An *in vitro* study to assess three different sterilising methods for infant feeding cups and bottles

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Thesis presented in fulfilment of the requirements for the degree Master of Nursing in the Faculty of Medicine and Health Sciences at Stellenbosch University.

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DECLARATION

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i

ABSTRACT

Background

Diarrhoea (frequent, loose, watery stools) is one of the major causes of morbidity and mortality globally and affects mainly infants and children under the age of five years. Unhygienic feeding practices and feeding utensils contribute to diarrhoeal incidences. The most common causes of acute gastroenteritis worldwide are infectious agents, such as viruses, bacteria and parasites

Aim

The aim of the study was to investigate which out of three particular sterilising methods is the most effective for sterilising feeding bottles and cups.

Methods

An experimental quantitative approach was most appropriate for the current study. An *in vitro* experimental study with a descriptive design was utilised under controlled laboratory conditions. The study was conducted at the University of the Western Cape (UWC) in April 2009.

Results

The sample size consisted of 16 samples, of which two were used for each method of sterilisation, namely: two (2) bottles and two (2) cups for sunlight; two (2) bottles and two (2) cups for Milton™; two (2) bottles and two (2) cups for Sunlight™ dishwashing liquid; and control utensils that consisted of two (2) bottles and two (2) cups. The target population for the study comprised infant feeding bottles and feeding cups. The analysis for the APC cultures that was compared in the cups vs. bottles, in order to see whether there was a significant difference between the mean bacteria counts, shows that the average bacteria count (on the ln scale) was 6 cfu/ml and 9 cfu/ml for the cups and bottles, respectively. The t-value was -1.17524. As the ρ-value was 0.2595, no significant difference was found between the cups and bottles.

The *E. coli* cultures were compared in the cups vs. bottles to see whether there was a significant difference between the mean bacteria counts.

The results show that the average bacteria count (on the ln scale) was 7 cfu/ml and 7.6 cfu/ml for cups and bottles, respectively. The t-value was -0.211902. The ρ-value was 0.835237, and therefore there was no significant difference between cups and bottles.

Conclusion

The current study showed no significant difference between the sterilising methods or between the use of either bottles or cups. Therefore, a study with a larger sample size is recommended for further research.

Recommendations

The researcher recommends that future researchers conduct broader studies, with a larger sample size on the topic. Studies with a larger sample size enabled the real differences to be large enough to be significant. The use of sunlight is recommended as a sterilisation method for infant feeding utensils, as it is both time- and cost-effective. Sunlight is an inexpensive and readily available method of sterilisation; therefore, it can be used by relatively under resourced socio-economic communities.

Keywords

Bacteria, *Escherichia coli*, Contamination, Cleaning, Feeding utensils, Feeding bottles, Feeding cups, *In vitro*, Infant, Sterilisation

OPSOMMING

Agtergrond

Diarree (gereelde, los, waterige stoelgang) is een van die hoofoorsake van morbiditeit en sterflikheid wêreldwyd en affekteer hoofsaaklik suigelinge en kinders onder die ouderdom van vyf jaar. Onhigiëniese voedingspraktyke en -gereedskap dra by tot die voorkoms van diarree-gevalle. Die mees algemene oorsake van akute gastroënteritus wêreldwyd word veroorsaak deur aansteeklike agente soos virusse, bakterieë en parasiete.

Doel

Die doel van hierdie studie is om ondersoek te doen na watter van die drie bepaalde steriliseringsmetodes die mees effektiewe is vir die sterilisering van bottels en koppies.

Metodes

'n Eksperimentele kwantitatiewe benadering is die mees geskikte een vir die huidige studie. 'n *In vitro*-eksperimentele studie met 'n deskriptiewe ontwerp is onder gekontroleerde laboratorium omstandighede aangewend. Die studie is by die Universitet van die Wes-Kaap (UWK) in April 2009 uitgevoer.

Resultate

Die steekproefgroote het bestaan uit 16 monsters waarvan twee gebruik is vir elke steriliseringsmetode, naamlik: twee (2) bottels en twee (2) koppies vir sonlig; twee (2) bottels en twee (2) koppies vir Milton™; twee (2) bottels en twee (2) koppies vir Sunlight™ skottelgoedopwasmiddel; en kontrole gereedskap wat bestaan het uit twee (2) bottels en twee (2) koppies. Die teikenbevolking vir die studie het bestaan uit voedingsbottels en - koppies vir suigelinge. Die analise vir die APC-kulture wat vergelyk is in die koppies vs. bottels om te bepaal of daar 'n beduidende verskil is tussen die gemiddelde bakterie-tellings, toon dat die gemiddelde bakterie-telling (op die ln-skaal) is 6 cfu/ml en 9 cfu/ml vir die koppies en bottels respektiewelik.

