new paradigm for understanding the gut as the "motor" of critical illness. Shock. 2007 Oct;28(4):384-93. doi: 10.1097/shk.0b013e31805569df. PMID: 17577136; PMCID: PMC2084394.

- Clarke RTJ. gut and its microorganisms. Microbial ecology of the gut. Edited by: Clarke RTJ, Bauchop T. 1977, New York: Academic Press, 35–71.

- Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell. 2012 Mar 16;148(6):1258-70. doi: 10.1016/j.cell.2012.01.035. PMID: 22424233; PMCID: PMC5050011.

- Codagnone MG, Stanton C, O'Mahony SM, Dinan TG, Cryan JF. Microbiota and Neurodevelopmental Trajectories: Role of Maternal and Early-Life Nutrition. Ann Nutr Metab. 2019;74 Suppl 2:16-27. doi: 10.1159/000499144. Epub 2019 Jun 24. PMID: 31234188.

- Cohen AL, Platts-Mills JA, Nakamura T, Operario DJ, Antoni S, Mwenda JM, Weldegebriel G, Rey-Benito G, De Oliveira LH, Ortiz C, Daniels DS. Aetiology and incidence of diarrhoea requiring hospitalisation in children under 5 years of age in 28 low-income and middle-income countries: findings from the Global Pediatric Diarrhea Surveillance network. BMJ Global Health. 2022 Sep 1;7(9):e009548. Doi:10.1136/bmjgh-2022-009548

- Coker MO, Hoen AG, Dade E, Lundgren S, Li Z, Wong AD, Zens MS, Palys TJ, Morrison HG, Sogin ML, Baker ER, Karagas MR, Madan JC. Specific class of intrapartum antibiotics relates to maturation of the infant gut microbiota: a prospective cohort study. BJOG. 2020 Jan;127(2):217-227. doi: 10.1111/1471-0528.15799. Epub 2019 Jun 5. PMID: 31006170; PMCID: PMC6803026.

- Collado MC, Cernada M, Baüerl C, Vento M, Pérez-Martínez G. Microbial ecology and host-microbiota interactions during early life stages. Gut Microbes. 2012 Jul-Aug;3(4):352-65. doi: 10.4161/gmic.21215. Epub 2012 Jun 29. Erratum in: Gut Microbes. 2014 Mar-Apr;5(2):271-2. PMID: 22743759; PMCID: PMC3463493.

- Collado MC, Isolauri E, Laitinen K, Salminen S. Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. Am J Clin Nutr. 2010 Nov;92(5):1023-30. doi: 10.3945/ajcn.2010.29877. Epub 2010 Sep 15. PMID: 20844065.

- Conway T, Cohen PS. Commensal and Pathogenic Escherichia coli Metabolism in the Gut. Microbiol Spectr. 2015 Jun;3(3):10.1128/microbiolspec.MBP-0006-2014. doi: 10.1128/microbiolspec.MBP-0006-2014. PMID: 26185077; PMCID: PMC4510460.

- Cooperstock MS, Zedd AJ. Intestinal flora of infants. Human Intestinal Microflora in Health and Disease. Edited by: Hentges DJ. 1983, New York: Academic Press, 1:79-99.

- Cortez V, Freiden P, Gu Z, Adderson E, Hayden R, Schultz-Cherry S. Persistent Infections with Diverse Co-Circulating Astroviruses in Pediatric Oncology Patients, Memphis, Tennessee, USA. Emerg Infect Dis. 2017 Feb;23(2):288-290. doi: 10.3201/eid2302.161436. PMID: 28098537; PMCID: PMC5324824.

- Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, Kim SG, Li H, Gao Z, Mahana D, Zárate Rodriguez JG, Rogers AB, Robine N, Loke P, Blaser MJ. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014 Aug 14;158(4):705-721. doi: 10.1016/j.cell.2014.05.052. PMID: 25126780; PMCID: PMC4134513.

- Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev. 2013 Oct;26(4):822-80. doi: 10.1128/CMR.00022-13. PMID: 24092857; PMCID: PMC3811233.

- Cruz JR, Bartlett AV, Herrmann JE, Cáceres P, Blacklow NR, Cano F. Astrovirus-associated diarrhea among Guatemalan ambulatory rural children. J Clin Microbiol. 1992 May;30(5):1140-4. doi: 10.1128/jcm.30.5.1140-1144.1992. PMID: 1583111; PMCID: PMC265238.

- Cunliffe NA, Gentsch JR, Kirkwood CD, Gondwe JS, Dove W, Nakagomi O, Nakagomi T, Hoshino Y, Bresee JS, Glass RI, Molyneux ME, Hart CA. Molecular and serologic characterization of novel serotype G8 human rotavirus strains detected in Blantyre, Malawi. Virology. 2000 Sep 1;274(2):309-20. doi: 10.1006/viro.2000.0456. PMID: 10964774.

- Cunnington AJ, Sim K, Deierl A, Kroll JS, Brannigan E, Darby J. "Vaginal seeding" of infants born by caesarean section. BMJ. 2016 Feb 23;352: i227. doi: 10.1136/bmj. i227. PMID: 26906151.

- Cutz E, Jackson A. Airway Inflammation and Peripheral Chemoreceptors. Sudden Infant Death Syndrome: Problems, Progress and Possibilities. Edited by: Byard RW, Krous HF, 2001, London: Arnold, 156–181.

- Daigle F, Harel J, Fairbrother JM, Lebel P. Expression and detection of pap-, sfa-, and afa-encoded fimbrial adhesin systems among uropathogenic Escherichia coli. Can J Microbiol. 1994 Apr;40(4):286-91. doi: 10.1139/m94-046. PMID: 7913657.

- Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. Nat Rev Gastroenterol Hepatol. 2019 Aug;16(8):461-478. doi: 10.1038/s41575-019-0157-3. PMID: 31123355.

- Dalton CB, Mintz ED, Wells JG, Bopp CA, Tauxe RV. Outbreaks of enterotoxigenic Escherichia coli infection in American adults: a clinical and epidemiologic profile. Epidemiol Infect. 1999 Aug;123(1):9-16. doi: 10.1017/s0950268899002526. PMID: 10487636; PMCID: PMC2810723.

- Daneman R, Prat A. The blood-brain barrier. Cold Spring Harb Perspect Biol. 2015 Jan 5;7(1): a020412. doi: 10.1101/cshperspect. a020412. PMID: 25561720; PMCID: PMC4292164.

- Davies DP. Cot death in Hong Kong: a rare problem? Lancet. 1985 Dec 14;2(8468):1346-9. doi: 10.1016/s0140-6736(85)92637-6. PMID: 2866397.

- de Jonge GA, Engelberts AC, Koomen-Liefting AJ, Kostense PJ. Cot death and prone sleeping position in The Netherlands. BMJ. 1989 Mar 18;298(6675):722. doi: 10.1136/bmj.298.6675.722. PMID: 2496821; PMCID: PMC1836032.

- De SN, Bhattacharya K, Sarkar JK. A study of the pathogenicity of strains of Bacterium coli from acute and chronic enteritis. J Pathol Bacteriol. 1956 Jan;71(1):201-9. doi: 10.1002/path.1700710126. PMID: 13307349.

- De Vlaminck I, Khush KK, Strehl C, Kohli B, Luikart H, Neff NF, Okamoto J, Snyder TM, Cornfield DN, Nicolls MR, Weill D, Bernstein D, Valantine HA, Quake SR. Temporal response of the human virome to immunosuppression and antiviral therapy. Cell. 2013 Nov 21;155(5):1178-87. doi: 10.1016/j.cell.2013.10.034. PMID: 24267896; PMCID: PMC4098717.

- Dehority BA. Foregut fermentation. Gastrointestinal Microbiology: Volume 1 Gastrointestinal Ecosystems and Fermentations. Edited by: Mackie RI, White BA, 1997, Boston: Chapman & Hall Microbiology Series. Springer, 39-83. doi.org/10.1007/978-1-4615-4111-0_3.

- Dempers JJ, Burger EH, Toit-Prinsloo LD, Verster J. A South African Perspective. In: Duncan JR, Byard RW, editors. SIDS Sudden Infant and Early Childhood Death: The Past, the Present and the Future. Adelaide (AU): University of Adelaide Press; 2018 May. Chapter 17. PMID: 30035954.

- Dempers JJ, Coldrey J, Burger EH, Thompson V, Wadee SA, Odendaal HJ, Sens MA, Randall BB, Folkerth RD, Kinney HC; PASS Network. The Institution of a Standardized Investigation Protocol for Sudden Infant Death in the Eastern Metropole, Cape Town, South Africa, J Forensic Sci. 2016 Nov;61(6):1508-1514. doi: 10.1111/1556-4029.13204. Epub 2016 Sep 27. PMID: 27677096; PMCID: PMC5663499.

- Deng J, Luo X, Wang R, Jiang L, Ding X, Hao W, Peng Y, Jiang C, Yu N, Che X. A comparison of Luminex xTAG® Gastrointestinal Pathogen Panel (xTAG GPP) and routine tests for the detection of enteropathogens

circulating in Southern China. Diagn Microbiol Infect Dis. 2015 Nov;83(3):325-30. doi: 10.1016/j.diagmicrobio.2015.07.024. Epub 2015 Jul 30. PMID: 26318973.

- Desmons A, Terrade C, Boulagnon C, Giusti D, Nguyen Y, Andreoletti L, Fornes P, Digeon B, Leveque N. Post-mortem diagnosis, of cytomegalovirus and varicella zoster virus co-infection by combined histology and tissue molecular biology, in a sudden unexplained infant death. J Clin Virol. 2013 Oct;58(2):486-9. doi: 10.1016/j.jcv.2013.08.007. Epub 2013 Aug 15. PMID: 24001883; PMCID: PMC7128706.

- Desselberger U, Gray J. Viral gastroenteritis. Medicine (Abingdon). 2009 Nov;37(11):594-598. doi: 10.1016/j.mpmed.2009.08.005. Epub 2009 Oct 31. PMID: 32288569; PMCID: PMC7108416.

- Desselberger U. Caliciviridae Other Than Noroviruses. Viruses. 2019 Mar 21;11(3):286. doi: 10.3390/v11030286. PMID: 30901945; PMCID: PMC6466229.

- Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008 Nov 18;6(11): e280. doi: 10.1371/journal.pbio.0060280. PMID: 19018661; PMCID: PMC2586385.

- Dettmeyer R, Baasner A, Schlamann M, Haag C, Madea B. Coxsackie B3 myocarditis in 4 cases of suspected sudden infant death syndrome: diagnosis by immunohistochemical and molecular-pathologic investigations. Pathol Res Pract. 2002;198(10):689-96. doi: 10.1078/0344-0338-00322. PMID: 12498225.

- Dettmeyer R, Baasner A, Schlamann M, Padosch SA, Haag C, Kandolf R, Madea B. Role of virus-induced myocardial affections in sudden infant death syndrome: a prospective postmortem study. Pediatr Res. 2004 Jun;55(6):947-52. doi: 10.1203/01.pdr.0000127022.45831.54. PMID: 15155864.

- Dettmeyer R, Kandolf R, Schmidt P, Schlamann M, Madea B. Lympho-monocytic enteroviral myocarditis: traditional, immunohistological and molecularpathological methods for diagnosis in a case of suspected sudden infant death syndrome (SIDS). Forensic Sci Int. 2001 Jun 1;119(1):141-4. doi: 10.1016/s0379-0738(00)00413-8. PMID: 11348811.

- Dettmeyer R, Sperhake JP, Müller J, Madea B. Cytomegalovirus-induced pneumonia and myocarditis in three cases of suspected sudden infant death syndrome (SIDS): diagnosis by immunohistochemical techniques and molecularpathologic methods. Forensic Sci Int. 2008 Jan 30;174(2-3):229-33. doi: 10.1016/j.forsciint.2007.05.009. Epub 2007 Jun 13. PMID: 17566679.

- Dhingra A, Hage E, Ganzenmueller T, Böttcher S, Hofmann J, Hamprecht K, Obermeier P, Rath B, Hausmann F, Dobner T, Heim A. Molecular Evolution of Human Adenovirus (HAdV) Species C. Sci Rep. 2019 Jan 31;9(1):1039. doi: 10.1038/s41598-018-37249-4. PMID: 30705303; PMCID: PMC6355881.

- Dhital S, Sherchand JB, Pokhrel BM, Parajuli K, Shah N, Mishra SK, Sharma S, Kattel HP, Khadka S, Khatiwada S, Parajuli N, Rijal B. Molecular epidemiology of Rotavirus causing diarrhea among children less than five years of age visiting national level children hospitals, Nepal. BMC Pediatr. 2017 Apr 7;17(1):101. doi: 10.1186/s12887-017-0858-0. PMID: 28388889; PMCID: PMC5385007.

- Di Mauro A, Neu J, Riezzo G, Raimondi F, Martinelli D, Francavilla R, Indrio F. Gastrointestinal function development and microbiota. Ital J Pediatr. 2013 Feb 24; 39:15. doi: 10.1186/1824-7288-39-15. PMID: 23433508; PMCID: PMC3615966.

- Diaz Heijtz R. Fetal, neonatal, and infant microbiome: Perturbations and subsequent effects on brain development and behavior. Semin Fetal Neonatal Med. 2016 Dec;21(6):410-417. doi: 10.1016/j.siny.2016.04.012. Epub 2016 May 30. PMID: 27255860.

- Dieterich CM, Felice JP, O'Sullivan E, Rasmussen KM. Breastfeeding and health outcomes for the mother-infant dyad. Pediatr Clin North Am. 2013 Feb;60(1):31-48. doi: 10.1016/j.pcl.2012.09.010. Epub 2012 Nov 3. PMID: 23178059; PMCID: PMC3508512.

- Diez-Valcarce M, Castro CJ, Marine RL, Halasa N, Mayta H, Saito M, Tsaknaridis L, Pan CY, Bucardo F, Becker-Dreps S, Lopez MR, Magaña LC, Ng TFF, Vinjé J. Genetic diversity of human sapovirus across the Americas. J Clin Virol. 2018 Jul; 104:65-72. doi: 10.1016/j.jcv.2018.05.003. Epub 2018 May 6. PMID: 29753103; PMCID: PMC8972816.

- Diez-Valcarce M, Montmayeur A, Tatusov R, Vinjé J. Near-Complete Human Sapovirus Genome Sequences from Kenya. Microbiol Resour Announc. 2019 Feb 14;8(7): e01602-18. doi: 10.1128/MRA.01602-18. PMID: 30801066; PMCID: PMC6376425.

- DiFranza JR, Savageau JA, Fletcher K, Ockene JK, Rigotti NA, McNeill AD, Coleman M, Wood C. Recollections and repercussions of the first inhaled cigarette. Addict Behav. 2004 Feb;29(2):261-72. doi: 10.1016/j.addbeh.2003.08.002. PMID: 14732415.

- DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, Gotsch F, Kim CJ, Erez O, Edwin S, Relman DA. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PLoS One. 2008 Aug 26;3(8): e3056. doi: 10.1371/journal.pone.0003056. PMID: 18725970; PMCID: PMC2516597.

- do Carmo GM, Yen C, Cortes J, Siqueira AA, de Oliveira WK, Cortez-Escalante JJ, Lopman B, Flannery B, de Oliveira LH, Carmo EH, Patel M. Decline in diarrhea mortality and admissions after routine childhood rotavirus immunization in Brazil: a time-series analysis. PLoS Med. 2011 Apr;8(4): e1001024. doi: 10.1371/journal.pmed.1001024. Epub 2011 Apr 19. PMID: 21526228; PMCID: PMC3079643.

- Doan YH, Nakagomi T, Cunliffe NA, Pandey BD, Sherchand JB, Nakagomi O. The occurrence of amino acid substitutions D96N and S242N in VP7 of emergent G2P[4] rotaviruses in Nepal in 2004-2005: a global and evolutionary perspective. Arch Virol. 2011 Nov;156(11):1969-78. doi: 10.1007/s00705-011-1083-z. Epub 2011 Aug 13. PMID: 21842161.

- Doll MK, Gagneur A, Tapiéro B, Charest H, Gonzales M, Buckeridge DL, Quach C. Temporal Changes in Pediatric Gastroenteritis after Rotavirus Vaccination in Quebec. Pediatr Infect Dis J. 2016 May;35(5):555-60. doi: 10.1097/INF.0000000000001077. PMID: 26855410.

- Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010 Jun 29;107(26):11971-5. doi: 10.1073/pnas.1002601107. Epub 2010 Jun 21. PMID: 20566857; PMCID: PMC2900693.

- Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, Gonzalez A, Bokulich NA, Song SJ, Hoashi M, Rivera-Vinas JI, Mendez K, Knight R, Clemente JC. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nat Med. 2016 Mar;22(3):250-3. doi: 10.1038/nm.4039. Epub 2016 Feb 1. PMID: 26828196; PMCID: PMC5062956.

- Donohue-Rolfe A, Kelley MA, Bennish M, Keusch GT. Enzyme-linked immunosorbent assay for shigella toxin. J Clin Microbiol. 1986 Jul;24(1):65-8. doi: 10.1128/jcm.24.1.65-68.1986. PMID: 3522627; PMCID: PMC268833.

- Doré J, Sghir A, Hannequart-Gramet G, Corthier G, Pochart P. Design and evaluation of a 16S rRNA-targeted oligonucleotide probe for specific detection and quantitation of human faecal Bacteroides populations. Syst Appl Microbiol. 1998 Mar;21(1):65-71. doi: 10.1016/S0723-2020(98)80009-X. PMID: 9741111.

- Dóró R, László B, Martella V, Leshem E, Gentsch J, Parashar U, Bányai K. Review of global rotavirus strain prevalence data from six years post vaccine licensure surveillance: is there evidence of strain selection from vaccine pressure? Infect Genet Evol. 2014 Dec;28:446-61. doi: 10.1016/j.meegid.2014.08.017. Epub 2014 Sep 16. PMID: 25224179; PMCID: PMC7976110.

- Doughty LA, Carlton S, Galen B, Cooma-Ramberan I, Chung CS, Ayala A. Activation of common antiviral pathways can potentiate inflammatory responses to septic shock. Shock. 2006 Aug;26(2):187-94. doi: 10.1097/01.shk.0000223129.79759.74. PMID: 16878028; PMCID: PMC1525211.

- Douglas TS, Fenton-Muir N, Kewana K, Ngema Y, Liebenberg L. Radiological findings at a South African forensic pathology laboratory in cases of sudden unexpected death in infants. SAJR. 2012 Feb 1;16(1):4-6. doi.org/10.4102/sajr. v16i1.222.

- Dove W, Cunliffe NA, Gondwe JS, Broadhead RL, Molyneux ME, Nakagomi O, Hart CA. Detection and characterization of human caliciviruses in hospitalized children with acute gastroenteritis in Blantyre, Malawi. J Med Virol. 2005 Dec;77(4):522-7. doi: 10.1002/jmv.20488. PMID: 16254959.

- Drexler JF, Baumgarte S, Eschbach-Bludau M, Simon A, Kemen C, Bode U, Eis-Hübinger AM, Madea B, Drosten C. Human cardioviruses, meningitis, and sudden infant death syndrome in children. Emerg Infect Dis. 2011 Dec;17(12):2313-5. doi: 10.3201/eid1712.111037. PMID: 22153118; PMCID: PMC3311172.

- Drexler JF, Luna LK, Stöcker A, Almeida PS, Ribeiro TC, Petersen N, Herzog P, Pedroso C, Huppertz HI, Ribeiro Hda C Jr, Baumgarte S, Drosten C. Circulation of 3 lineages of a novel Saffold cardiovirus in humans. Emerg Infect Dis. 2008 Sep;14(9):1398-405. doi: 10.3201/eid1409.080570. PMID: 18760006; PMCID: PMC2603095.

- D'Souza RM, Hall G, Becker NG. Climatic factors associated with hospitalizations for rotavirus diarrhoea in children under 5 years of age. Epidemiol Infect. 2008 Jan;136(1):56-64. doi: 10.1017/S0950268807008229. Epub 2007 Mar 12. PMID: 17352836; PMCID: PMC2870768.

- du Toit-Prinsloo L, Dempers J, Verster J, Hattingh C, Nel H, Brandt VD, Jordaan J, Saayman G. Toward a standardized investigation protocol in sudden unexpected deaths in infancy in South Africa: a multicenter study of medico-legal investigation procedures and outcomes. Forensic Sci Med Pathol. 2013 Sep;9(3):344-50. doi: 10.1007/s12024-013-9427-5. Epub 2013 Apr 6. PMID: 23564060.

- du Toit-Prinsloo L, Dempers JJ, Wadee SA, Saayman G. The medico-legal investigation of sudden, unexpected and/or unexplained infant deaths in South Africa: where are we--and where are we going? Forensic Sci Med Pathol. 2011 Mar;7(1):14-20. doi: 10.1007/s12024-010-9184-7. Epub 2010 Jul 22. PMID: 20652450.

- Duijts L, Jaddoe VW, Hofman A, Moll HA. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. Pediatrics. 2010 Jul;126(1): e18-25. doi: 10.1542/peds.2008-3256. Epub 2010 Jun 21. PMID: 20566605.

- Dulguer MV, Fabbricotti SH, Bando SY, Moreira-Filho CA, Fagundes-Neto U, Scaletsky IC. Atypical enteropathogenic Escherichia coli strains: phenotypic and genetic profiling reveals a strong association between enteroaggregative *E. coli* heat-stable enterotoxin and diarrhea. J Infect Dis. 2003 Dec 1;188(11):1685-94. doi: 10.1086/379666. Epub 2003 Nov 14. PMID: 14639540.

- Duncan JR, Byard RW, editors. SIDS Sudden Infant and Early Childhood Death: The Past, the Present and the Future. Adelaide (AU): University of Adelaide Press; 2018 May. PMID: 30024688.

- Dunlop AL, Mulle JG, Ferranti EP, Edwards S, Dunn AB, Corwin EJ. Maternal Microbiome and Pregnancy Outcomes That Impact Infant Health: A Review. Adv Neonatal Care. 2015 Dec;15(6):377-85. doi: 10.1097/ANC.0000000000000218. PMID: 26317856; PMCID: PMC4658310.

- Duvallet C, Gibbons SM, Gurry T, Irizarry RA, Alm EJ. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. Nat Commun. 2017 Dec 5;8(1):1784. doi: 10.1038/s41467-017-01973-8. PMID: 29209090; PMCID: PMC5716994.

- Dwyer T, Ponsonby AL, Blizzard L, Newman NM, Cochrane JA. The contribution of changes in the prevalence of prone sleeping position to the decline in sudden infant death syndrome in Tasmania. JAMA. 1995 Mar 8;273(10):783-9. PMID: 7861572.

- Edwards PR, Ewing WH. Edward's and Ewing's Identification of Enterobacteriaceae, 4th ed. 1986, New York: Elsevier Science, 135–172.

- Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. WHO Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. Lancet. 2000 Feb 5;355(9202):451-5. Erratum in: Lancet 2000 Mar 25;355(9209):1104. PMID: 10841125.

- Elfving K, Andersson M, Msellem MI, Welinder-Olsson C, Petzold M, Björkman A, Trollfors B, Mårtensson A, Lindh M. Real-time PCR threshold cycle cutoffs help to identify agents causing acute childhood diarrhea in Zanzibar. J Clin Microbiol. 2014 Mar;52(3):916-23. doi: 10.1128/JCM.02697-13. Epub 2014 Jan 8. PMID: 24403298; PMCID: PMC3957757.

- Elliott AJ, Kinney HC, Haynes RL, Dempers JD, Wright C, Fifer WP, Angal J, Boyd TK, Burd L, Burger E, Folkerth RD, Groenewald C, Hankins G, Hereld D, Hoffman HJ, Holm IA, Myers MM, Nelsen LL, Odendaal HJ, Petersen J, Randall BB, Roberts DJ, Robinson F, Schubert P, Sens MA, Sullivan LM, Tripp T, Van Eerden P, Wadee S, Willinger M, Zaharie D, Dukes KA. Concurrent prenatal drinking and smoking increases risk for SIDS: Safe Passage Study report. EClinicalMedicine. 2020 Jan 20; 19:100247. doi: 10.1016/j.eclinm.2019.100247. PMID: 32140668; PMCID: PMC7046523.

- Elliott EJ. Acute gastroenteritis in children. BMJ. 2007 Jan 6;334(7583):35-40. doi: 10.1136/bmj.39036.406169.80. PMID: 17204802; PMCID: PMC1764079.

- Ellis SJ, Crossman LC, McGrath CJ, Chattaway MA, Hölken JM, Brett B, Bundy L, Kay GL, Wain J, Schüller S. Identification and characterisation of enteroaggregative Escherichia coli subtypes associated with human disease. Sci Rep. 2020 May 4;10(1):7475. Doi: 10.1038/s41598-020-64424-3.

- Ellwardt E, Walsh JT, Kipnis J, Zipp F. Understanding the Role of T Cells in CNS Homeostasis. Trends Immunol. 2016 Feb;37(2):154-165. doi: 10.1016/j.it.2015.12.008. Epub 2016 Jan 14. PMID: 26775912.

- Engelhardt B, Carare RO, Bechmann I, Flügel A, Laman JD, Weller RO. Vascular, glial, and lymphatic immune gateways of the central nervous system. Acta Neuropathol. 2016 Sep;132(3):317-38. doi: 10.1007/s00401-016-1606-5. Epub 2016 Aug 13. PMID: 27522506; PMCID: PMC4992028.

- Engelhardt B, Liebner S. Novel insights into the development and maintenance of the blood-brain barrier. Cell Tissue Res. 2014 Mar;355(3):687-99. doi: 10.1007/s00441-014-1811-2. Epub 2014 Mar 4. PMID: 24590145; PMCID: PMC3972432.

- Enweronu-Laryea CC, Sagoe KW, Glover-Addy H, Asmah RH, Mingle JA, Armah GE. Prevalence of severe acute rotavirus gastroenteritis and intussusceptions in Ghanaian children under 5 years of age. J Infect Dev Ctries. 2012 Feb 13;6(2):148-55. doi: 10.3855/jidc.1667. PMID: 22337844.

- Ercolini D. High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. Appl Environ Microbiol. 2013 May;79(10):3148-55. doi: 10.1128/AEM.00256-13. Epub 2013 Mar 8. PMID: 23475615; PMCID: PMC3685257.

- Escher M, Scavia G, Morabito S, Tozzoli R, Maugliani A, Cantoni S, Fracchia S, Bettati A, Casa R, Gesu GP, Torresani E, Caprioli A. A severe foodborne outbreak of diarrhoea linked to a canteen in Italy caused by enteroinvasive Escherichia coli, an uncommon agent. Epidemiol Infect. 2014 Dec;142(12):2559-66. doi: 10.1017/S0950268814000181. Epub 2014 Feb 13. PMID: 24534429; PMCID: PMC9151301.

- Esona MD, Gautam R. Rotavirus. Clin Lab Med. 2015 Jun;35(2):363-91. doi: 10.1016/j.cll.2015.02.012. PMID: 26004648.

- Esona MD, Geyer A, Page N, Trabelsi A, Fodha I, Aminu M, Agbaya VA, Tsion B, Kerin TK, Armah GE, Steele AD, Glass RI, Gentsch JR. Genomic characterization of human rotavirus G8 strains from the African rotavirus network: relationship to animal rotaviruses. J Med Virol. 2009 May;81(5):937-51. doi: 10.1002/jmv.21468. PMID: 19319943.

- Estes MK, Cohen J. Rotavirus gene structure and function. Microbiol Rev. 1989 Dec;53(4):410-49. doi: 10.1128/mr.53.4.410-449.1989. PMID: 2556635; PMCID: PMC372748.

- Estes MK. Rotaviruses and their replication. In Fields Virology. 4th edition. Edited by: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, 2001, Philadelphia: Lippincott, Williams and Wilkins, 1747–1785.

- Estes, M.K.; Kapikian, A. Rotaviruses. In Fields Virology. Wolters Kluwer Health. Edited by: Lippincott KD, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, 2007, Philadelphia: Williams and Wilkins, 1917–1975.

- Estrada-Garcia T, Navarro-Garcia F. Enteroaggregative Escherichia coli pathotype: a genetically heterogeneous emerging foodborne enteropathogen. FEMS Immunol Med Microbiol. 2012 Dec;66(3):281-98. doi: 10.1111/j.1574-695X.2012.01008. x. Epub 2012 Jul 31. PMID: 22775224.

- European Centre for Disease Prevention and Control. ECDC Expert opinion on rotavirus vaccination in infancy. Stockholm: ECDC. [Website]. 2017. Available from: https://www.ecdc.europa.eu/en/publications-data/expert-opinion-rotavirus-vaccination-infancy [Date accessed 11 May 2023].

- Ewing WH, Gravatti JL. Shigella types encountered in the mediterranean area. Journal of Bacteriology. 1947 Feb;53(2):191-5.

- Faith DP. Conservation evaluation and phylogenetic diversity. Biol Cons. 1992 Jan 1;61(1):1-10. doi.org/10.1016/0006-3207(92)91201-3.

- Farahmand M, Moghoofei M, Dorost A, Shoja Z, Ghorbani S, Kiani SJ, Khales P, Esteghamati A, Sayyahfar S, Jafarzadeh M, Minaeian S, Khanaliha K, Naghdalipour M, Tavakoli A. Global prevalence and genotype distribution of norovirus infection in children with gastroenteritis: A meta-analysis on 6 years of research from 2015 to 2020. Rev Med Virol. 2022 Jan;32(1): e2237. doi: 10.1002/rmv.2237. Epub 2021 Apr 1. PMID: 33793023.

- Fedor A, Bojanowski I, Korzeniewski K. Gastrointestinal infections in returned travelers. Int Marit Health. 2019;70(4):244-251. doi: 10.5603/IMH.2019.0039. PMID: 31891179.

- Feng Y, Zhou Z, Liu Z, Zheng C, Feng F, Xie F, Wu ZD. Interleukin 17A Derived from γδ T Cell Induces Demyelination of the Brain in Angiostrongylus cantonensis Infection. Mol Neurobiol. 2021 Aug;58(8):3968-3982. doi: 10.1007/s12035-021-02366-1. Epub 2021 Apr 26. PMID: 33904019.

- Fernández-Rodríguez A, Ballesteros S, de Ory F, Echevarría JE, Alvarez-Lafuente R, Vallejo G, Gómez J. Virological analysis in the diagnosis of sudden children death: a medico-legal approach. Forensic Sci Int. 2006 Aug 10;161(1):8-14. doi: 10.1016/j.forsciint.2005.10.012. Epub 2005 Nov 18. PMID: 16300916.

- Fernández-Rodríguez A, Burton JL, Andreoletti L, Alberola J, Fornes P, Merino I, Martínez MJ, Castillo P, Sampaio-Maia B, Caldas IM, Saegeman V, Cohen MC; ESGFOR and the ESP. Post-mortem microbiology in sudden death: sampling protocols proposed in different clinical settings. Clin Microbiol Infect. 2019 May;25(5):570-579. doi: 10.1016/j.cmi.2018.08.009. Epub 2018 Aug 24. PMID: 30145399.

- Ferreira MS, Victoria M, Carvalho-Costa FA, Vieira CB, Xavier MP, Fioretti JM, Andrade J, Volotão EM, Rocha M, Leite JP, Miagostovich MP. Surveillance of norovirus infections in the state of Rio De Janeiro, Brazil 2005-2008. J Med Virol. 2010 Aug;82(8):1442-8. doi: 10.1002/jmv.21831. PMID: 20572088.

- Filho EP, da Costa Faria NR, Fialho AM, de Assis RS, Almeida MMS, Rocha M, Galvão M, Dos Santos FB, Barreto ML, Leite JPG. Adenoviruses associated with acute gastroenteritis in hospitalized and community children up to 5 years old in Rio de Janeiro and Salvador, Brazil. J Med Microbiol. 2007 Mar;56(Pt 3):313-319. doi: 10.1099/jmm.0.46685-0. PMID: 17314359.

- Filiano JJ, Kinney HC. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. Biol Neonate. 1994;65(3-4):194-7. doi: 10.1159/000244052. PMID: 8038282.

- Finley SJ, Benbow ME, Javan GT. Microbial communities associated with human decomposition and their potential use as postmortem clocks. Int J Legal Med. 2015 May;129(3):623-32. doi: 10.1007/s00414-014-1059-0. Epub 2014 Aug 17. PMID: 25129823.

- Fish EN. The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol. 2008 Sep;8(9):737-44. doi: 10.1038/nri2394. PMID: 18728636; PMCID: PMC7097214.

- Fitzgerald DA, Jeffery H, Arbuckle S, du Toit-Prinsloo L, O'Sullivan T, Waters K. Sudden Unexpected Death in Infancy [SUDI]: What the clinician, pathologist, coroner and researchers want to know. Paediatr Respir Rev. 2022 Mar; 41:14-20. doi: 10.1016/j.prrv.2021.08.002. Epub 2021 Aug 13. PMID: 34998675.

- Flahault A, Hanslik T. Epidémiologie des gastroentérites virales en France et en Europe [Epidemiology of viral gastroenteritis in France and Europe]. Bull Acad Natl Med. 2010 Nov;194(8):1415-24; discussion 1424-5. French. PMID: 22046706.

- Fleming PJ, Blair PS, Bacon C, Bensley D, Smith I, Taylor E, Berry J, Golding J, Tripp J. Environment of infants during sleep and risk of the sudden infant death syndrome: results of 1993-5 case-control study for confidential inquiry into stillbirths and deaths in infancy. Confidential Enquiry into Stillbirths and Deaths Regional Coordinators and Researchers. BMJ. 1996 Jul 27;313(7051):191-5. doi: 10.1136/bmj.313.7051.191. PMID: 8696193; PMCID: PMC2351639.

- Fleming PJ, Blair PS, Pease A. Sudden unexpected death in infancy: aetiology, pathophysiology, epidemiology and prevention in 2015. Arch Dis Child. 2015 Oct;100(10):984-8. doi: 10.1136/archdischild-2014-306424. Epub 2015 Feb 19. PMID: 25699563

- Fleming PJ, Blair PS, Pollard K, Platt MW, Leach C, Smith I, Berry PJ, Golding J. Pacifier use and sudden infant death syndrome: results from the CESDI/SUDI case control study. CESDI SUDI Research Team. Arch Dis Child. 1999 Aug;81(2):112-6. doi: 10.1136/adc.81.2.112. PMID: 10490514; PMCID: PMC1718026.

- Fleming PJ, Gilbert R, Azaz Y, Berry PJ, Rudd PT, Stewart A, Hall E. Interaction between bedding and sleeping position in the sudden infant death syndrome: a population-based case-control study. BMJ. 1990 Jul 14;301(6743):85-9. doi: 10.1136/bmj.301.6743.85. PMID: 2390588; PMCID: PMC1663432.

- Fong TT, Lipp EK. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Microbiol Mol Biol Rev. 2005 Jun;69(2):357-71. doi: 10.1128/MMBR.69.2.357-371.2005. PMID: 15944460; PMCID: PMC1197419.

- Forsthuber T, Yip HC, Lehmann PV. Induction of TH1 and TH2 immunity in neonatal mice. Science. 1996 Mar 22;271(5256):1728-30. doi: 10.1126/science.271.5256.1728. PMID: 8596934.

- Fouhy F, Ross RP, Fitzgerald GF, Stanton C, Cotter PD. Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. Gut Microbes. 2012 May-Jun;3(3):203-20. doi: 10.4161/gmic.20169. Epub 2012 May 1. PMID: 22572829; PMCID: PMC3427213.

- França EL, Bitencourt RV, Fujimori M, de Morais TC, Calderon Ide M, Honorio-França AC. Human colostral phagocytes eliminate enterotoxigenic Escherichia coli opsonized by colostrum supernatant. J Microbiol Immunol Infect. 2011 Feb;44(1):1-7. doi: 10.1016/j.jmii.2011.01.002. Epub 2011 Jan 12. PMID: 21531345.

- Franco P, Groswasser J, Hassid S, Lanquart JP, Scaillet S, Kahn A. Prenatal exposure to cigarette smoking is associated with a decrease in arousal in infants. J Pediatr. 1999 Jul;135(1):34-8. doi: 10.1016/s0022-3476(99)70324-0. PMID: 10393601.

- Franco P, Scaillet S, Wermenbol V, Valente F, Groswasser J, Kahn A. The influence of a pacifier on infants' arousals from sleep. J Pediatr. 2000 Jun;136(6):775-9. PMID: 10839876.

- Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007 May 11;316(5826):889-94. doi: 10.1126/science.1141634. Epub 2007 Apr 12. PMID: 17434869; PMCID: PMC2646098.

- Freeman K, Mistry H, Tsertsvadze A, Royle P, McCarthy N, Taylor-Phillips S, Manuel R, Mason J. Multiplex tests to identify gastrointestinal bacteria, viruses, and parasites in people with suspected infectious gastroenteritis: a systematic review and economic analysis. Health Technol Assess. 2017 Apr;21(23):1-188. doi: 10.3310/hta21230. PMID: 28619124; PMCID: PMC5494512.

- Freemantle CJ, Read AW, de Klerk NH, McAullay D, Anderson IP, Stanley FJ. Sudden infant death syndrome and unascertainable deaths: trends and disparities among Aboriginal and non-Aboriginal infants born in Western Australia from 1980 to 2001 inclusive. J Paediatr Child Health. 2006 Jul-Aug;42(7-8):445-51. doi: 10.1111/j.1440-1754.2006.00895. x. PMID: 16898883.