Die t-waarde is -1.17524. Aangesien die p-waarde 0.2595 is, is daar geen beduidende verskil gevind tussen die koppies en die bottels nie. Die *E. coli*-kulture is vergelyk in die koppies vs. bottels om te bepaal of daar 'n beduidende verskil tussen die gemiddelde bakterie-tellings is. Die uitslae wys dat die gemiddelde bakterie-telling (op die In-skaal) is 7cfu/ml en 7.6 cfu/ml vir koppies en bottels respektiewelik. Die t-waarde is -0.211902. Die p-waarde is 0.835237 en dus is daar geen beduidende verskil tussen koppies en bottels nie.

Gevolgtrekking

Die huidige studie toon dat daar geen beduidende verskil tussen die steriliseringsmetodes of tussen die gebruik van of bottels of koppies is nie. Dus, 'n studie met 'n groter steekproefgrootte word aanbeveel vir toekomstige navorsing.

Aanbevelings

Die navorser beveel aan dat toekomstige navorsers meer omvattende studies met 'n groter steekproefgrootte oor die onderwerp uitvoer. Studies met 'n groter steekproefgrootte sal veroorsaak dat die werklike verskille vanweë hul grootte genoegsaam sal wees, om beduidend te wees. Die gebruik van sonlig as 'n steriliseringsmetode vir die gereedskap van suigelinge word aanbeveel, aangesen dit beide tyd- en kostebesparend is. Sonlig is 'n goedkoop en maklik verkrygbare metode van sterilisasie; dus kan dit gebruik word deur gemeenskappe wat nie oor die nodige middele beskik nie, vanweë hul sosio-ekonomiese situasies.

DEDICATION

I hereby dedicate this thesis to my late grandfather, Maarman Maloy and to my uncle, Jerry Christo Maloy.

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I am extremely grateful to my supervisor, Janet Bell, for her unfailing patience, support, care, and invaluable insights, as well as for believing in me when I felt at my lowest and thought that I would not be able to complete the thesis.

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TABLE OF CONTENTS

Page

CHAPTER ONE: ORIENTATION TO THE STUDY

CHAPTER FOUR: DATA ANALYSIS AND DISCUSSION

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURES

List of Annexure

LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ANOVA	analysis of variance
AOAC	The Scientific Association Dedicated to Analytical Excellence
APC	aerobic plate count
ATP	adenosine triphosphate
CFU	colony-forming unit
CINAHL	Cumulative Index to Nursing and Allied Health Literature
HIV	Human immunodeficiency virus
NCSS	Number Cruncher Statistical System
NUFU	Norwegian Programme for Development, Research and Education
PMTCT	Prevention of Mother-to-Child Transmission
REVEAL	Rotavirus gastroenteritis epidemiology and viral types in Europe accounting for losses in public health and society
SAGLP	South African Good Clinical Laboratory Practice
SU	Stellenbosch University
TSB	Tryptic Soy Broth
UNICEF	United Nations Children's Fund
UWC	University of the Western Cape
WHO	World Health Organisation

CHAPTER ONE
INTRODUCTION TO STUDY

1.1   INTRODUCTION

Acute diarrhoea due to gastroenteritis is commonly self-limiting, but if medical help is not sought in time, it can result in death due to dehydration, electrolyte imbalance and acid-base disturbance. Diarrhoea (marked by frequent, loose, watery stools) is one of the major causes of morbidity and mortality globally and affects mainly infants and children under the age of five years (Dalby-Payne & Elliott, 2009:314). Anything from 3 to 5 billion cases of diarrhoea are reported each year and between 1.4 and 2.5 million children die every year due to diarrhoea (Vreeman, 2009:337-341).