- Fricke WF. The more the merrier? Reduced fecal microbiota diversity in preterm infants treated with antibiotics. J Pediatr. 2014 Jul;165(1):8-10. doi: 10.1016/j.jpeds.2014.03.022. Epub 2014 Apr 21. PMID: 24750805.

- Frøen JF, Akre H, Stray-Pedersen B, Saugstad OD. Adverse effects of nicotine and interleukin-1beta on autoresuscitation after apnea in piglets: implications for sudden infant death syndrome. Pediatrics. 2000 Apr;105(4): E52. doi: 10.1542/peds.105.4. e52. PMID: 10742373.

- Froggatt P, Lynas MA, Marshall TK. Sudden unexpected death in infants ("cot death"). Report of a collaborative study in northern Ireland. Ulster Med J. 1971;40(2):116-35. PMID: 5150053; PMCID: PMC2385282.

- Fujita MQ, Zhu BL, Ishida K, Quan L, Oritani S, Maeda H. Serum C-reactive protein levels in postmortem blood--an analysis with special reference to the cause of death and survival time. Forensic Sci Int. 2002 Dec 4;130(2-3):160-6. doi: 10.1016/s0379-0738(02)00381-x. PMID: 12477638.

- Fulmer M, Zachritz W, Posencheg MA. Intensive Care Neonates and Evidence to Support the Elimination of Hats for Safe Sleep. Adv Neonatal Care. 2020 Jun;20(3):229-232. doi: 10.1097/ANC.0000000000000695. PMID: 31842154.

- Furukawa I, Suzuki M, Masaoka T, Nakajima N, Mitani E, Tasaka M, Teranishi H, Matsumoto Y, Koizumi M, Ogawa A, Oota Y, Homma S, Sasaki K, Satoh H, Sato K, Muto S, Anan Y, Kuroki T. Outbreak of Enterohemorrhagic Escherichia coli O157:H7 Infection Associated with Minced Meat Cutlets Consumption in Kanagawa, Japan. Jpn J Infect Dis. 2018 Nov 22;71(6):436-441. doi: 10.7883/yoken.JJID.2017.495. Epub 2018 Jul 31. PMID: 30068886.

- Fyfe KL, Yiallourou SR, Wong FY, Odoi A, Walker AM, Horne RS. Cerebral oxygenation in preterm infants. Pediatrics. 2014 Sep;134(3):435-45. doi: 10.1542/peds.2014-0773. PMID: 25157010.

- Galley JD, Bailey M, Kamp Dush C, Schoppe-Sullivan S, Christian LM. Maternal obesity is associated with alterations in the gut microbiome in toddlers. PLoS One. 2014 Nov 19;9(11): e113026. doi: 10.1371/journal.pone.0113026. PMID: 25409177; PMCID: PMC4237395.

- Gallien P. Detection and subtyping of Shiga toxin-producing Escherichia coli (STEC). Methods Mol Biol. 2003; 216:163-84. doi: 10.1385/1-59259-344-5:163. PMID: 12512363.

- Gao R, Zhu C, Li H, Yin M, Pan C, Huang L, Kong C, Wang X, Zhang Y, Qu S, Qin H. Dysbiosis Signatures of Gut Microbiota Along the Sequence from Healthy, Young Patients to Those with Overweight and Obesity. Obesity (Silver Spring). 2018 Feb;26(2):351-361. doi: 10.1002/oby.22088. Epub 2017 Dec 27. PMID: 29280312.

- García C, DuPont HL, Long KZ, Santos JI, Ko G. Asymptomatic norovirus infection in Mexican children. J Clin Microbiol. 2006 Aug;44(8):2997-3000. doi: 10.1128/JCM.00065-06. PMID: 16891526; PMCID: PMC1594604.

- García MG, Pérez-Cárceles MD, Osuna E, Legaz I. Impact of the Human Microbiome in Forensic Sciences: a Systematic Review. Appl Environ Microbiol. 2020 Oct 28;86(22): e01451-20. doi: 10.1128/AEM.01451-20. PMID: 32887714; PMCID: PMC7642070.

- Garcia-Mantrana I, Collado MC. Obesity and overweight: Impact on maternal and milk microbiome and their role for infant health and nutrition. Mol Nutr Food Res. 2016 Aug;60(8):1865-75. doi: 10.1002/mnfr.201501018. Epub 2016 Jun 30. PMID: 27159888.

- Gardiner D, Gobin M, Verlander NQ, Oliver I, Hawker J. Use of an ingredient-based analysis to investigate a national outbreak of Escherichia coli O157, United Kingdom, July 2016. Euro Surveill. 2018 Jun;23(26):1700627. doi: 10.2807/1560-7917.ES.2018.23.26.1700627. PMID: 29970217; PMCID: PMC6030876.

- Gascón J, Vargas M, Quintó L, Corachán M, Jimenez de Anta MT, Vila J. Enteroaggregative Escherichia coli strains as a cause of traveler's diarrhea: a case-control study. J Infect Dis. 1998 May;177(5):1409-12. doi: 10.1086/517826. PMID: 9593036.

- Gastañaduy AS, Bégué RE. Acute Gastroenteritis Viruses. Infectious Diseases. 2017:1390–1398.e3. doi: 10.1016/B978-0-7020-6285-8.00162-3. Epub 2016 Aug 12. PMCID: PMC7173516.

- Gastañaduy PA, Steenhoff AP, Mokomane M, Esona MD, Bowen MD, Jibril H, Pernica JM, Mazhani L, Smieja M, Tate JE, Parashar UD, Goldfarb DM. Effectiveness of Monovalent Rotavirus Vaccine After Programmatic Implementation in Botswana: A Multisite Prospective Case-Control Study. Clin Infect Dis. 2016 May 1;62 Suppl 2: S161-7. doi: 10.1093/cid/civ1207. PMID: 27059351.

- Gates L, Klein NJ, Sebire NJ, Alber DG. Characterising Post-mortem Bacterial Translocation Under Clinical Conditions Using 16S rRNA Gene Sequencing in Two Animal Models. Front Microbiol. 2021 May 31; 12:649312. doi: 10.3389/fmicb.2021.649312. PMID: 34135873; PMCID: PMC8200633.

- GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015 Jan 10;385(9963):117-71. doi: 10.1016/S0140-6736(14)61682-2. Epub 2014 Dec 18. PMID: 25530442; PMCID: PMC4340604.

- GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016 Oct 8;388(10053):1459-1544. doi: 10.1016/S0140-6736(16)31012-1. Erratum in: Lancet. 2017 Jan 7;389(10064): e1. PMID: 27733281; PMCID: PMC5388903.

- GBD 2016 Diarrhoeal Disease Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis. 2018 Nov;18(11):1211-1228. doi: 10.1016/S1473-3099(18)30362-1. Epub 2018 Sep 19. PMID: 30243583; PMCID: PMC6202444.

- GBD 2016 Lower Respiratory Infections Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis. 2018 Nov;18(11):1191-1210. doi: 10.1016/S1473-3099(18)30310-4. Epub 2018 Sep 19. PMID: 30243584; PMCID: PMC6202443.

- GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018 Nov 10;392(10159):1736-1788. doi: 10.1016/S0140-6736(18)32203-7. Epub 2018 Nov 8.

Erratum in: Lancet. 2019 Jun 22;393(10190): e44. Erratum in: Lancet. 2018 Nov 17;392(10160):2170. PMID: 30496103; PMCID: PMC6227606.

- GBD Diarrhoeal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis. 2017 Sep;17(9):909-948. doi: 10.1016/S1473-3099(17)30276-1. Epub 2017 Jun 1. Erratum in: Lancet Infect Dis. 2017 Sep;17(9):897. PMID: 28579426; PMCID: PMC5589208.

- Geertinger P, Bodenhoff J, Helweg-Larsen K, Lund A. Endogenous alcohol production by intestinal fermentation in sudden infant death. Z Rechtsmed. 1982;89(3):167-72. doi: 10.1007/BF01873798. PMID: 6760604.

- Gehlbach SH, MacCormack JN, Drake BM, Thompson WV. Spread of disease by fecal-oral route in day nurseries. Health Serv Rep. 1973 Apr;88(4):320-2. PMID: 4574421; PMCID: PMC1616047.

- Gelaw A, Pietsch C, Liebert UG. Genetic diversity of human adenovirus and human astrovirus in children with acute gastroenteritis in Northwest Ethiopia. Arch Virol. 2019 Dec;164(12):2985-2993. doi: 10.1007/s00705-019-04421-8. Epub 2019 Sep 30. PMID: 31570995.

- Gelfer P, Tatum M. Sudden infant death syndrome. J Pediatr Health Care. 2014 Sep-Oct;28(5):470-4. doi: 10.1016/j.pedhc.2014.04.007. Epub 2014 Jun 16. PMID: 24944089.

- Geoba se: Gazetter - The World at Your Fingertips. South Africa [Website]. 2021. Available from: http://www.geoba.se/country.php?cc=ZA&year=2021. [Date accessed: 17 February 2022].

- GERMS-SA Annual Report. [Website]. 2017. Available from: http://www.nicd.ac.za/index.php/publications/germs-annual-reports. [Date Accessed: 10 May 2023].

- GERMS-SA Annual Report. [Website]. 2018. Available from: http://www.nicd.ac.za/index.php/publications/germs-annual-reports/. [Date Accessed: 10 May 2023].

- Ghebremedhin B. Human adenovirus: Viral pathogen with increasing importance. Eur J Microbiol Immunol (Bp). 2014 Mar;4(1):26-33. doi: 10.1556/EuJMI.4.2014.1.2. Epub 2014 Mar 14. PMID: 24678403; PMCID: PMC3955829.

- Gianelli Castiglione A, Greenwald M, Stephens BG. Infant death in San Francisco 1989-1990. J Forensic Sci. 1993 Jul;38(4):821-30. PMID: 8355003.

- Gibson MK, Wang B, Ahmadi S, Burnham CA, Tarr PI, Warner BB, Dantas G. Developmental dynamics of the preterm infant gut microbiota and antibiotic resistome. Nat Microbiol. 2016 Mar 7; 1:16024. doi: 10.1038/nmicrobiol.2016.24. PMID: 27572443; PMCID: PMC5031140.

- Gilbert R, Rudd P, Berry PJ, Fleming PJ, Hall E, White DG, Oreffo VO, James P, Evans JA. Combined effect of infection and heavy wrapping on the risk of sudden unexpected infant death. Arch Dis Child. 1992 Feb;67(2):171-7. doi: 10.1136/adc.67.2.171. PMID: 1543374; PMCID: PMC1793423.

- Gillevet PM, Sikaroodi M, Torzilli AP. Analyzing salt-marsh fungal diversity: comparing ARISA fingerprinting with clone sequencing and pyrosequencing. Fungal Ecol, 2009 Nov 1;2(4):160- 167. doi: 10.1016/j.funeco.2009.04.001.

- Gilligan PH. Escherichia coli. EAEC, EHEC, EIEC, ETEC. Clin Lab Med. 1999 Sep;19(3):505-21. PMID: 10549423.

- Girón JA, Qadri F, Azim T, Jarvis KJ, Kaper JB, Albert MJ. Monoclonal antibodies specific for the bundle-forming pilus of enteropathogenic Escherichia coli. Infect Immun. 1995 Dec;63(12):4949-52. doi: 10.1128/iai.63.12.4949-4952.1995. PMID: 7591164; PMCID: PMC173713.

- Gismero-Ordoñez J, Dall'Agnol M, Trabulsi LR, Girón JA. Expression of the bundle-forming pilus by enteropathogenic Escherichia coli strains of heterologous serotypes. J Clin Microbiol. 2002 Jun;40(6):2291-6. doi: 10.1128/JCM.40.6.2291-2296.2002. PMID: 12037114; PMCID: PMC130815.

- Glass RI, Noel J, Mitchell D, Herrmann JE, Blacklow NR, Pickering LK, Dennehy P, Ruiz-Palacios G, de Guerrero ML, Monroe SS. The changing epidemiology of astrovirus-associated gastroenteritis: a review. Arch Virol Suppl. 1996; 12:287-300. doi: 10.1007/978-3-7091-6553-9_31. PMID: 9015126.

- Gleeson M, Clancy RL, Cox AJ, Gulliver SA, Hall ST, Cooper DM. Mucosal immune responses to infections in infants with acute life-threatening events classified as 'near-miss' sudden infant death syndrome. FEMS Immunol Med Microbiol. 2004 Sep 1;42(1):105-18. doi: 10.1016/j.femsim.2004.06.019. PMID: 15325403.

- Global Alliance for Vaccines and Immunizations (GAVI) Detailed Product Profiles/Gavi, the Vaccine Alliance. Available from: https://www.gavi.org/news/document-library/detailed-product-profiles. [Date Accessed: 25 May 2023].

- Goldstein RD, Kinney HC, Willinger M. Sudden Unexpected Death in Fetal Life Through Early Childhood. Pediatrics. 2016 Jun;137(6): e20154661. doi: 10.1542/peds.2015-4661. PMID: 27230764; PMCID: PMC4894250.362.

- Goldwater P, Bettelheim K. SIDS risk factors: time for new interpretations. The role of bacteria. Pediatrics Res Intern J. 2013.doi: 10.5171/2013.867520.

- Goldwater PN, Bettelheim KA. Curliated Escherichia coli, soluble curlin and the sudden infant death syndrome (SIDS). J Med Microbiol. 2002 Nov;51(11):1009-1012. doi: 10.1099/0022-1317-51-11-1009. PMID: 12448686.

- Goldwater PN. Gut Microbiota and Immunity: Possible Role in Sudden Infant Death Syndrome. Front Immunol. 2015 Jun 3; 6:269. doi: 10.3389/fimmu.2015.00269. PMID: 26089821; PMCID: PMC4453473.

- Goldwater PN. Sterile site infection at autopsy in sudden unexpected deaths in infancy. Arch Dis Child. 2009 Apr;94(4):303-7. doi: 10.1136/adc.2007.135939. Epub 2008 Sep 15. PMID: 18794179.

- Gomes TA, Elias WP, Scaletsky IC, Guth BE, Rodrigues JF, Piazza RM, Ferreira LC, Martinez MB. Diarrheagenic Escherichia coli. Braz J Microbiol. 2016 Dec;47 Suppl 1(Suppl 1):3-30. doi: 10.1016/j.bjm.2016.10.015. Epub 2016 Nov 5. PMID: 27866935; PMCID: PMC5156508.

- Gomes TA, Vieira MA, Wachsmuth IK, Blake PA, Trabulsi LR. Serotype-specific prevalence of Escherichia coli strains with EPEC adherence factor genes in infants with and without diarrhea in São Paulo, Brazil. J Infect Dis. 1989 Jul;160(1):131-5. doi: 10.1093/infdis/160.1.131. PMID: 2567320.

- Gomes TA, Yamamoto D, Vieira MAM, Hernandes RT.Atypical enteropathogenic Escherichia coli. In: Torres AG, ed.Escherichia coli in the Americas. Springer InternationalPublishing; 2016:77–96.

- Gomes TAT, González-Pedrajo B. EnteropathogenicEscherichia coli (EPEC). In: Torres AG, ed. PathogenicEscherichia coli in Latin America. Sharjah, United ArabEmirates: Betham Science Publishers Ltd.; 2010:66–126.16

- Gomez-Duarte OG, Romero-Herazo YC, Paez-Canro CZ, Eslava-Schmalbach JH, Arzuza O. Enterotoxigenic Escherichia coli associated with childhood diarrhoea in Colombia, South America. J Infect Dev Ctries. 2013 May 13;7(5):372-81. doi: 10.3855/jidc.2667. PMID: 23669426.

- Gomez-Llorente C, Plaza-Diaz J, Aguilera M, Muñoz-Quezada S, Bermudez-Brito M, Peso-Echarri P, Martinez-Silla R, Vasallo-Morillas MI, Campaña-Martin L, Vives-Piñera I, Ballesta-Martinez MJ, Gil A. Three main factors define changes in fecal microbiota associated with feeding modality in infants. J Pediatr Gastroenterol Nutr. 2013 Oct;57(4):461-6. doi: 10.1097/MPG.0b013e31829d519a. PMID: 23752082.

- Gonzales L, Joffre E, Rivera R, Sjöling Å, Svennerholm AM, Iñiguez V. Prevalence, seasonality and severity of disease caused by pathogenic Escherichia coli in children with diarrhoea in Bolivia. J Med Microbiol. 2013 Nov;62(Pt 11):1697-1706. doi: 10.1099/jmm.0.060798-0. Epub 2013 Jul 12. PMID: 23851188.

- Gonzalez-Perez G, Hicks AL, Tekieli TM, Radens CM, Williams BL, Lamousé-Smith ES. Maternal Antibiotic Treatment Impacts Development of the Neonatal Intestinal Microbiome and Antiviral Immunity. J Immunol. 2016 May 1;196(9):3768-79. doi: 10.4049/jimmunol.1502322. Epub 2016 Apr 1. PMID: 27036912.

- Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, Spector TD, Bell JT, Clark AG, Ley RE. Genetic Determinants of the Gut Microbiome in UK Twins. Cell Host Microbe. 2016 May 11;19(5):731-43. doi: 10.1016/j.chom.2016.04.017. PMID: 27173935; PMCID: PMC4915943.

- Gould LH, Bopp C, Strockbine N, Atkinson R, Baselski V, Body B, Carey R, Crandall C, Hurd S, Kaplan R, Neill M, Shea S, Somsel P, Tobin-D'Angelo M, Griffin PM, Gerner-Smidt P; Centers for Disease Control and Prevention (CDC). Recommendations for diagnosis of shiga toxin--producing Escherichia coli infections by clinical laboratories. MMWR Recomm Rep. 2009 Oct 16;58(RR-12):1-14. PMID: 19834454.

- Govender N, Perovic O, Meiring S, Kleynhans J, von Gottberg A, Thomas J, Ismail F, Ismail N, Page NA, Hunt G. GERMS-SA Annual Report 2018. National Institute for Communicable Diseases. 2017:37-8.

- Green KY. Caliciviridae: the noroviruses. In Fields Virology, 6th edition, vol 1. Edited by: Knipe DM, Howley PM, Cohen JI, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B, 2013, Philadelphia: Lippincott, Williams and Wilkins, 582–608.

- Greer FR, Sicherer SH, Burks AW; American Academy of Pediatrics Committee on Nutrition; American Academy of Pediatrics Section on Allergy and Immunology. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics. 2008 Jan;121(1):183-91. doi: 10.1542/peds.2007-3022. PMID: 18166574.

- Grimwood K, Carzino R, Barnes GL, Bishop RF. Patients with enteric adenovirus gastroenteritis admitted to an Australian pediatric teaching hospital from 1981 to 1992. J Clin Microbiol. 1995 Jan;33(1):131-6. doi: 10.1128/jcm.33.1.131-136.1995. PMID: 7699028; PMCID: PMC227894.

- Gritz EC, Bhandari V. The human neonatal gut microbiome: a brief review. Front Pediatr. 2015 Mar 5; 3:17. doi: 10.3389/fped.2015.00017. Erratum in: Front Pediatr. 2015; 3:60. PMID: 25798435; PMCID: PMC4350424.

- Grohmann GS, Glass RI, Pereira HG, Monroe SS, Hightower AW, Weber R, Bryan RT. Enteric viruses and diarrhea in HIV-infected patients. Enteric Opportunistic Infections Working Group. N Engl J Med. 1993 Jul 1;329(1):14-20. doi: 10.1056/NEJM199307013290103. PMID: 8099429.

- Grönlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. J Pediatr Gastroenterol Nutr. 1999 Jan;28(1):19-25. doi: 10.1097/00005176-199901000-00007. PMID: 9890463.

- Groome MJ, Page N, Cortese MM, Moyes J, Zar HJ, Kapongo CN, Mulligan C, Diedericks R, Cohen C, Fleming JA, Seheri M, Mphahlele J, Walaza S, Kahn K, Chhagan M, Steele AD, Parashar UD, Zell ER, Madhi SA. Effectiveness of monovalent human rotavirus vaccine against admission to hospital for acute rotavirus diarrhoea in South African children: a case-control study. Lancet Infect Dis. 2014 Nov;14(11):1096-1104. doi: 10.1016/S1473-3099(14)70940-5. Epub 2014 Oct 7. Erratum in: Lancet Infect Dis. 2014 Nov;14(11):1040. PMID: 25303843.

- Groome MJ, Zell ER, Solomon F, Nzenze S, Parashar UD, Izu A, Madhi SA. Temporal Association of Rotavirus Vaccine Introduction and Reduction in All-Cause Childhood Diarrheal Hospitalizations in South Africa. Clin Infect Dis. 2016 May 1;62 Suppl 2:S188-95. doi: 10.1093/cid/civ1204. PMID: 27059355.

- Groswasser J, Simon T, Scaillet S, Franco P, Kahn A. Reduced arousals following obstructive apneas in infants sleeping prone. Pediatr Res. 2001 Mar;49(3):402-6. doi: 10.1203/00006450-200103000-00015. PMID: 11228267.

- Guaraldi F, Salvatori G. Effect of breast and formula feeding on gut microbiota shaping in newborns. Front Cell Infect Microbiol. 2012 Oct 16; 2:94. doi: 10.3389/fcimb.2012.00094. PMID: 23087909; PMCID: PMC3472256.

- Guntheroth WG, Lohmann R, Spiers PS. A seasonal association between SIDS deaths and kindergarten absences. Public Health Rep. 1992 May-Jun;107(3):319-23. PMID: 1594742; PMCID: PMC1403653.

- Gunzburg ST, Chang BJ, Elliott SJ, Burke V, Gracey M. Diffuse and enteroaggregative patterns of adherence of enteric Escherichia coli isolated from aboriginal children from the Kimberley region of Western Australia. J Infect Dis. 1993 Mar;167(3):755-8. doi: 10.1093/infdis/167.3.755. PMID: 8440943.

- Guth BEC, Picheth CF, Gomes TAT. Escherichia coli situation in Brazil. Pathogenic Escherichia coli in Latin America. Edited by: Torres AG, 2010, Sharjah, United Arab Emirates: Betham Science Publishers Ltd, 162–178.

- Gyles CL. Shiga toxin-producing Escherichia coli: an overview. J Anim Sci. 2007 Mar;85(13 Suppl): E45-62. doi: 10.2527/jas.2006-508. Epub 2006 Nov 3. PMID: 17085726.

- Haglund B. Cigarette smoking and sudden infant death syndrome: some salient points in the debate. Acta Paediatr Suppl. 1993 Jun;82 Suppl 389:37-9. doi: 10.1111/j.1651-2227. 1993.tb12872. x. PMID: 8374189.

- Hakeem GF, Oddy L, Holcroft CA, Abenhaim HA. Incidence and determinants of sudden infant death syndrome: a population-based study on 37 million births. World J Pediatr. 2015 Feb;11(1):41-7. doi: 10.1007/s12519-014-0530-9. Epub 2014 Dec 1. PMID: 25447630.

- Hall AJ, Lopman BA, Payne DC, Patel MM, Gastañaduy PA, Vinjé J, Parashar UD. Norovirus disease in the United States. Emerg Infect Dis. 2013 Aug;19(8):1198-205. doi: 10.3201/eid1908.130465. PMID: 23876403; PMCID: PMC3739528.

- Hameed JM, McCaffrey RL, McCoy A, Brannock T, Martin GJ, Scouten WT, Brooks K, Putnam SD, Riddle MS. Incidence, Etiology and Risk Factors for Travelers' Diarrhea during a Hospital Ship-Based Military Humanitarian Mission: Continuing Promise 2011. PLoS One. 2016 May 12;11(5): e0154830. doi: 10.1371/journal.pone.0154830. PMID: 27171433; PMCID: PMC4865142.

- Hanson LA, Hahn-Zoric M, Berndes M, Ashraf R, Herias V, Jalil F, Bhutta TI, Laeeq A, Mattsby-Baltzer I. Breast feeding: overview and breast milk immunology. Acta Paediatr Jpn. 1994 Oct;36(5):557-61. doi: 10.1111/j.1442-200x.1994.tb03246. x. PMID: 7825463.

- Hanzlick RL, Jentzen JM, Clark SC. Sudden, Unexplained Infant Death Investigation, Infant Death Investigation: Guidelines for the Scene Investigator. [Website]. Atlanta, GA: Department of Health and Human Services (US), Centers for Disease Control. 2007. Available from: https://stacks.cdc.gov/view/cdc/25331. [Date accessed: 7 May 2018].

- Harcke HT. The case for postmortem imaging. Pediatr Radiol. 2010 Feb;40(2):138-40. doi: 10.1007/s00247-009-1433-0. Epub 2009 Dec 16. PMID: 20013259.

- Harder T, Bergmann R, Kallischnigg G, Plagemann A. Duration of breastfeeding and risk of overweight: a meta-analysis. Am J Epidemiol. 2005 Sep 1;162(5):397-403. doi: 10.1093/aje/kwi222. Epub 2005 Aug 2. PMID: 16076830.

- Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection

methods. J Pediatr Gastroenterol Nutr. 2000 Jan;30(1):61-7. doi: 10.1097/00005176-200001000-00019. PMID: 10630441.

- Harper RM. Sudden infant death syndrome. Encyclopedia of respiratory medicine. Edited by: Laurent GJ and Shapiro SD. 2006, Oxford: Elsevier, 132-134.

- Hascoët JM, Hubert C, Rochat F, Legagneur H, Gaga S, Emady-Azar S, Steenhout PG. Effect of formula composition on the development of infant gut microbiota. J Pediatr Gastroenterol Nutr. 2011 Jun;52(6):756-62. doi: 10.1097/MPG.0b013e3182105850. PMID: 21593648.

- Hasegawa M, Kishino H, Yano T. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol. 1985;22(2):160-74. doi: 10.1007/BF02101694. PMID: 3934395.

- Hassan F, Kanwar N, Harrison CJ, Halasa NB, Chappell JD, Englund JA, Klein EJ, Weinberg GA, Szilagyi PG, Moffatt ME, Oberste MS, Nix WA, Rogers S, Bowen MD, Vinjé J, Wikswo ME, Parashar UD, Payne DC, Selvarangan R. Viral Etiology of Acute Gastroenteritis in <2-Year-Old US Children in the Post-Rotavirus Vaccine Era. J Pediatric Infect Dis Soc. 2019 Nov 6;8(5):414-421. doi: 10.1093/jpids/piy077. PMID: 30184153.

- Hauck FR, Herman SM, Donovan M, Iyasu S, Merrick Moore C, Donoghue E, Kirschner RH, Willinger M. Sleep environment and the risk of sudden infant death syndrome in an urban population: the Chicago Infant Mortality Study. Pediatrics. 2003 May;111(5 Pt 2):1207-14. PMID: 12728140.

- Hauck FR, Moore CM, Herman SM, Donovan M, Kalelkar M, Christoffel KK, Hoffman HJ, Rowley D. The contribution of prone sleeping position to the racial disparity in sudden infant death syndrome: the Chicago Infant Mortality Study. Pediatrics. 2002 Oct;110(4):772-80. doi: 10.1542/peds.110.4.772. PMID: 12359794.

- Hauck FR, Omojokun OO, Siadaty MS. Do pacifiers reduce the risk of sudden infant death syndrome? A meta-analysis. Pediatrics. 2005 Nov;116(5): e716-23. doi: 10.1542/peds.2004-2631. Epub 2005 Oct 10. PMID: 16216900.

- Hauck FR, Tanabe KO. International trends in sudden infant death syndrome and other sudden unexpected deaths in infancy: need for better diagnostic standardization. Curr Pediatr Rev, 2010 Feb 1;6(1):95-101. doi: 10.2174/157339610791317241

- Hauck FR, Tanabe KO. International trends in sudden infant death syndrome: stabilization of rates requires further action. Pediatrics. 2008 Sep;122(3):660-6. doi: 10.1542/peds.2007-0135. PMID: 18762537.

- Hauck FR. Changing epidemiology. Sudden Infant Death Syndrome: Problems, Progress and Possibilities. Edited by: Byard RWand Krous HF. 2001, London: Arnold Press, 31-57.

- Hauther KA, Cobaugh KL, Jantz LM, Sparer TE, DeBruyn JM. Estimating Time Since Death from Postmortem Human Gut Microbial Communities. J Forensic Sci. 2015 Sep;60(5):1234-40. doi: 10.1111/1556-4029.12828. Epub 2015 Jun 21. PMID: 26096156.

- Heathfield LJ, Martin LJ, Ramesar R. A 5-year retrospective analysis of infant death at Salt River Mortuary, Cape Town. S Afr j child health. 2020 Sep;14(3):148-154. doi: 10.7196/SAJCH. 2020.v14i3.1720.

- Hebbelstrup Jensen B, Olsen KE, Struve C, Krogfelt KA, Petersen AM. Epidemiology and clinical manifestations of enteroaggregative Escherichia coli. Clin Microbiol Rev. 2014 Jul;27(3):614-30. doi: 10.1128/CMR.00112-13. PMID: 24982324; PMCID: PMC4135892.

- Hedberg CW, Savarino SJ, Besser JM, Paulus CJ, Thelen VM, Myers LJ, Cameron DN, Barrett TJ, Kaper JB, Osterholm MT. An outbreak of foodborne illness caused by Escherichia coli O39: NM, an agent not fitting into the existing scheme for classifying diarrheogenic *E. coli*. J Infect Dis. 1997 Dec;176(6):1625-8. doi: 10.1086/517342. PMID: 9395379.

- Heikkilä MP, Saris PE. Inhibition of Staphylococcus aureus by the commensal bacteria of human milk. J Appl Microbiol. 2003;95(3):471-8. doi: 10.1046/j.1365-2672.2003.02002. x. PMID: 12911694.

- Heimesaat MM, Boelke S, Fischer A, Haag LM, Loddenkemper C, Kühl AA, Göbel UB, Bereswill S. Comprehensive postmortem analyses of intestinal microbiota changes and bacterial translocation in human flora associated mice. PLoS One. 2012;7(7): e40758. doi: 10.1371/journal.pone.0040758. Epub 2012 Jul 12. PMID: 22808253; PMCID: PMC3395637.

- Hemming M, Räsänen S, Huhti L, Paloniemi M, Salminen M, Vesikari T. Major reduction of rotavirus, but not norovirus, gastroenteritis in children seen in hospital after the introduction of RotaTeq vaccine into the National Immunization Programme in Finland. Eur J Pediatr. 2013 Jun;172(6):739-46. doi: 10.1007/s00431-013-1945-3. Epub 2013 Jan 30. PMID: 23361964; PMCID: PMC7086648.

- Herbert A, Gerry NP, McQueen MB, Heid IM, Pfeufer A, Illig T, Wichmann HE, Meitinger T, Hunter D, Hu FB, Colditz G, Hinney A, Hebebrand J, Koberwitz K, Zhu X, Cooper R, Ardlie K, Lyon H, Hirschhorn JN, Laird NM, Lenburg ME, Lange C, Christman MF. A common genetic variant is associated with adult and childhood obesity. Science. 2006 Apr 14;312(5771):279-83. doi: 10.1126/science.1124779. PMID: 16614226.

- Hernandes RT, Elias WP, Vieira MA, Gomes TA. An overview of atypical enteropathogenic Escherichia coli. FEMS Microbiol Lett. 2009 Aug;297(2):137-49. doi: 10.1111/j.1574-6968.2009.01664. x. Epub 2009 May 27. PMID: 19527295.

- Heron M, Hoyert DL, Murphy SL, Xu J, Kochanek KD, Tejada-Vera B. Deaths: final data for 2006. Natl Vital Stat Rep. 2009 Apr 17;57(14):1-134. PMID: 19788058.

- Hespell RB, Akin DE, Dehority BA, Mackie R, White B, Isaacson R. Bacteria, fungi, and protozoa of the rumen. Gastrointestinal microbiology. Vol 2. Edited by: Mackie RI, White BA, Isaacson RE. 1997, New York: Chapman and Hall, 59–141.

- Hesselmar B, Sjöberg F, Saalman R, Aberg N, Adlerberth I, Wold AE. Pacifier cleaning practices and risk of allergy development. Pediatrics. 2013 Jun;131(6): e1829-37. doi: 10.1542/peds.2012-3345. Epub 2013 May 6. PMID: 23650304.

- Highet AR, Berry AM, Bettelheim KA, Goldwater PN. Gut microbiome in sudden infant death syndrome (SIDS) differs from that in healthy comparison babies and offers an explanation for the risk factor of prone position. Int J Med Microbiol. 2014 Jul;304(5-6):735-41. doi: 10.1016/j.ijmm.2014.05.007. Epub 2014 May 29. PMID: 24951305.

- Highet AR, Goldwater PN. Staphylococcal enterotoxin genes are common in Staphylococcus aureus intestinal flora in Sudden Infant Death Syndrome (SIDS) and live comparison infants. FEMS Immunol Med Microbiol. 2009 Nov;57(2):151-5. doi: 10.1111/j.1574-695X.2009.00592.x. Epub 2009 Aug 3. PMID: 19702876.

- Highet AR. An infectious aetiology of sudden infant death syndrome. J Appl Microbiol. 2008 Sep;105(3):625-35. doi: 10.1111/j.1365-2672.2008.03747.x. Epub 2008 Feb 6. PMID: 18266695.

- Hoa Tran TN, Trainor E, Nakagomi T, Cunliffe NA, Nakagomi O. Molecular epidemiology of noroviruses associated with acute sporadic gastroenteritis in children: global distribution of genogroups, genotypes and GII.4 variants. J Clin Virol. 2013 Mar;56(3):185-93. doi: 10.1016/j.jcv.2012.11.011. Epub 2012 Dec 5. PMID: 23218993.

- Hochwald C, Kivela L. Rotavirus vaccine, live, oral, tetravalent (RotaShield). Pediatr Nurs. 1999 Mar-Apr;25(2):203-4, 207. PMID: 10532018.

- Hodkinson BP, Grice EA. Next-Generation Sequencing: A Review of Technologies and Tools for Wound Microbiome Research. Adv Wound Care (New Rochelle). 2015 Jan 1;4(1):50-58. doi: 10.1089/wound.2014.0542. PMID: 25566414; PMCID: PMC4281878.

- Hoffman HJ, Damus K, Hillman L, Krongrad E. Risk factors for SIDS: Results of the National Institute of Child Health and Human Development SIDS cooperative epidemiological study. Ann N Y Acad Sci. 1988; 533:13-30. doi: 10.1111/j.1749-6632. 1988.tb37230. x.

- Hoffman HJ, Hillman LS. Epidemiology of the sudden infant death syndrome: maternal, neonatal, and postneonatal risk factors. Clin Perinatol. 1992 Dec;19(4):717-37. PMID: 1464187.

- Högberg U, Bergström E. Suffocated prone: the iatrogenic tragedy of SIDS. Am J Public Health. 2000 Apr;90(4):527-31. doi: 10.2105/ajph.90.4.527. PMID: 10754964; PMCID: PMC1446204.

- Honda K, Takeda K. Regulatory mechanisms of immune responses to intestinal bacteria. Mucosal Immunol. 2009 May;2(3):187-96. doi: 10.1038/mi.2009.8. Epub 2009 Mar 4. PMID: 19262502.

- Horne RS, Ferens D, Watts AM, Vitkovic J, Lacey B, Andrew S, Cranage SM, Chau B, Adamson TM. The prone sleeping position impairs arousability in term infants. J Pediatr. 2001 Jun;138(6):811-6. doi: 10.1067/mpd.2001.114475. PMID: 11391321.

- Horne RS, Franco P, Adamson TM, Groswasser J, Kahn A. Influences of maternal cigarette smoking on infant arousability. Early Hum Dev. 2004 Aug;79(1):49-58. doi: 10.1016/j.earlhumdev.2004.04.005. PMID: 15282122.

- Horne RS. Effects of prematurity on heart rate control: implications for sudden infant death syndrome. Expert Rev Cardiovasc Ther. 2006 May;4(3):335-43. doi: 10.1586/14779072.4.3.335. PMID: 16716094.

- Horne RSC. Sudden infant death syndrome: current perspectives. Intern Med J. 2019 Apr;49(4):433-438. doi: 10.1111/imj.14248. PMID: 30957377.

- Hoyert DL, Arias E, Smith BL, Murphy SL, Kochanek KD. Deaths: final data for 1999. Natl Vital Stat Rep. 2001 Sep 21;49(8):1-113. PMID: 11591077.

- Hu J, Nomura Y, Bashir A, Fernandez-Hernandez H, Itzkowitz S, Pei Z, Stone J, Loudon H, Peter I. Diversified microbiota of meconium is affected by maternal diabetes status. PLoS One. 2013 Nov 6;8(11): e78257. doi: 10.1371/journal.pone.0078257. PMID: 24223144; PMCID: PMC3819383.

- Hu J, Torres AG. Enteropathogenic Escherichia coli: foe or innocent bystander? Clin Microbiol Infect. 2015 Aug;21(8):729-34. doi: 10.1016/j.cmi.2015.01.015. Epub 2015 Jan 28. PMID: 25726041; PMCID: PMC4497942.

- Huang DB, Mohanty A, DuPont HL, Okhuysen PC, Chiang T. A review of an emerging enteric pathogen: enteroaggregative Escherichia coli. J Med Microbiol. 2006 Oct;55(Pt 10):1303-1311. doi: 10.1099/jmm.0.46674-0. Erratum in: J Med Microbiol. 2007 Jan;56(Pt 1):142. PMID: 17005776.