The most common causes of acute gastroenteritis worldwide are infectious agents, such as viruses, bacteria and parasites. Viruses, primarily rotavirus species, are responsible for 70% to 80% of infectious diarrhoeal cases in the developed world. Ten to 20% of cases are due to various bacterial pathogens. Another 10% of cases may be due to diarrheagenic *Escherichia coli* (Cohen, Nataro, Bernstein, Hawkins, Roberts & Staat, 2005:54-61). The distribution of diarrheal episodes is affected by climate and season and the winter rotavirus infections account for more than 50% of hospitalisations for paediatric gastroenteritis (Talley & Martin, 2006:204).

Acute watery diarrhoea is associated with a significant fluid loss and rapid dehydration. The diarrhoea usually lasts for several hours or days. Pathogens that generally cause acute watery diarrhoea include *Vibrio cholerae* or *E. coli* bacteria, as well as rotaviruses.

Bloody diarrhoea or dysentery is identifiable through visible blood in stools. Dysentery is associated with intestinal damage and nutrient losses. The most common cause of bloody diarrhoea is the *Shigella* species of bacteria and it also gives rise to the most prominent of severe cases.

1

Persistent diarrhoea is an episode of diarrhoea, with or without blood, that continues for at least 14 days. Undernourished children and children with acquired immunodeficiency syndrome (AIDS) are more likely to develop persistent diarrhoea that, in turn, is likely to worsen their condition (United Nations Children's Fund/World Health Organisation, 2009:10).

In 2004, rotavirus accounted for 527 000 deaths in children, and for 29% of all deaths due to diarrhoea in children under five year of age (Parashar, Gibson, Bresse & Glass, 2006:304-306). The virus has been shown to cause 40% to 50% of all severe acute diarrhoea in young children worldwide. In addition, more than 600 000 young children die and nearly 2.4 million children are hospitalised annually from rotavirus disease, mainly in South East Asia and in sub-Saharan Africa (Mukherjee & Chawla-Sarkar, 2011:11-23). Between 2003 and 2005, of all the children under five years of age admitted to Dr George Mukhari Hospital in Gauteng, an estimated 5.5% had rotavirus diarrhoea (Mapaseka, Dewar, van der Merwe, Geyer, Tumbo, Zweygarth, Bos, Esona, Steele & Sommerfelt, 2010:131-138).

Utensils, such as feeding bottles, are difficult to clean. Inadequately cleaned feeding bottles and formula feed that is prepared with contaminated water cause diarrhoea. Feeding bottles that are not thoroughly cleaned can increase the risk of diarrhoea in those under the age of five years who are fed via feeding bottles, as compared to those children who are fed via feeding cups (Kelly, Khanfir, David, Arata & Kleinau, 1999:7; Okertcho, Nyaruhucha, Tayabali & Karimuribo, 2012:1-14).

The current *in vitro* study was conducted to determine which of three sterilising methods applied to feeding cups and bottles was most effective in limiting the growth of diarrhoea-causing micro-organisms.

1.2   RATIONALE OF THE STUDY

Although death due to diarrhoea is less common in developed countries, dehydration secondary to the condition is still a significant cause of morbidity and hospital admission (Dalby-Payne & Elliott, 2009:314). In South Africa, diarrhoeal disease is a major cause of

morbidity and mortality in children under five years of age and accounted for 10.2% of deaths in 2000 (Bradshaw, Groenewald, Laubscher , Nannan, Nojilana, Norman, Pieterse & Schneider, 2003).

Seventy-eight babies died from complications of diarrhoea in the Ukhahlamba district of the Eastern Cape in January to March 2008. Eighty percent of the children concerned lived in households with no sanitation. The local tap water was found to be contaminated with *E. coli* (Lake & Reynolds, 2009:4-13). During the 2009 diarrhoea season in the Western Cape province (November to May), the number of children diagnosed with acute gastroenteritis had risen by 26.1% as compared to the number diagnosed with the same complaint in the 2008 diarrhoea season. Deteriorating conditions in informal settlements and inadequate penetration of preventative and promotion messages was ruled to be the cause of the increased incidence of diarrhoea (Western Cape Department of Health, 2009-2010).

Diarrhoea due to gastroenteritis is also associated with the human immunodeficiency virus (HIV) and is a leading cause of illness and death in HIV-infected children in Africa (Steele, Cunliffle, Tumbo, Madhi, De Vos & Bouckenooghe, 2009:S57).