- Huang Y, Hauck FR, Signore C, Yu A, Raju TN, Huang TT, Fein SB. Influence of bedsharing activity on breastfeeding duration among US mothers. JAMA Pediatr. 2013 Nov;167(11):1038-44. doi: 10.1001/jamapediatrics.2013.2632. PMID: 24061708.

- Huffnagle GB, Noverr MC. The emerging world of the fungal microbiome. Trends Microbiol. 2013 Jul;21(7):334-41. doi: 10.1016/j.tim.2013.04.002. Epub 2013 May 17. PMID: 23685069; PMCID: PMC3708484.

- Huh JW, Kim WH, Moon SG, Lee JB, Lim YH. Viral etiology and incidence associated with acute gastroenteritis in a 5-year survey in Gyeonggi province, South Korea. J Clin Virol. 2009 Feb;44(2):152-6. doi: 10.1016/j.jcv.2008.11.016. Epub 2009 Jan 7. PMID: 19131274.

- Hunt CE, Hauck FR. Sudden infant death syndrome. CMAJ. 2006 Jun 20;174(13):1861-9. doi: 10.1503/cmaj.051671. PMID: 16785462; PMCID: PMC1475900.

- Hurtado JC, Quintó L, Castillo P, Carrilho C, Fernandes F, Jordao D, Lovane L, Navarro M, Casas I, Bene R, Nhampossa T, Santos Ritchie P, Bandeira S, Sambo C, Chicamba V, Mocumbi S, Jaze Z, Mabota F, Ismail MR, Lorenzoni C, Guisseve A, Rakislova N, Marimon L, Castrejon N, Sanz A, Cossa A, Mandomando I, Munguambe K, Maixenchs M, Muñoz-Almagro C, Macete E, Alonso P, Vila J, Bassat Q, Menéndez C, Martínez MJ, Ordi J. Postmortem Interval and Diagnostic Performance of the Autopsy Methods. Sci Rep. 2018 Oct 31;8(1):16112. doi: 10.1038/s41598-018-34436-1. PMID: 30382145; PMCID: PMC6208334.

- Hwang BM, Jung S, Jeong HJ, Chung GT, Kang YH, Yang SJ, Seo NY, Shin TH, Yoo CK, Lee DY. Outbreak of astrovirus in adults with acute gastroenteritis in Korea. J. Gastrointest. Dig. Syst. 2015; 13:1-4. doi:10.4172/2161-069X.1000S13-004

- Iijima Y, Oundo JO, Hibino T, Saidi SM, Hinenoya A, Osawa K, Shirakawa T, Osawa R, Yamasaki S. High Prevalence of Diarrheagenic Escherichia coli among Children with Diarrhea in Kenya. Jpn J Infect Dis. 2017 Jan 24;70(1):80-83. doi: 10.7883/yoken.JJID.2016.064. Epub 2016 May 9. PMID: 27169953.

- Iizuka S, Oka T, Tabara K, Omura T, Katayama K, Takeda N, Noda M. Detection of sapoviruses and noroviruses in an outbreak of gastroenteritis linked genetically to shellfish. J Med Virol. 2010 Jul;82(7):1247-54. doi: 10.1002/jmv.21791. PMID: 20513092.

- Institute for Health Metrics and Evaluation (IHME). GBD 2019 Cause and Risk Summary: Sudden infant death syndrome - Level 3 cause. 2020, Seattle: IHME, University of Washington. Available from: https://www.healthdata.org/results/gbd_summaries/2019/sudden-infant-death-syndrome-level-3-cause. [Date accessed: 17 February 2022].

- International Committee on Taxonomy of Viruses (ICTV). [Website] Updated in July 2021. Available from: https://ictv.global/report/chapter/sedoreoviridae/sedoreoviridae/rotavirus\. [Date accessed on 1 March 2023].

- International Committee on Taxonomy of Viruses (ICTV). Virus Taxonomy: 2019 Release. 2019. Available from: https://talk.ictvonline.org/taxonomy/. [Date accessed:25 February 2022].

- Ison MG. Adenovirus infections in transplant recipients. Clin Infect Dis. 2006 Aug 1;43(3):331-9. doi: 10.1086/505498. Epub 2006 Jun 19. PMID: 16804849.

- Itoh Y, Nagano I, Kunishima M, Ezaki T. Laboratory investigation of enteroaggregative Escherichia coli O untypeable:H10 associated with a massive outbreak of gastrointestinal illness. J Clin Microbiol. 1997 Oct;35(10):2546-50. doi: 10.1128/jcm.35.10.2546-2550.1997. PMID: 9316905; PMCID: PMC230008.

- Iturriza-Gómara M, Jere KC, Hungerford D, Bar-Zeev N, Shioda K, Kanjerwa O, Houpt ER, Operario DJ, Wachepa R, Pollock L, Bennett A, Pitzer VE, Cunliffe NA. Etiology of Diarrhea Among Hospitalized Children in Blantyre, Malawi, Following Rotavirus Vaccine Introduction: A Case-Control Study. J Infect Dis. 2019 Jun 19;220(2):213-218. doi: 10.1093/infdis/jiz084. PMID: 30816414; PMCID: PMC6581894.

- Iturriza-Gómara M, Kang G, Gray J. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. J Clin Virol. 2004 Dec;31(4):259-65. doi: 10.1016/j.jcv.2004.04.009. PMID: 15494266.

- Iyaloo S, Mapuroma F, Seheri M, Peenze I, Kruger T, Walaza S, Cohen C, Page N. Rotavirus surveillance in South Africa, 2012. Commun Dis Surveill Bull. 2013;11:37-41.

- Jaakkonen A, Salmenlinna S, Rimhanen-Finne R, Lundström H, Heinikainen S, Hakkinen M, Hallanvuo S. Severe Outbreak of Sorbitol-Fermenting Escherichia coli O157 via Unpasteurized Milk and Farm Visits, Finland 2012. Zoonoses Public Health. 2017 Sep;64(6):468-475. doi: 10.1111/zph.12327. Epub 2017 Jan 3. PMID: 28045227.

- Jafari F, Shokrzadeh L, Hamidian M, Salmanzadeh-Ahrabi S, Zali MR. Acute diarrhea due to enteropathogenic bacteria in patients at hospitals in Tehran. Jpn J Infect Dis. 2008 Jul;61(4):269-73. PMID: 18653967.

- Jain N, Walker WA. Diet and host-microbial crosstalk in postnatal intestinal immune homeostasis. Nat Rev Gastroenterol Hepatol. 2015 Jan;12(1):14-25. doi: 10.1038/nrgastro.2014.153. Epub 2014 Sep 9. PMID: 25201040.

- Jakab F, Péterfai J, Meleg E, Bányai K, Mitchell DK, Szucs G. Comparison of clinical characteristics between astrovirus and rotavirus infections diagnosed in 1997 to 2002 in Hungary. Acta Paediatr. 2005 Jun;94(6):667-71. doi: 10.1111/j.1651-2227. 2005.tb01962. x. PMID: 16188766.

- Jakeman KJ, Rushton DI, Smith H, Sweet C. Exacerbation of bacterial toxicity to infant ferrets by influenza virus: possible role in sudden infant death syndrome. J Infect Dis. 1991 Jan;163(1):35-40. doi: 10.1093/infdis/163.1.35. Erratum in: J Infect Dis 1991 Jul;164(1):232. PMID: 1984474.

- Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, Björkstén B, Engstrand L, Andersson AF. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. Gut. 2014 Apr;63(4):559-66. doi: 10.1136/gutjnl-2012-303249. Epub 2013 Aug 7. PMID: 23926244.

- Janda JM, Abbott SL. The Changing Face of the Family Enterobacteriaceae (Order: "Enterobacterales"): New Members, Taxonomic Issues, Geographic Expansion, and New Diseases and Disease Syndromes. Clin Microbiol Rev. 2021 Feb 24;34(2):e00174-20. doi: 10.1128/CMR.00174-20. PMID: 33627443; PMCID: PMC8262773.

- Jarchow-Macdonald AA, Halley S, Chandler D, Gunson R, Shepherd SJ, Parcell BJ. First report of an astrovirus type 5 gastroenteritis outbreak in a residential elderly care home identified by sequencing. J Clin Virol. 2015 Dec; 73:115-119. doi: 10.1016/j.jcv.2015.11.006. Epub 2015 Nov 5. PMID: 26590691.

- Jašarević E, Howard CD, Misic AM, Beiting DP, Bale TL. Stress during pregnancy alters temporal and spatial dynamics of the maternal and offspring microbiome in a sex-specific manner. Sci Rep. 2017 Mar 7; 7:44182. doi: 10.1038/srep44182. PMID: 28266645; PMCID: PMC5339804.

- Jenkins C, Chart H, Willshaw GA, Cheasty T, Smith HR. Genotyping of enteroaggregative Escherichia coli and identification of target genes for the detection of both typical and atypical strains. Diagn Microbiol Infect Dis. 2006 May;55(1):13-9. doi: 10.1016/j.diagmicrobio.2005.10.019. Epub 2006 Feb 23. PMID: 16500068.

- Jenkins C, Chart H, Willshaw GA, Cheasty T, Tompkins DS. Association of putative pathogenicity genes with adherence characteristics and fimbrial genotypes in typical enteroaggregative Escherichia coli from patients with and without diarrhoea in the United Kingdom. Eur J Clin Microbiol Infect Dis. 2007 Dec;26(12):901-6. doi: 10.1007/s10096-007-0388-z. PMID: 17899229.

- Jenkins C. Enteroaggregative Escherichia coli. Curr Top Microbiol Immunol. 2018; 416:27-50. doi: 10.1007/82_2018_105. PMID: 30232602.

- Jenkins RO, Sherburn RE. Used cot mattresses as potential reservoirs of bacterial infection: nutrient availability within polyurethane foam. J Appl Microbiol. 2008 Feb;104(2):526-33. doi: 10.1111/j.1365-2672.2007.03609. x. Epub 2007 Nov 26. PMID: 18042213.

- Jenkins TA, Nguyen JC, Polglaze KE, Bertrand PP. Influence of Tryptophan and Serotonin on Mood and Cognition with a Possible Role of the Gut-Brain Axis. Nutrients. 2016 Jan 20;8(1):56. doi: 10.3390/nu8010056. PMID: 26805875; PMCID: PMC4728667.

- Jere KC, Mlera L, O'Neill HG, Peenze I, van Dijk AA. Whole genome sequence analyses of three African bovine rotaviruses reveal that they emerged through multiple reassortment events between rotaviruses from different mammalian species. Vet Microbiol. 2012 Sep 14;159(1-2):245-50. doi: 10.1016/j.vetmic.2012.03.040. Epub 2012 Apr 6. PMID: 22541163.

- Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J. 2007 May;1(1):56-66. doi: 10.1038/ismej.2007.3. Erratum in: ISME J. 2013 Feb;7(2):456. PMID: 18043614.

- Jesser KJ, Levy K. Updates on defining and detecting diarrheagenic Escherichia coli pathotypes. Curr Opin Infect Dis. 2020 Oct;33(5):372-380. doi: 10.1097/QCO.0000000000000665. PMID: 32773499; PMCID: PMC7819864.

- Jiang X, Wang M, Wang K, Estes MK. Sequence and genomic organization of Norwalk virus. Virology. 1993 Jul;195(1):51-61. doi: 10.1006/viro.1993.1345. PMID: 8391187.

- Jiang ZD, Lowe B, Verenkar MP, Ashley D, Steffen R, Tornieporth N, von Sonnenburg F, Waiyaki P, DuPont HL. Prevalence of enteric pathogens among international travelers with diarrhea acquired in Kenya (Mombasa), India (Goa), or Jamaica (Montego Bay). J Infect Dis. 2002 Feb 15;185(4):497-502. doi: 10.1086/338834. Epub 2002 Jan 22. PMID: 11865402.

- Jiménez E, Fernández L, Marín ML, Martín R, Odriozola JM, Nueno-Palop C, Narbad A, Olivares M, Xaus J, Rodríguez JM. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol. 2005 Oct;51(4):270-4. doi: 10.1007/s00284-005-0020-3. Epub 2005 Sep 20. PMID: 16187156.

- Jiménez E, Marín ML, Martín R, Odriozola JM, Olivares M, Xaus J, Fernández L, Rodríguez JM. Is meconium from healthy newborns actually sterile? Res Microbiol. 2008 Apr;159(3):187-93. doi: 10.1016/j.resmic.2007.12.007. Epub 2008 Jan 11. PMID: 18281199.

- Johne R, Tausch SH, Grützke J, Falkenhagen A, Patzina-Mehling C, Beer M, Höper D, Ulrich RG. Distantly Related Rotaviruses in Common Shrews, Germany, 2004-2014. Emerg Infect Dis. 2019 Dec;25(12):2310-2314. doi: 10.3201/eid2512.191225. PMID: 31742508; PMCID: PMC6874240.

- Jørgensen T, Biering-Sørensen F, Hilden J. Sudden infant death in Copenhagen 1956--1971. III. Perinatal and perimortal factors. Acta Paediatr Scand. 1979 Jan;68(1):11-22. doi: 10.1111/j.1651-2227. 1979.tb04423. x. PMID: 758724

- Jost T, Lacroix C, Braegger CP, Chassard C. New insights in gut microbiota establishment in healthy breast fed neonates. PLoS One. 2012;7(8): e44595. doi: 10.1371/journal.pone.0044595. Epub 2012 Aug 30. PMID: 22957008; PMCID: PMC3431319.

- Jung B, Hoilat GJ. MacConkey Medium. [Updated 2022 Sep 26]. Edited by: StatPearls [Website]. 2022, Treasure Island: StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557394/

- Kabue JP, Meader E, Hunter PR, Potgieter N. Human Norovirus prevalence in Africa: a review of studies from 1990 to 2013. Trop Med Int Health. 2016 Jan;21(1):2-17. doi: 10.1111/tmi.12617. Epub 2015 Nov 9. PMID: 26436862.

- Kaneko M, Takanashi S, Thongprachum A, Hanaoka N, Fujimoto T, Nagasawa K, Kimura H, Okitsu S, Mizuguchi M, Ushijima H. Identification of vaccine-derived rotavirus strains in children with acute gastroenteritis in Japan, 2012-2015. PLoS One. 2017 Sep 13;12(9):e0184067. doi: 10.1371/journal.pone.0184067. PMID: 28902863; PMCID: PMC5597190.

- Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nat Rev Microbiol. 2004 Feb;2(2):123-40. doi: 10.1038/nrmicro818. PMID: 15040260.

- Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol. 1972 Nov;10(5):1075-81. doi: 10.1128/JVI.10.5.1075-1081.1972. PMID: 4117963; PMCID: PMC356579.

- Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, Nielsen J, Bäckhed F. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature. 2013 Jun 6;498(7452):99-103. doi: 10.1038/nature12198. Epub 2013 May 29. PMID: 23719380.

- Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing Escherichia coli in stools. Lancet. 1983 Mar 19;1(8325):619-20. doi: 10.1016/s0140-6736(83)91795-6. PMID: 6131302.

- Kattwinkel J, Brooks J, Myerberg D. American Academy of Pediatrics AAP Task Force on infant positioning and SIDS: positioning and SIDS. Pediatrics. 1992 Jun;89(6 Pt 1):1120-6. PMID: 1503575.

- Kattwinkel, J. & Brooks, J.G. & Keenan, M.E. & Malloy, M. & Willinger, M. & Scheers, N (2000). Changing concepts of sudden infant death syndrome: implications for infant sleeping environment and sleep position. Pediatrics. 2000 Mar;105(3 Pt 1):650-6. doi: 10.1542/peds.105.3.650. PMID: 10699127.

- Keddy KH, Smith AM, Page NA. GEMS extend understanding of childhood diarrhoea. Lancet. 2016 Sep 24;388(10051):1252-4. doi: 10.1016/S0140-6736(16)31664-6. PMID: 27673454.

- Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Kynurenine pathway metabolism and the microbiota-gut-brain axis. Neuropharmacology. 2017 Jan;112(Pt B):399-412. doi: 10.1016/j.neuropharm.2016.07.002. Epub 2016 Jul 5. PMID: 27392632.

- Kenyon S, Pike K, Jones DR, Brocklehurst P, Marlow N, Salt A, Taylor DJ. Childhood outcomes after prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of the ORACLE II trial. Lancet. 2008 Oct 11;372(9646):1319-27. doi: 10.1016/S0140-6736(08)61203-9. Epub 2008 Sep 17. PMID: 18804276.

- Khalil IA, Troeger C, Blacker BF, Rao PC, Brown A, Atherly DE, Brewer TG, Engmann CM, Houpt ER, Kang G, Kotloff KL, Levine MM, Luby SP, MacLennan CA, Pan WK, Pavlinac PB, Platts-Mills JA, Qadri F, Riddle MS, Ryan ET, Shoultz DA, Steele AD, Walson JL, Sanders JW, Mokdad AH, Murray CJL, Hay SI, Reiner RC Jr.

Morbidity and mortality due to shigella and enterotoxigenic Escherichia coli diarrhoea: the Global Burden of Disease Study 1990-2016. Lancet Infect Dis. 2018 Nov;18(11):1229-1240. doi: 10.1016/S1473-3099(18)30475-4. Epub 2018 Sep 25. Erratum in: Lancet Infect Dis. 2018 Oct 30; PMID: 30266330; PMCID: PMC6202441.

- Khanal S, Ghimire P, Dhamoon AS. The Repertoire of Adenovirus in Human Disease: The Innocuous to the Deadly. Biomedicines. 2018 Mar 7;6(1):30. doi: 10.3390/biomedicines6010030. PMID: 29518985; PMCID: PMC5874687.

- Khandoker N, Thongprachum A, Takanashi S, Okitsu S, Nishimura S, Kikuta H, Yamamoto A, Sugita K, Baba T, Kobayashi M, Hayakawa S, Mizuguchi M, Ushijima H. Molecular epidemiology of rotavirus gastroenteritis in Japan during 2014-2015: Characterization of re-emerging G2P[4] after rotavirus vaccine introduction. J Med Virol. 2018 Jun;90(6):1040-1046. doi: 10.1002/jmv.25067. Epub 2018 Mar 13. PMID: 29488230.

- Kim S, Kim H, Yim YS, Ha S, Atarashi K, Tan TG, Longman RS, Honda K, Littman DR, Choi GB, Huh JR. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. Nature. 2017 Sep 28;549(7673):528-532. doi: 10.1038/nature23910. Epub 2017 Sep 13. PMID: 28902840; PMCID: PMC5870873.

- Kinney HC, Thach BT. The sudden infant death syndrome. N Engl J Med. 2009 Aug 20;361(8):795-805. doi: 10.1056/NEJMra0803836. PMID: 19692691; PMCID: PMC3268262.

- Kipnis J. Multifaceted interactions between adaptive immunity and the central nervous system. Science. 2016 Aug 19;353(6301):766-71. doi: 10.1126/science. aag2638. PMID: 27540163; PMCID: PMC5590839.

- Kirkwood CD. Genetic and antigenic diversity of human rotaviruses: potential impact on vaccination programs. J Infect Dis. 2010 Sep 1;202 Suppl:S43-8. doi: 10.1086/653548. PMID: 20684716.

- Kitajima M, Haramoto E, Phanuwan C, Katayama H. Genotype distribution of human sapoviruses in wastewater in Japan. Appl Environ Microbiol. 2011 Jun;77(12):4226-9. doi: 10.1128/AEM.00088-11. Epub 2011 Apr 22. PMID: 21515720; PMCID: PMC3131659.

- Kiulia NM, Mwenda JM, Nyachieo A, Nyaundi JK, Steele AD, Taylor MB. Astrovirus infection in young Kenyan children with diarrhoea. J Trop Pediatr. 2007 Jun;53(3):206-9. doi: 10.1093/tropej/fml093. Epub 2007 Feb 17. PMID: 17308325.

- Knights D, Silverberg MS, Weersma RK, Gevers D, Dijkstra G, Huang H, Tyler AD, van Sommeren S, Imhann F, Stempak JM, Huang H, Vangay P, Al-Ghalith GA, Russell C, Sauk J, Knight J, Daly MJ, Huttenhower C, Xavier RJ. Complex host genetics influence the microbiome in inflammatory bowel disease. Genome Med. 2014 Dec 2;6(12):107. doi: 10.1186/s13073-014-0107-1. PMID: 25587358; PMCID: PMC4292994.

- Knobel GJ, Flash G, Bowie GF. Lodox Statscan proves to be invaluable in forensic medicine. S Afr Med J. 2006 Jul;96(7):593-4, 596. PMID: 16909178.

- Kobayashi S, Fujiwara N, Yasui Y, Yamashita T, Hiramatsu R, Minagawa H. A foodborne outbreak of sapovirus linked to catered box lunches in Japan. Arch Virol. 2012 Oct;157(10):1995-7. doi: 10.1007/s00705-012-1394-8. Epub 2012 Jun 30. PMID: 22752792.

- Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A. 2011 Mar 15;108 Suppl 1(Suppl 1):4578-85. doi: 10.1073/pnas.1000081107. Epub 2010 Jul 28. PMID: 20668239; PMCID: PMC3063592.

- Kongmuang U, Honda T, Miwatani T. Enzyme-linked immunosorbent assay to detect Shiga toxin of Shigella dysenteriae and related toxins. J Clin Microbiol. 1987 Jan;25(1):115-8. doi: 10.1128/jcm.25.1.115-118.1987. PMID: 3539985; PMCID: PMC265836.

- Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of Escherichia coli. Infect Immun. 1977 Dec;18(3):775-9. doi: 10.1128/iai.18.3.775-779.1977. PMID: 338490; PMCID: PMC421302.

- Koo HL, Neill FH, Estes MK, Munoz FM, Cameron A, DuPont HL, Atmar RL. Noroviruses: The Most Common Pediatric Viral Enteric Pathogen at a Large University Hospital After Introduction of Rotavirus Vaccination. J Pediatric Infect Dis Soc. 2013 Mar;2(1):57-60. doi: 10.1093/jpids/pis070. Epub 2012 Aug 3. PMID: 23687584; PMCID: PMC3656546.

- Korpela K, de Vos WM. Early life colonization of the human gut: microbes matter everywhere. Curr Opin Microbiol. 2018 Aug; 44:70-78. doi: 10.1016/j.mib.2018.06.003. Epub 2018 Aug 4. PMID: 30086431.

- Korpela K, Helve O, Kolho KL, Saisto T, Skogberg K, Dikareva E, Stefanovic V, Salonen A, Andersson S, de Vos WM. Maternal Fecal Microbiota Transplantation in Cesarean-Born Infants Rapidly Restores Normal Gut Microbial Development: A Proof-of-Concept Study. Cell. 2020 Oct 15;183(2):324-334.e5. doi: 10.1016/j.cell.2020.08.047. Epub 2020 Oct 1. PMID: 33007265.

- Korpela K, Salonen A, Virta LJ, Kekkonen RA, Forslund K, Bork P, de Vos WM. Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. Nat Commun. 2016 Jan 26; 7:10410. doi: 10.1038/ncomms10410. PMID: 26811868; PMCID: PMC4737757.

- Kotloff KL, Losonsky GA, Morris JG Jr, Wasserman SS, Singh-Naz N, Levine MM. Enteric adenovirus infection and childhood diarrhea: an epidemiologic study in three clinical settings. Pediatrics. 1989 Aug;84(2):219-25. PMID: 2546121.

- Kotloff KL, Nasrin D, Blackwelder WC, Wu Y, Farag T, Panchalingham S, Sow SO, Sur D, Zaidi AKM, Faruque ASG, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ahmed S, Qureshi S, Quadri F, Hossain A, Das SK, Antonio M, Hossain MJ, Mandomando I, Acácio S, Biswas K, Tennant SM, Verweij JJ, Sommerfelt H, Nataro JP, Robins-Browne RM, Levine MM. The incidence, aetiology, and adverse clinical consequences of less severe diarrhoeal episodes among infants and children residing in low-income and middle-income countries: a 12-month case-control study as a follow-on to the Global Enteric Multicenter Study (GEMS). Lancet Glob Health. 2019 May;7(5): e568-e584. doi: 10.1016/S2214-109X (19)30076-2. PMID: 31000128; PMCID: PMC6484777.

- Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola

RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 2013 Jul 20;382(9888):209-22. doi: 10.1016/S0140-6736(13)60844-2. Epub 2013 May 14. PMID: 23680352.

- Kotloff KL. The Burden and Etiology of Diarrheal Illness in Developing Countries. Pediatr Clin North Am. 2017 Aug;64(4):799-814. doi: 10.1016/j.pcl.2017.03.006. PMID: 28734511.

- Kotloff KL. The Burden and Etiology of Diarrheal Illness in Developing Countries. Pediatr Clin North Am. 2017 Aug;64(4):799-814. doi: 10.1016/j.pcl.2017.03.006. PMID: 28734511.

- Krause G, Frank C, Gilsdorf A, Mielke M, Schaade L, Stark K, Burger R. The 2011 HUS epidemic in Germany: Challenges for disease control: what should be improved?. Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz. 2013 Jan;56:56-66. Doi:10.1007/s00103-012-1585-1

- Krous HF, Beckwith JB, Byard RW, Rognum TO, Bajanowski T, Corey T, Cutz E, Hanzlick R, Keens TG, Mitchell EA. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. Pediatrics. 2004 Jul;114(1):234-8. doi: 10.1542/peds.114.1.234. PMID: 15231934.

- Krous HF, Ferandos C, Masoumi H, Arnold J, Haas EA, Stanley C, Grossfeld PD. Myocardial inflammation, cellular death, and viral detection in sudden infant death caused by SIDS, suffocation, or myocarditis. Pediatr Res. 2009 Jul;66(1):17-21. doi: 10.1203/PDR.0b013e3181a290a7. PMID: 19287341.

- Kubitschek HE. Cell volume increase in Escherichia coli after shifts to richer media. J Bacteriol. 1990 Jan;172(1):94-101. doi: 10.1128/jb.172.1.94-101.1990. PMID: 2403552; PMCID: PMC208405.

- Kumthip K, Khamrin P, Ushijima H, Maneekarn N. Enteric and non-enteric adenoviruses associated with acute gastroenteritis in pediatric patients in Thailand, 2011 to 2017. PLoS One. 2019 Aug 1;14(8): e0220263. doi: 10.1371/journal.pone.0220263. PMID: 31369615; PMCID: PMC6675392.

- La Rosa G, Della Libera S, Petricca S, Iaconelli M, Donia D, Saccucci P, Cenko F, Xhelilaj G, Divizia M. Genetic Diversity of Human Adenovirus in Children with Acute Gastroenteritis, Albania, 2013-2015. Biomed Res Int. 2015; 2015:142912. doi: 10.1155/2015/142912. Epub 2015 Aug 3. PMID: 26339589; PMCID: PMC4538309.

- La Rosa PS, Warner BB, Zhou Y, Weinstock GM, Sodergren E, Hall-Moore CM, Stevens HJ, Bennett WE Jr, Shaikh N, Linneman LA, Hoffmann JA, Hamvas A, Deych E, Shands BA, Shannon WD, Tarr PI. Patterned progression of bacterial populations in the premature infant gut. Proc Natl Acad Sci U S A. 2014 Aug 26;111(34):12522-7. doi: 10.1073/pnas.1409497111. Epub 2014 Aug 11. Erratum in: Proc Natl Acad Sci U S A. 2014 Dec 2;111(48):17336. PMID: 25114261; PMCID: PMC4151715.

- Lääveri T, Pakkanen SH, Kirveskari J, Kantele A. Travellers' diarrhoea: Impact of TD definition and control group design on study results. Travel Med Infect Dis. 2018 Jul-Aug; 24:37-43. doi: 10.1016/j.tmaid.2018.01.006. Epub 2018 Feb 2. PMID: 29409749.

- Ladomenou F, Moschandreas J, Kafatos A, Tselentis Y, Galanakis E. Protective effect of exclusive breastfeeding against infections during infancy: a prospective study. Arch Dis Child. 2010 Dec;95(12):1004-8. doi: 10.1136/adc.2009.169912. Epub 2010 Sep 27. PMID: 20876557.

- Lalioui L, Jouve M, Gounon P, Le Bouguenec C. Molecular cloning and characterization of the afa-7 and afa-8 gene clusters encoding afimbrial adhesins in Escherichia coli strains associated with diarrhea or septicemia in calves. Infect Immun. 1999 Oct;67(10):5048-59. doi: 10.1128/IAI.67.10.5048-5059.1999. PMID: 10496877; PMCID: PMC96852.

- Lan R, Reeves PR. Escherichia coli in disguise: molecular origins of Shigella. Microbes Infect. 2002 Sep;4(11):1125-32. doi: 10.1016/s1286-4579(02)01637-4. PMID: 12361912.

- Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci U S A. 1985 Oct;82(20):6955-9. doi: 10.1073/pnas.82.20.6955. PMID: 2413450; PMCID: PMC391288.

- Lane DJ. 16S/23S rRNA sequencing. Nucleic Acid Techniques in Bacterial Systematic. Edited by: Stackebrandt, E. and Goodfellow, M. 1991, New York: John Wiley & Sons, 115–175.

- Langendorf C, Le Hello S, Moumouni A, Gouali M, Mamaty AA, Grais RF, Weill FX, Page AL. Enteric bacterial pathogens in children with diarrhea in Niger: diversity and antimicrobial resistance. PLoS One. 2015 Mar 23;10(3): e0120275. doi: 10.1371/journal.pone.0120275. PMID: 25799400; PMCID: PMC4370739.

- Larragoite ET, Spivak AM. Down but not out. Elife. 2019 Dec 23;8:e53363. doi: 10.7554/eLife.53363. PMID: 31868581; PMCID: PMC6927739.

- Laubereau B, Filipiak-Pittroff B, von Berg A, Grübl A, Reinhardt D, Wichmann HE, Koletzko S; GINI Study Group. Caesarean section and gastrointestinal symptoms, atopic dermatitis, and sensitisation during the first year of life. Arch Dis Child. 2004 Nov;89(11):993-7. doi: 10.1136/adc.2003.043265. PMID: 15499049; PMCID: PMC1719727.

- Laursen MF, Zachariassen G, Bahl MI, Bergström A, Høst A, Michaelsen KF, Licht TR. Having older siblings is associated with gut microbiota development during early childhood. BMC Microbiol. 2015 Aug 1; 15:154. doi: 10.1186/s12866-015-0477-6. PMID: 26231752; PMCID: PMC4522135.

- Lazić G, Grubač S, Lupulović D, Bugarski D, Lazić S, Knežević P, Petrović T. Presence of Human and Animal Viruses in Surface Waters in Vojvodina Province of Serbia. Food Environ Virol. 2015 Feb 17. doi: 10.1007/s12560-015-9187-3. Epub ahead of print. PMID: 25687987.

- le Roux DM, Myer L, Nicol MP, Zar HJ. Incidence of childhood pneumonia: facility-based surveillance estimate compared to measured incidence in a South African birth cohort study. BMJ Open. 2015 Dec 18;5(12): e009111. doi: 10.1136/bmjopen-2015-009111. PMID: 26685027; PMCID: PMC4691755.

- Ledwaba SE, Kabue JP, Barnard TG, Traore AN, Potgieter N. Enteric pathogen co-infections in the paediatric population from rural communities in the Vhembe District, South Africa. South African Journal of Child Health. 2018;12(4):170-4. DOI:10.7196/SAJCH.2018.V12I4.1550.

- Lee A. Neglected niches: the microbial ecology of the gastrointestinal tract. Advances in microbial ecology. Edited by: Marshall K. 1984, New York: Plenum Press, 115–162.

- Lee J, Choi EH, Lee HJ. Comprehensive serotyping and epidemiology of human adenovirus isolated from the respiratory tract of Korean children over 17 consecutive years (1991-2007). J Med Virol. 2010 Apr;82(4):624-31. doi: 10.1002/jmv.21701. PMID: 20166176.

- Lee LE, Cebelinski EA, Fuller C, Keene WE, Smith K, Vinjé J, Besser JM. Sapovirus outbreaks in long-term care facilities, Oregon and Minnesota, USA, 2002-2009. Emerg Infect Dis. 2012 May;18(5):873-6. doi: 10.3201/eid1805.111843. PMID: 22516204; PMCID: PMC3358050.

- Lee S, Barson AJ, Drucker DB, Morris JA, Telford DR. Lethal challenge of gnotobiotic weanling rats with bacterial isolates from cases of sudden infant death syndrome (SIDS). J Clin Pathol. 1987 Dec;40(12):1393-6. doi: 10.1136/jcp.40.12.1393. PMID: 3323245; PMCID: PMC1141271.

- Lee SH, Yun JW, Lee JH, Jung YH, Lee DH. Trends in recent waterborne and foodborne disease outbreaks in South Korea, 2015-2019. Osong Public Health Res Perspect. 2021 Apr;12(2):73-79. doi: 10.24171/j.phrp.2021.12.2.04. Epub 2021 Apr 29. PMID: 33979997; PMCID: PMC8102876.

- Lekana-Douki SE, Kombila-Koumavor C, Nkoghe D, Drosten C, Drexler JF, Leroy EM. Molecular epidemiology of enteric viruses and genotyping of rotavirus A, adenovirus and astrovirus among children under 5 years old in Gabon. Int J Infect Dis. 2015 May; 34:90-5. doi: 10.1016/j.ijid.2015.03.009. Epub 2015 Mar 18. PMID: 25796432.

- Leong LEX, Taylor SL, Shivasami A, Goldwater PN, Rogers GB. Intestinal Microbiota Composition in Sudden Infant Death Syndrome and Age-Matched Controls. J Pediatr. 2017 Dec; 191:63-68. e1. doi: 10.1016/j.jpeds.2017.08.070. PMID: 29173325.

- Leotta GA, Chinen I, Epszteyn S, Miliwebsky E, Melamed IC, Motter M, Ferrer M, Marey E, Rivas M. Validación de una técnica de PCR múltiple para la detección de Escherichia coli productor de toxina Shiga [Validation of a multiplex PCR for detection of Shiga toxin-producing Escherichia coli]. Rev Argent Microbiol. 2005 Jan-Mar;37(1):1-10. Spanish. PMID: 15991473.

- Leung AK, Kellner JD, Davies HD. Rotavirus gastroenteritis. Adv Ther. 2005 Sep-Oct;22(5):476-87. doi: 10.1007/BF02849868. PMID: 16418157.

- Levi Mortera S, Del Chierico F, Vernocchi P, Rosado MM, Cavola A, Chierici M, Pieroni L, Urbani A, Carsetti R, Lante I, Dallapiccola B, Putignani L. Monitoring Perinatal Gut Microbiota in Mouse Models by Mass Spectrometry Approaches: Parental Genetic Background and Breastfeeding Effects. Front Microbiol. 2016 Sep 26; 7:1523. doi: 10.3389/fmicb.2016.01523. PMID: 27725814; PMCID: PMC5036385.

- Levine MM, Edelman R. Enteropathogenic Escherichia coli of classic serotypes associated with infant diarrhea: epidemiology and pathogenesis. Epidemiol Rev. 1984; 6:31-51. doi: 10.1093/oxfordjournals.epirev.a036274. PMID: 6386503.

- Levy J, Habib RH, Liptsen E, Singh R, Kahn D, Steele AM, Courtney SE. Prone versus supine positioning in the well preterm infant: effects on work of breathing and breathing patterns. Pediatr Pulmonol. 2006 Aug;41(8):754-8. doi: 10.1002/ppul.20435. PMID: 16779849.

- Lewak N, van den Berg BJ, Beckwith JB. Sudden infant death syndrome risk factors. Prospective data review. Clin Pediatr (Phila). 1979 Jul;18(7):404-11. doi: 10.1177/000992287901800704. PMID: 455873.

- Lewis KW, Bosque EM. Deficient hypoxia awakening response in infants of smoking mothers: possible relationship to sudden infant death syndrome. J Pediatr. 1995 Nov;127(5):691-9. doi: 10.1016/s0022-3476(95)70155-9. PMID: 7472818.

- Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006 Dec 21;444(7122):1022-3. doi: 10.1038/4441022a. PMID: 17183309.

- L'Hoir MP, Engelberts AC, van Well GT, Damsté PH, Idema NK, Westers P, Mellenbergh GJ, Wolters WH, Huber J. Dummy use, thumb sucking, mouth breathing and cot death. Eur J Pediatr. 1999 Nov;158(11):896-901. doi: 10.1007/s004310051237. PMID: 10541944.

- Li DK, Petitti DB, Willinger M, McMahon R, Odouli R, Vu H, Hoffman HJ. Infant sleeping position and the risk of sudden infant death syndrome in California, 1997-2000. Am J Epidemiol. 2003 Mar 1;157(5):446-55. doi: 10.1093/aje/kwf226. PMID: 12615609.

- Li DK, Willinger M, Petitti DB, Odouli R, Liu L, Hoffman HJ. Use of a dummy (pacifier) during sleep and risk of sudden infant death syndrome (SIDS): population based case-control study. BMJ. 2006 Jan 7;332(7532):18-22. doi: 10.1136/bmj.38671.640475.55. Epub 2005 Dec 9. PMID: 16339767; PMCID: PMC1325127.