Studies have shown that few women in South Africa who live in peri-urban informal settlements practise adequate personal and domestic hygienic behaviour (Lin, Puckree & Ntshangase, 2002:252-253). Most of the women in the described environment lack knowledge about the prevention of diarrhoea in their children (Lin et al., 2002:252-253). The mothers' lack of knowledge about the causes of diarrhoea, as well as unsafe water, the unhygienic handling of food, and poor domestic and personal hygiene are all associated with the preparation and managing of bottle-feeding that, in turn, relates to the development of diarrhoeal disease (Singh, 2010:404-422).

As a result of the above, throughout the current study, the researcher will determine an adequate and cost-effective sterilisation method that can be used in all communities to try to reduce the prevalence of gastroenteritis/diarrhoea. Following the method to be advocated will specifically benefit those parents and babies living in poorly resourced areas.

3

1.3   RESEARCH PROBLEM

Gastroenteritis, which is a global problem, results in high morbidity and mortality rates in infants, especially when mothers choose to bottle-feed their infants (Wright, Parkinson & Drewett, 2004:813-816). The World Health Organisation (WHO) recommends that parents should use cup feeding rather than bottle-feeding, as cups are presumed to be easier to clean (World Health Organisation, 2006/2010). However, it is not known whether micro-organisms are found less prevalently in feeding cups as compared to feeding bottles, and whether it is easier to clean feeding cups than it is to clean feeding bottles.

1.4   RESEARCH QUESTION

Based on the discussion provided above, the following research questions were posed:

- Are feeding cups easier to sterilise than feeding bottles?
- Is exposure of a feeding cup or bottle to sunlight an adequate method of sterilising such utensils?

1.5   RESEARCH AIM

The aim of the study was to investigate which out of three particular sterilising methods is the most effective to sterilise feeding bottles and cups.

1.6   RESEARCH OBJECTIVES

The objective of the current study was to determine the efficacy of three different methods of feeding utensil sterilisation commonly used in resource-poor areas, namely:

- chemical sterilisation using Milton™;
- dishwashing liquid using Sunlight™ dishwashing liquid; and
- natural sunlight.

1.7   METHODOLOGY

1.7.1 Approach and design

An experimental quantitative approach was considered to be most appropriate for the current study. An *in vitro* experimental study with a descriptive design was utilised under controlled laboratory conditions. The study was conducted at the University of the Western Cape (UWC) in April 2009.

1.7.2 Population and population sample

The population for the current study comprised infant feeding bottles and feeding cups. The study sample comprised a total of 16 infant feeding bottles and feeding cups, with eight of each utensil. The bottles were purchased locally at a supermarket and the cups were provided to the researcher by a local hospital milk kitchen. The cups had never before been used and were sealed in a polypropylene bag.

Two samples were used for each method of sterilisation, namely:
- 2 bottles and 2 cups for sunlight;
- 2 bottles and 2 cups for Milton™;
- 2 bottles and 2 cups for Sunlight™ dishwashing liquid; and
- 2 bottles and 2 cups as control utensils.

1.7.3 Data collection and management

Data were collected by making use of structured observational measurements. All the study feeding bottles and feeding cups were sterilised in boiling water for five minutes. *E. coli* was used as the contaminant, as the bacteria are commonly associated with the contamination of infant feeding utensils. One hundred µl of *E. coli* spp. was grown up in 10 ml of Tryptic Soy Broth (TSB) and incubated at 37ºC for 24 hours. Doing so allowed the *E. coli* spp. to approach the stationary phase of growth at a concentration of approximately 10^{-6} colony-forming units (cfu) per ml. For the experimental utensils, 100 µl of *E. coli* spp. culture was inoculated into six infant feeding bottles and six feeding cups, each of which contained 100 ml of formula milk (Lactogen). The inoculated utensils were then incubated at 37ºC for 24 hours, after which the formula milk was discarded from each infant feeding bottle and cup. The feeding utensils were then sterilised using one of the three identified methods, namely direct sunlight, Sunlight™ dishwashing liquid and Milton™.

The two control utensils were inoculated with formula milk containing no *E. coli* spp. (two infant feeding bottles and two feeding cups). Nine millilitres of quarter-strength Ringers solution were aseptically transferred into each infant feeding bottle and feeding cup. One millilitre from each infant feeding bottle and cup was aseptically transferred into 9 ml of quarter-strength Ringers solution, followed by serial dilutions up to 10^{-6}. One millilitre of each serial dilution was plated onto an aerobic plate count (APC) and *E. coli* petrifilm and incubated at 37ºC for 24 hours.

1.7.4   Statistical analysis

The researcher observed the relationships between the contamination and the effect of the sterilising methods used in the study. In the current thesis, data are given in frequencies and correlations of the contamination. The analysis was done in cooperation with the Stellenbosch University (SU) Department of Statistics and Actuarial Science.

1.8   VALIDITY AND RELIABILITY

Validity and reliability were ensured by using standard calibrated equipment. Carefully measured numbers of bacteria were used in each of the samples, under controlled laboratory conditions. Bacterial counts in each sample were done in controlled laboratory conditions. The South African Good Clinical Laboratory Practice (SAGLP), as well as UWC precaution laboratory policy, were followed to ensure the safety and accuracy of the results obtained.

1.9   ETHICAL CONSIDERATIONS

The current research took the form of an *in vitro* study, with no participant consent being required. Consent to conduct the study was obtained from SU, with laboratory work for the study being conducted in the microbiology laboratory at UWC, with the permission of the facility manager, Prof Pieter Gouws.

Ethical research requires the consideration of, and precaution measures for, any safety risks. UWC precaution laboratory policy was followed by ensuring that the researcher was aware of any potential biosafety risks in the laboratory, such as biological risks including

6

exposure to potential infectious pathogens like *E. coli*. Measures to ensure the safety of the researcher and the laboratory were taken, such as training in activities related to first aid, chemical spills, health and safety precautions, and fire safety. In the laboratory up to three trained microbiology students were available to assist the researcher. The researcher attended updating sessions on laboratory practice to ensure that the most appropriate techniques and processes were implemented. For personal protection, sterile gloves and a clean laboratory coat and closed shoes were worn at all times to avoid contamination from any chemical spills that might have occurred. Masks were worn when working in the laboratory.

1.10   DISSEMINATION OF RESULTS

Results of the current study were to be published in peer-reviewed journals.

1.11   BUDGET

The supervisor received funding from the Norwegian Programme for Development, Research and Education (NUFU), which covered all the costs, including the student's class fees (see Table 1.1 below).

Table 1.1: Expenditure covered by NUFU funding

Expense item	Expense
Laboratory fees	R5 000.00
Printing & copying	R5 000.00
Language editor	R3 000.00
Class fees	R15 000.00
Total expenditure	R28 000.00

1.12   CHAPTERS OF THE THESIS

The chapters of the thesis consist of the following:

- Chapter One: An overview of the research;

- Chapter Two: Literature review;
- Chapter Three: Research design and methodology;
- Chapter Four: Analysis and interpretation of results; and
- Chapter Five: Study conclusions and recommendations.

1.13   DEFINITION OF TERMS

1.13.1   Bacteria

Bacteria are one-celled, plant-like micro-organisms that are visible only under a microscope. They are recognised according to shape, growth needs, staining reactions and loci of infection in the body. Some bacteria live off dead organic matter, whereas others live off tissue that is alive (Blackwells, 2002).

1.13.2   *Escherichia coli*

E. coli is a species of coliform bacteria of the family Enterobacteriaceae, which is normally present in the intestines and which is common in water, milk, and soil. *E. coli* is the most frequent cause of urinary tract infection, and is a serious gram-negative pathogen in humans (Anderson, Keith, Novak & Elliott, 2002).

1.13.3   Contamination

Contamination is a condition of being soiled, stained, touched or otherwise exposed to harmful agents, making an object potentially unsafe for use without barrier techniques (Anderson et al., 2002).

1.13.4   Cleaning

Cleaning is a process in which one physically removes contamination, but which does not necessarily destroy micro-organisms. The reduction of microbial contamination is not routinely quantified and depends upon many factors, including the efficiency of the cleaning process and the initial bio burden involved. Cleaning removes micro-organisms and the organic material on which they thrive. It is a necessary prerequisite for effective disinfection or sterilisation (Horton & Parker, 2002).

1.13.5   Feeding utensils

Feeding utensils are devices used in infant feeding, such as bottles, teats, covers and cups (World Health Organisation, 2009).

1.13.6 Feeding bottle

A feeding bottle, which is a device for infants or young children, consists of a container with a rubber teat on the end which is used to provide milk formula or clear feeds (Anderson et al., 2002).