- Li L, Deng X, Linsuwanon P, Bangsberg D, Bwana MB, Hunt P, Martin JN, Deeks SG, Delwart E. AIDS alters the commensal plasma virome. J Virol. 2013 Oct;87(19):10912-5. doi: 10.1128/JVI.01839-13. Epub 2013 Jul 31. PMID: 23903845; PMCID: PMC3807392.

- Li L, Wang F, Liu Y, Gu F. Intestinal microbiota dysbiosis in children with recurrent respiratory tract infections. Microb Pathog. 2019 Nov; 136:103709. doi: 10.1016/j.micpath.2019.103709. Epub 2019 Sep 5. PMID: 31494301.

- Light JS, Hodes HL. Studies on Epidemic Diarrhea of the New-born: Isolation of a Filtrable Agent Causing Diarrhea in Calves. Am J Public Health Nations Health. 1943 Dec;33(12):1451-4. doi: 10.2105/ajph.33.12.1451. PMID: 18015921; PMCID: PMC1527675.

- Lim ES, Zhou Y, Zhao G, Bauer IK, Droit L, Ndao IM, Warner BB, Tarr PI, Wang D, Holtz LR. Early life dynamics of the human gut virome and bacterial microbiome in infants. Nat Med. 2015 Oct;21(10):1228-34. doi: 10.1038/nm.3950. Epub 2015 Sep 14. PMID: 26366711; PMCID: PMC4710368.

- Lindsay JA, Mach AS, Wilkinson MA, Martin LM, Wallace FM, Keller AM, Wojciechowski LM. Clostridium perfringens type A cytotoxic-enterotoxin (s) as triggers for death in the sudden infant death syndrome: development of a toxico-infection hypothesis. Curr Microbiol. 1993 Jul; 27:51-9. doi.org/10.1007/BF01576834.

- Linhares AC, Pinheiro FP, Freitas RB, Gabbay YB, Shirley JA, Beards GM. An outbreak of rotavirus diarrhea among a nonimmune, isolated South American Indian community. Am J Epidemiol. 1981 Jun;113(6):703-10. doi: 10.1093/oxfordjournals.aje.a113151. PMID: 6263087.

- Linhares AC, Stupka JA, Ciapponi A, Bardach AE, Glujovsky D, Aruj PK, Mazzoni A, Rodriguez JA, Rearte A, Lanzieri TM, Ortega-Barria E, Colindres R. Burden and typing of rotavirus group A in Latin America and the Caribbean: systematic review and meta-analysis. Rev Med Virol. 2011 Mar;21(2):89-109. doi: 10.1002/rmv.682. Epub 2011 Mar 7. PMID: 21384462.

- Liste MB, Natera I, Suarez JA, Pujol FH, Liprandi F, Ludert JE. Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children. J Clin Microbiol. 2000 Aug;38(8):2873-7. doi: 10.1128/JCM.38.8.2873-2877.2000. PMID: 10921942; PMCID: PMC87134.

- Liu J, Kibiki G, Maro V, Maro A, Kumburu H, Swai N, Taniuchi M, Gratz J, Toney D, Kang G, Houpt E. Multiplex reverse transcription PCR Luminex assay for detection and quantitation of viral agents of gastroenteritis. J Clin Virol. 2011 Apr;50(4):308-13. doi: 10.1016/j.jcv.2010.12.009. Epub 2011 Jan 21. PMID: 21256076; PMCID: PMC3059344.

- Liu J, Platts-Mills JA, Juma J, Kabir F, Nkeze J, Okoi C, Operario DJ, Uddin J, Ahmed S, Alonso PL, Antonio M, Becker SM, Blackwelder WC, Breiman RF, Faruque AS, Fields B, Gratz J, Haque R, Hossain A, Hossain MJ, Jarju S, Qamar F, Iqbal NT, Kwambana B, Mandomando I, McMurry TL, Ochieng C, Ochieng JB, Ochieng M, Onyango C, Panchalingam S, Kalam A, Aziz F, Qureshi S, Ramamurthy T, Roberts JH, Saha D, Sow SO, Stroup SE, Sur D, Tamboura B, Taniuchi M, Tennant SM, Toema D, Wu Y, Zaidi A, Nataro JP, Kotloff KL, Levine MM, Houpt ER. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet. 2016 Sep 24;388(10051):1291-301. doi: 10.1016/S0140-6736(16)31529-X. PMID: 27673470; PMCID: PMC5471845.

- Liu X, Jahuira H, Gilman RH, Alva A, Cabrera L, Okamoto M, Xu H, Windle HJ, Kelleher D, Varela M, Verastegui M, Calderon M, Sanchez G, Sarabia V, Ballard SB, Bern C, Mayta H, Crabtree JE, Cama V, Saito M, Oshitani H. Etiological Role and Repeated Infections of Sapovirus among Children Aged Less than 2 Years in a Cohort Study in a Peri-urban Community of Peru. J Clin Microbiol. 2016 Jun;54(6):1598-1604. doi: 10.1128/JCM.03133-15. Epub 2016 Apr 13. PMID: 27076657; PMCID: PMC4879303.

- Liu X, Yamamoto D, Saito M, Imagawa T, Ablola A, Tandoc AO 3rd, Segubre-Mercado E, Lupisan SP, Okamoto M, Furuse Y, Saito M, Oshitani H. Molecular detection and characterization of sapovirus in hospitalized children with acute gastroenteritis in the Philippines. J Clin Virol. 2015 Jul; 68:83-8. doi: 10.1016/j.jcv.2015.05.001. Epub 2015 May 6. PMID: 26071343.

- Liu Y, Xu ZQ, Zhang Q, Jin M, Yu JM, Li JS, Liu N, Cui SX, Kong XY, Wang H, Li HY, Cheng WX, Ma XJ, Duan ZJ. Simultaneous detection of seven enteric viruses associated with acute gastroenteritis by a multiplexed

Luminex-based assay. J Clin Microbiol. 2012 Jul;50(7):2384-9. doi: 10.1128/JCM.06790-11. Epub 2012 Apr 18. PMID: 22518865; PMCID: PMC3405628.

- Livesey A. A multiagency protocol for responding to sudden unexpected death in infancy: descriptive study. BMJ. 2005 Jan 29;330(7485):227-8. doi: 10.1136/bmj.38323. 652523.F7. Epub 2004 Dec 13. PMID: 15596417; PMCID: PMC546068.

- Lobmaier IV, Vege A, Gaustad P, Rognum TO. Bacteriological investigation--significance of time lapse after death. Eur J Clin Microbiol Infect Dis. 2009 Oct;28(10):1191-8. doi: 10.1007/s10096-009-0762-0. Epub 2009 Jun 6. PMID: 19504136.

- Logan C, O'Leary JJ, O'Sullivan N. Real-time reverse transcription-PCR for detection of rotavirus and adenovirus as causative agents of acute viral gastroenteritis in children. J Clin Microbiol. 2006 Sep;44(9):3189-95. doi: 10.1128/JCM.00915-06. PMID: 16954246; PMCID: PMC1594742.

- Lönnerdal B. Nutritional and physiologic significance of human milk proteins. Am J Clin Nutr. 2003 Jun;77(6):1537S-1543S. doi: 10.1093/ajcn/77.6.1537S. PMID: 12812151.

- Lopman BA, Trivedi T, Vicuña Y, Costantini V, Collins N, Gregoricus N, Parashar U, Sandoval C, Broncano N, Vaca M, Chico ME, Vinjé J, Cooper PJ. Norovirus Infection and Disease in an Ecuadorian Birth Cohort: Association of Certain Norovirus Genotypes With Host FUT2 Secretor Status. J Infect Dis. 2015 Jun 1;211(11):1813-21. doi: 10.1093/infdis/jiu672. Epub 2014 Dec 10. PMID: 25505295; PMCID: PMC4425937.

- Lozer DM, Souza TB, Monfardini MV, Vicentini F, Kitagawa SS, Scaletsky IC, Spano LC. Genotypic and phenotypic analysis of diarrheagenic Escherichia coli strains isolated from Brazilian children living in low socioeconomic level communities. BMC Infect Dis. 2013 Sep 8;13:418. doi: 10.1186/1471-2334-13-418. PMID: 24010735; PMCID: PMC3846636.

- Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005 Dec;71(12):8228-35. doi: 10.1128/AEM.71.12.8228-8235.2005. PMID: 16332807; PMCID: PMC1317376.

- Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. Appl Environ Microbiol. 2007 Mar;73(5):1576-85. doi: 10.1128/AEM.01996-06. Epub 2007 Jan 12. PMID: 17220268; PMCID: PMC1828774.

- Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012 Sep 13;489(7415):220-30. doi: 10.1038/nature11550. PMID: 22972295; PMCID: PMC3577372.

- Lu T, Stroot PG, Oerther DB. Reverse transcription of 16S rRNA to monitor ribosome-synthesizing bacterial populations in the environment. Appl Environ Microbiol. 2009 Jul;75(13):4589-98. doi: 10.1128/AEM.02970-08. Epub 2009 Apr 24. PMID: 19395563; PMCID: PMC2704851.

- Lundgren O, Svensson L. Pathogenesis of rotavirus diarrhea. Microbes Infect. 2001 Nov;3(13):1145-56. doi: 10.1016/s1286-4579(01)01475-7. PMID: 11709295; PMCID: PMC7128947.

- Luoto R, Collado MC, Salminen S, Isolauri E. Reshaping the gut microbiota at an early age: functional impact on obesity risk? Ann Nutr Metab. 2013;63 Suppl 2:17-26. doi: 10.1159/000354896. Epub 2013 Nov 8. PMID: 24217033.

- Mabika RM, Liabagui SL, Moundounga HK, Mounioko F, Souza A, Yala JF. Molecular prevalence and epidemiological characteristics of diarrheagenic *E. coli* in children under 5 years old in the City of Koula-Moutou, East-Central Gabon. Open Journal of Medical Microbiology. 2021 Aug 5;11(3):157-75. doi: 10.4236/ojmm.2021.113013.

- MacDorman MF, Cnattingius S, Hoffman HJ, Kramer MS, Haglund B. Sudden infant death syndrome and smoking in the United States and Sweden. Am J Epidemiol. 1997 Aug 1;146(3):249-57. doi: 10.1093/oxfordjournals.aje.a009260. PMID: 9247009.

- Macfarlane GT, McBain AJ. (1999). The Human Colonic Microbiota. Colonic Microbiota, Nutrition and Health. Edited by: Gibson GR, Roberfroid MB. 1999, Dordrecht: Springer, 1-25. doi.org/10.1007/978-94-017-1079-4_1.

- Machaalani R, Waters KA. Neuronal cell death in the Sudden Infant Death Syndrome brainstem and associations with risk factors. Brain. 2008 Jan;131(Pt 1):218-28. doi: 10.1093/brain/awm290. PMID: 18084013.

- Mackenzie AM, Lebel P, Orrbine E, Rowe PC, Hyde L, Chan F, Johnson W, McLaine PN. Sensitivities and specificities of premier *E. coli* O157 and premier EHEC enzyme immunoassays for diagnosis of infection with verotxin (Shiga-like toxin)-producing Escherichia coli. The SYNSORB Pk Study investigators. J Clin Microbiol. 1998 Jun;36(6):1608-11. doi: 10.1128/JCM.36.6.1608-1611.1998. PMID: 9620386; PMCID: PMC104886.

- Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr. 1999 May;69(5):1035S-1045S. doi: 10.1093/ajcn/69.5.1035s. PMID: 10232646.

- Maclachlan NJ, Dubovi EJ, Barthold SW, Swayne DE, Winton JR. Fenner's Veterinary Virology (Fifth ed.). 2017, Amsterdam: Elsevier/Academic Press.

- Madan JC, Farzan SF, Hibberd PL, Karagas MR. Normal neonatal microbiome variation in relation to environmental factors, infection and allergy. Curr Opin Pediatr. 2012 Dec;24(6):753-9. doi: 10.1097/MOP.0b013e32835a1ac8. PMID: 23111681; PMCID: PMC3914299.

- Madan JC, Hoen AG, Lundgren SN, Farzan SF, Cottingham KL, Morrison HG, Sogin ML, Li H, Moore JH, Karagas MR. Association of Cesarean Delivery and Formula Supplementation With the Intestinal Microbiome of 6-Week-Old Infants. JAMA Pediatr. 2016 Mar;170(3):212-9. doi: 10.1001/jamapediatrics.2015.3732. PMID: 26752321; PMCID: PMC4783194.

- Madeley CR, Cosgrove BP. Letter: Viruses in infantile gastroenteritis. Lancet. 1975 Jul 19;2(7925):124. doi: 10.1016/s0140-6736(75)90020-3. PMID: 49708.

- Madigan MT, Martinko JM. Microorganisms and microbiology. Brock biology of microorganisms. 11th ed. 2006, New Jersey: Pearson Prentice Hall,1-20.

- Magagula NB, Esona MD, Nyaga MM, Stucker KM, Halpin RA, Stockwell TB, Seheri ML, Steele AD, Wentworth DE, Mphahlele MJ. Whole genome analyses of G1P[8] rotavirus strains from vaccinated and non-vaccinated South African children presenting with diarrhea. J Med Virol. 2015 Jan;87(1):79-101. doi: 10.1002/jmv.23971. Epub 2014 May 20. PMID: 24841697; PMCID: PMC5831395.

- Magwalivha M, Wolfaardt M, Kiulia NM, van Zyl WB, Mwenda JM, Taylor MB. High prevalence of species D human adenoviruses in fecal specimens from Urban Kenyan children with diarrhea. J Med Virol. 2010 Jan;82(1):77-84. doi: 10.1002/jmv.21673. PMID: 19950234.

- Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, Yeung DH, Kirk MD. Global incidence of human Shiga toxin-producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathog Dis. 2014 Jun;11(6):447-55. doi: 10.1089/fpd.2013.1704. Epub 2014 Apr 21. PMID: 24750096; PMCID: PMC4607253.

- Makhaola K, Moyo S, Kebaabetswe LP. Distribution and Genetic Variability of Sapoviruses in Africa. Viruses. 2020 Apr 27;12(5):490. doi: 10.3390/v12050490. PMID: 32349380; PMCID: PMC7291139.

- Malakalinga JJ, Misinzo G, Msalya GM, Kazwala RR. Rotavirus Burden, Genetic Diversity and Impact of Vaccine in Children under Five in Tanzania. Pathogens. 2019 Oct 29;8(4):210. doi: 10.3390/pathogens8040210. PMID: 31671824; PMCID: PMC6963457.

- Malam JE, Carrick GF, Telford DR, Morris JA. Staphylococcal toxins and sudden infant death syndrome. J Clin Pathol. 1992 Aug;45(8):716-21. doi: 10.1136/jcp.45.8.716. PMID: 1401186; PMCID: PMC495153.

- Maldonado Y, Cantwell M, Old M, Hill D, Sanchez ML, Logan L, Millan-Velasco F, Valdespino JL, Sepulveda J, Matsui S. Population-based prevalence of symptomatic and asymptomatic astrovirus infection in rural Mayan infants. J Infect Dis. 1998 Aug;178(2):334-9. doi: 10.1086/515625. PMID: 9697712.

- Maldonado YA, Yolken RH. Rotavirus. Baillieres Clin Gastroenterol. 1990 Sep;4(3):609-25. doi: 10.1016/0950-3528(90)90052-i. PMID: 1962726.

- Malloy MH, Freeman DH Jr. Birth weight- and gestational age-specific sudden infant death syndrome mortality: United States, 1991 versus 1995. Pediatrics. 2000 Jun;105(6):1227-31. doi: 10.1542/peds.105.6.1227. PMID: 10835061.

- Malloy MH, Hoffman HJ. Prematurity, sudden infant death syndrome, and age of death. Pediatrics. 1995 Sep;96(3 Pt 1):464-71. PMID: 7651779.

- Manolov DG. A new type of the genus Shigella--"Shigella 13". J Hyg Epidemiol Microbiol Immunol. 1959;3:184-90. PMID: 14420729.

- Mansour A, Shaheen HI, Amine M, Hassan K, Sanders JW, Riddle MS, Armstrong AW, Svennerholm AM, Sebeny PJ, Klena JD, Young SY, Frenck RW. Diarrhea burden due to natural infection with enterotoxigenic Escherichia coli in a birth cohort in a rural Egyptian community. J Clin Microbiol. 2014 Jul;52(7):2595-603. doi: 10.1128/JCM.00215-14. Epub 2014 May 14. PMID: 24829232; PMCID: PMC4097688.

- Mare AD, Ciurea CN, Man A, Tudor B, Moldovan V, Decean L, Toma F. Enteropathogenic Escherichia coli—A Summary of the Literature. Gastroenterology Insights. 2021 Mar;12(1):28-40. doi.org/10.3390/gastroent12010004.

- Mark E. The state and use of autopsy around the world. Symposium 11. Who wants autopsies and who does not ─ lessons learned from the rise and fall of the autopsy over the past 100 years. Histopathology 2002;41(Suppl. 2):197-213.

- Markestad T, Skadberg B, Hordvik E, Morild I, Irgens LM. Sleeping position and sudden infant death syndrome (SIDS): effect of an intervention programme to avoid prone sleeping. Acta Paediatr. 1995 Apr;84(4):375-8. doi: 10.1111/j.1651-2227. 1995.tb13653. x. PMID: 7795344.

- Markkula J, Hemming-Harlo M, Salminen MT, Savolainen-Kopra C, Pirhonen J, Al-Hello H, Vesikari T. Rotavirus epidemiology 5-6 years after universal rotavirus vaccination: persistent rotavirus activity in older children and elderly. Infect Dis (Lond). 2017 May;49(5):388-395. doi: 10.1080/23744235.2016.1275773. Epub 2017 Jan 9. PMID: 28067093.

- Marrier R, Wells JG, Swanson RC, Callahan W, Mehlman IJ. An outbreak of enteropathogenic Escherichia coli foodborne disease traced to imported french cheese. Lancet. 1973;2:1376-8. Doi:10.1016/S0140-6736(73)93335-7.

- Martín R, Langa S, Reviriego C, Jimínez E, Marín ML, Xaus J, Fernández L, Rodríguez JM. Human milk is a source of lactic acid bacteria for the infant gut. J Pediatr. 2003 Dec;143(6):754-8. doi: 10.1016/j.jpeds.2003.09.028. PMID: 14657823.

- Martin R, Nauta AJ, Ben Amor K, Knippels LM, Knol J, Garssen J. Early life: gut microbiota and immune development in infancy. Benef Microbes. 2010 Nov;1(4):367-82. doi: 10.3920/BM2010.0027. PMID: 21831776.

- Mathew S, Smatti MK, Al Ansari K, Nasrallah GK, Al Thani AA, Yassine HM. Mixed Viral-Bacterial Infections and Their Effects on Gut Microbiota and Clinical Illnesses in Children. Sci Rep. 2019 Jan 29;9(1):865. doi: 10.1038/s41598-018-37162-w. PMID: 30696865; PMCID: PMC6351549.

- Mathews TJ, Menacker F, MacDorman MF; Centers for Disease Control and Prevention, National Center for Health Statistics. Infant mortality statistics from the 2002 period: linked birth/infant death data set. Natl Vital Stat Rep. 2004 Nov 24;53(10):1-29. PMID: 15622996.

- Matsui SM, Greenberg HB. 2001. Astroviruses. In Fields Virology, 4th ed. Edited by: Knipe DM and Howley PM. 2001, Philadelphia: Lippincott-Ravan, 875–893.

- Matsui SM, Kiang D, Ginzton N, Chew T, Geigenmüller-Gnirke U. Molecular biology of astroviruses: selected highlights. Novartis Found Symp. 2001; 238:219-33; discussion 233-6. doi: 10.1002/0470846534.ch13. PMID: 11444028.

- Matthijnssens J, Attoui H, Bányai K, Brussaard CPD, Danthi P, Del Vas M, Dermody TS, Duncan R, Fāng 方勤 Q, Johne R, Mertens PPC, Mohd Jaafar F, Patton JT, Sasaya 笹谷孝英 T, Suzuki 鈴木信弘 N, Wei 魏太云 T. ICTV Virus Taxonomy Profile: *Sedoreoviridae* 2022. J Gen Virol. 2022 Oct;103(10). doi: 10.1099/jgv.0.001782. PMID: 36215107.

- Matthijnssens J, Bilcke J, Ciarlet M, Martella V, Bányai K, Rahman M, Zeller M, Beutels P, Van Damme P, Van Ranst M. Rotavirus disease and vaccination: impact on genotype diversity. Future Microbiol. 2009 Dec;4(10):1303-16. doi: 10.2217/fmb.09.96. PMID: 19995190.

- Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Bányai K, Brister JR, Buesa J, Esona MD, Estes MK, Gentsch JR, Iturriza-Gómara M, Johne R, Kirkwood CD, Martella V, Mertens PP, Nakagomi O, Parreño V, Rahman M, Ruggeri FM, Saif LJ, Santos N, Steyer A, Taniguchi K, Patton JT, Desselberger U, Van Ranst M. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch Virol. 2011 Aug;156(8):1397-413. doi: 10.1007/s00705-011-1006-z. Epub 2011 May 20. PMID: 21597953; PMCID: PMC3398998.

- Matthijnssens J, Van Ranst M. Genotype constellation and evolution of group A rotaviruses infecting humans. Curr Opin Virol. 2012 Aug;2(4):426-33. doi: 10.1016/j.coviro.2012.04.007. Epub 2012 Jun 9. PMID: 22683209.

- Matussek A, Dienus O, Djeneba O, Simpore J, Nitiema L, Nordgren J. Molecular characterization and genetic susceptibility of sapovirus in children with diarrhea in Burkina Faso. Infect Genet Evol. 2015 Jun; 32:396-400. doi: 10.1016/j.meegid.2015.03.039. Epub 2015 Apr 4. PMID: 25847694.

- Mayo MA. ICTV at the Paris ICV: results of the plenary session and the binomial ballot. Arch Virol. 2002 May;147(11):2254-60. doi.org/10.1007/s007050200052.

- McAtee CL, Webman R, Gilman RH, Mejia C, Bern C, Apaza S, Espetia S, Pajuelo M, Saito M, Challappa R, Soria R, Ribera JP, Lozano D, Torrico F. Burden of Norovirus and Rotavirus in Children After Rotavirus Vaccine Introduction, Cochabamba, Bolivia. Am J Trop Med Hyg. 2016 Jan;94(1):212-7. doi: 10.4269/ajtmh.15-0203. Epub 2015 Nov 23. PMID: 26598569; PMCID: PMC4710432.

- McElvania TeKippe E, Wylie KM, Deych E, Sodergren E, Weinstock G, Storch GA. Increased prevalence of anellovirus in pediatric patients with fever. PLoS One. 2012;7(11): e50937. doi: 10.1371/journal.pone.0050937. Epub 2012 Nov 30. PMID: 23226428; PMCID: PMC3511395.

- Mebus CA, Wyatt RG, Sharpee RL, Sereno MM, Kalica AR, Kapikian AZ, Twiehaus MJ. Diarrhea in gnotobiotic calves caused by the reovirus-like agent of human infantile gastroenteritis. Infect Immun. 1976 Aug;14(2):471-4. doi: 10.1128/iai.14.2.471-474.1976. PMID: 184047; PMCID: PMC420908.

- Meliopoulos VA, Kayali G, Burnham A, Oshansky CM, Thomas PG, Gray GC, Beck MA, Schultz-Cherry S. Detection of antibodies against Turkey astrovirus in humans. PLoS One. 2014 May 14;9(5): e96934. doi: 10.1371/journal.pone.0096934. PMID: 24826893; PMCID: PMC4020816.

- Melton-Celsa AR. Shiga Toxin (Stx) Classification, Structure, and Function. Microbiol Spectr. 2014 Aug;2(4): EHEC-0024-2013. doi: 10.1128/microbiolspec.EHEC-0024-2013. PMID: 25530917; PMCID: PMC4270005.

- Mendes-Ledesma MR, Rocha LB, Bueris V, Krause G, Beutin L, Franzolin MR, Trabulsi LR, Elias WP, Piazza RM. Production, and characterization of rabbit polyclonal sera against Shiga toxins Stx1 and Stx2 for detection of Shiga toxin-producing Escherichia coli. Microbiol Immunol. 2008 Oct;52(10):484-91. doi: 10.1111/j.1348-0421.2008.00068. x. PMID: 18822082.

- Meraz IM, Arikawa K, Nakamura H, Ogasawara J, Hase A, Nishikawa Y. Association of IL-8-inducing strains of diffusely adherent Escherichia coli with sporadic diarrheal patients with less than 5 years of age. Braz J Infect Dis. 2007 Feb;11(1):44-9. doi: 10.1590/s1413-86702007000100012. PMID: 17625726.

- Mertens KL, Kalsbeek A, Soeters MR, Eggink HM. Bile Acid Signaling Pathways from the Enterohepatic Circulation to the Central Nervous System. Front Neurosci. 2017 Nov 7; 11:617. doi: 10.3389/fnins.2017.00617. PMID: 29163019; PMCID: PMC5681992.

- Meyer CT, Bauer IK, Antonio M, Adeyemi M, Saha D, Oundo JO, Ochieng JB, Omore R, Stine OC, Wang D, Holtz LR. Prevalence of classic, MLB-clade and VA-clade Astroviruses in Kenya and The Gambia. Virol J. 2015 May 15; 12:78. doi: 10.1186/s12985-015-0299-z. PMID: 25975198; PMCID: PMC4465002.

- Michelacci V, Prosseda G, Maugliani A, Tozzoli R, Sanchez S, Herrera-León S, Dallman T, Jenkins C, Caprioli A, Morabito S. Characterization of an emergent clone of enteroinvasive Escherichia coli circulating in Europe. Clin Microbiol Infect. 2016 Mar;22(3):287.e11-9. doi: 10.1016/j.cmi.2015.10.025. Epub 2015 Nov 10. PMID: 26551840.

- Michelangeli F, Ruiz MC. I, 2. Physiology and pathophysiology of the gut in relation to viral diarrhea. Perspect Med Virol. 2003; 9:23-50. doi: 10.1016/S0168-7069(03)09003-7. Epub 2004 Sep 14. PMID: 32308248; PMCID: PMC7157931.

- Mikhail AFW, Jenkins C, Dallman TJ, Inns T, Douglas A, Martín AIC, Fox A, Cleary P, Elson R, Hawker J. An outbreak of Shiga toxin-producing Escherichia coli O157:H7 associated with contaminated salad leaves: epidemiological, genomic and food trace back investigations. Epidemiol Infect. 2018 Jan;146(2):187-196. doi: 10.1017/S0950268817002874. Epub 2017 Dec 17. Erratum in: Epidemiol Infect. 2018 Oct;146(14):1879. PMID: 29248018; PMCID: PMC9134740.

- Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, Belzer C, Delgado Palacio S, Arboleya Montes S, Mancabelli L, Lugli GA, Rodriguez JM, Bode L, de Vos W, Gueimonde M, Margolles A, van Sinderen D, Ventura M. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. Microbiol Mol Biol Rev. 2017 Nov 8;81(4): e00036-17. doi: 10.1128/MMBR.00036-17. PMID: 29118049; PMCID: PMC5706746.

- Miliwebsky E, Schelotto F, Varela G, Luz D, Chinen I, Piazza RMF. Human diarrheal infections: diagnosis of diarrheagenic Escherichia coli pathotypes. Escherichia coli in the Americas. Edited by: Torres AG. 2016, Switzerland: Springer International Publishing, 343–69. doi: 10.1007/978-3-319-45092-6_15.

- Miller JE, Wu C, Pedersen LH, de Klerk N, Olsen J, Burgner DP. Maternal antibiotic exposure during pregnancy and hospitalization with infection in offspring: a population-based cohort study. Int J Epidemiol. 2018 Apr 1;47(2):561-571. doi: 10.1093/ije/dyx272. PMID: 29415232.

- Mitchell EA, Brunt JM, Everard C. Reduction in mortality from sudden infant death syndrome in New Zealand: 1986-92. Arch Dis Child. 1994 Apr;70(4):291-4. doi: 10.1136/adc.70.4.291. PMID: 8185361; PMCID: PMC1029781.

- Mitchell EA, Freemantle J, Young J, Byard RW. Scientific consensus forum to review the evidence underpinning the recommendations of the Australian SIDS and Kids Safe Sleeping Health Promotion Programme--October 2010. J Paediatr Child Health. 2012 Aug;48(8):626-33. doi: 10.1111/j.1440-1754.2011.02215. x. Epub 2011 Nov 3. PMID: 22050484.

- Mitchell EA, Krous HF. Sudden unexpected death in infancy: a historical perspective. J Paediatr Child Health. 2015 Jan;51(1):108-12. doi: 10.1111/jpc.12818. PMID: 25586853.

- Mitchell EA, Milerad J. Smoking and the sudden infant death syndrome. Rev Environ Health. 2006 Apr-Jun;21(2):81-103. doi: 10.1515/reveh.2006.21.2.81. PMID: 16898673.

- Mitchell EA, Taylor BJ, Ford RP, Stewart AW, Becroft DM, Thompson JM, Scragg R, Hassall IB, Barry DM, Allen EM, et al. Dummies and the sudden infant death syndrome. Arch Dis Child. 1993 Apr;68(4):501-4. doi: 10.1136/adc.68.4.501. PMID: 8503676; PMCID: PMC1029275.

- Mitchell EA, Taylor BJ, Ford RP, Stewart AW, Becroft DM, Thompson JM, Scragg R, Hassall IB, Barry DM, Allen EM, et al. Four modifiable and other major risk factors for cot death: the New Zealand study. J Paediatr Child Health. 1992;28 Suppl 1: S3-8. doi: 10.1111/j.1440-1754. 1992.tb02729. x. PMID: 1524879.

- Mitchell EA, Tuohy PG, Brunt JM, Thompson JM, Clements MS, Stewart AW, Ford RP, Taylor BJ. Risk factors for sudden infant death syndrome following the prevention campaign in New Zealand: a prospective study. Pediatrics. 1997 Nov;100(5):835-40. doi: 10.1542/peds.100.5.835. PMID: 9346984.

- Mitsuoka T. Intestinal flora and human health. Asia Pac J Clin Nutr. 1996 Mar;5(1):2-9. PMID: 24394457.

- Mittal R, Debs LH, Patel AP, Nguyen D, Patel K, O'Connor G, Grati M, Mittal J, Yan D, Eshraghi AA, Deo SK, Daunert S, Liu XZ. Neurotransmitters: The Critical Modulators Regulating Gut-Brain Axis. J Cell Physiol. 2017 Sep;232(9):2359-2372. doi: 10.1002/jcp.25518. Epub 2017 Apr 10. PMID: 27512962; PMCID: PMC5772764.

- Mohammad HA, Madi NM, Al-Nakib W. Analysis of viral diversity in stool samples from infants and children with acute gastroenteritis in Kuwait using Metagenomics approach. Virol J. 2020 Jan 30;17(1):10. doi: 10.1186/s12985-020-1287-5. PMID: 32000795; PMCID: PMC6993487.

- Molony N, Blackwell CC, Busuttil A. The effect of prone posture on nasal temperature in children in relation to induction of staphylococcal toxins implicated in sudden infant death syndrome. FEMS Immunol Med Microbiol. 1999 Aug 1;25(1-2):109-13. doi: 10.1111/j.1574-695X.1999.tb01333.x. PMID: 10443498.

- Monastiri A, Aouni M, Guix S, Mechri B, Lopez-Roig M, Abid NBS, Gueddiche N, Hamami S, Boughzala L, Serra-Cobo J. Clinical surveillance for human astrovirus in Monastir region, Tunisia. BMC Public Health. 2016 Jan 21; 16:57. doi: 10.1186/s12889-016-2726-5. PMID: 26796330; PMCID: PMC4722761.

- Monroe SS, Carter MJ, Herrmann JE, Kurtz JB, and Matsui SM. Astroviridae. Virus taxonomy: classification and nomenclature of viruses. Sixth report of the International Committee on Taxonomy of Viruses. Edited by: Murphy FA, Bishop DHL, Ghabrail SA, Jarvis AW, Martielli GP, Mayo MA, and Summers MD. 1995, Vienna, Austria: Springer-Verlag, 364–384

- Monroe SS, Jiang B, Stine SE, Koopmans M, Glass RI. Subgenomic RNA sequence of human astrovirus supports classification of Astroviridae as a new family of RNA viruses. J Virol. 1993 Jun;67(6):3611-4. doi: 10.1128/JVI.67.6.3611-3614.1993. PMID: 8497068; PMCID: PMC237710.

- Montoya-Williams D, Lemas DJ, Spiryda L, Patel K, Carney OO, Neu J, Carson TL. The Neonatal Microbiome and Its Partial Role in Mediating the Association between Birth by Cesarean Section and Adverse Pediatric Outcomes. Neonatology. 2018;114(2):103-111. doi: 10.1159/000487102. Epub 2018 May 22. PMID: 29788027; PMCID: PMC6532636.

- Moon RY, Carlin RF, Hand I; TASK FORCE ON SUDDEN INFANT DEATH SYNDROME and THE COMMITTEE ON FETUS AND NEWBORN. Evidence Base for 2022 Updated Recommendations for a Safe Infant Sleeping Environment to Reduce the Risk of Sleep-Related Infant Deaths. Pediatrics. 2022 Jul 1;150(1):e2022057991. doi: 10.1542/peds.2022-057991. PMID: 35921639.

- Moon RY, Horne RS, Hauck FR. Sudden infant death syndrome. Lancet. 2007 Nov 3;370(9598):1578-87. doi: 10.1016/S0140-6736(07)61662-6. PMID: 17980736.

- Moon RY, Tanabe KO, Yang DC, Young HA, Hauck FR. Pacifier use and SIDS: evidence for a consistently reduced risk. Matern Child Health J. 2012 Apr;16(3):609-14. doi: 10.1007/s10995-011-0793-x. PMID: 21505778.

- Moon RY; TASK FORCE ON SUDDEN INFANT DEATH SYNDROME. SIDS and Other Sleep-Related Infant Deaths: Evidence Base for 2016 Updated Recommendations for a Safe Infant Sleeping Environment. Pediatrics. 2016 Nov;138(5): e20162940. doi: 10.1542/peds.2016-2940. PMID: 27940805.

- Morais TB, Gomes TA, Sigulem DM. Enteroaggregative Escherichia coli in infant feeding bottles. Lancet. 1997 May 17;349(9063):1448-9. doi: 10.1016/S0140-6736(05)63724-5. PMID: 9164322.

- Morgan DJ, Vargas SL, Reyes-Mugica M, Walterspiel JN, Carver W, Gigliotti F. Identification of Pneumocystis carinii in the lungs of infants dying of sudden infant death syndrome. Pediatr Infect Dis J. 2001 Mar;20(3):306-9. doi: 10.1097/00006454-200103000-00017. PMID: 11303835.

- Moriel DG, Rosini R, Seib KL, Serino L, Pizza M, Rappuoli R. Escherichia coli: great diversity around a common core. mBio. 2012 Jun 5;3(3):e00118-12. doi: 10.1128/mBio.00118-12. PMID: 22669628; PMCID: PMC3374390.

- Morris JA, Harrison LM, Partridge SM. Postmortem bacteriology: a re-evaluation. J Clin Pathol. 2006 Jan;59(1):1-9. doi: 10.1136/jcp.2005.028183. PMID: 16394274; PMCID: PMC1860254.

- Morris JA, Harrison LM, Partridge SM. Practical and theoretical aspects of postmortem bacteriology. Curr. Diagn. Pathol. 2007 Feb 1;13(1):65-74. Doi:10.1016/j.cdip.2006.07.005.

- Morris JA. The common bacterial toxins hypothesis of sudden infant death syndrome. FEMS Immunol Med Microbiol. 1999 Aug 1;25(1-2):11-7. doi: 10.1111/j.1574-695X.1999.tb01322.x. PMID: 10443487.

- Morrison JL, Regnault TR. Nutrition in Pregnancy: Optimising Maternal Diet and Fetal Adaptations to Altered Nutrient Supply. Nutrients. 2016 Jun 4;8(6):342. doi: 10.3390/nu8060342. PMID: 27271666; PMCID: PMC4924183.

- Moscovis SM, Hall ST, Burns CJ, Scott RJ, Blackwell CC. The male excess in sudden infant deaths. Innate Immun. 2014 Jan;20(1):24-9. doi: 10.1177/1753425913481071. Epub 2013 Apr 22. PMID: 23608823.

- Mosko S, Richard C, McKenna J. Infant arousals during mother-infant bed sharing: implications for infant sleep and sudden infant death syndrome research. Pediatrics. 1997 Nov;100(5):841-9. doi: 10.1542/peds.100.5.841. PMID: 9346985.

- Moure UAE, Banga-Mingo V, Gody JC, Mwenda JM, Fandema J, Waku-Kouomou D, Manengu C, Koyazegbe TD, Esona MD, Bowen MD, Gouandijka-Vasilache I. Emergence of G12 and G9 rotavirus genotypes in the Central African Republic, January 2014 to February 2016. BMC Res Notes. 2018 Jan 5;11(1):5. doi: 10.1186/s13104-017-3122-7. PMID: 29304830; PMCID: PMC5755012.

- Mphahlele MJ, Groome MJ, Page NA, Bhagwandin N, Mwenda JM, Steele AD. A decade of rotavirus vaccination in Africa - Saving lives and changing the face of diarrhoeal diseases: Report of the 12th African Rotavirus Symposium. Vaccine. 2021 Apr 22;39(17):2319-2324. doi: 10.1016/j.vaccine.2021.03.014. Epub 2021 Mar 26. PMID: 33775436.