1.13.7 Feeding cups

Feeding cups are devices that are used to provide infant feeds, such as formula milk. In addition to differing in shape, feeding cups differ from feeding bottles in that the former do not use a rubber teat. Rather, the infant sips from the lip of the cup (Abouelfettoh, Dowling, Dabash, Elguindy & Seoud, 2008).

1.13.8 *In vitro*

In vitro refers to a study that is conducted in glass, such as in a test tube. It is conducted outside the living body, and normally in a laboratory (Blackwells, 2002).

1.13.9 Infant

An infant is a baby from birth to about two years of age, or until the child is able to walk and talk (Blackwells, 2002).

1.13.10 Sterilisation

Sterilisation is a process that is used to render an object free from viable micro-organisms, including viruses and spores. Sterilisation is required where small numbers of residual organisms on an item could be sufficient to cause disease, where exceptionally virulent organisms are suspected, or where surviving organisms might multiply on an item and reach an infective dose before it is used (Horton & Parker, 2002).

1.14 CONCLUSION

Health care workers promote formula feeding for infants of HIV-positive women in order to reduce mother–to–child transmission of the virus. However, many risks are involved with formula feeding, of which a significant one is the development of diarrhoeal disease

through contaminated feeding utensils, as infants are more vulnerable to micro-organisms. Children affected by diarrhoea come mainly from relatively poor communities, where limited utensil sterilisation methods are available. By assisting in determining effective methods, which are also cost-effective, to sterilise bottle and feeding cups, the current study will contribute to the health of vulnerable infants.

CHAPTER TWO
LITERATURE REVIEW

2.1 INTRODUCTION

The current review of literature summarises information gleaned from theoretical and empirical sources in order to generate a picture of what is known and not known about a particular problem (Burns & Grove, 2007:545). A literature review builds a logical case for the proposed study and includes the description of the current knowledge about the problem; the review must identify the gaps in the knowledge base and must contribute information to the study. Critical judgement of the evidence published in relation to the topic is required (Polit & Beck, 2006:142-143).

For the purpose of the current study, the researcher consulted international data on gastroenteritis in children. The data were used to supplement the relevant research that was available about gastroenteritis in the South African context at the time of the study. Further to the above, data regarding bottle- and cup-feeding practices were sought. According to Mouton (2001:87), a review of the existing scholarship and the available body of knowledge is necessary:

- to prevent duplication of a previous study;
- to discover the most recent, authoritative theory about the subject;
- to find the most widely accepted empirical findings in the study field;
- to identify the available instrumentation to prove validity and reliability; and
- to obtain the most widely accepted definitions of key concepts in the field.

The purpose of the current literature review was to gain information concerning the morbidity and mortality rates of gastroenteritis and diarrhoea in infants and young children. The purpose included establishing which feeding utensil and sterilisation method was more effective in the prevention of gastroenteritis.

11

The literature for which a search was undertaken included both national and international literature. Literature for the review was obtained through searches of data sources that were available in the library at SU. Relevant internet health sciences databases (Cumulative Index to Nursing and Allied Health Literature [CINAHL[®]], PubMed[®] and Medline) and various books were consulted. The time span of the literature review was from 2001-2012.

Key words that were used in the initial literature search were:

- gastro;
- gastroenteritis;
- feeding bottle contamination;
- infant feeding bottles; and
- infant feeding cups.

According to Mouton (2001:91), a literature review should be well-organised, structured and logical, thus the current review is structured as follows:

- conceptual framework;
- general overview of the incidence and prevention of diarrhoea; and
- prevention and control of diarrhoea in the Western Cape.

2.2   CONCEPTUAL FRAMEWORK

A conceptual framework deals with concepts that are assembled because of their relevance to a common theme. Such a framework provides a conceptual perspective regarding interrelated phenomena, but is more loosely structured than is a theory and does not link concepts within logically derived deductive systems. The framework presents a broad understanding of the phenomenon of interest and reflects the assumptions and philosophical views of the researcher (Polit & Beck, 2006:155).

The researcher combined the concepts describing phenomena contributing to diarrhoea with the environment, organism and host to generate a conceptual framework to guide the research project. The combination concerned enabled the researcher to stay focused on the process and the outcome of the research question asked.