- Msimang VM, Page N, Groome MJ, Moyes J, Cortese MM, Seheri M, Kahn K, Chagan M, Madhi SA, Cohen C. Impact of rotavirus vaccine on childhood diarrheal hospitalization after introduction into the South African public immunization program. Pediatr Infect Dis J. 2013 Dec;32(12):1359-64. doi: 10.1097/INF.0b013e3182a72fc0. PMID: 24569308.

- Msolo L, Iweriebor BC, Okoh AI. Antimicrobial Resistance Profiles of Diarrheagenic *E. coli* (DEC) and Salmonella Species Recovered from Diarrheal Patients in Selected Rural Communities of the Amathole District Municipality, Eastern Cape Province, South Africa. Infect Drug Resist. 2020a Dec 22; 13:4615-4626. doi: 10.2147/IDR.S269219. PMID: 33376367; PMCID: PMC7764871.a

- Msolo L, Iweriebor BC, Okoh AI. Pervasiveness of diarrheagenic *E. coli* pathotypes and Salmonella species among gastroenteritis patients in some selected pastoral hinterlands of the Amathole district municipality, Eastern Cape, South Africa. Human Microbiome Journal. 2020b Aug 1;17: 100074.Doi.org/10.1016/j.humic.2020.100074.

- Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. The infant microbiome development: mom matters. Trends Mol Med. 2015 Feb;21(2):109-17. doi: 10.1016/j.molmed.2014.12.002. Epub 2014 Dec 11. PMID: 25578246; PMCID: PMC4464665.

- Murray TY, Nadan S, Page NA, Taylor MB. Diverse sapovirus genotypes identified in children hospitalised with gastroenteritis in selected regions of South Africa. J Clin Virol. 2016 Mar; 76:24-9. doi: 10.1016/j.jcv.2016.01.003. Epub 2016 Jan 6. PMID: 26803285.

- Murrell WG, Stewart BJ, O'Neill C, Siarakas S, Kariks S. Enterotoxigenic bacteria in the sudden infant death syndrome. J Med Microbiol. 1993 Aug;39(2):114-27. doi: 10.1099/00222615-39-2-114. PMID: 8345506.

- Mwenda JM, Ntoto KM, Abebe A, Enweronu-Laryea C, Amina I, Mchomvu J, Kisakye A, Mpabalwani EM, Pazvakavambwa I, Armah GE, Seheri LM, Kiulia NM, Page N, Widdowson MA, Steele AD. Burden and epidemiology of rotavirus diarrhea in selected African countries: preliminary results from the African Rotavirus Surveillance Network. J Infect Dis. 2010 Sep 1;202 Suppl: S5-S11. doi: 10.1086/653557. PMID: 20684718.

- Nadan S, Taylor MB, Groome MJ, Cohen C, Madhi SA, Page NA. Epidemiology of human astroviruses among children younger than 5 years: Prospective hospital-based sentinel surveillance in South Africa, 2009-2014. J Med Virol. 2019 Feb;91(2):225-234. doi: 10.1002/jmv.25308. Epub 2018 Oct 9. PMID: 30192398.

- Nair GB, Ramamurthy T, Bhattacharya MK, Krishnan T, Ganguly S, Saha DR, Rajendran K, Manna B, Ghosh M, Okamoto K, Takeda Y. Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India. Gut Pathog. 2010 Jun 5;2(1):4. doi: 10.1186/1757-4749-2-4. PMID: 20525383; PMCID: PMC2901208.

- Nataro JP, Kaper JB, Robins-Browne R, Prado V, Vial P, Levine MM. Patterns of adherence of diarrheagenic Escherichia coli to HEp-2 cells. Pediatr Infect Dis J. 1987 Sep;6(9):829-31. doi: 10.1097/00006454-198709000-00008. PMID: 3313248.

- Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev. 1998 Jan;11(1):142-201. doi: 10.1128/CMR.11.1.142. Erratum in: Clin Microbiol Rev 1998 Apr;11(2):403. PMID: 9457432; PMCID: PMC121379.

- Nataro JP, Mai V, Johnson J, Blackwelder WC, Heimer R, Tirrell S, Edberg SC, Braden CR, Glenn Morris J Jr, Hirshon JM. Diarrheagenic Escherichia coli infection in Baltimore, Maryland, and New Haven, Connecticut. Clin Infect Dis. 2006 Aug 15;43(4):402-7. doi: 10.1086/505867. Epub 2006 Jul 11. PMID: 16838226.

- National Center for Chronic Disease Prevention and Health Promotion, Division of Reproductive Health, and Centers for Disease Control and Prevention. [Website] Sudden unexplained infant death investigation: a systematic training program for the professional infant death investigation specialist. Atlanta, GA: Centers for Disease Control and Prevention. 2017. Available from: http://www.cdc.gov/sids/TrainingMaterial.htm#manual. [Date accessed: 26 January 2018].

- Naudé PJW, Claassen-Weitz S, Gardner-Lubbe S, Botha G, Kaba M, Zar HJ, Nicol MP, Stein DJ. Association of maternal prenatal psychological stressors and distress with maternal and early infant faecal bacterial profile. Acta Neuropsychiatr. 2020 Feb;32(1):32-42. doi: 10.1017/neu.2019.43. Epub 2019 Dec 20. PMID: 31753055; PMCID: PMC7116644.

- Neter E, Westphal O, Luderitz O, Gino RM, Gorzynski EA. Demonstration of antibodies against enteropathogenic Escherichia coli in sera of children of various ages. Pediatrics. 1955 Dec;16(6):801-8. PMID: 13273119.

- Neuwirth C, Francois C, Laurent N, Pechinot A. Myocarditis due to Salmonella virchow and sudden infant death. Lancet. 1999 Sep 18;354(9183):1004. doi: 10.1016/S0140-6736(99)03795-2. PMID: 10501372.

- Newitt S, MacGregor V, Robbins V, Bayliss L, Chattaway MA, Dallman T, Ready D, Aird H, Puleston R, Hawker J. Two Linked Enteroinvasive Escherichia coli Outbreaks, Nottingham, UK, June 2014. Emerg Infect Dis. 2016 Jul;22(7):1178-84. doi: 10.3201/eid2207.152080. PMID: 27314432; PMCID: PMC4918187.

- Nguyen TV, Le Van P, Le Huy C, Gia KN, Weintraub A. Detection and characterization of diarrheagenic Escherichia coli from young children in Hanoi, Vietnam. J Clin Microbiol. 2005 Feb;43(2):755-60. doi: 10.1128/JCM.43.2.755-760.2005. PMID: 15695676; PMCID: PMC548099.

- Ni J, Friedman H, Boyd BC, McGurn A, Babinski P, Markossian T, Dugas LR. Early antibiotic exposure and development of asthma and allergic rhinitis in childhood. BMC Pediatr. 2019 Jul 5;19(1):225. doi: 10.1186/s12887-019-1594-4. PMID: 31277618; PMCID: PMC6612173.

- Nicholls SM, Quick JC, Tang S, Loman NJ. Ultra-deep, long read nanopore sequencing of mock microbial community standards. Gigascience. 2019 May 1;8(5): giz043. doi: 10.1093/gigascience/giz043. PMID: 31089679; PMCID: PMC6520541.

- Nimri LF, Elnasser Z, Batchoun R. Polymicrobial infections in children with diarrhoea in a rural area of Jordan. FEMS Immunol Med Microbiol. 2004 Oct 1;42(2):255-9. doi: 10.1016/j.femsim.2004.05.014. PMID: 15364112.

- Nkosi V, Haman T, Naicker N, Mathee A. Overcrowding and health in two impoverished suburbs of Johannesburg, South Africa. BMC Public Health. 2019 Oct 24;19(1):1358. doi: 10.1186/s12889-019-7665-5. PMID: 31651269; PMCID: PMC6813127.

- Noel JS, Fankhauser RL, Ando T, Monroe SS, Glass RI. Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. J Infect Dis. 1999 Jun;179(6):1334-44. doi: 10.1086/314783. PMID: 10228052.

- Noverr MC, Falkowski NR, McDonald RA, McKenzie AN, Huffnagle GB. Development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase: role of host genetics, antigen, and interleukin-13. Infect Immun. 2005 Jan;73(1):30-8. doi: 10.1128/IAI.73.1.30-38.2005. PMID: 15618138; PMCID: PMC538952.

- Novicki TJ, Daly JA, Mottice SL, Carroll KC. Comparison of sorbitol MacConkey agar and a two-step method which utilizes enzyme-linked immunosorbent assay toxin testing and a chromogenic agar to detect and isolate enterohemorrhagic Escherichia coli. J Clin Microbiol. 2000 Feb;38(2):547-51. doi: 10.1128/JCM.38.2.547-551.2000. PMID: 10655343; PMCID: PMC86145.

- Nyaga MM, Tan Y, Seheri ML, Halpin RA, Akopov A, Stucker KM, Fedorova NB, Shrivastava S, Duncan Steele A, Mwenda JM, Pickett BE, Das SR, Jeffrey Mphahlele M. Whole-genome sequencing and analyses identify high

genetic heterogeneity, diversity and endemicity of rotavirus genotype P[6] strains circulating in Africa. Infect Genet Evol. 2018 Sep; 63:79-88. doi: 10.1016/j.meegid.2018.05.013. Epub 2018 May 18. PMID: 29782933; PMCID: PMC6364979.

- Nyanga PL, Onyuka J, Webale MK, Were T, Budambula V. Escherichia coli pathotypes and Shigella sero-groups in diarrheic children in Nairobi city, Kenya. Gastroenterol Hepatol Bed Bench. 2017 Summer;10(3):220-228. PMID: 29118939; PMCID: PMC5660273.

- O'Brien AD, LaVeck GD, Thompson MR, Formal SB. Production of Shigella dysenteriae type 1-like cytotoxin by Escherichia coli. J Infect Dis. 1982 Dec;146(6):763-9. doi: 10.1093/infdis/146.6.763. PMID: 6754826.

- Ochoa TJ, Barletta F, Contreras C, Mercado E. New insights into the epidemiology of enteropathogenic Escherichia coli infection. Trans R Soc Trop Med Hyg. 2008 Sep;102(9):852-6. doi: 10.1016/j.trstmh.2008.03.017. Epub 2008 May 2. PMID: 18455741; PMCID: PMC2575077.

- Oden RP, Joyner BL, Ajao TI, Moon RY. Factors influencing African American mothers' decisions about sleep position: a qualitative study. J Natl Med Assoc. 2010 Oct;102(10):870-2, 875-80. doi: 10.1016/s0027-9684(15)30705-7. PMID: 21053701.

- Odetoyin BW, Hofmann J, Aboderin AO, Okeke IN. Diarrhoeagenic Escherichia coli in mother-child Pairs in Ile-Ife, South Western Nigeria. BMC Infect Dis. 2016 Jan 25; 16:28. doi: 10.1186/s12879-016-1365-x. PMID: 26809819; PMCID: PMC4727348.

- O'Donnell C, Woodford N. Post-mortem radiology--a new sub-speciality? Clin Radiol. 2008 Nov;63(11):1189-94. doi: 10.1016/j.crad.2008.05.008. Epub 2008 Sep 3. PMID: 18929036.

- Oh JZ, Ravindran R, Chassaing B, Carvalho FA, Maddur MS, Bower M, Hakimpour P, Gill KP, Nakaya HI, Yarovinsky F, Sartor RB, Gewirtz AT, Pulendran B. TLR5-mediated sensing of gut microbiota is necessary for antibody responses to seasonal influenza vaccination. Immunity. 2014 Sep 18;41(3):478-492. doi: 10.1016/j.immuni.2014.08.009. Epub 2014 Sep 11. PMID: 25220212; PMCID: PMC4169736.

- Oh KJ, Lee SE, Jung H, Kim G, Romero R, Yoon BH. Detection of ureaplasmas by the polymerase chain reaction in the amniotic fluid of patients with cervical insufficiency. J Perinat Med. 2010 May;38(3):261-8. doi: 10.1515/jpm.2010.040. PMID: 20192887; PMCID: PMC3085903.

- Oka T, Wang Q, Katayama K, Saif LJ. Comprehensive review of human sapoviruses. Clin Microbiol Rev. 2015 Jan;28(1):32-53. doi: 10.1128/CMR.00011-14. PMID: 25567221; PMCID: PMC4284302.

- O'Keefe SJ. Diet, microorganisms and their metabolites, and colon cancer. Nat Rev Gastroenterol Hepatol. 2016 Dec;13(12):691-706. doi: 10.1038/nrgastro.2016.165. Epub 2016 Nov 16. PMID: 27848961; PMCID: PMC6312102.

- Olin A, Henckel E, Chen Y, Lakshmikanth T, Pou C, Mikes J, Gustafsson A, Bernhardsson AK, Zhang C, Bohlin K, Brodin P. Stereotypic Immune System Development in Newborn Children. Cell. 2018 Aug 23;174(5):1277-1292.e14. doi: 10.1016/j.cell.2018.06.045. PMID: 30142345; PMCID: PMC6108833.

- Oliveira M, Amorim A. Microbial forensics: new breakthroughs and future prospects. Appl Microbiol Biotechnol. 2018 Dec;102(24):10377-10391. doi: 10.1007/s00253-018-9414-6. Epub 2018 Oct 9. PMID: 30302518; PMCID: PMC7080133.

- Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR, Stahl DA. Microbial ecology and evolution: a ribosomal RNA approach. Annu Rev Microbiol. 1986; 40:337-65. doi: 10.1146/annurev.mi.40.100186.002005. PMID: 2430518.

- Omatola CA, Ogunsakin RE, Olaniran AO. Prevalence, Pattern and Genetic Diversity of Rotaviruses among Children under 5 Years of Age with Acute Gastroenteritis in South Africa: A Systematic Review and Meta-Analysis. Viruses. 2021 Sep 23;13(10):1905. doi: 10.3390/v13101905. PMID: 34696335; PMCID: PMC8538439.

- Operario DJ, Platts-Mills JA, Nadan S, Page N, Seheri M, Mphahlele J, Praharaj I, Kang G, Araujo IT, Leite JPG, Cowley D, Thomas S, Kirkwood CD, Dennis F, Armah G, Mwenda JM, Wijesinghe PR, Rey G, Grabovac V, Berejena C, Simwaka CJ, Uwimana J, Sherchand JB, Thu HM, Galagoda G, Bonkoungou IJO, Jagne S, Tsolenyanu E, Diop A, Enweronu-Laryea C, Borbor SA, Liu J, McMurry T, Lopman B, Parashar U, Gentsch J, Steele AD, Cohen A, Serhan F, Houpt ER. Etiology of Severe Acute Watery Diarrhea in Children in the Global Rotavirus Surveillance Network Using Quantitative Polymerase Chain Reaction. J Infect Dis. 2017 Jul 15;216(2):220-227. doi: 10.1093/infdis/jix294. Erratum in: J Infect Dis. 2017 Nov 15;216(8):1048. PMID: 28838152; PMCID: PMC5853801.

- Oppong TB, Yang H, Amponsem-Boateng C, Kyere EKD, Abdulai T, Duan G, Opolot G. Enteric pathogens associated with gastroenteritis among children under 5 years in sub-Saharan Africa: a systematic review and meta-analysis. Epidemiol Infect. 2020 Mar 2;148: e64. doi: 10.1017/S0950268820000618. PMID: 32115003; PMCID: PMC7118358.

- Osei-Poku GK, Thomas S, Mwananyanda L, Lapidot R, Elliott PA, Macleod WB, Somwe SW, Gill CJ. A systematic review of the burden and risk factors of sudden infant death syndrome (SIDS) in Africa. J Glob Health. 2021 Dec 25; 11:04075. doi: 10.7189/jogh.11.04075. PMID: 35003713; PMCID: PMC8719309.

- Ouédraogo N, Kaplon J, Bonkoungou IJ, Traoré AS, Pothier P, Barro N, Ambert-Balay K. Prevalence and Genetic Diversity of Enteric Viruses in Children with Diarrhea in Ouagadougou, Burkina Faso. PLoS One. 2016 Apr 19;11(4): e0153652. doi: 10.1371/journal.pone.0153652. PMID: 27092779; PMCID: PMC4836733.

- Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. JAMA. 2002 Aug 28;288(8):963-72. doi: 10.1001/jama.288.8.963. PMID: 12190366.

- Oyen N, Markestad T, Skaerven R, Irgens LM, Helweg-Larsen K, Alm B, Norvenius G, Wennergren G. Combined effects of sleeping position and prenatal risk factors in sudden infant death syndrome: the Nordic Epidemiological SIDS Study. Pediatrics. 1997 Oct;100(4):613-21. doi: 10.1542/peds.100.4.613. PMID: 9310514.

- Page N, Groome MJ, Murray T, Nadan S, Netshikweta R, Keddy KH, Poonsamy B, Moyes J, Walaza S, Kahn K, Kuonza L, Taylor MB, Madhi SA, Cohen C. Sapovirus prevalence in children less than five years of age

hospitalised for diarrhoeal disease in South Africa, 2009-2013. J Clin Virol. 2016b May; 78:82-8. doi: 10.1016/j.jcv.2016.03.013. Epub 2016 Mar 19. PMID: 27002709.

- Page N, Kruger T, Seheri M, Peenze I, Quan V, Groome M, Madhi S. rotavirus surveillance report, South Africa, 2014-2015: a comparison with previous rotavirus seasons. Commun. Dis. Surv. Bull. 2016a;14:126-36. Available from: https://www.nicd.ac.za/wp-content/uploads/2017/03/CommDisBull-144-November-2016.pdf. (Date Accessed: 10 May 2023).

- Page NA, Groome MJ, Nadan S, Netshikweta R, Keddy KH, Poonsamy B, Moyes J, Walaza S, Kahn K, Madhi SA, Taylor MB, Mans J, Cohen C. Norovirus epidemiology in South African children <5 years hospitalised for diarrhoeal illness between 2009 and 2013. Epidemiol Infect. 2017 Jul;145(9):1942-1952. doi: 10.1017/S0950268817000668. Epub 2017 Apr 10. PMID: 28393756; PMCID: PMC9203319.

- Page NA, Seheri LM, Groome MJ, Moyes J, Walaza S, Mphahlele J, Kahn K, Kapongo CN, Zar HJ, Tempia S, Cohen C, Madhi SA. Temporal association of rotavirus vaccination and genotype circulation in South Africa: Observations from 2002 to 2014. Vaccine. 2018 Nov 12;36(47):7231-7237. doi: 10.1016/j.vaccine.2017.10.062. Epub 2017 Oct 27. PMID: 29110933; PMCID: PMC6497815.

- Page NA, Steele AD. Antigenic and genetic characterization of serotype G2 human rotavirus strains from South Africa from 1984 to 1998. J Med Virol. 2004 Feb;72(2):320-7. doi: 10.1002/jmv.10571. PMID: 14695677.

- Pai M, Kang G, Ramakrishna BS, Venkataraman A, Muliyil J. An epidemic of diarrhoea in south India caused by enteroaggregative Escherichia coli. Indian J Med Res. 1997 Jul; 106:7-12. PMID: 9248208.

- Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. PLoS Biol. 2007 Jul;5(7): e177. doi: 10.1371/journal.pbio.0050177. Epub 2007 Jun 26. PMID: 17594176; PMCID: PMC1896187.

- Palmiere C, Egger C, Prod'Hom G, Greub G. Bacterial Translocation and Sample Contamination in Postmortem Microbiological Analyses. J Forensic Sci. 2016 Mar;61(2):367-374. doi: 10.1111/1556-4029.12991. Epub 2015 Dec 31. PMID: 27404610.

- Palmiere C, Vanhaebost J, Ventura F, Bonsignore A, Bonetti LR. Cerebrospinal fluid PCR analysis and biochemistry in bodies with severe decomposition. J Forensic Leg Med. 2015 Feb;30:21-4. doi: 10.1016/j.jflm.2014.12.012. Epub 2014 Dec 20. PMID: 25623190.

- Panaretto KS, Whitehall JF, McBride G, Patole S, Whitehall JS. Sudden infant death syndrome in Indigenous and non-Indigenous infants in north Queensland, 1990-1998. J Paediatr Child Health. 2002 Apr;38(2):135-9. doi: 10.1046/j.1440-1754.2002.00760. x. PMID: 12030993.

- Pang XL, Honma S, Nakata S, Vesikari T. Human caliciviruses in acute gastroenteritis of young children in the community. J Infect Dis. 2000 May;181 Suppl 2: S288-94. doi: 10.1086/315590. PMID: 10804140.

- Pang XL, Lee BE, Tyrrell GJ, Preiksaitis JK. Epidemiology and genotype analysis of sapovirus associated with gastroenteritis outbreaks in Alberta, Canada: 2004-2007. J Infect Dis. 2009 Feb 15;199(4):547-51. doi: 10.1086/596210. PMID: 19099483.

- Pankovics P, Boros Á, Rovács M, Nagy E, Krisztián E, Vollain M, Reuter G. Humán astrovirus okozta gastroenteritisjárvány első hazai igazolása [First detection of human astrovirus in gastroenteritis outbreak in Hungary]. Orv Hetil. 2011 Jan 9;152(2):45-50. Hungarian. doi: 10.1556/OH.2011.29013. PMID: 21177230.

- Pantoja-Feliciano IG, Clemente JC, Costello EK, Perez ME, Blaser MJ, Knight R, Dominguez-Bello MG. Biphasic assembly of the murine intestinal microbiota during early development. ISME J. 2013 Jun;7(6):1112-5. doi: 10.1038/ismej.2013.15. Epub 2013 Mar 28. PMID: 23535917; PMCID: PMC3660675.

- Parker EP, Ramani S, Lopman BA, Church JA, Iturriza-Gómara M, Prendergast AJ, Grassly NC. Causes of impaired oral vaccine efficacy in developing countries. Future Microbiol. 2018 Jan;13(1):97-118. doi: 10.2217/fmb-2017-0128. Epub 2017 Dec 8. PMID: 29218997; PMCID: PMC7026772.

- Parsons BD, Zelyas N, Berenger BM, Chui L. Detection, Characterization, and Typing of Shiga Toxin-Producing Escherichia coli. Front Microbiol. 2016 Apr 12; 7:478. doi: 10.3389/fmicb.2016.00478. PMID: 27148176; PMCID: PMC4828450.

- Patel MM, Glass R, Desai R, Tate JE, Parashar UD. Fulfilling the promise of rotavirus vaccines: how far have we come since licensure? Lancet Infect Dis. 2012 Jul;12(7):561-70. doi: 10.1016/S1473-3099(12)70029-4. PMID: 22742639.

- Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinjé J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis. 2008 Aug;14(8):1224-31. doi: 10.3201/eid1408.071114. PMID: 18680645; PMCID: PMC2600393.

- Paterson DS, Trachtenberg FL, Thompson EG, Belliveau RA, Beggs AH, Darnall R, Chadwick AE, Krous HF, Kinney HC. Multiple serotonergic brainstem abnormalities in sudden infant death syndrome. JAMA. 2006 Nov 1;296(17):2124-32. doi: 10.1001/jama.296.17.2124. PMID: 17077377.

- Pavlovic M, Luze A, Konrad R, Berger A, Sing A, Busch U, Huber I. Development of a duplex real-time PCR for differentiation between E. coli and Shigella spp. J Appl Microbiol. 2011 May;110(5):1245-51. doi: 10.1111/j.1365-2672.2011.04973.x. Epub 2011 Mar 14. PMID: 21332893.

- Payne DC, Edwards KM, Bowen MD, Keckley E, Peters J, Esona MD, Teel EN, Kent D, Parashar UD, Gentsch JR. Sibling transmission of vaccine-derived rotavirus (RotaTeq) associated with rotavirus gastroenteritis. Pediatrics. 2010 Feb;125(2):e438-41. doi: 10.1542/peds.2009-1901. Epub 2010 Jan 25. PMID: 20100758.

- Payne DC, Parashar UD, Lopman BA. Developments in understanding acquired immunity and innate susceptibility to norovirus and rotavirus gastroenteritis in children. Curr Opin Pediatr. 2015 Feb;27(1):105-9. doi: 10.1097/MOP.0000000000000166. PMID: 25490691; PMCID: PMC4618547.

- Payne DC, Vinjé J, Szilagyi PG, Edwards KM, Staat MA, Weinberg GA, Hall CB, Chappell J, Bernstein DI, Curns AT, Wikswo M, Shirley SH, Hall AJ, Lopman B, Parashar UD. Norovirus and medically attended gastroenteritis in U.S. children. N Engl J Med. 2013 Mar 21;368(12):1121-30. doi: 10.1056/NEJMsa1206589. PMID: 23514289; PMCID: PMC4618551.

- Pearce JL, Bettelheim KA, Luke RK, Goldwater PN. Serotypes of Escherichia coli in sudden infant death syndrome. J Appl Microbiol. 2010 Feb;108(2):731-5. doi: 10.1111/j.1365-2672.2009.04473. x. Epub 2009 Jul 13. PMID: 19674183.

- Pearce JL, Luke RK, Bettelheim KA. Infection and food: a factor in sudden infant death syndrome? FEMS Immunol Med Microbiol. 2004 Sep 1;42(1):66-75. doi: 10.1016/j.femsim.2004.06.017. PMID: 15325399.

- Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, Stobberingh EE. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006 Aug;118(2):511-21. doi: 10.1542/peds.2005-2824. PMID: 16882802.

- Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr. 2009 Sep;139(9):1619-25. doi: 10.3945/jn.109.104638. Epub 2009 Jul 22. PMID: 19625695; PMCID: PMC2728689.

- Peng Z, Ling L, Stratton CW, Li C, Polage CR, Wu B, Tang YW. Advances in the diagnosis and treatment of Clostridium difficile infections. Emerg Microbes Infect. 2018 Feb 7;7(1):15. doi: 10.1038/s41426-017-0019-4. PMID: 29434201; PMCID: PMC5837143.

- Petriz BA, Franco OL. Metaproteomics as a Complementary Approach to Gut Microbiota in Health and Disease. Front Chem. 2017 Jan 26; 5:4. doi: 10.3389/fchem.2017.00004. PMID: 28184370; PMCID: PMC5266679.

- Pettengill EA, Pettengill JB, Binet R. Phylogenetic Analyses of Shigella and Enteroinvasive Escherichia coli for the Identification of Molecular Epidemiological Markers: Whole-Genome Comparative Analysis Does Not Support Distinct Genera Designation. Front Microbiol. 2016 Jan 19;6:1573. doi: 10.3389/fmicb.2015.01573. Erratum in: Front Microbiol. 2018 Jan 09;8:2598. PMID: 26834722; PMCID: PMC4718091.

- Phan TG, Nordgren J, Ouermi D, Simpore J, Nitiema LW, Deng X, Delwart E. New astrovirus in human feces from Burkina Faso. J Clin Virol. 2014 Jun;60(2):161-4. doi: 10.1016/j.jcv.2014.03.024. Epub 2014 Apr 13. PMID: 24775733; PMCID: PMC4047031.

- Phua KB, Quak SH, Lee BW, Emmanuel SC, Goh P, Han HH, De Vos B, Bock HL. Evaluation of RIX4414, a live, attenuated rotavirus vaccine, in a randomized, double-blind, placebo-controlled phase 2 trial involving 2464 Singaporean infants. J Infect Dis. 2005 Sep 1;192 Suppl 1:S6-S16. doi: 10.1086/431511. PMID: 16088807.

- Piacentini G, Peroni D, Bessi E, Morelli L. Molecular characterization of intestinal microbiota in infants fed with soymilk. J Pediatr Gastroenterol Nutr. 2010 Jul;51(1):71-6. doi: 10.1097/MPG.0b013e3181dc8b02. PMID: 20543718.

- Platts-Mills JA, Babji S, Bodhidatta L, Gratz J, Haque R, Havt A, McCormick BJ, McGrath M, Olortegui MP, Samie A, Shakoor S, Mondal D, Lima IF, Hariraju D, Rayamajhi BB, Qureshi S, Kabir F, Yori PP, Mufamadi B, Amour C, Carreon JD, Richard SA, Lang D, Bessong P, Mduma E, Ahmed T, Lima AA, Mason CJ, Zaidi AK, Bhutta ZA, Kosek M, Guerrant RL, Gottlieb M, Miller M, Kang G, Houpt ER; MAL-ED Network Investigators. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health. 2015 Sep;3(9): e564-75. doi: 10.1016/S2214-109X (15)00151-5. Epub 2015 Jul 19. PMID: 26202075; PMCID: PMC7328884.

- Platts-Mills JA, Liu J, Rogawski ET, Kabir F, Lertsethtakarn P, Siguas M, Khan SS, Praharaj I, Murei A, Nshama R, Mujaga B, Havt A, Maciel IA, McMurry TL, Operario DJ, Taniuchi M, Gratz J, Stroup SE, Roberts JH, Kalam A, Aziz F, Qureshi S, Islam MO, Sakpaisal P, Silapong S, Yori PP, Rajendiran R, Benny B, McGrath M, McCormick BJJ, Seidman JC, Lang D, Gottlieb M, Guerrant RL, Lima AAM, Leite JP, Samie A, Bessong PO, Page N, Bodhidatta L, Mason C, Shrestha S, Kiwelu I, Mduma ER, Iqbal NT, Bhutta ZA, Ahmed T, Haque R, Kang G, Kosek MN, Houpt ER; MAL-ED Network Investigators. Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhoea in children in low-resource settings: a reanalysis of the MAL-ED cohort study. Lancet Glob Health. 2018 Dec;6(12): e1309-e1318. doi: 10.1016/S2214-109X (18)30349-8. Epub 2018 Oct 1. PMID: 30287127; PMCID: PMC6227251.

- Pongsuwanna Y, Tacharoenmuang R, Prapanpoj M, Sakon N, Komoto S, Guntapong R, Taniguchi K. Monthly Distribution of Norovirus and Sapovirus Causing Viral Gastroenteritis in Thailand. Jpn J Infect Dis. 2017 Jan 24;70(1):84-86. doi: 10.7883/yoken.JJID.2015.440. Epub 2016 Mar 18. PMID: 27000453.

- Ponsonby AL, Dwyer T, Gibbons LE, Cochrane JA, Wang YG. Factors potentiating the risk of sudden infant death syndrome associated with the prone position. N Engl J Med. 1993 Aug 5;329(6):377-82. doi: 10.1056/NEJM199308053290601. PMID: 8326970.

- Poroyko V, Morowitz M, Bell T, Ulanov A, Wang M, Donovan S, Bao N, Gu S, Hong L, Alverdy JC, Bergelson J, Liu DC. Diet creates metabolic niches in the "immature gut" that shape microbial communities. Nutr Hosp. 2011 Nov-Dec;26(6):1283-95. doi: 10.1590/S0212-16112011000600015. PMID: 22411374.

- Potgieter N, Heine L, Ngandu JPK, Ledwaba SE, Zitha T, Mudau LS, Becker P, Traore AN, Barnard TG. High Burden of Co-Infection with Multiple Enteric Pathogens in Children Suffering with Diarrhoea from Rural and Peri-Urban Communities in South Africa. Pathogens. 2023 Feb 14;12(2):315. doi: 10.3390/pathogens12020315. PMID: 36839587; PMCID: PMC9959912.

- Power SE, O'Toole PW, Stanton C, Ross RP, Fitzgerald GF. Intestinal microbiota, diet and health. Br J Nutr. 2014 Feb;111(3):387-402. doi: 10.1017/S0007114513002560. Epub 2013 Aug 12. PMID: 23931069.

- Praharaj I, Platts-Mills JA, Taneja S, Antony K, Yuhas K, Flores J, Cho I, Bhandari N, Revathy R, Bavdekar A, Rongsen-Chandola T, McMurry T, Houpt ER, Kang G. Diarrheal Etiology and Impact of Coinfections on Rotavirus Vaccine Efficacy Estimates in a Clinical Trial of a Monovalent Human-Bovine (116E) Oral Rotavirus Vaccine, Rotavac, India. Clin Infect Dis. 2019 Jul 2;69(2):243-250. doi: 10.1093/cid/ciy896. PMID: 30335135; PMCID: PMC6603264.

- Prasad BV, Chiu W. Structure of rotavirus. Curr Top Microbiol Immunol. 1994; 185:9-29. doi: 10.1007/978-3-642-78256-5_2. PMID: 8050286.

- Prescott SL. Early-life environmental determinants of allergic diseases and the wider pandemic of inflammatory noncommunicable diseases. J Allergy Clin Immunol. 2013 Jan;131(1):23-30. doi: 10.1016/j.jaci.2012.11.019. PMID: 23265694.

- Presterl E, Zwick RH, Reichmann S, Aichelburg A, Winkler S, Kremsner PG, Graninger W. Frequency and virulence properties of diarrheagenic Escherichia coli in children with diarrhea in Gabon. Am J Trop Med Hyg. 2003 Oct;69(4):406-10. PMID: 14640501.

- Primo D, Pacheco GT, Timenetsky MDCST, Luchs A. Surveillance and molecular characterization of human adenovirus in patients with acute gastroenteritis in the era of rotavirus vaccine, Brazil, 2012-2017. J Clin Virol. 2018 Dec; 109:35-40. doi: 10.1016/j.jcv.2018.10.010. Epub 2018 Nov 1. PMID: 30399502.

- Probert WS, Miller GM, Ledin KE. Contaminated Stream Water as Source for Escherichia coli O157 Illness in Children. Emerg Infect Dis. 2017 Jul;23(7):1216-1218. doi: 10.3201/eid2307.170226. PMID: 28628436; PMCID: PMC5512484.

- Pryce JW, Weber MA, Hartley JC, Ashworth MT, Malone M, Sebire NJ. Difficulties in interpretation of post-mortem microbiology results in unexpected infant death: evidence from a multidisciplinary survey. J Clin Pathol. 2011 Aug;64(8):706-10. doi: 10.1136/jclinpath-2011-200056. Epub 2011 May 10. PMID: 21561893.

- Qadri F, Saha A, Ahmed T, Al Tarique A, Begum YA, Svennerholm AM. Disease burden due to enterotoxigenic Escherichia coli in the first 2 years of life in an urban community in Bangladesh. Infect Immun. 2007 Aug;75(8):3961-8. doi: 10.1128/IAI.00459-07. Epub 2007 Jun 4. PMID: 17548483; PMCID: PMC1951985.

- Qadri F, Svennerholm AM, Faruque AS, Sack RB. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin Microbiol Rev. 2005 Jul;18(3):465-83. doi: 10.1128/CMR.18.3.465-483.2005. PMID: 16020685; PMCID: PMC1195967.

- Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J; MetaHIT Consortium; Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010 Mar 4;464(7285):59-65. doi: 10.1038/nature08821. PMID: 20203603; PMCID: PMC3779803.

- Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R,

Pedersen O, Kristiansen K, Wang J. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012 Oct 4;490(7418):55-60. doi: 10.1038/nature11450. Epub 2012 Sep 26. PMID: 23023125.

- Qiu FZ, Shen XX, Li GX, Zhao L, Chen C, Duan SX, Guo JY, Zhao MC, Yan TF, Qi JJ, Wang L, Feng ZS, Ma XJ. Adenovirus associated with acute diarrhea: a case-control study. BMC Infect Dis. 2018 Sep 3;18(1):450. doi: 10.1186/s12879-018-3340-1. PMID: 30176819; PMCID: PMC6122197.

- Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. Nat Biotechnol. 2017 Sep 12;35(9):833-844. doi: 10.1038/nbt.3935. Erratum in: Nat Biotechnol. 2017 Dec 8;35(12):1211. PMID: 28898207.

- Rahman M, Matthijnssens J, Yang X, Delbeke T, Arijs I, Taniguchi K, Iturriza-Gómara M, Iftekharuddin N, Azim T, Van Ranst M. Evolutionary history and global spread of the emerging g12 human rotaviruses. J Virol. 2007 Mar;81(5):2382-90. doi: 10.1128/JVI.01622-06. Epub 2006 Dec 13. PMID: 17166908; PMCID: PMC1865926.

- Rajendran P, Ajjampur SS, Chidambaram D, Chandrabose G, Thangaraj B, Sarkar R, Samuel P, Rajan DP, Kang G. Pathotypes of diarrheagenic Escherichia coli in children attending a tertiary care hospital in South India. Diagn Microbiol Infect Dis. 2010 Oct;68(2):117-22. doi: 10.1016/j.diagmicrobio.2010.06.003. PMID: 20846583; PMCID: PMC3918514.

- Rajeshwari K, Beena U, Singh R, Tiwari G, Ajay KM. Multi drug resistant enteropathogenic *E. coli* diarrhea in children. Am J Res Commun. 2015;3(9):27-48.

- Rajilić-Stojanović M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev. 2014 Sep;38(5):996-1047. doi: 10.1111/1574-6976.12075. Epub 2014 Jun 27. PMID: 24861948; PMCID: PMC4262072.

- Ramani S, Atmar RL, Estes MK. Epidemiology of human noroviruses and updates on vaccine development. Curr Opin Gastroenterol. 2014 Jan;30(1):25-33. doi: 10.1097/MOG.0000000000000022. PMID: 24232370; PMCID: PMC3955997.

- Rambaut, A. FigTree v1.4.4. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. 2018. Available from: http://tree.bio.ed.ac.uk/software/figtree/. [Date accessed: 2 May 2023].