12

In the current conceptual framework, the environment was represented by the feeding utensils. Inadequate personal hygiene, unclean water, inappropriate storage of formula milk and inadequate sanitation can result in the contamination of feeding utensils and in the ineffective sterilisation of the utensils. The feeding bottle is readily contaminated with micro-organisms, leading to milk contamination, infection of infants, and diarrhoea. The hosts in the present study were infant humans. An infant's immune system is not adequately developed to resist microbial contamination, making an infant more susceptible to infection. The organisms were the micro-organisms that contaminate unclean feeding utensils. A safe sterilisation method was, therefore, needed to keep feeding utensils free from harmful bacteria and to prevent infants from developing diseases as a result of exposure to such bacteria.

The arrows in the model in Figure 2.1 show the development from a position where diarrhoea can be contracted from the feeding utensils used by infants to one where a safe, effective and affordable sterilisation method is used to reduce diarrhoeal incidences.

ENVIRONMENT:
FEEDING UTENSILS
(Cups, bottles)

POOR HYGIENE

GOOD HYGIENE

(SAFE, EFFECTIVE AND AFFORDABLE STERILISATION METHOD)

Organism:

Bacteria

Host:

Infant:

DIARRHOEA

NO DIARRHOEA

(GOOD HEALTH)
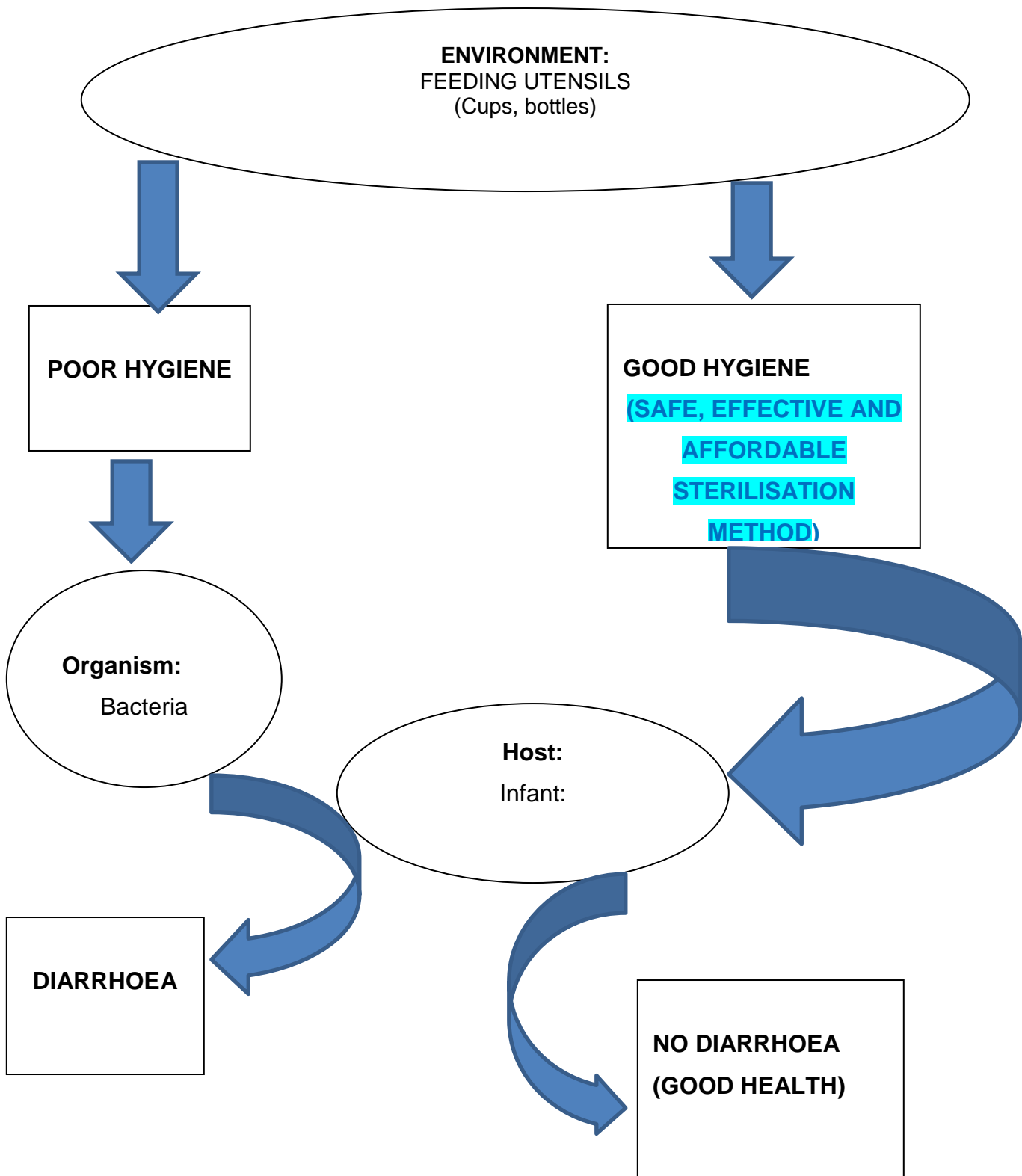
Figure 2.1: Conceptual framework model