- Rappelli P, Folgosa E, Solinas ML, Dacosta JL, Pisanu C, Sidat M, Melo J, Cappuccinelli P, Colombo MM. Pathogenic enteric Escherichia coli in children with and without diarrhea in Maputo, Mozambique. FEMS Immunol Med Microbiol. 2005 Jan 1;43(1):67-72. doi: 10.1016/j.femsim.2004.07.006. PMID: 15607638.

- Räsänen S, Lappalainen S, Kaikkonen S, Hämäläinen M, Salminen M, Vesikari T. Mixed viral infections causing acute gastroenteritis in children in a waterborne outbreak. Epidemiol Infect. 2010 Sep;138(9):1227-34. doi: 10.1017/S0950268809991671. Epub 2010 Jan 22. PMID: 20092670.

- Rautava S, Luoto R, Salminen S, Isolauri E. Microbial contact during pregnancy, intestinal colonization and human disease. Nat Rev Gastroenterol Hepatol. 2012 Oct;9(10):565-76. doi: 10.1038/nrgastro.2012.144. Epub 2012 Aug 14. PMID: 22890113.

- Raza MW, Blackwell CC. Sudden infant death syndrome, virus infections and cytokines. FEMS Immunol Med Microbiol. 1999 Aug 1;25(1-2):85-96. doi: 10.1111/j.1574-695X.1999.tb01330.x. PMID: 10443495.

- Read JS, Clemens JD, Klebanoff MA. Moderate low birth weight and infectious disease mortality during infancy and childhood. Am J Epidemiol. 1994 Oct 15;140(8):721-33. doi: 10.1093/oxfordjournals.aje.a117320. PMID: 7942774.

- Reddington K, Tuite N, Minogue E, Barry T. A current overview of commercially available nucleic acid diagnostics approaches to detect and identify human gastroenteritis pathogens. Biomol Detect Quantif. 2014 Aug 14;1(1):3-7. doi: 10.1016/j.bdq.2014.07.001. PMID: 27920992; PMCID: PMC5129431.

- Reidpath DD, Allotey P. Infant mortality rate as an indicator of population health. J Epidemiol Community Health. 2003 May;57(5):344-6. doi: 10.1136/jech.57.5.344. PMID: 12700217; PMCID: PMC1732453.

- Reis TA, Assis AS, do Valle DA, Barletta VH, de Carvalho IP, Rose TL, Portes SA, Leite JP, da Rosa e Silva ML. The role of human adenoviruses type 41 in acute diarrheal disease in Minas Gerais after rotavirus vaccination. Braz J Microbiol. 2016 Jan-Mar;47(1):243-50. doi: 10.1016/j.bjm.2015.11.011. Epub 2016 Jan 27. PMID: 26887251; PMCID: PMC4822746.

- Reyman M, van Houten MA, van Baarle D, Bosch AATM, Man WH, Chu MLJN, Arp K, Watson RL, Sanders EAM, Fuentes S, Bogaert D. Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life. Nat Commun. 2019 Nov 1;10(1):4997. doi: 10.1038/s41467-019-13014-7. Erratum in: Nat Commun. 2019 Nov 25;10(1):5352. PMID: 31676793; PMCID: PMC6825150.

- Riddle MS, Sanders JW, Putnam SD, Tribble DR. Incidence, etiology, and impact of diarrhea among long-term travelers (US military and similar populations): a systematic review. Am J Trop Med Hyg. 2006 May;74(5):891-900. PMID: 16687698.

- Ridge JP, Fuchs EJ, Matzinger P. Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. Science. 1996 Mar 22;271(5256):1723-6. doi: 10.1126/science.271.5256.1723. PMID: 8596932.

- Roach SN, Langlois RA. Intra- and Cross-Species Transmission of Astroviruses. Viruses. 2021 Jun 11;13(6):1127. doi: 10.3390/v13061127. PMID: 34208242; PMCID: PMC8230745.

- Robert Koch Institut. Bericht: Abschließende Darstellung und Bewertung der epidemiologischen Erkenntnisse im EHEC O104: H4 Ausbruch, Deutschland 2011.[Website].2011. Available at: http://edoc.rki.de/documents/rki_ab/reeFNxULvsdZo/PDF/262b4Pk2TGGs.pdf. [Date accessed: 16 July 2023].

- Robins-Browne RM. Traditional enteropathogenic Escherichia coli of infantile diarrhea. Rev Infect Dis. 1987 Jan-Feb;9(1):28-53. doi: 10.1093/clinids/9.1.28. PMID: 3547577.

- Rocha LB, Luz DE, Moraes CT, Caravelli A, Fernandes I, Guth BE, Horton DS, Piazza RM. Interaction between Shiga toxin and monoclonal antibodies: binding characteristics and in vitro neutralizing abilities. Toxins (Basel). 2012 Sep;4(9):729-47. doi: 10.3390/toxins4090729. Epub 2012 Sep 18. PMID: 23105978; PMCID: PMC3475226.

- Rocha LB, Piazza RM. Production of Shiga toxin by Shiga toxin-expressing Escherichia coli (STEC) in broth media: from divergence to definition. Lett Appl Microbiol. 2007 Oct;45(4):411-7. doi: 10.1111/j.1472-765X.2007.02214. x. PMID: 17897384.

- Roczo-Farkas S, Kirkwood CD, Cowley D, Barnes GL, Bishop RF, Bogdanovic-Sakran N, Boniface K, Donato CM, Bines JE. The Impact of Rotavirus Vaccines on Genotype Diversity: A Comprehensive Analysis of 2 Decades of Australian Surveillance Data. J Infect Dis. 2018 Jul 13;218(4):546-554. doi: 10.1093/infdis/jiy197. Erratum in: J Infect Dis. 2019 Mar 15;219(7):1172. PMID: 29790933.

- Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, Avershina E, Rudi K, Narbad A, Jenmalm MC, Marchesi JR, Collado MC. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis. 2015 Feb 2; 26:26050. doi: 10.3402/mehd. v26.26050. PMID: 25651996; PMCID: PMC4315782.

- Rognum TO, Saugstad OD. Biochemical and immunological studies in SIDS victims. Clues to understanding the death mechanism. Acta Paediatr Suppl. 1993 Jun;82 Suppl 389:82-5. doi: 10.1111/j.1651-2227. 1993.tb12886.x. PMID: 8374202.

- Rohayem J, Berger S, Juretzek T, Herchenröder O, Mogel M, Poppe M, Henker J, Rethwilm A. A simple and rapid single-step multiplex RT-PCR to detect Norovirus, Astrovirus and Adenovirus in clinical stool samples. J Virol Methods. 2004 Jun 1;118(1):49-59. doi: 10.1016/j.jviromet.2004.01.016. PMID: 15158068.

- Romani S, Azimzadeh P, Mohebbi SR, Bozorgi SM, Zali N, Jadali F. Prevalence of sapovirus infection among infant and adult patients with acute gastroenteritis in Tehran, Iran. Gastroenterol Hepatol Bed Bench. 2012 Winter;5(1):43-8. PMID: 24834197; PMCID: PMC4017447.

- Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nat Rev Immunol. 2016 May 27;16(6):341-52. doi: 10.1038/nri.2016.42. PMID: 27231050; PMCID: PMC5541232.

- Rossouw E, Brauer M, Meyer P, du Plessis NM, Avenant T, Mans J. Virus Etiology, Diversity and Clinical Characteristics in South African Children Hospitalised with Gastroenteritis. Viruses. 2021 Jan 30;13(2):215. doi: 10.3390/v13020215. PMID: 33573340; PMCID: PMC7911269.

- Rossouw E. The molecular epidemiology and diversity of gastroenteritis viruses in HIV- infected, -exposed and -unexposed children under the age of five years in Pretoria, South Africa. [Master's Dissertation]. Pretoria: University of Pretoria; 2020. Available from: https://repository.up.ac.za/handle/2263/75468.

- Roussel C, Cordonnier C, Livrelli V, Van de Wiele T, Blanquet-Diot S. Enterotoxigenic and enterohemorrhagic Escherichia coli: Survival and modulation of virulence in the human gastrointestinal tract. Escherichia coli-Recent Advances on Physiology, Pathogenesis and Biotechnological Applications. [Website]. 2017, London: Intech. Available from: https://www.intechopen.com/chapters/54926 doi: 10.5772/intechopen.68309. [Date accessed: 21 Apr 2022].

- Rowe B, Gross RJ, Woodroof DP. Proposal to recognise seroovar 145/46 (synonyms: 147, Shigella 13, Shigella sofia, and Shigella manolovii) as a New Escherichia coli O group, O164. International Journal of Systematic and Evolutionary Microbiology. 1977 Jan;27(1):15-8. Doi.org/10.1099/00207713-27-1-15.

- Röytiö H, Mokkala K, Vahlberg T, Laitinen K. Dietary intake of fat and fibre according to reference values relates to higher gut microbiota richness in overweight pregnant women. Br J Nutr. 2017 Sep;118(5):343-352. doi: 10.1017/S0007114517002100. Erratum in: Br J Nutr. 2018 Sep;120(5):599-600. PMID: 28901891.

- Royuela E, Negredo A, Sánchez-Fauquier A. Development of a one-step real-time RT-PCR method for sensitive detection of human astrovirus. J Virol Methods. 2006 Apr;133(1):14-9. doi: 10.1016/j.jviromet.2005.10.012. PMID: 16321452.

- Rubino S, Cappuccinelli P, Kelvin DJ. Escherichia coli (STEC) serotype O104 outbreak causing haemolytic syndrome (HUS) in Germany and France. J Infect Dev Ctries. 2011 Jul 4;5(6):437-40. doi: 10.3855/jidc.2172. PMID: 21727641.

- Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, Cheuvart B, Espinoza F, Gillard P, Innis BL, Cervantes Y, Linhares AC, López P, Macías-Parra M, Ortega-Barría E, Richardson V, Rivera-Medina DM, Rivera L, Salinas B, Pavía-Ruz N, Salmerón J, Rüttimann R, Tinoco JC, Rubio P, Nuñez E, Guerrero ML, Yarzábal JP, Damaso S, Tornieporth N, Sáez-Llorens X, Vergara RF, Vesikari T, Bouckenooghe A, Clemens R, De Vos B, O'Ryan M; Human Rotavirus Vaccine Study Group. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med. 2006 Jan 5;354(1):11-22. doi: 10.1056/NEJMoa052434. PMID: 16394298.

- Rusen ID, Liston R, Wen SW, Bartholomew S. Special Report on Maternal Mortality and Severe Morbidity in Canada. Enhanced Surveillance. The Path to Prevention. Health Canda. Canadian Perinatal Surveillance System. [Website]. 2004. Available at http://publications.gc.ca/Collection/H39-4-44-2004E.pdf. [Date accessed: 21 February 2019].

- Rutayisire E, Huang K, Liu Y, Tao F. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. BMC Gastroenterol. 2016 Jul 30;16(1):86. doi: 10.1186/s12876-016-0498-0. PMID: 27475754; PMCID: PMC4967522.

- Sabey KA, Song SJ, Jolles A, Knight R, Ezenwa VO. Coinfection and infection duration shape how pathogens affect the African buffalo gut microbiota. ISME J. 2021 May;15(5):1359-1371. doi: 10.1038/s41396-020-00855-0. Epub 2020 Dec 17. PMID: 33328653; PMCID: PMC8115229.

- Sack RB. Diarrhea producing factors in cultures of Escherichia coli. Proceedings of the 4th Joint Conference, Japan-US Cooperative Medical Science Program. 1968, 23-25.

- Sadeharju K, Knip M, Virtanen SM, Savilahti E, Tauriainen S, Koskela P, Akerblom HK, Hyöty H; Finnish TRIGR Study Group. Maternal antibodies in breast milk protect the child from enterovirus infections. Pediatrics. 2007 May;119(5):941-6. doi: 10.1542/peds.2006-0780. PMID: 17473095.

- Saeed A, Abd H, Sandstrom G. Microbial aetiology of acute diarrhoea in children under five years of age in Khartoum, Sudan. J Med Microbiol. 2015 Apr;64(Pt 4):432-437. doi: 10.1099/jmm.0.000043. Epub 2015 Feb 24. PMID: 25713206; PMCID: PMC4635512.

- Saegeman V, Cohen MC, Alberola J, Ziyade N, Farina C; ESCMID Study Group for Forensic and Postmortem Microbiology; Cornaglia G, Fernández-Rodríguez A. How is post-mortem microbiology appraised by pathologists? Results from a practice survey conducted by ESGFOR. Eur J Clin Microbiol Infect Dis. 2017 Aug;36(8):1381-1385. doi: 10.1007/s10096-017-2943-6. Epub 2017 Feb 24. PMID: 28236029.

- Saegeman V, Cohen MC, Burton JL, Martinez MJ, Rakislova N, Offiah AC, Fernandez-Rodriguez A. Microbiology in minimally invasive autopsy: best techniques to detect infection. ESGFOR (ESCMID study group of forensic and post-mortem microbiology) guidelines. Forensic Sci Med Pathol. 2021 Mar;17(1):87-100. doi: 10.1007/s12024-020-00337-x. Epub 2021 Jan 19. PMID: 33464531; PMCID: PMC7814172.

- Sakai Y, Nakata S, Honma S, Tatsumi M, Numata-Kinoshita K, Chiba S. Clinical severity of Norwalk virus and Sapporo virus gastroenteritis in children in Hokkaido, Japan. Pediatr Infect Dis J. 2001 Sep;20(9):849-53. doi: 10.1097/00006454-200109000-00005. PMID: 11734762.

- Sakazaki R, Tamura K, Saito M. Enteropathogenic Escherichia coli associated with diarrhea in children and adults. Jpn J Med Sci Biol. 1967 Oct;20(5):387-99. doi: 10.7883/yoken1952.20.387. PMID: 4866680.

- Salli K, Anglenius H, Hirvonen J, Hibberd AA, Ahonen I, Saarinen MT, Tiihonen K, Maukonen J, Ouwehand AC. The effect of 2'-fucosyllactose on simulated infant gut microbiome and metabolites; a pilot study in comparison to GOS and lactose. Sci Rep. 2019 Sep 13;9(1):13232. doi: 10.1038/s41598-019-49497-z. PMID: 31520068; PMCID: PMC6744565.

- Salvadori M, Bertoni E. Update on hemolytic uremic syndrome: Diagnostic and therapeutic recommendations. World J Nephrol. 2013 Aug 6;2(3):56-76. doi: 10.5527/wjn. v2.i3.56. PMID: 24255888; PMCID: PMC3832913.

- Sánchez GJ, Mayta H, Pajuelo MJ, Neira K, Xiaofang L, Cabrera L, Ballard SB, Crabtree JE, Kelleher D, Cama V, Bern C, Oshitani H, Gilman RH, Saito M; Sapovirus Working Group. Epidemiology of Sapovirus Infections in a Birth Cohort in Peru. Clin Infect Dis. 2018 Jun 1;66(12):1858-1863. doi: 10.1093/cid/cix1103. PMID: 29309577; PMCID: PMC5982808.

- Santona S, Diaz N, Fiori PL, Francisco M, Sidat M, Cappuccinelli P, Rappelli P. Genotypic and phenotypic features of enteropathogenic Escherichia coli isolated in industrialized and developing countries. J Infect Dev Ctries. 2013 Mar 14;7(3):214-9. doi: 10.3855/jidc.3054. PMID: 23492999.

- Santos FS, Sousa Junior EC, Guerra SFS, Lobo PS, Penha Junior ET, Lima ABF, Vinente CBG, Chagas EHN, Justino MCA, Linhares AC, Matthijnssens J, Soares LS, Mascarenhas JDP. G1P[8] Rotavirus in children with severe diarrhea in the post-vaccine introduction era in Brazil: Evidence of reassortments and structural modifications of the antigenic VP7 and VP4 regions. Infect Genet Evol. 2019 Apr;69:255-266. doi: 10.1016/j.meegid.2019.02.009. Epub 2019 Feb 11. PMID: 30763774.

- Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Reviews in medical virology. 2005 Jan;15(1):29-56 . Doi.org/10.1002/rmv.448

- Santos VS, Berezin EN, Gurgel RQ. Rotavirus in Latin America: Current Situation and Perspectives. J Pediatric Infect Dis Soc. 2017 Mar 1;6(1):1-2. doi: 10.1093/jpids/piw071. PMID: 28391328.

- Sarangi AN, Goel A, Aggarwal R. Methods for Studying Gut Microbiota: A Primer for Physicians. J Clin Exp Hepatol. 2019 Jan-Feb;9(1):62-73. doi: 10.1016/j.jceh.2018.04.016. Epub 2018 May 4. PMID: 30774267; PMCID: PMC6363981.

- Sarzotti M, Robbins DS, Hoffman PM. Induction of protective CTL responses in newborn mice by a murine retrovirus. Science. 1996 Mar 22;271(5256):1726-8. doi: 10.1126/science.271.5256.1726. PMID: 8596933.

- Sassi HP, Sifuentes LY, Koenig DW, Nichols E, Clark-Greuel J, Wong LF, McGrath K, Gerba CP, Reynolds KA. Control of the spread of viruses in a long-term care facility using hygiene protocols. Am J Infect Control. 2015 Jul 1;43(7):702-6. doi: 10.1016/j.ajic.2015.03.012. Epub 2015 May 2. PMID: 25944726

- Satokari R, Grönroos T, Laitinen K, Salminen S, Isolauri E. Bifidobacterium and Lactobacillus DNA in the human placenta. Lett Appl Microbiol. 2009 Jan;48(1):8-12. doi: 10.1111/j.1472-765X.2008.02475. x. Epub 2008 Oct 17. PMID: 19018955.

- Sattar SBA, Singh S. Bacterial Gastroenteritis. 2021 Aug 11. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 30020667.

- Scaletsky IC, Fabbricotti SH, Carvalho RL, Nunes CR, Maranhão HS, Morais MB, Fagundes-Neto U. Diffusely adherent Escherichia coli as a cause of acute diarrhea in young children in Northeast Brazil: a case-control study. J Clin Microbiol. 2002a Feb;40(2):645-8. doi: 10.1128/JCM.40.2.645-648.2002. PMID: 11825986; PMCID: PMC153384.

- Scaletsky IC, Fabbricotti SH, Silva SO, Morais MB, Fagundes-Neto U. HEp-2-adherent Escherichia coli strains associated with acute infantile diarrhea, São Paulo, Brazil. Emerg Infect Dis. 2002b Aug;8(8):855-8. doi: 10.3201/eid0808.010492. PMID: 12141974; PMCID: PMC2732515.

- Scaletsky IC, Pedroso MZ, Oliva CA, Carvalho RL, Morais MB, Fagundes-Neto U. A localized adherence-like pattern as a second pattern of adherence of classic enteropathogenic Escherichia coli to HEp-2 cells that is associated with infantile diarrhea. Infect Immun. 1999 Jul;67(7):3410-5. doi: 10.1128/IAI.67.7.3410-3415.1999. PMID: 10377120; PMCID: PMC116525.

- Scallan E, Mahon BE, Hoekstra RM, Griffin PM. Estimates of illnesses, hospitalizations and deaths caused by major bacterial enteric pathogens in young children in the United States. Pediatr Infect Dis J. 2013 Mar;32(3):217-21. doi: 10.1097/INF.0b013e31827ca763. PMID: 23249909.

- Scavia G, Staffolani M, Fisichella S, Striano G, Colletta S, Ferri G, Escher M, Minelli F, Caprioli A. Enteroaggregative Escherichia coli associated with a foodborne outbreak of gastroenteritis. J Med Microbiol. 2008 Sep;57(Pt 9):1141-1146. doi: 10.1099/jmm.0.2008/001362-0. PMID: 18719185.

- Scheutz F, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sanchez M, Persson S, O'Brien AD. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. J Clin Microbiol. 2012 Sep;50(9):2951-63. doi: 10.1128/JCM.00860-12. Epub 2012 Jul 3. PMID: 22760050; PMCID: PMC3421821.

- Schoendorf KC, Kiely JL. Relationship of sudden infant death syndrome to maternal smoking during and after pregnancy. Pediatrics. 1992 Dec;90(6):905-8. PMID: 1437432.

- Schuffenecker I, Javouhey E, Gillet Y, Kugener B, Billaud G, Floret D, Lina B, Morfin F. Human parechovirus infections, Lyon, France, 2008-10: evidence for severe cases. J Clin Virol. 2012 Aug;54(4):337-41. doi: 10.1016/j.jcv.2012.04.016. Epub 2012 May 17. PMID: 22608363.

- Schwartz S, Friedberg I, Ivanov IV, Davidson LA, Goldsby JS, Dahl DB, Herman D, Wang M, Donovan SM, Chapkin RS. A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response. Genome Biol. 2012 Apr 30;13(4): r32. doi: 10.1186/gb-2012-13-4-r32. PMID: 22546241; PMCID: PMC3446306.

- Scragg R, Mitchell EA, Taylor BJ, Stewart AW, Ford RP, Thompson JM, Allen EM, Becroft DM. Bed sharing, smoking, and alcohol in the sudden infant death syndrome. New Zealand Cot Death Study Group. BMJ. 1993 Nov 20;307(6915):1312-8. doi: 10.1136/bmj.307.6915.1312. PMID: 8257885; PMCID: PMC1679405.

- Seheri LM, Magagula NB, Peenze I, Rakau K, Ndadza A, Mwenda JM, Weldegebriel G, Steele AD, Mphahlele MJ. Rotavirus strain diversity in Eastern and Southern African countries before and after vaccine introduction. Vaccine. 2018 Nov 12;36(47):7222-7230. doi: 10.1016/j.vaccine.2017.11.068. Epub 2017 Dec 2. PMID: 29203181.

- Seheri M, Nemarude L, Peenze I, Netshifhefhe L, Nyaga MM, Ngobeni HG, Maphalala G, Maake LL, Steele AD, Mwenda JM, Mphahlele JM. Update of rotavirus strains circulating in Africa from 2007 through 2011. Pediatr Infect Dis J. 2014 Jan;33 Suppl 1: S76-84. doi: 10.1097/INF.0000000000000053. PMID: 24343619.

- Séreny B. Biochemical reactions and virulence of E. Coli O124: K72 (17). Acta Microbiol Acad Sci Hung. 1963;10:11-8. PMID: 14043490.

- Servin AL. Pathogenesis of human diffusely adhering Escherichia coli expressing Afa/Dr adhesins (Afa/Dr DAEC): current insights and future challenges. Clin Microbiol Rev. 2014 Oct;27(4):823-69. doi: 10.1128/CMR.00036-14. PMID: 25278576; PMCID: PMC4187631.

- Shah N, DuPont HL, Ramsey DJ. Global etiology of travelers' diarrhea: systematic review from 1973 to the present. Am J Trop Med Hyg. 2009 Apr;80(4):609-14. PMID: 19346386.

- Shah T, Sullivan K, Carter J. Sudden infant death syndrome and reported maternal smoking during pregnancy. Am J Public Health. 2006 Oct;96(10):1757-9. doi: 10.2105/AJPH.2005.073213. PMID: 17008569; PMCID: PMC1586150.

- Shaheen HI, Khalil SB, Rao MR, Abu Elyazeed R, Wierzba TF, Peruski LF Jr, Putnam S, Navarro A, Morsy BZ, Cravioto A, Clemens JD, Svennerholm AM, Savarino SJ. Phenotypic profiles of enterotoxigenic Escherichia coli associated with early childhood diarrhea in rural Egypt. J Clin Microbiol. 2004 Dec;42(12):5588-95. doi: 10.1128/JCM.42.12.5588-5595.2004. PMID: 15583286; PMCID: PMC535259.

- Shapiro-Mendoza CK, Tomashek KM, Anderson RN, Wingo J. Recent national trends in sudden, unexpected infant deaths: more evidence supporting a change in classification or reporting. Am J Epidemiol. 2006 Apr 15;163(8):762-9. doi: 10.1093/aje/kwj117. PMID: 16582034.

- Sharapov UM, Wendel AM, Davis JP, Keene WE, Farrar J, Sodha S, Hyytia-Trees E, Leeper M, Gerner-Smidt P, Griffin PM, Braden C; OUTBREAK INVESTIGATION TEAM. Multistate Outbreak of Escherichia coli O157:H7 Infections Associated with Consumption of Fresh Spinach: United States, 2006. J Food Prot. 2016 Dec;79(12):2024-2030. doi: 10.4315/0362-028X.JFP-15-556. PMID: 28221950.

- Shepherd KL, Yiallourou SR, Odoi A, Yeomans E, Willis S, Horne RSC, Wong FY. When does prone sleeping improve cardiorespiratory status in preterm infants in the NICU? Sleep. 2020 Apr 15;43(4):zsz256. doi: 10.1093/sleep/zsz256. PMID: 31691829.

- Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. Curr Opin Gastroenterol. 2015 Jan;31(1):69-75. doi: 10.1097/MOG.0000000000000139. PMID: 25394236; PMCID: PMC4290017.

- Shrivastava AK, Kumar S, Mohakud NK, Suar M, Sahu PS. Multiple etiologies of infectious diarrhea and concurrent infections in a pediatric outpatient-based screening study in Odisha, India. Gut Pathog. 2017 Apr 11; 9:16. doi: 10.1186/s13099-017-0166-0. PMID: 28400860; PMCID: PMC5387278.

- Siebenga JJ, Vennema H, Zheng DP, Vinjé J, Lee BE, Pang XL, Ho EC, Lim W, Choudekar A, Broor S, Halperin T, Rasool NB, Hewitt J, Greening GE, Jin M, Duan ZJ, Lucero Y, O'Ryan M, Hoehne M, Schreier E, Ratcliff RM, White PA, Iritani N, Reuter G, Koopmans M. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001-2007. J Infect Dis. 2009 Sep 1;200(5):802-12. doi: 10.1086/605127. PMID: 19627248.

- Silva PA, Stark K, Mockenhaupt FP, Reither K, Weitzel T, Ignatius R, Saad E, Seidu-Korkor A, Bienzle U, Schreier E. Molecular characterization of enteric viral agents from children in northern region of Ghana. J Med Virol. 2008 Oct;80(10):1790-8. doi: 10.1002/jmv.21231. PMID: 18712819.

- Silva RM, Toledo MR, Trabulsi LR. Biochemical and cultural characteristics of invasive Escherichia coli. J Clin Microbiol. 1980 May;11(5):441-4. doi: 10.1128/jcm.11.5.441-444.1980. PMID: 6991526; PMCID: PMC273427.

- Simmonds MK, Armah G, Asmah R, Banerjee I, Damanka S, Esona M, Gentsch JR, Gray JJ, Kirkwood C, Page N, Iturriza-Gómara M. New oligonucleotide primers for P-typing of rotavirus strains: Strategies for typing previously

untypeable strains. J Clin Virol. 2008 Aug;42(4):368-73. doi: 10.1016/j.jcv.2008.02.011. Epub 2008 Apr 2. PMID: 18378188.

- Singh AP. Genomic Techniques Used to Investigate the Human Gut Microbiota. Human Microbiome. Edited by: Beloborodova NV, Grechko AV. [Website]. 2021, London: IntechOpen; 2021 Available from: https://www.intechopen.com/chapters/71502 doi: 10.5772/intechopen.91808. [Date accessed: 13 May 2022].

- Sjögren YM, Jenmalm MC, Böttcher MF, Björkstén B, Sverremark-Ekström E. Altered early infant gut microbiota in children developing allergy up to 5 years of age. Clin Exp Allergy. 2009 Apr;39(4):518-26. doi: 10.1111/j.1365-2222.2008.03156. x. Epub 2009 Feb 9. PMID: 19220322.

- Skonieczna-Żydecka K, Marlicz W, Misera A, Koulaouzidis A, Łoniewski I. Microbiome-The Missing Link in the Gut-Brain Axis: Focus on Its Role in Gastrointestinal and Mental Health. J Clin Med. 2018 Dec 7;7(12):521. doi: 10.3390/jcm7120521. PMID: 30544486; PMCID: PMC6306769.

- Smith AM, Tau NP, Kalule BJ, Nicol MP, McCulloch M, Jacobs CA, McCarthy KM, Ismail A, Allam M, Kleynhans J. Shiga toxin-producing Escherichia coli O26:H11 associated with a cluster of haemolytic uraemic syndrome cases in South Africa, 2017. Access Microbiol. 2019 Sep 12;1(9):e000061. doi: 10.1099/acmi.0.000061. PMID: 32974561; PMCID: PMC7472548.

- Søvik S, Lossius K, Walløe L. Heart rate response to transient chemoreceptor stimulation in term infants is modified by exposure to maternal smoking. Pediatr Res. 2001 Apr;49(4):558-65. doi: 10.1203/00006450-200104000-00019. PMID: 11264441.

- Spano LC, Sadovsky ADI, Segui PN, Saick KW, Kitagawa SMS, Pereira FEL, Fagundes-Neto U, Scaletsky ICA. Age-specific prevalence of diffusely adherent Escherichia coli in Brazilian children with acute diarrhoea. J Med Microbiol. 2008 Mar;57(Pt 3):359-363. doi: 10.1099/jmm.0.47660-0. PMID: 18287300.

- Squire SA, Ryan U. Cryptosporidium and Giardia in Africa: current and future challenges. Parasit Vectors. 2017 Apr 20;10(1):195. doi: 10.1186/s13071-017-2111-y. PMID: 28427454; PMCID: PMC5397716.

- Stark PL, Lee A. The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. J Med Microbiol. 1982 May;15(2):189-203. doi: 10.1099/00222615-15-2-189. PMID: 7143428.

- Statistics South Africa. Mortality and causes of death in South Africa: Findings from death notification. [Website]. 2018a. Available from: https://www.statssa.gov.za/publications/P03093/P030932018.pdf. [Date accessed: 28 February 2022].

- Statistics South Africa. Tourism and migration: December 2017. [Website].2018b. Available from: /http://www.statssa.gov.za/publications/P0351/P0351December2017.pdf. [Date accessed: 13 May 2023].

- Steel JH, Malatos S, Kennea N, Edwards AD, Miles L, Duggan P, Reynolds PR, Feldman RG, Sullivan MH. Bacteria and inflammatory cells in fetal membranes do not always cause preterm labor. Pediatr Res. 2005 Mar;57(3):404-11. doi: 10.1203/01.PDR.0000153869.96337.90. Epub 2005 Jan 19. PMID: 15659699.

- Steele AD, Groome MJ. Measuring Rotavirus Vaccine Impact in Sub-Saharan Africa. Clin Infect Dis. 2020 May 23;70(11):2314-2316. doi: 10.1093/cid/ciz918. PMID: 31544209; PMCID: PMC7245150.

- Steele AD, Peenze I, de Beer MC, Pager CT, Yeats J, Potgieter N, Ramsaroop U, Page NA, Mitchell JO, Geyer A, Bos P, Alexander JJ. Anticipating rotavirus vaccines: epidemiology and surveillance of rotavirus in South Africa. Vaccine. 2003 Jan 17;21(5-6):354-60. doi: 10.1016/s0264-410x (02)00615-1. PMID: 12531632.

- Steffen R, Hill DR, DuPont HL. Traveler's diarrhea: a clinical review. JAMA. 2015 Jan 6;313(1):71-80. doi: 10.1001/jama.2014.17006. PMID: 25562268.

- Steger CL, Boudreaux CE, LaConte LE, Pease JB, McDonald SM. Group A Rotavirus VP1 Polymerase and VP2 Core Shell Proteins: Intergenotypic Sequence Variation and In Vitro Functional Compatibility. J Virol. 2019 Jan 4;93(2): e01642-18. doi: 10.1128/JVI.01642-18. PMID: 30355692; PMCID: PMC6321916.

- Stewart CJ, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, Ross MC, Lloyd RE, Doddapaneni H, Metcalf GA, Muzny D, Gibbs RA, Vatanen T, Huttenhower C, Xavier RJ, Rewers M, Hagopian W, Toppari J, Ziegler AG, She JX, Akolkar B, Lernmark A, Hyoty H, Vehik K, Krischer JP, Petrosino JF. Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature. 2018 Oct;562(7728):583-588. doi: 10.1038/s41586-018-0617-x. Epub 2018 Oct 24. PMID: 30356187; PMCID: PMC6415775.

- Stiemsma LT, Turvey SE. Asthma and the microbiome: defining the critical window in early life. Allergy Asthma Clin Immunol. 2017 Jan 6; 13:3. doi: 10.1186/s13223-016-0173-6. PMID: 28077947; PMCID: PMC5217603.

- Stinson LF, Payne MS, Keelan JA. A Critical Review of the Bacterial Baptism Hypothesis and the Impact of Cesarean Delivery on the Infant Microbiome. Front Med (Lausanne). 2018 May 4; 5:135. doi: 10.3389/fmed.2018.00135. PMID: 29780807; PMCID: PMC5945806.

- Stokholm J, Thorsen J, Chawes BL, Schjørring S, Krogfelt KA, Bønnelykke K, Bisgaard H. Cesarean section changes neonatal gut colonization. J Allergy Clin Immunol. 2016 Sep;138(3):881-889.e2. doi: 10.1016/j.jaci.2016.01.028. Epub 2016 Apr 1. PMID: 27045582.

- Strandberg-Larsen K, Grønboek M, Andersen AM, Andersen PK, Olsen J. Alcohol drinking pattern during pregnancy and risk of infant mortality. Epidemiology. 2009 Nov;20(6):884-91. doi: 10.1097/EDE.0b013e3181bbd46c. PMID: 19797967.

- Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, Kubo C, Koga Y. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol. 2004 Jul 1;558(Pt 1):263-75. doi: 10.1113/jphysiol.2004.063388. Epub 2004 May 7. PMID: 15133062; PMCID: PMC1664925.

- Swidsinski A, Loening-Baucke V, Verstraelen H, Osowska S, Doerffel Y. Biostructure of fecal microbiota in healthy subjects and patients with chronic idiopathic diarrhea. Gastroenterology. 2008 Aug;135(2):568-79. doi : 10.1053/j.gastro.2008.04.017. Epub 2008 Apr 18. PMID: 18570896.

- Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. Nat Med. 2016 Jul 7;22(7):713-22. doi: 10.1038/nm.4142. PMID: 27387886.

- Tan M. Norovirus Vaccines: Current Clinical Development and Challenges. Pathogens. 2021 Dec 19;10(12):1641. doi: 10.3390/pathogens10121641. PMID: 34959596; PMCID: PMC8709042.

- Tan Y, He WT, Chen MM, Mo JJ, Ju Y, Chen M. An outbreak of human astrovirus lineage 1b in a middle school in Guangxi, Southern China in 2017. Chin Med J (Engl). 2019 Feb 5;132(3):336-338. doi: 10.1097/CM9.0000000000000072. PMID: 30681500; PMCID: PMC6595803.

- Tanih NF, Samie A, Nyathi E, Barrett L, Guerrant R, Bessong P. Prevalence of diarrheagenic Escherichia coli in young children from rural South Africa: The Mal-ED cohort. International Journal of Infectious Diseases. 2014 Apr 1; 21:149. doi: 10.1016/j.ijid.2014.03.735.

- Tappin D, Ecob R, Brooke H. Bedsharing, roomsharing, and sudden infant death syndrome in Scotland: a case-control study. J Pediatr. 2005 Jul;147(1):32-7. doi: 10.1016/j.jpeds.2005.01.035. PMID: 16027691.

- Tau NP, Smith AM, Sooka A, Keddy KH, For The Group For Enteric Respiratory And Meningeal Disease Surveillance In South Africa Germs-Sa. Molecular characterization of extended-spectrum β-lactamase-producing Shigella isolates from humans in South Africa, 2003-2009. J Med Microbiol. 2012 Jan;61(Pt 1):162-164. doi: 10.1099/jmm.0.033142-0. Epub 2011 Sep 8. PMID: 21903820.

- Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal Escherichia coli. Nat Rev Microbiol. 2010 Mar;8(3):207-17. doi: 10.1038/nrmicro2298. PMID: 20157339.

- Tennant SM, Tauschek M, Azzopardi K, Bigham A, Bennett-Wood V, Hartland EL, Qi W, Whittam TS, Robins-Browne RM. Characterisation of atypical enteropathogenic *E. coli* strains of clinical origin. BMC Microbiol. 2009 Jun 3; 9:117. doi: 10.1186/1471-2180-9-117. PMID: 19490652; PMCID: PMC2700815.

- Thanh HD, Tran VT, Lim I, Kim W. Emergence of Human G2P[4] Rotaviruses in the Post-vaccination Era in South Korea: Footprints of Multiple Interspecies Re-assortment Events. Sci Rep. 2018 Apr 16;8(1):6011. doi: 10.1038/s41598-018-24511-y. PMID: 29662148; PMCID: PMC5902508.

- Thomas JP, Raine T, Reddy S, Belteki G. Probiotics for the prevention of necrotising enterocolitis in very low-birth-weight infants: a meta-analysis and systematic review. Acta Paediatr. 2017 Nov;106(11):1729-1741. doi: 10.1111/apa.13902. Epub 2017 Jun 9. PMID: 28471478.

- Titford M. The long history of hematoxylin. Biotech Histochem. 2005 Mar-Apr;80(2):73-8. doi: 10.1080/10520290500138372. PMID: 16195172.

- Tokuda K, Nishi J, Imuta N, Fujiyama R, Kamenosono A, Manago K, Kawano Y. Characterization of typical and atypical enteroaggregative escherichia coli in Kagoshima, Japan: biofilm formation and acid resistance. Microbiol Immunol. 2010 Jun;54(6):320-9. doi: 10.1111/j.1348-0421.2010.00210. x. PMID: 20536730.

- Toledo MR, Trabulsi LR. Correlation between biochemical and serological characteristics of Escherichia coli and results of the Serény test. J. Clin. Microbiol. 1983 Mar;17(3):419-21. DOI: 10.1128/jcm.17.3.419-421.1983

- Tormo-Badia N, Håkansson Å, Vasudevan K, Molin G, Ahrné S, Cilio CM. Antibiotic treatment of pregnant non-obese diabetic mice leads to altered gut microbiota and intestinal immunological changes in the offspring. Scand J Immunol. 2014 Oct;80(4):250-60. doi: 10.1111/sji.12205. PMID: 24965690.

- Torrazza RM, Neu J. The altered gut microbiome and necrotizing enterocolitis. Clin Perinatol. 2013 Mar;40(1):93-108. doi: 10.1016/j.clp.2012.12.009. PMID: 23415266; PMCID: PMC3589666.

- Trabulsi LR, Keller R, Tardelli Gomes TA. Typical and atypical enteropathogenic Escherichia coli. Emerg Infect Dis. 2002 May;8(5):508-13. doi: 10.3201/eid0805.010385. PMID: 11996687; PMCID: PMC2732489.

- Trachtenberg FL, Haas EA, Kinney HC, Stanley C, Krous HF. Risk factor changes for sudden infant death syndrome after initiation of Back-to-Sleep campaign. Pediatrics. 2012 Apr;129(4):630-8. doi: 10.1542/peds.2011-1419. Epub 2012 Mar 26. PMID: 22451703; PMCID: PMC3356149.

- Troeger C, Khalil IA, Rao PC, Cao S, Blacker BF, Ahmed T, Armah G, Bines JE, Brewer TG, Colombara DV, Kang G, Kirkpatrick BD, Kirkwood CD, Mwenda JM, Parashar UD, Petri WA Jr, Riddle MS, Steele AD, Thompson RL, Walson JL, Sanders JW, Mokdad AH, Murray CJL, Hay SI, Reiner RC Jr. Rotavirus Vaccination and the Global Burden of Rotavirus Diarrhea Among Children Younger Than 5 Years. JAMA Pediatr. 2018 Oct 1;172(10):958-965. doi: 10.1001/jamapediatrics.2018.1960. Erratum in: JAMA Pediatr. 2022 Feb 1;176(2):208. PMID: 30105384; PMCID: PMC6233802.

- Tulloch EF Jr, Ryan KJ, Formal SB, Franklin FA. Invasive enteropathic Escherichia coli dysentery. An outbreak in 28 adults. Ann Intern Med. 1973 Jul;79(1):13-7. doi: 10.7326/0003-4819-79-1-13. PMID: 4578638.

- Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. Nature. 2009 Jan 22;457(7228):480-4. doi: 10.1038/nature07540. Epub 2008 Nov 30. PMID: 19043404; PMCID: PMC2677729.

- Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature. 2007 Oct 18;449(7164):804-10. doi: 10.1038/nature06244. PMID: 17943116; PMCID: PMC3709439.

- Turroni F, Peano C, Pass DA, Foroni E, Severgnini M, Claesson MJ, Kerr C, Hourihane J, Murray D, Fuligni F, Gueimonde M, Margolles A, De Bellis G, O'Toole PW, van Sinderen D, Marchesi JR, Ventura M. Diversity of bifidobacteria within the infant gut microbiota. PLoS One. 2012;7(5): e36957. doi: 10.1371/journal.pone.0036957. Epub 2012 May 11. PMID: 22606315; PMCID: PMC3350489.

- Tuttle AR, Trahan ND, Son MS. Growth and Maintenance of Escherichia coli Laboratory Strains. Curr Protoc. 2021 Jan;1(1): e20. doi: 10.1002/cpz1.20. Erratum in: Curr Protoc. 2022 Aug;2(8): e552. Erratum in: Curr Protoc. 2022 Aug;2(8): e551. PMID: 33484484; PMCID: PMC8006063.

- Uhnoo I, Wadell G, Svensson L, Johansson ME. Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. J Clin Microbiol. 1984 Sep;20(3):365-72. doi: 10.1128/jcm.20.3.365-372.1984. PMID: 6092424; PMCID: PMC271331.

- UN-Habitat. United Nations Human Settlements Programme. SDG indicator metadata. [Website]. 2021. Available from: https://unstats.un.org/sdgs/metadata/files/Metadata-11-01-01.pdf. [Date accessed 1 February 2022].

- Valentini SR, Gomes TA, Falcão DP. Lack of virulence factors in Escherichia coli strains of enteropathogenic serogroups isolated from water. Appl Environ Microbiol. 1992 Jan;58(1):412-4. doi: 10.1128/aem.58.1.412-414.1992. PMID: 1539989; PMCID: PMC195225.

- Vallès Y, Artacho A, Pascual-García A, Ferrús ML, Gosalbes MJ, Abellán JJ, Francino MP. Microbial succession in the gut: directional trends of taxonomic and functional change in a birth cohort of Spanish infants. PLoS Genet. 2014 Jun 5;10(6):e1004406. doi: 10.1371/journal.pgen.1004406. PMID: 24901968; PMCID: PMC4046925.

- Van den Beld MJ, Reubsaet FA. Differentiation between Shigella, enteroinvasive Escherichia coli (EIEC) and noninvasive Escherichia coli. Eur J Clin Microbiol Infect Dis. 2012 Jun;31(6):899-904. doi: 10.1007/s10096-011-1395-7. Epub 2011 Sep 7. PMID: 21901636.

- Varela MF, Rivadulla E, Lema A, Romalde JL. Human Sapovirus among Outpatients with Acute Gastroenteritis in Spain: A One-Year Study. Viruses. 2019 Feb 8;11(2):144. doi: 10.3390/v11020144. PMID: 30744057; PMCID: PMC6409837.

- Vargas SL, Ponce CA, Gallo M, Pérez F, Astorga JF, Bustamante R, Chabé M, Durand-Joly I, Iturra P, Miller RF, Aliouat el M, Dei-Cas E. Near-universal prevalence of Pneumocystis and associated increase in mucus in the lungs of infants with sudden unexpected death. Clin Infect Dis. 2013 Jan;56(2):171-9. doi: 10.1093/cid/cis870. Epub 2012 Oct 16. PMID: 23074306; PMCID: PMC3526255.

- Vargas SL, Ponce CA, Gálvez P, Ibarra C, Haas EA, Chadwick AE, Krous HF. Pneumocystis is not a direct cause of sudden infant death syndrome. Pediatr Infect Dis J. 2007 Jan;26(1):81-3. doi: 10.1097/01.inf.0000247071. 40739.fd. PMID: 17195713.

- Vennemann MM, Findeisen M, Butterfass-Bahloul T, Jorch G, Brinkmann B, Köpcke W, Bajanowski T, Mitchell EA; GeSID Group. Modifiable risk factors for SIDS in Germany: results of GeSID. Acta Paediatr. 2005 Jun;94(6):655-60. doi: 10.1111/j.1651-2227. 2005.tb01960. x. PMID: 16188764.

- Verthelyi D. Sex hormones as immunomodulators in health and disease. Int Immunopharmacol. 2001 Jun;1(6):983-93. doi: 10.1016/s1567-5769(01)00044-3. PMID: 11407317.

- Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, Dallas MJ, Heyse JF, Goveia MG, Black SB, Shinefield HR, Christie CD, Ylitalo S, Itzler RF, Coia ML, Onorato MT, Adeyi BA, Marshall GS, Gothefors L, Campens D, Karvonen A, Watt JP, O'Brien KL, DiNubile MJ, Clark HF, Boslego JW, Offit PA, Heaton PM; Rotavirus Efficacy and Safety Trial (REST) Study Team. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med. 2006 Jan 5;354(1):23-33. doi: 10.1056/NEJMoa052664. PMID: 16394299.

- Vial PA, Mathewson JJ, DuPont HL, Guers L, Levine MM. Comparison of two assay methods for patterns of adherence to HEp-2 cells of Escherichia coli from patients with diarrhea. J Clin Microbiol. 1990 May;28(5):882-5. doi: 10.1128/jcm.28.5.882-885.1990. PMID: 2191011; PMCID: PMC267829.

- Vieira MA, Andrade JR, Trabulsi LR, Rosa AC, Dias AM, Ramos SR, Frankel G, Gomes TA. Phenotypic and genotypic characteristics of Escherichia coli strains of non-enteropathogenic *E. coli* (EPEC) serogroups that carry EAE and lack the EPEC adherence factor and Shiga toxin DNA probe sequences. J Infect Dis. 2001 Mar 1;183(5):762-72. doi: 10.1086/318821. Epub 2001 Feb 8. PMID: 11181153.

- Vieira N, Bates SJ, Solberg OD, Ponce K, Howsmon R, Cevallos W, Trueba G, Riley L, Eisenberg JN. High prevalence of enteroinvasive Escherichia coli isolated in a remote region of northern coastal Ecuador. Am J Trop Med Hyg. 2007 Mar;76(3):528-33. PMID: 17360879; PMCID: PMC2396511.

- Vinjé J, Estes MK, Esteves P, Green KY, Katayama K, Knowles NJ, L'Homme Y, Martella V, Vennema H, White PA, Ictv Report Consortium. ICTV Virus Taxonomy Profile: Caliciviridae. J Gen Virol. 2019 Nov;100(11):1469-1470. doi: 10.1099/jgv.0.001332. PMID: 31573467; PMCID: PMC7011698.

- Vinjé J, Green J, Lewis DC, Gallimore CI, Brown DW, Koopmans MP. Genetic polymorphism across regions of the three open reading frames of "Norwalk-like viruses". Arch Virol. 2000;145(2):223-41. doi: 10.1007/s007050050020. PMID: 10752550.

- Virgin HW. The virome in mammalian physiology and disease. Cell. 2014 Mar 27;157(1):142-50. doi: 10.1016/j.cell.2014.02.032. PMID: 24679532; PMCID: PMC3977141.

- Vizzi E, Piñeros OA, Oropeza MD, Naranjo L, Suárez JA, Fernández R, Zambrano JL, Celis A, Liprandi F. Human rotavirus strains circulating in Venezuela after vaccine introduction: predominance of G2P[4] and reemergence of G1P[8]. Virol J. 2017 Mar 21;14(1):58. doi: 10.1186/s12985-017-0721-9. PMID: 28320411; PMCID: PMC5359893.

- Vorontsov IM, Kelmanson IA. Generalized view of the origins of the sudden infant death syndrome. Med Hypotheses. 1990 Nov;33(3):187-92. doi: 10.1016/0306-9877(90)90174-d. PMID: 2292983.

- Vu DL, Bosch A, Pintó RM, Guix S. Epidemiology of Classic and Novel Human Astrovirus: Gastroenteritis and Beyond. Viruses. 2017 Feb 18;9(2):33. doi: 10.3390/v9020033. PMID: 28218712; PMCID: PMC5332952.

- Vygen-Bonnet S, Rosner B, Wilking H, Fruth A, Prager R, Kossow A, Lang C, Simon S, Seidel J, Faber M, Schielke A, Michaelis K, Holzer A, Kamphausen R, Kalhöfer D, Thole S, Mellmann A, Flieger A, Stark K. Ongoing haemolytic uraemic syndrome (HUS) outbreak caused by sorbitol-fermenting (SF) Shiga toxin-producing Escherichia coli (STEC) O157, Germany, December 2016 to May 2017. Euro Surveill. 2017 May 25;22(21):30541. doi: 10.2807/1560-7917.ES.2017.22.21.30541. PMID: 28597831; PMCID: PMC5479985.

- Walker WA, Iyengar RS. Breast milk, microbiota, and intestinal immune homeostasis. Pediatr Res. 2015 Jan;77(1-2):220-8. doi: 10.1038/pr.2014.160. Epub 2014 Oct 13. PMID: 25310762.

- Wang J, Li F, Wei H, Lian ZX, Sun R, Tian Z. Respiratory influenza virus infection induces intestinal immune injury via microbiota-mediated Th17 cell-dependent inflammation. J Exp Med. 2014 Nov 17;211(12):2397-410. doi: 10.1084/jem.20140625. Epub 2014 Nov 3. Erratum in: J Exp Med. 2014 Dec 15;211(13):2683. Erratum in: J Exp Med. 2014 Nov 17;211(12):2396-7. PMID: 25366965; PMCID: PMC4235643.

- Wani SA, Nabi A, Fayaz I, Ahmad I, Nishikawa Y, Qureshi K, Khan MA, Chowdhary J. Investigation of diarrhoeic faecal samples for enterotoxigenic, Shiga toxin-producing and typical or atypical enteropathogenic Escherichia coli in Kashmir, India. FEMS Microbiol Lett. 2006 Aug;261(2):238-44. doi: 10.1111/j.1574-6968.2006.00354.x. PMID: 16907727.

- Webb A, Starr M. Acute gastroenteritis in children. Aust Fam Physician. 2005 Apr;34(4):227-31. PMID: 15861741.

- Webb PM, Knight T, Greaves S, Wilson A, Newell DG, Elder J, Forman D. Relation between infection with Helicobacter pylori and living conditions in childhood: evidence for person-to-person transmission in early life. BMJ. 1994 Mar 19;308(6931):750-3. doi: 10.1136/bmj.308.6931.750. PMID: 8142828; PMCID: PMC2539652.

- Weber MA, Ashworth MT, Risdon RA, Hartley JC, Malone M, Sebire NJ. The role of post-mortem investigations in determining the cause of sudden unexpected death in infancy. Arch Dis Child. 2008 Dec;93(12):1048-53. doi: 10.1136/adc.2007.136739. Epub 2008 Jun 30. PMID: 18591183.

- Weber MA, Hartley JC, Ashworth MT, Malone M, Sebire NJ. Virological investigations in sudden unexpected deaths in infancy (SUDI). Forensic Sci Med Pathol. 2010 Dec;6(4):261-7. doi: 10.1007/s12024-010-9181-x. Epub 2010 Jul 11. PMID: 20623342.

- Weber MA, Hartley JC, Brooke I, Lock PE, Klein NJ, Malone M, Sebire NJ. Post-mortem interval and bacteriological culture yield in sudden unexpected death in infancy (SUDI). Forensic Sci Int. 2010 May 20;198(1-3):121-5. doi: 10.1016/j.forsciint.2010.02.002. Epub 2010 Mar 11. PMID: 20226606.

- Weber MA, Hartley JC, Klein NJ, Risdon RA, Malone M, Sebire NJ. Staphylococcal toxins in sudden unexpected death in infancy: experience from a single specialist centre. Forensic Sci Med Pathol. 2011 Jun;7(2):141-7. doi: 10.1007/s12024-010-9199-0. Epub 2010 Nov 10. PMID: 21063809.

- Weber MA, Klein NJ, Hartley JC, Lock PE, Malone M, Sebire NJ. Infection and sudden unexpected death in infancy: a systematic retrospective case review. Lancet. 2008 May 31;371(9627):1848-53. doi: 10.1016/S0140-6736(08)60798-9. PMID: 18514728.

- Weber MA, Sebire NJ. Postmortem investigation of sudden unexpected death in infancy: current issues and autopsy protocol Diagn. Histopathol. 2009 Nov 1;15(11):510-23. doi: 10.1016/j.mpdhp.2009.08.003.

- Wedgwood RJ. Review of USA experience. Sudden and Unexpected Death in Infancy (Cot Deaths). Edited by: Camps FE, Carpenter RG. 1972, England: Wright, 22–28.

- Weinbaum FI, Lavie S, Danek M, Sixsmith D, Heinrich GF, Mills SS. Doing it right the first time: quality improvement and the contaminant blood culture. J Clin Microbiol. 1997 Mar;35(3):563-5. doi: 10.1128/jcm.35.3.563-565.1997. PMID: 9041389; PMCID: PMC229627.

- Weinstein MP. Blood culture contamination: persisting problems and partial progress. J Clin Microbiol. 2003 Jun;41(6):2275-8. doi: 10.1128/JCM.41.6.2275-2278.2003. PMID: 12791835; PMCID: PMC156489.

- Weng M, Walker WA. The role of gut microbiota in programming the immune phenotype. J Dev Orig Health Dis. 2013 Jun;4(3):203-14. doi: 10.1017/S2040174412000712. PMID: 24353893; PMCID: PMC3864895.

- Weng SF, Redsell SA, Swift JA, Yang M, Glazebrook CP. Systematic review and meta-analyses of risk factors for childhood overweight identifiable during infancy. Arch Dis Child. 2012 Dec;97(12):1019-26. doi: 10.1136/archdischild-2012-302263. Epub 2012 Oct 29. PMID: 23109090; PMCID: PMC3512440.

- What is Autolysis? - Definition & Histology [Website]. Available from: https://study.com/academy/lesson/what-is-autolysis-definition-histology.html. [Date accessed: 22 Jan 2022].

- Whitacre CC. Sex differences in autoimmune disease. Nat Immunol. 2001 Sep;2(9):777-80. doi: 10.1038/ni0901-777. PMID: 11526384.

- WHO Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. (2000). Effect of breast-feeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis Lancet. 2000 Feb 5;355(9202):451-5. Erratum in: Lancet 2000 Mar 25;355(9209):1104. PMID: 10841125.

- Wilhelmi I, Roman E, Sánchez-Fauquier A. Viruses causing gastroenteritis. Clin Microbiol Infect. 2003 Apr;9(4):247-62. doi: 10.1046/j.1469-0691.2003.00560. x. PMID: 12667234; PMCID: PMC7129320.

- Willcocks MM, Carter MJ. Identification and sequence determination of the capsid protein gene of human astrovirus serotype 1. FEMS Microbiol Lett. 1993 Nov 15;114(1):1-7. doi: 10.1016/0378-1097(93)90133-m. PMID: 8293952; PMCID: PMC7110315.

- Willinger M, Hoffman HJ, Hartford RB. Infant sleep position and risk for sudden infant death syndrome: report of meeting held January 13 and 14, 1994, National Institutes of Health, Bethesda, MD. Pediatrics. 1994 May;93(5):814-9. PMID: 8165085.

- Willinger M, James LS, Catz C. Defining the sudden infant death syndrome (SIDS): deliberations of an expert panel convened by the National Institute of Child Health and Human Development. Pediatr Pathol. 1991 Sep-Oct;11(5):677-84. doi: 10.3109/15513819109065465. PMID: 1745639.

- Willinger M, Ko CW, Hoffman HJ, Kessler RC, Corwin MJ; National Infant Sleep Position study. Trends in infant bed sharing in the United States, 1993-2000: the National Infant Sleep Position study. Arch Pediatr Adolesc Med. 2003 Jan;157(1):43-9. doi: 10.1001/archpedi.157.1.43. PMID: 12517193.

- Wilson D, Dolan G, Aird H, Sorrell S, Dallman TJ, Jenkins C, Robertson L, Gorton R. Farm-to-fork investigation of an outbreak of Shiga toxin-producing Escherichia coli O157. Microb Genom. 2018 Mar;4(3):e000160. doi: 10.1099/mgen.0.000160. Epub 2018 Feb 28. PMID: 29488865; PMCID: PMC5885012.

- Wilson SJ, Wilson ML, Reller LB. Diagnostic utility of postmortem blood cultures. Arch Pathol Lab Med. 1993 Oct;117(10):986-8. PMID: 8215840.

- Winterbach M, Hattingh C, Heathfield LJ. Retrospective study of sudden unexpected death of infants in the Garden Route and Central Karoo districts of South Africa: Causes of death and epidemiological factors. S Afr j child health. 2021 Jun 1;15(2):74-82. doi:10.7196/SAJCH. 2021.v15i2.01729.

- Witcombe NB, Yiallourou SR, Walker AM, Horne RS. Blood pressure and heart rate patterns during sleep are altered in preterm-born infants: implications for sudden infant death syndrome. Pediatrics. 2008 Dec;122(6): e1242-8. doi: 10.1542/peds.2008-1400. PMID: 19047224.

- Wong FY, Witcombe NB, Yiallourou SR, Yorkston S, Dymowski AR, Krishnan L, Walker AM, Horne RS. Cerebral oxygenation is depressed during sleep in healthy term infants when they sleep prone. Pediatrics. 2011 Mar;127(3): e558-65. doi: 10.1542/peds.2010-2724. Epub 2011 Feb 28. PMID: 21357341.

- Wood LF, Brown BP, Lennard K, Karaoz U, Havyarimana E, Passmore JS, Hesseling AC, Edlefsen PT, Kuhn L, Mulder N, Brodie EL, Sodora DL, Jaspan HB. Feeding-Related Gut Microbial Composition Associates With Peripheral T-Cell Activation and Mucosal Gene Expression in African Infants. Clin Infect Dis. 2018 Sep 28;67(8):1237-1246. doi: 10.1093/cid/ciy265. PMID: 29659737; PMCID: PMC6455922.

- World Health Organization. Coronavirus disease (COVID-19): How is it transmitted? [Website]. (2021). Available from: https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease-covid-19-how-is-it-transmitted. [Date accessed: 28 July 2022].

- World Health Organization. Diarrheal Disease—Key Facts. [Website] 2017. Available from: http://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease. [Date accessed: 06 May 2021].

- World Health Organization. Meeting of the immunization Strategic Advisory Group of Experts, April 2009—conclusions and recommendations. Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire. 2009;84(23):220-36.

- World Health Organization. Rotavirus Vaccines: WHO Position Paper–July 2021. Wkly. Epidemiol. Rec. 2021 Jul 16;96(28):301-219.

- Wu MH. Sudden death in pediatric populations. Korean Circ J. 2010 Jun;40(6):253-7. doi: 10.4070/kcj.2010.40.6.253. Epub 2010 Jun 29. PMID: 20589195; PMCID: PMC2893363.

- Yadav M, Sujatha R, Kumar A. Characterization of Enterotoxigenic and Enterohemorrhagic Escherichia coli in Paediatric Patients. J. Pure Appl. Microbiol., 2020; 14(1): 375-381. https://doi.org/10.22207/JPAM.14.1.38

- Yamamoto-Hanada K, Yang L, Narita M, Saito H, Ohya Y. Influence of antibiotic use in early childhood on asthma and allergic diseases at age 5. Ann Allergy Asthma Immunol. 2017 Jul;119(1):54-58. doi: 10.1016/j.anai.2017.05.013. PMID: 28668240.

- Yan H, Nguyen TA, Phan TG, Okitsu S, Li Y, Ushijima H. Development of RT-multiplex PCR assay for detection of adenovirus and group A and C rotaviruses in diarrheal fecal specimens from children in China. Kansenshogaku Zasshi. 2004 Aug;78(8):699-709. doi: 10.11150/kansenshogakuzasshi1970.78.699. PMID: 15478645.

- Yang R, Gao R, Cui S, Zhong H, Zhang X, Chen Y, Wang J, Qin H. Dynamic signatures of gut microbiota and influences of delivery and feeding modes during the first 6 months of life. Physiol Genomics. 2019 Aug 1;51(8):368-378. doi: 10.1152/physiolgenomics.00026.2019. Epub 2019 Jun 21. PMID: 31226006.

- Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell. 2015 Apr 9;161(2):264-76. doi: 10.1016/j.cell.2015.02.047. Erratum in: Cell. 2015 Sep 24; 163:258. PMID: 25860609; PMCID: PMC4393509.

- Yao Y, Cai X, Ye Y, Wang F, Chen F, Zheng C. The Role of Microbiota in Infant Health: From Early Life to Adulthood. Front Immunol. 2021 Oct 7; 12:708472. doi: 10.3389/fimmu.2021.708472. PMID: 34691021; PMCID: PMC8529064.

- Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. Human gut microbiome viewed across age and geography. Nature. 2012 May 9;486(7402):222-7. doi: 10.1038/nature11053. PMID: 22699611; PMCID: PMC3376388

- Yatsuyanagi J, Saito S, Sato H, Miyajima Y, Amano K, Enomoto K. Characterization of enteropathogenic and enteroaggregative Escherichia coli isolated from diarrheal outbreaks. J Clin Microbiol. 2002 Jan;40(1):294-7. doi: 10.1128/JCM.40.1.294-297.2002. PMID: 11773137; PMCID: PMC120118.

- Yiallourou SR, Walker AM, Horne RS. Effects of sleeping position on development of infant cardiovascular control. Arch Dis Child. 2008 Oct;93(10):868-72. doi: 10.1136/adc.2007.132860. Epub 2008 May 2. PMID: 18456690.

- Yiallourou SR, Walker AM, Horne RS. Prone sleeping impairs circulatory control during sleep in healthy term infants: implications for SIDS. Sleep. 2008 Aug;31(8):1139-46. PMID: 18714786; PMCID: PMC2542960.

- Yoshioka H, Iseki K, Fujita K. Development and differences of intestinal flora in the neonatal period in breast-fed and bottle-fed infants. Pediatrics. 1983 Sep;72(3):317-21. PMID: 6412205.

- Young J, Shipstone R. Shared Sleeping Surfaces and Dangerous Sleeping Environments. In: Duncan JR, Byard RW, editors. SIDS Sudden Infant and Early Childhood Death: The Past, the Present and the Future. Adelaide (AU): University of Adelaide Press; 2018 May. Chapter 11. PMID: 30035939.

- Yu AC, Loo JF, Yu S, Kong SK, Chan TF. Monitoring bacterial growth using tunable resistive pulse sensing with a pore-based technique. Appl Microbiol Biotechnol. 2014 Jan;98(2):855-62. doi: 10.1007/s00253-013-5377-9. Epub 2013 Nov 29. PMID: 24287933.

- Yu J, Jing H, Lai S, Xu W, Li M, Wu J, Liu W, Yuan Z, Chen Y, Zhao S, Wang X, Zhao Z, Ran L, Wu S, Klena JD, Feng L, Li F, Ye X, Qiu Y, Wang X, Yu H, Li Z, Yang W. Etiology of diarrhea among children under the age five in China: Results from a five-year surveillance. J Infect. 2015 Jul;71(1):19-27. doi: 10.1016/j.jinf.2015.03.001. Epub 2015 Mar 6. PMID: 25753104; PMCID: PMC4667737.

- Yuan M, Jiang Z, Bi G, Nomura K, Liu M, Wang Y, Cai B, Zhou JM, He SY, Xin XF. Pattern-recognition receptors are required for NLR-mediated plant immunity. Nature. 2021 Apr;592(7852):105-109. doi: 10.1038/s41586-021-03316-6. Epub 2021 Mar 10. PMID: 33692546; PMCID: PMC8016741.

- Zahorsky J. Hyperemesis hiemis or the winter vomiting disease. Arch Pediatr. 1929 Jan 1; 46:391-5.

- Zeller M, Donato C, Trovão NS, Cowley D, Heylen E, Donker NC, McAllen JK, Akopov A, Kirkness EF, Lemey P, Van Ranst M, Matthijnssens J, Kirkwood CD. Genome-Wide Evolutionary Analyses of G1P[8] Strains Isolated Before and After Rotavirus Vaccine Introduction. Genome Biol Evol. 2015 Aug 8;7(9):2473-83. doi: 10.1093/gbe/evv157. PMID: 26254487; PMCID: PMC4607516.

- Zeller M, Rahman M, Heylen E, De Coster S, De Vos S, Arijs I, Novo L, Verstappen N, Van Ranst M, Matthijnssens J. Rotavirus incidence and genotype distribution before and after national rotavirus vaccine introduction in Belgium. Vaccine. 2010 Nov 3;28(47):7507-13. doi: 10.1016/j.vaccine.2010.09.004. Epub 2010 Sep 17. PMID: 20851085.

- Zhang M, Differding MK, Benjamin-Neelon SE, Østbye T, Hoyo C, Mueller NT. Association of prenatal antibiotics with measures of infant adiposity and the gut microbiome. Ann Clin Microbiol Antimicrob. 2019 Jun 21;18(1):18. doi: 10.1186/s12941-019-0318-9. PMID: 31226994; PMCID: PMC6587281.

- Zheng J, Xiao X, Zhang Q, Mao L, Yu M, Xu J. The Placental Microbiome Varies in Association with Low Birth Weight in Full-Term Neonates. Nutrients. 2015 Aug 17;7(8):6924-37. doi: 10.3390/nu7085315. PMID: 26287241; PMCID: PMC4555154.

- Zhou P, Zhou Y, Liu B, Jin Z, Zhuang X, Dai W, Yang Z, Feng X, Zhou Q, Liu Y, Xu X, Zhang L. Perinatal Antibiotic Exposure Affects the Transmission between Maternal and Neonatal Microbiota and Is Associated with Early-Onset Sepsis. mSphere. 2020 Feb 19;5(1):e00984-19. doi: 10.1128/mSphere.00984-19. PMID: 32075882; PMCID: PMC7031618.

- Zhou X, Wang YH, Pang BB, Chen N, Kobayashi N. Surveillance of Human Rotavirus in Wuhan, China (2011-2019): Predominance of G9P[8] and Emergence of G12. Pathogens. 2020 Oct 2;9(10):810. doi: 10.3390/pathogens9100810. PMID: 33023203; PMCID: PMC7600066.

- Zorgani A, Essery SD, Madani OA, Bentley AJ, James VS, MacKenzie DA, Keeling JW, Rambaud C, Hilton J, Blackwell CC, Weir DM, Busuttil A. Detection of pyrogenic toxins of Staphylococcus aureus in sudden infant death syndrome. FEMS Immunol Med Microbiol. 1999 Aug 1;25(1-2):103-8. doi: 10.1111/j.1574-695X.1999.tb01332.x. PMID: 10443497.

- Zou W, Cui D, Wang X, Guo H, Yao X, Jin M, Huang Q, Gao M, Wen X. Clinical characteristics and molecular epidemiology of noroviruses in outpatient children with acute gastroenteritis in Huzhou of China. PLoS One. 2015 May 26;10(5): e0127596. doi: 10.1371/journal.pone.0127596. PMID: 26011043; PMCID: PMC4444205.

- Zou ZH, Liu D, Li HD, Zhu DP, He Y, Hou T, Yu JL. Prenatal and postnatal antibiotic exposure influences the gut microbiota of preterm infants in neonatal intensive care units. Ann Clin Microbiol Antimicrob. 2018 Mar 19;17(1):9. doi: 10.1186/s12941-018-0264-y. PMID: 29554907; PMCID: PMC5858143.

Appendices

Appendix A: Ethics Approval

Stellenbosch UNIVERSITY IYUNIVESITHI UNIVERSITEIT

forward together
sonke siya phambili
saam vorentoe

Approval Letter
Progress Report

13/02/2023

Project ID: 2130

Ethics Reference No: S16/10/214

Project Title: Investigating pathogens of the gastrointestinal tract (GIT) in Sudden and Unexpected Death in Infancy (SUDI) cases at the Tygerberg Medico-Legal Mortuary, compared to an age- matched healthy control group

Dear Ms DT Cupido

We refer to your request for an extension/annual renewal of ethics approval dated 16/01/2023 15:23 .

The Health Research Ethics Committee reviewed and approved the annual progress report through an expedited review process.

The approval of this project is extended for a further year.

Approval date: 13 February 2023

Expiry date: 12 February 2024

Kindly be reminded to submit progress reports two (2) months before expiry date.

Where to submit any documentation

Kindly note that the HREC uses an electronic ethics review management system, *Infonetica*, to manage ethics applications and ethics review process. To submit any documentation to HREC, please click on the following link: https://applyethics.sun.ac.za.

Please remember to use your Project Id 2130 and ethics reference number S16/10/214 on any documents or correspondence with the HREC concerning your research protocol.

Please note that for studies involving the use of questionnaires, the final copy should be uploaded on Infonetica.

Yours sincerely,

Miss EL Rohland
Health Research Ethics Committee 1 (HREC 1)

National Health Research Ethics Council (NHREC) Registration Number:
REC-130408-012 (HREC1)•REC-230208-010 (HREC2)

Federal Wide Assurance Number: 00001372
Office of Human Research Protections (OHRP) Institutional Review Board (IRB) Number:
IRB0005240 (HREC1)•IRB0005239 (HREC2)

The Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical norms and principles for research, established by the World Medical Association (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects; the South African Department of Health (2006).Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition); as well as the Department of Health (2015). Ethics in Health Research: Principles, Processes and Structures (2nd edition).

The Health Research Ethics Committee reviews research involving human subjects conducted or supported by the Department of Health and Human Services, or other federal departments or agencies that apply the Federal Policy for the Protection of Human Subjects to such research (United States Code of Federal Regulations Title 45 Part 46); and/or clinical investigations regulated by the Food and Drug Administration (FDA) of the Department of Health and Human Services.

Appendix B: Provincial Government of the Western Cape, Forensic Pathology Service, SUDI Questionnaire, FPS006(b)

DEPARTMENT of HEALTH
Provincial Government of the Western Cape

FPS006(b)

FORENSIC PATHOLOGY SERVICE

SUDI (Complete If A Baby Should Suddenly And Unexpectedly Die)

FPS laboratory_____ WC _____

Name of baby _____

Part 1: Scene Questionnaire and Observations

Date: Time: Name of Forensic officer:

Section A. **Who gives the history/ information in this case e.g. mother/father/granny/grandpa/other relative(give details)**	
Name:	Relationship:
Address:	Contact telephone number:
ID Number:	
Infants full name:	
Home Address:	
Age of Baby	Date of birth:
Race:	Sex:

Section B **Person(s) at/called to the scene and relationship**		
Name/relationship	Date	Time
Name/relationship	Date	Time
Name/relationship	Date	Time
Police response/name	Date	Time
Paramedic response/name	Date	Time
When was the death certified/by whom	Date	Time
If the baby was taken to hospital		
Name of hospital		

WC_____

Date of arrival: Time of arrival:			
Name of doctor seen / declared death:			
Comment: Get copies of doctors notes			
Was resuscitation done on the baby by the paramedic or the doctors at the hospital?			
Section C **Household environment:**			
Place where baby lives: house shack other –			
Number of bedrooms Is the room in which the baby is found well ventilated?			
Odour(s) present in the room the baby slept in?	Yes	No	
Peeling paint in the room the baby slept in?	Yes	No	
Fungal growth (mould) in the room the baby slept in?	Yes	No	
Did people smoke cigarettes in the room the baby slept?	Yes	No	
Are there pets in the house? If yes – type and number:	Yes	No	
Did caregiver use alcohol or drugs on the night baby died?	Yes	No	
Was there a heater or open fire or galley blik or other heating device in room where baby slept?	Yes	No	
In what position was the baby found lying? Has the baby been moved? Were there any covers/ clothing etc over the baby's head?			
Was the baby squashed/wedged between anything (object)?	Yes	No	
Was there overlaying (someone lay on top of the baby)?	Yes	No	
Comments from forensic officer who attended the scene:			

WC_____

Part 2: Facility Questionnaire

Date: ***Time:*** ***Name of Forensic officer:***

Section D
Circumstances of death / details about events before death

1. When was the baby last seen alive				Date	Time
2. Who last saw the baby alive					
3. When was the baby found dead				Date	Time
4. Who found the baby dead at the scene					
5. Was the baby ill?				Yes	No
a) If yes – What was wrong and for how long?					
b) Was the baby taken to the doctor or pharmacy or clinic or traditional healer for the illness? When (date and time)?				Yes	No
c) If not, why not:					
d) Was the baby admitted to a hospital or clinic for the illness: When (date and time)?				Yes	No
e) If not, do you know why not?					
f) What medication was given (names please					
6. Where was the baby found dead	Bed	Couch	Cot	Floor	Other
Other:					
7. Did the baby sustain any injuries – eg by falling or being hit: If yes:				Yes	No
a) When did it happen?					
b) How did it happen?					
c) Where did it happen?					
d) What did the caretaker do about it?					

WC_____

8. a) On what was the baby placed to sleep	Bed with a pillow	Bed without a pillow	Couch with a pillow	Couch without pillow	Cot with pillow
	Cot without pillow	Floor with pillow	Floor without pillow	Other	
b) If placed on a bed/cot, what was the mattress type			Foam rubber	Inner spring	Other
c) Was the mattress covered with a blanket or sheet				Yes	No
d) What position was the baby placed when put to sleep?		Back	Stomach	Side	Other
Other -					
e) what was used to cover the baby: List items					
e) What position was the baby found dead?		Back	Stomach	Side	Other
Other –					
f) Has the baby been moved?				Yes	No
g) Face position when the baby was found dead			To the left	To the right	Face down
			Face up	Unknown	
h) Face and or chest squashed / wedged between any object(s) when the baby was found dead?			Yes	No	Unknown
If yes – details please –					
i) Was the nose and mouth of the baby covered by anything – eg blankets or anything else			Yes	No	Unknown
j) Were there other items in contact with the baby – eg pillow			Yes	No	Unknown
k) Did the baby use a Dummy (pacifier)?				Yes	No
l) Did the baby sleep in the same bed as the mother?				Yes	No
m) Did the baby sleep in her arms?				Yes	No
n) Did the baby sleep on her chest?				Yes	No
o) Did the baby sleep with the mother on a couch?				Yes	No
p) How many other people slept on the same bed as the baby at the time the baby died?					
q) Was anyone found on top of the baby while in the bed (Overlaying)?				Yes	No
r) Was the window where the baby slept on the day /night the baby died				Open	Closed
s) Did the mother or anyone in the house smoke while the baby slept on the night/day of death?					
t) When was the baby last fed?				Date	Time

4

WC_____

u) Did the mother/caregiver use alcohol before going to bed with the baby on the night/day the baby was found dead? If yes, how much?			Yes	No
v) Did the mother/caregiver use drugs before going to bed with the baby on the night/day the baby was found dead? If yes, what drugs?			Yes	No
w) Did the mother/caregiver give the baby medication on the night/day of death? If yes, name of medication:			Yes	No
Section E **About the baby**				
1. Where was the baby born?	Hospital	Clinic	Home	Other
Name of hospital/clinic/other				
2. How was the baby born?			Normal vaginal delivery	Caesarian section
3. How much did the baby weigh at birth?				
4. Was the baby		Premature	Full term	Post dates (Overdue)
5. If the baby was premature, how premature was it?				
6. Did the baby receive Kangaroo care (KMC)			Yes	No
7. Did the mother carry the baby on her back?			Yes	No
8. Was the baby		Breast fed	Bottle/formula fed	Both breast and bottle fed
If formula, name of the milk –				
9. Was boiling water used to make the bottle?			Yes	No
10. What other food was use to feed the baby?				
11. Does the mother have the clinic card?			Yes	No
If yes – keep the card for the pathologist. If no – ask the mother to bring it to the facility				
12. Was the baby sick before it died?			Yes	No
If yes	<24h	>24h	> 2 weeks	Never
a) Did the baby have a cold/ runny nose?				
b) was the baby coughing?				
c) did the baby have diarrhea (runny tummy)?				

WC_____

d) Was the baby unusually restless / irritable?				
e) Was the baby crying more than usual?				
f) Was there a difference /change in the appetite / feeding?				
g) Was the baby vomiting?				
h) Any fits / seizures?				
i) did the baby have a fever / showed increased sweating?				
j) Was the baby listless? (floppy)				
k) did the baby turn blue?				
13. Was the now deceased baby taken to	Hospital	clinic	doctor	Pharmacy
	Traditional healer	Other		
14. Did the baby come in contact with someone who is sick in the past two weeks?			Yes	No
If yes – who?				
15. Did the baby ever suddenly stopped breathing?		Yes	No	Unknown
16. When was the baby's last vaccination?				
18. Is the baby known to be allergic to anything?		Yes	No	Unknown
If yes, what?				
19. Did the family visit another country prior to the death of the baby?			Yes	No
If yes, give details				
20. Was the baby admitted to hospital in the past week before the death?			Yes	No
a) If yes, for how long and where:				
b) Why?				
c) Discharge date?				
d) Condition of baby after discharge:				
e) Medication after discharge from the hospital (names please)				
21. Was the baby taken to a traditional healer?			Yes	No
a)If yes, date when the baby was taken to the healer:				

WC_____

b) What was given?		
c) Ask for the medication to be given to the pathologist.		
d) Condition of the baby after going to the healer?		
21. What did the baby wear when it died? (list clothing)		
Section F **About the mother**		
1. Is the mother	Married	Single
2. Is the mother employed?	Yes	No
3. Age of the mother?		
4. What standard of schooling did she achieve?		
5. Was she on contraception before she fell pregnant?	Yes	No
6. Did she take iron and vitamin tablets during her pregnancy?	Yes	No
7. Did she receive antenatal care?	Yes	No
8. Did the mother have diabetes in pregnancy?	Yes	No
9. Did the mother have high blood pressure in pregnancy?	Yes	No
10. Did the mother gain weight adequately in pregnancy?	Yes	No
11. Was she diagnosed with any illness during the pregnancy eg. HIV?	Yes	No
12. Was the mother on any medication during the pregnancy?	Yes	No
If yes, what medication:		
13. Were there any difficulties during the delivery?	Yes	No
If yes, what?		
14. Were there any problems with the baby after the delivery?	Yes	No
If yes, what?		

WC_____

15. Was any specific instruction given about specific health care for the baby?			Yes	No
If yes, what?				
16. Was she depressed after the pregnancy?			Yes	No
17. Did she get any treatment?			Yes	No
18. How many babyren does she have?				
19. How old are they?				
20. Are they healthy?			Yes	No
21. Do any of the babyren have learning disability?			Yes	No
22. Do the living babyren have the same father as the deceased baby?			Yes	No
23. Does she look after the baby?			Yes	No
24. If not, who looks after the baby?				
25. Why is the mother unable to look after the baby?				
26. Did the mother smoke during the pregnancy?			Yes	No
If yes, how many per day?				
27. Did the mother drink during the pregnancy?			Yes	No
a) What did she drink?	Beer	Wine	Spirits	Other
b) how much did she drink?	Every day		Now and again	Weekends
1 glass	Every day		Now and again	Weekends
> 1 glass	Every day		Now and again	Weekends
A bottle of alcohol	Every day		Now and again	Weekends
> 1 bottle	Every day		Now and again	Weekends
28. Does she use drugs?			Yes	No
a) If yes, what drugs does she use?	Tik	Cocaine / Heroin	Mandrax	Other
b) How often does she use drugs?	Every day		Now and again	Weekends
29. Does the mother smoke after the pregnancy?			Yes	No
30. Does the mother know that smoking harms the unborn baby?			Yes	No
31. Does the husband/partner drinks?			Yes	No

8

WC_____

32. Does the mother drink after the pregnancy?	Yes	No
33. Do the parents of the mother drink?	Yes	No
34. Does the mother know that alcohol harms the unborn baby?	Yes	No
35. Did the mother have a previous baby that died suddenly?	Yes	No
a) If yes, how many died?		
b) At what age?		
c) Was a PM done?	Yes	No
If yes, where was it done?		
36. Did the mother have a previous stillbirth?	Yes	No

Section G
Household environment

1. Place where the baby lives	House		Shack	Other
2. Number of bedrooms?				
3. Is the room in which the baby was found well ventilated?			Yes	No
4. Odour(s) present in the room the baby slept in?			Yes	No
5. Peeling paint in the room the baby slept in?			Yes	No
6. Fungal growth (mould) in the room the baby slept in?			Yes	No
7. Are there pets in the house?			Yes	No
If yes, type and number:				
8. Was the following in the room where the baby slept to heat the room?	Electric heater	"Galley"	Fire	Other
Describe other –				
9. Number of adults in the dwelling?				
10. Number of babyren in the dwelling?				
11. Total number of people in the dwelling?				
12. Estimated monthly income?				
13. Number of smokers in the dwelling?				
14. Are there mentally retarded/ challenged people in the dwelling?			Yes	No

9

WC_____

COMMENTS TO PATHOLOGIST FROM THE FORENSIC OFFICER WHO ATTENDED THE SCENE AND INTERVIEWED DURING ID PROCESS:

ITEMS RETAINED AT THE SCENE OR FROM THE MOTHER DURING INTERVIEW

Date:

Signature / Thumbprint of deponent

I certify that the above statement was taken down by myself and that the deponent has acknowledged that he / she knows and understands the contents hereof.

Date _____ Time: _____

Place: _____

_____

Department of Health
Forensic Pathology Laboratory

10

Appendix C: Informed consent and Questionnaire – Control cases

UNIVERSITEIT•STELLENBOSCH•UNIVERSITY
jou kennisvennoot • your knowledge partner

STELLENBOSCH UNIVERSITY

CONSENT TO PARTICIPATE IN RESEARCH

INVESTIGATING PATHOGENS OF THE GASTROINTESTINAL TRACT (GIT) IN SUDDEN AND UNEXPECTED DEATH IN INFANCY (SUDI) CASES AT THE TYGERBERG MEDICO-LEGAL MORTUARY, COMPARED TO AN AGE-MATCHED HEALTHY CONTROL GROUP.

HREC Number S16/10/214 **Approved on 30/01/2018.**

Sudden and unexpected death in infancy (SUDI) is where an infant dies suddenly and without obvious cause, or cot-death, before the age of 1 year.

You are hereby invited to take part in a research study conducted by Ms Danielle Cupido [BSc (Medical Bioscience), BSc (Honours) (Medical Bioscience), MPhil (Biomedical Forensic Science)], from the Department of Pathology, Division of Medical Virology at Stellenbosch University. The results from this research will be used to write a research dissertation and articles in medical and/or scientific journals.

Your infant was selected as a healthy control to collect information on the bacteria and viruses present in the digestive tract of infants. This will be compared to the bacteria and viruses that are found in cot-death cases to try and understand what makes certain infants more prone to cot-death than others. This is the first study of its kind in South Africa.

1. PURPOSE OF THE STUDY

The goal of this study is to identify pathogens that are present in the digestive tract of infants that died as a result of cot-death and were admitted to the Tygerberg Medico-Legal Mortuary. Stool samples will be collected from healthy control infants and the results will be compared to the pathogens found in digestive tract of the cot-death cases.

2. PROCEDURES

If you agree to take part in this study, the following will be done:

Once your infant's nappy has been soiled, the informed consent form will be read and explained to you. If you understand and agree to take part in the study, you will be asked to sign the form. A copy of the signed form will be given to you.

A small amount of the stool inside the nappy will be collected directly from the nappy and placed into a special container. Where it isn't possible to collect stool, a swab sample will be taken from the soiled nappy. No samples will be collected from your baby.

You will be asked specific questions and your answers will be written down on a questionnaire. These questions include general information, such as date of birth, gender (male or female), type of birth (normal birth or caesarean section), gestational age (at how many weeks pregnancy was the baby born), feeding (breast vs bottle), home and sleeping environment (type of home and room, type of bedding, sharing of beds, position in which the baby sleeps), etc. Other information that might be important for us will also be collected, such as any medication (e.g., antibiotics) your baby has

received or is currently using, signs and symptoms of any illness (e.g., diarrhoea, cold, coughing, etc) over the last 3 to 4 weeks, vaccinations received to date, etc.

3. POTENTIAL RISKS AND DISCOMFORTS

There are no risks involved, because we will only collect stool from the nappy and not directly from the baby.

4. POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY

You will most personally benefit from this study, but we hope to identify risk factors that increase the chances of cot-deaths. If early detection can lead to prevention of sudden infant deaths, it could lead to a decrease in the number of cot-deaths in the community. If any treatable condition of your baby is identified through our research, we will inform you immediately to enable you to seek medical care.

5. PAYMENT FOR PARTICIPATION

There will be no payment received for participation in this study.

6. CONFIDENTIALITY

All information collected from you or obtained from your infant's stool sample that can identify you will remain confidential and will only be made known with your permission or as required by law. Confidentiality will be ensured by using patient numbers instead of names and only the research team will have access to this information.

7. PARTICIPATION AND WITHDRAWAL

If you prefer not to have your infant take part or withdraw after the stool sample was collected, it will not affect your or your child's treatment at any healthcare facility in any way.

8. IDENTIFICATION OF INVESTIGATORS

If you have any questions or concerns about the research, please feel free to contact:

Ms Danielle Cupido (Ph.D. Candidate/ Principal Investigator)
Division of Medical Virology, Faculty of Medicine and Health Sciences
Stellenbosch University/ NHLS, PO Box 241; CAPE TOWN, 8000, South Africa
Cell: 0727996254, Email: daniellec@sun.ac.az

Prof Corena de Beer (Supervisor)
Division of Medical Virology, Faculty of Medicine and Health Sciences
Stellenbosch University / NHLS, PO Box 241; CAPE TOWN, 8000, South Africa
Tel: +27 21 938-9453; Fax: +27 86 6213268
Email: cdeb@sun.ac.za

Prof Andrew Whitelaw (Co-supervisor)
Division of Medical Microbiology, Faculty of Medicine and Health Sciences
Stellenbosch University / NHLS, PO Box 241; CAPE TOWN, 8000, South Africa
Tel: +27 21 938-4032; Fax: +27 21 938-4005
Email: awhitelaw@sun.ac.za

5. RIGHTS OF RESEARCH SUBJECTS

You may change your mind at any time and withdraw from the study. You are not waiving any legal claims, rights or remedies because of your participation in this research study. If you have questions regarding your rights as a research subject, contact Ms Maléne Fouché [mfouche@sun.ac.za; 021 808 4622] at the Division for Research Development.

SIGNATURE OF RESEARCH SUBJECT OR LEGAL REPRESENTATIVE

The information above was described to [*me/the subject/the participant]* by _____ [*name of relevant person]* in [*Afrikaans/English/Xhosa/other]* and [*I am/the subject is/the participant is]* in command of this language or it was satisfactorily translated to [*me/him/her*]. [*I/the participant/the subject]* was given the opportunity to ask questions and these questions were answered to *[my/his/her]* satisfaction.

I hereby consent that the subject/participant may participate in this study. I have been given a copy of this form.

_____ _____

(specify)

Name of person giving consent **Capacity of person giving consent (parent/guardian/caregiver)**

_____

Name of Legal Representative (if applicable)

_____ _____

Signature of Parent / Guardian or Legal Representative **Date**

SIGNATURE OF INVESTIGATOR

I declare that I explained the information given in this document to _____ [*name of the subject/participant]* and/or [his/her] representative _____ [*name of the representative]. [He*/she] was encouraged and given ample time to ask me any questions. This conversation was conducted in [*Afrikaans/*English/*Xhosa/*Other*] and [*no translator was used/this conversation was translated into* _____ by _____].

_____ _____

Signature of Investigator Date

Stellenbosch University
Faculty of Medicine and Health Sciences

Infant Healthcare and Socio-Demographics Questionnaire

SECTION A:

About the infant

1. Patient number:				
2. Gender:				
3. Race:				
4. Date of birth:				
5. Age:				
6. Where was the baby born?	Hospital	Clinic	Home	Other
How was the baby born?		Normal vaginal delivery		Caesarean section
7. Name of hospital / clinic / other				
8. How much did the baby weigh?				
9. Was the baby		Premature	Full Term	Post-dates (Overdue)
10. If the baby was premature, how premature was it?				
11. Does the mother carry the baby on her back?				
12. Is the baby	Breast fed	Bottle / formula fed		Both
13. What other food is used to feed the baby?				
14. Is the baby placed on a bed / cot to sleep?				
15. If placed on a bed / cot, what type of mattress				
16. Was the mattress covered with a blanket or sheet?			Yes	No
17. In what position is the baby placed when put to sleep?	Back	Stomach	Side	Other
18. Does the baby sleep in the same bed as the mother?				
19. How many other people sleep on the same bed as the baby?				

SECTION B:

Infant's medical history

1. At any time in the infant's life, does s/he have a history of? a. Allergies (food, medication, or other) b. Abnormal growth or weight gain/ loss) c. Others	**Unknown**	**Yes**	**No**
2. Does the infant have any birth defect(s)?		Yes	No

3. Describe
4. Is the baby ill? If yes, what is wrong and for how long?
5. Has the baby received any medication (e.g., antibiotics) or is the baby currently using any medication? If yes, what medication?
6. Which vaccinations has the baby received to date?

Appendix D: Blast results for all obtained sequences from the screening of the VP7 and VP4 genes.

Sample I	Rotavirus Specie	(%) identit	Accession numb	Country	Host	Strain	Isolation sourc	Year	Reference	Journal	Segment/Gen
897	Rotavirus A	99.76	MW392029 .1	South Africa	Homo sapiens	NS0190 (Isolates)	Stool	2017	Rossouw et al., 2021	unpublished	4/VP4
897	Rotavirus A	99.52	MZ094320.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KLF0616/2012/G2P[4]	Stool	2012	Lambisia et al., 2021	unpublished	4/VP4
897	Rotavirus A	100.00	MW392039.1	South Africa	Homo sapiens	NS0203(Isolates)	Stool	2017	Rossouw et al., 2021	unpublished	4/VP4
897	Rotavirus A	99.76	MW392041.1	South Africa	Homo sapiens	NS0219(Isolates)	Stool	2017	Rossouw et al., 2021	unpublished	4/VP4
897	Rotavirus A	99.76	MW392006.1	South Africa	Homo sapiens	NS0021(Isolates)	Stool	2016	Rossouw et al., 2021	unpublished	4/VP4
1174	Rotavirus A	99.82	MW552576.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD13791/2014/G2P[4]	Stool	2014	Nyaga et al., 2021	unpublished	4/VP4
1174	Rotavirus A	99.63	MW552642.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD15070/2015/G2P[4]	Stool	2015	Nyaga et al., 2021	unpublished	4/VP4
1174	Rotavirus A	99.82	MW552554	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD13522/2014/G2P[4]	Stool	2014	Nyaga et al., 2021	unpublished	4/VP4
1174	Rotavirus A	100.00	MW392003.1	South Africa	Homo sapiens	NS0009(Isolates)	Stool	2016	Rossouw et al., 2021	unpublished	4/VP4
1174	Rotavirus A	100.00	MZ096973.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KLF1033/2018/G2P[4]	Stool	2018	Lambisia et al., 2021	unpublished	4/VP4
1174	Rotavirus A	100.00	MW552686.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD18920/2017/G2P[4]	Stool	2017	Nyaga et al., 2021	unpublished	4/VP4
1525	Rotavirus A	99.75	MW552576.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD13791/2014/G2P[4]	Stool	2014	Nyaga et al., 2021	unpublished	4/VP4
1525	Rotavirus A	99.51	MW552642.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD15070/2015/G2P[4]	Stool	2015	Nyaga et al., 2021	unpublished	4/VP4
1525	Rotavirus A	99.75	MW552554.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD13522/2014/G2P[4]	Stool	2014	Nyaga et al., 2021	unpublished	4/VP4
1525	Rotavirus A	100.00	MW392003.1	South Africa	Homo sapiens	NS0009(Isolates)	Stool	2016	Rossouw et al., 2021	unpublished	4/VP4
1525	Rotavirus A	100.00	MZ096973.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KLF1033/2018/G2P[4]	Stool	2018	Lambisia et al., 2021	unpublished	4/VP4
1525	Rotavirus A	99.75	MW552686.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD18920/2017/G2P[4]	Stool	2017	Nyaga et al., 2021	unpublished	4/VP4
1366	Rotavirus A	99.82	MW552576.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD13791/2014/G2P[4]	Stool	2014	Nyaga et al., 2021	unpublished	4/VP4
1366	Rotavirus A	99.63	MW552642.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD15070/2015/G2P[4]	Stool	2015	Nyaga et al., 2021	unpublished	4/VP4
1366	Rotavirus A	99.82	MW552554.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD13522/2014/G2P[4]	Stool	2014	Nyaga et al., 2021	unpublished	4/VP4
1366	Rotavirus A	100.00	MW392003.1	South Africa	Homo sapiens	NS0009(Isolates)	Stool	2016	Rossouw et al., 2021	unpublished	4/VP4
1366	Rotavirus A	99.82	MZ096973.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KLF1033/2018/G2P[4]	Stool	2018	Lambisia et al., 2021	unpublished	4/VP4
1366	Rotavirus A	99.82	MW552686.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD18920/2017/G2P[4]	Stool	2017	Nyaga et al., 2021	unpublished	4/VP4
H12	Rotavirus A	100.00	MN366066.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/RJ_24920/2015/G1P[8]	Stool	2015	Cantelli et al., 2020	unpublished	4/VP4
H12	Rotavirus A	100.00	MG590348.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/CE0196/2010/P[8]		2010	Pankov et al., 2018	unpublished	4/VP4
H12	Rotavirus A	100.00	MN366074.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/RJ_24025/2014/G1P[8]	Stool	2014	Cantelli et al., 2020	unpublished	4/VP4
H12	Rotavirus A	100.00	MN366057.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/RJ_26479/2017/G1P[8]	Stool	2017	Cantelli et al., 2020	unpublished	4/VP4
H12	Rotavirus A	100.00	MN478758.1	USA	Homo sapiens	RVA/Human-wt/USA/3000378272/2016/G1P[8]	Stool	2016	Esona et al., 2021	unpublished	4/VP4
H12	Rotavirus A	100.00	MW718952.1	Tanzania	Homo sapiens	VAC2P(Isolates)	Stool	2019	Malakalinga et al., 2022	Direct Submission	4/VP4
R31	Rotavirus A	99.82	MN366066.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/RJ_24920/2015/G1P[8]	Stool	2015	Cantelli et al., 2020	unpublished	4/VP4
R31	Rotavirus A	100.00	MG590348.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/CE0196/2010/P[8]		2010	Pankov et al., 2018	unpublished	4/VP4
R31	Rotavirus A	100.00	MN366074.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/RJ_24025/2014/G1P[8]	Stool	2014	Cantelli et al., 2020	unpublished	4/VP4
R31	Rotavirus A	100.00	MN366057.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/RJ_24920/2015/G1P[8]	Stool	2017	Cantelli et al., 2020	unpublished	4/VP4
R31	Rotavirus A	100.00	MN478758.1	USA	Homo sapiens	RVA/Human-wt/USA/3000378272/2016/G1P[8]	Stool	2016	Esona et al., 2021	unpublished	4/VP4
R31	Rotavirus A	100.00	MW718952.1	Tanzania	Homo sapiens	VAC2P(Isolates)	Stool	2019	Malakalinga et al., 2022	Direct Submission	4/VP4
1677	Rotavirus A	99.45	MN527952.1	China	Homo sapiens	RVA/Human-wt/CHN/P13291065/2013/G9P8	Stool	2013	Gao et al., 2022	unpublished	4/VP4
1677	Rotavirus A	99.45	MN527836.1	China	Homo sapiens	RVA/Human-wt/CHN/P15112036/2015/G9P8	Stool	2015	Gao et al., 2022	unpublished	4/VP4
1677	Rotavirus A	99.45	MN527845.1	China	Homo sapiens	RVA/Human-wt/CHN/P14011114/2014/G9P8	Stool	2014	Gao et al., 2022	unpublished	4/VP4
1677	Rotavirus A	99.45	MN527837.1	China	Homo sapiens	RVA/Human-wt/CHN/P15081103/2015/G9P8	Stool	2015	Gao et al., 2022	unpublished	4/VP4
1677	Rotavirus A	100.00	MW392028.1	South Africa	Homo sapiens	NS0189(Isolates)	Stool	2017	Rossouw et al., 2021	unpublished	4/VP4
Outgroup			HQ650119.1	USA	Homo sapiens	Rotavirus A RVA/Human-tc/USA/DS-1/1976/G2P[4]		1976	Mlera et al., 2011	published	4/VP4

Sample I	Rotavirus Specie	(%) identit	Accession numbe	Country	Host	Strain	Isolation source	Year	Reference	Journal	Segment/Gen
1174	Rotavirus A	99.86	MW552597.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD13907/2014/G2P[4]	Stool	2014	Nyaga et al., 2021	unpublished	9/VP7
1174	Rotavirus A	99.86	MW552146.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-MRC-DPRU198/2013/G2P[4]	Stool	2013	Nyaga et al., 2021	unpublished	9/VP7
1174	Rotavirus A	100.00	MW552685.1	South Africa	Homo sapiens	RVA/Human-wt/ZAF/UFS-NGS-NICD18920/2017/G2P[4]	Stool	2017	Nyaga et al., 2021	unpublished	9/VP7
1174	Rotavirus A	99.86	MZ096975.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KLF1033/2018/G2P[4]	Stool	2018	Lambisia et al., 2021	unpublished	9/VP7
1174	Rotavirus A	99.86	MN194385.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KLF0906/2018/G2P[8]		2018	Mwanga et al., 2020	unpublished	9/VP7
897	Rotavirus A	99.12	MH402590.1	Kenya	Homo sapiens	KEN/0231/2009 (Isolates)		2009	Owor et al., 2018	unpublished	9/VP7
897	Rotavirus A	99.12	MK434785.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KBR177/2012/G8P[4]	Stool	2012	Mwanga et al., 2019	unpublished	9/VP7
897	Rotavirus A	99.12	MZ093894.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KLF0563/2012/G8P[4]	Stool	2012	Lambisia et al., 2021	unpublished	9/VP7
897	Rotavirus A	99.12	MK434784.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KBR178/2012/G8P[4]	Stool	2012	Mwanga et al., 2019	unpublished	9/VP7
897	Rotavirus A	99.12	MK434780.1	Kenya	Homo sapiens	RVA/Human-wt/KEN/KBR171/2012/G8P[4]	Stool	2012	Mwanga et al., 2019	unpublished	9/VP7
H16	Rotavirus A	99.17	KP753098.1	Zimbabwe	Homo sapiens	RVA/Human-wt/ZWE/MRC-DPRU1855/2011/G9P[8]		2011	Das et al., 2015	Direct Submission	9/VP7
H16	Rotavirus A	99.31	KJ752797.1	Zimbabwe	Homo sapiens	RVA/Human-wt/ZWE/MRC-DPRU1841/2009/G9P[8]		2009	Wentworth et al., 2015	Direct Submission	9/VP7
H16	Rotavirus A	99.17	KJ753710.1	Zimbabwe	Homo sapiens	RVA/Human-wt/ZWE/MRC-DPRU3348/2010/G9P[8]		2010	Wentworth et al., 2015	Direct Submission	9/VP7
H16	Rotavirus A	99.17	KJ753539.1	Zimbabwe	Homo sapiens	RVA/Human-wt/ZWE/MRC-DPRU3367/2010/9P[8]		2010	Wentworth et al., 2015	Direct Submission	9/VP7
H16	Rotavirus A	99.58	MN787037.1	South Africa	Homo sapiens	AK262 (Isolates)	Stool	2018	Kuča and Mans, 2021	unpublished	9/VP7
1606	Rotavirus A	99.87	ON855111.1	Belgium	Homo sapiens	RVA/Human-wt/BEL/F03589/2011/G1P8		2009/2019	Simsek et al., 2022	published	9/VP7
1606	Rotavirus A	99.87	ON855125.1	Belgium	Homo sapiens	RVA/Human-wt/BEL/F09066/2017/G1P8		2009/2019	Simsek et al., 2022	published	9/VP7
1606	Rotavirus A	100.00	MG571803.1	Venezuela	Homo sapiens	clone="RVA/Human-wt/VEN/VME8A/2015/G1P[8]	Stool	2015	Siqueira et al., 2018	unpublished	9/VP7
1606	Rotavirus A	100.00	KY616899.1	Japan	Homo sapiens	RVA/Human-wt/JPN/JP11786/2013/G1P[8]	Stool	2013	Kaneko et al., 2018	published	9/VP7
1606	Rotavirus A	100.00	MG922993.1	South Korea	Homo sapiens	CAU95/RVA/KOR/2016 (Isolates)		2016	Tran & Kim, 2018	unpublished	9/VP7
1606	Rotavirus A	100.00	MG922997.1	South Korea	Homo sapiens	CAU242/RVA/KOR/2016 (Isolates)		2016	Tran & Kim, 2018	unpublished	9/VP7
1606	Rotavirus A	100.00	MT633136.1	Brazil	Homo sapiens	TO-237 (Isolates)	Stool	2014	Silva-Sales et al., 2021	published	9/VP7
1606	Rotavirus A	100.00	MG590364.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/CE0158/2010/G1		2010	Pankov et al., 2018	unpublished	9/VP7
1606	Rotavirus A	100.00	MG590362.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/CE0045/2010/G1		2010	Pankov et al., 2018	unpublished	9/VP7
124	Rotavirus A	99.69	MH884610.1	Brazil	Homo sapiens	RVA/Vaccine/Rotarix/ETH/BD445/2016/G1P[8]	Stool	2016	Gelaw et al., 2019	unpublished	9/VP7
124	Rotavirus A	99.84	ON855125.1	Belgium	Homo sapiens	RVA/Human-wt/BEL/F09066/2017/G1P8		2009/2019	Simsek et al., 2022	published	9/VP7
124	Rotavirus A	99.84	ON855111.1	Belgium	Homo sapiens	RVA/Human-wt/BEL/F03589/2011/G1P8		2009/2019	Simsek et al., 2022	published	9/VP7
124	Rotavirus A	99.69	MG571803.1	Venezuela	Homo sapiens	clone="RVA/Human-wt/VEN/VME8A/2015/G1P[8]	Stool	2015	Siqueira et al., 2018	unpublished	9/VP7
124	Rotavirus A	99.69	KY616899.1	Japan	Homo sapiens	RVA/Human-wt/JPN/JP11786/2013/G1P[8]	Stool	2013	Kaneko et al., 2018	published	9/VP7
124	Rotavirus A	99.69	MG922993.1	South Korea	Homo sapiens	CAU95/RVA/KOR/2016 (Isolates)		2016	Tran & Kim, 2018	unpublished	9/VP7
124	Rotavirus A	99.69	MG922997.1	South Korea	Homo sapiens	CAU242/RVA/KOR/2016 (Isolates)		2016	Tran & Kim, 2018	unpublished	9/VP7
124	Rotavirus A	99.69	MT633136.1	Brazil	Homo sapiens	TO-237 (Isolates)	Stool	2014	Silva-Sales et al., 2021	published	9/VP7
124	Rotavirus A	99.69	MG590364.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/CE0158/2010/G1		2010	Pankov et al., 2018	unpublished	9/VP7
124	Rotavirus A	99.69	MG590362.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/CE0045/2010/G1		2010	Pankov et al., 2018	unpublished	9/VP7
H12	Rotavirus A	100.00	MH884610.1	Brazil	Homo sapiens	RVA/Vaccine/Rotarix/ETH/BD445/2016/G1P[8]	Stool	2016	Gelaw et al., 2019	unpublished	9/VP7
H12	Rotavirus A	99.86	ON855125.1	Belgium	Homo sapiens	RVA/Human-wt/BEL/F09066/2017/G1P8		2009/2019	Simsek et al., 2022	published	9/VP7
H12	Rotavirus A	99.86	ON855111.1	Belgium	Homo sapiens	RVA/Human-wt/BEL/F03589/2011/G1P8		2009/2019	Simsek et al., 2022	published	9/VP7
H12	Rotavirus A	100.00	MG571803.1	Venezuela	Homo sapiens	clone="RVA/Human-wt/VEN/VME8A/2015/G1P[8]	Stool	2015	Siqueira et al., 2018	unpublished	9/VP7
H12	Rotavirus A	100.00	KY616899.1	Japan	Homo sapiens	RVA/Human-wt/JPN/JP11786/2013/G1P[8]	Stool	2013	Kaneko et al., 2018	published	9/VP7
H12	Rotavirus A	100.00	MG922993.1	South Korea	Homo sapiens	CAU95/RVA/KOR/2016 (Isolates)		2016	Tran & Kim, 2018	unpublished	9/VP7
H12	Rotavirus A	100.00	MG922997.1	South Korea	Homo sapiens	CAU242/RVA/KOR/2016 (Isolates)		2016	Tran & Kim, 2018	unpublished	9/VP7
H12	Rotavirus A	100.00	MT633136.1	Brazil	Homo sapiens	TO-237 (Isolates)	Stool	2014	Silva-Sales et al., 2021	published	9/VP7
H12	Rotavirus A	100.00	MG590364.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/CE0158/2010/G1		2010	Pankov et al., 2018	unpublished	9/VP7
H12	Rotavirus A	100.00	MG590362.1	Brazil	Homo sapiens	RVA/Human-wt/BRA/CE0045/2010/G1		2010	Pankov et al., 2018	unpublished	9/VP7
	Outgroup		HQ650124_1	USA	Homo sapiens	Rotavirus A RVA/Human-tc/USA/DS-1/1976/G2P[4]		1976	Mlera et al., 2011	published	9/VP7

Appendix E: QIIME input commands

```
#Import sequence reads (fastq) as a qiime artifact
qiime tools import \
        --type 'SampleData[SequencesWithQuality]' \
        --input-path danielle-manifest.txt \
        --output-path single-end-demux.qza \
        --input-format SingleEndFastqManifestPhred33V2

#Denoise pacbio data and chimaera removal with dada2; also generates ASVs and the resulting feature table and representative sequences

#The sequences given are the 27F and 1492R 16S primers used during for the sequencing run
qiime dada2 denoise-ccs \
        --i-demultiplexed-seqs single-end-demux.qza \
        --p-front AGRGTTYGATYMTGGCTCAG \
        --p-adapter RGYTACCTTGTTACGACTT \
        --p-n-threads 10 \
        --o-table table.qza \
        --o-representative-sequences rep-seqs.qza \
        --o-denoising-stats stats.qza

#Generate statistics from the dada2 run
qiime metadata tabulate \
         --m-input-file stats.qza \
         --o-visualization stats.qzv

#Generate statistics from the dada2 run - tells you how much data passed filters
qiime metadata tabulate \
         --m-input-file stats.qza \
         --o-visualization stats.qzv

#Generate visualisations for the feature table and representative sequences
qiime feature-table summarize \
         --i-table table.qza \
         --o-visualization table.qzv \
         --m-sample-metadata-file danielle-metadata.tsv
qiime feature-table tabulate-seqs \
         --i-data rep-seqs.qza \
         --o-visualization rep-seqs.qzv

#Assign taxonomy to sequences using a classifier trained on the full-length 16S region from the SILVA database
qiime feature-classifier classify-sklearn \
         --i-classifier silva-138-99-nb-classifier.qza \
         --i-reads rep-seqs.qza \
         --o-classification taxonomy.qza
qiime metadata tabulate \
         --m-input-file taxonomy.qza \
```

```
        --o-visualization taxonomy.qzv
qiime taxa barplot \
        --i-table table.qza \
        --i-taxonomy taxonomy.qza \
        --m-metadata-file danielle-metadata.tsv \
        --o-visualization taxa-bar-plots.qzv

# Feature tables must be created in order to filter data

# Create a text file that contains only the sample-ids to be kept in the table
qiime feature-table filter-samples \
        --i-table table.qza \
        --m-metadata-file samples-only.tsv \
        --o-filtered-table samples-only-filtered-table.qza
 qiime taxa barplot \
        --i-table samples-only-filtered-table.qza \
        --i-taxonomy taxonomy.qza \
        --m-metadata-file danielle-metadata-unknown-blank.tsv \
        --o-visualization samples-only-taxa-bar-plots.qzv

#Generate a phylogenetic tree - needed for some of the diversity metrics
qiime phylogeny align-to-tree-mafft-fasttree \
        --i-sequences rep-seqs.qza \
        --o-alignment aligned-rep-seqs.qza \
        --o-masked-alignment masked-aligned-rep-seqs.qza \
        --o-tree unrooted-tree.qza \
        --o-rooted-tree rooted-tree.qza

# Determine whether you need to rarefy your data and if so, at what sequencing depth
qiime diversity alpha-rarefaction \
        --i-table table.qza \
        --i-phylogeny rooted-tree.qza \
        --p-max-depth 28303 \
        --m-metadata-file danielle-metadata.tsv \
        --o-visualization alpha-rarefaction.qzv

#Run the core diversity metrics
qiime diversity core-metrics-phylogenetic \
        --i-phylogeny rooted-tree.qza \
        --i-table samples-only-filtered-table.qza \
        --p-sampling-depth 8062 \
        --m-metadata-file danielle-metadata-unknown-blank.tsv \
        --output-dir core-metrics-results

# Additional statistical testing for each group, based on metrics and groups of interest
qiime diversity alpha-group-significance \
        --i-alpha-diversity core-metrics-results/shannon_vector.qza \
        --m-metadata-file danielle-metadata-unknown-blank.tsv \
```

```
    --o-visualization core-metrics-results/shannon-group-significance.qzv
qiime diversity alpha-group-significance \
    --i-alpha-diversity core-metrics-results/observed_features_vector.qza \
    --m-metadata-file danielle-metadata-unknown-blank.tsv \
    --o-visualization core-metrics-results/observed_features_vector.qzv
qiime diversity beta-group-significance \
    --i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \
    --m-metadata-file danielle-metadata.tsv \
    --m-metadata-column status \
    --o-visualization core-metrics-results/unweighted-unifrac-status-significance.qzv \
    --p-pairwise
qiime diversity beta-group-significance \
    --i-distance-matrix core-metrics-results/weighted_unifrac_distance_matrix.qza \
    --m-metadata-file danielle-metadata.tsv \
    --m-metadata-column mode-of-birth \
    --o-visualization core-metrics-results/weighted-unifrac-birth-significance.qzv \
    --p-pairwise

#Differential abundance with ANCOM

#ANCOM recommends that features be present in at least 25% of samples and at least 20 times overall (p-min-frequency) in order to remove filters
qiime feature-table filter-features \
    --i-table samples-only-filtered-table.qza \
    --p-min-frequency 20 \
    --p-min-samples 11 \
    --o-filtered-table feature-frequency-filtered-table.qza

#Add pseudocount (ANCOM does not like 0s)
    qiime composition add-pseudocount \
    --i-table samples-only-filtered-table.qza \
    --o-composition-table comp-samples-only-filtered-table.qza

#Run ANCOM based on different metadata column

#Create a filtered table that excludes samples with no information for that column, and then run ancom on this table
qiime composition ancom \
    --i-table comp-samples-only-filtered-table.qza \
    --m-metadata-file danielle-metadata.tsv \
    --m-metadata-column status \
    --o-visualization ancom-status.qzv

#Run ANCOM at different taxonomic levels (e.g., L2=Phylum, L6=genus)
qiime taxa collapse \
    --i-table feature-frequency-filtered-table.qza \
    --i-taxonomy taxonomy.qza \
    --p-level 6 \
    --o-collapsed-table samples-only-filtered-table-l6.qza
```

```
qiime composition add-pseudocount \
        --i-table samples-only-filtered-table-l6.qza \
        --o-composition-table comp-samples-only-filtered-table-l6.qza
qiime composition ancom \
        --i-table comp-samples-only-filtered-table-l6.qza \
        --m-metadata-file danielle-metadata.tsv \
        --m-metadata-column status \
        --o-visualization l6-ancom-status.qzv
```