14

2.3   AN OVERVIEW OF DIARRHOEA

As was discussed in Chapter One, diarrhoea is one of the major causes of morbidity and mortality globally (Dalby-Payne & Elliott, 2009:314). Infants and children under the age of five years are mainly affected by diarrhoea, with between 1.4 and 2.5 million children dying every year from diarrhoea (Vreeman, 2009:337-341).

2.3.1 Risk factors and their impact

Various risk factors are associated with the outcome of diarrhoea in infants. Some of the factors include food and environment contamination, maternal education and employment, an infant's birth weight, unsafe water and poor basic sanitary conditions, and the duration of maternal breastfeeding (Al Jarousha, El Jarou & El Qouqa, 2011:165-170). Furthermore, the Western Cape Department of Health (2009-2010) added poor personal hygiene, contaminated water and poor hygiene to the factors contributing to infantile diarrhoea in the preparation of formula feed.

Most diarrhoeal episodes occur during the first two years of life. Incidences of diarrhoea are higher in the 6- to 11-month age group, as said period is when weaning from breastfeeding often occurs (World Health Organisation, 2003). Such incidences are the result of declining levels of maternally acquired antibodies, the lack of active immunity in infants, the introduction of food that may be contaminated with faecal bacteria, and direct contact with human or animal faeces when the infant starts to crawl (Maharjan, Lekhak, Shrestha & Shrestha, 2007:23-26). The rotavirus gastroenteritis epidemiology and viral types in Europe accounting for losses in public health and society (REVEAL) study in Europe showed that rotavirus gastroenteritis cases tend to occur more frequently in the 6- to 23-month age group (Giaquinto & Van Damme, 2010:142-147). In a review of epidemiology and surveillance of South African children hospitalised for rotavirus infection, more than 95% of cases were found to occur in children under the age of 18 months (Steele, Peenze, de Beer, Pager, Yeats, Potgieter, Ramsaroop, Page, Mitchell, Geyer, Bos, & Alexander, 2003:354-360).

A significant association was found between hospitalisation for acute gastroenteritis due to rotavirus and low birth weight. Undernourished mothers have often been found to deliver

15

low-birth weight infants, which combined with a compromised breastfeeding capacity, results in the mother resorting to bottle-feeding, thus leading to increased risk of exposure to diarrhoea-causing bacteria (Nahar, Ahmed, Brown & Iqbul Hossain, 2010:476-483).

Although breast milk is the best and safest for young infants, the incidence of breastfeeding is declining in most developing countries. The reason for the decline includes the belief that bottle-feeding is the modern method of feeding, with other influencing factors being the aggressive promotion of infant formulas, the need for mothers to work away from their children, the lack of facilities for breastfeeding at places of work, the fear of not being able to breastfeed adequately, and a lack of nursing support for mothers who wish to breastfeed (Singh, 2010:404-422).

Infants that are not breast fed, particularly in low-resource settings where water supplies are unsafe and sustainable replacement feeding is impossible, are at risk of mortality due to malnutrition and severe gastroenteritis (Kafulafula, Hoover, Taha, Thigpen, Li, Fowler, Kumwenda, Nkanaunena, Mipando & Mofenson, 2010:6-13). Infants that are not breastfed are up to 25 times more likely to die from diarrhoea than are exclusively breastfed infants (Thapar & Sanderson, 2004:641-653). A study conducted by Plenge-Bönig, Soto-Ramírez, Karmaus, Petersen, Davis & Forster (2010:1471-1476) has shown that breastfeeding protects against the contraction of rotavirus-related gastroenteritis, especially in infants six months and younger. Breastfeeding appears to enhance the development of the immune system (Thapar & Sanderson, 2004:641-653). Infants who are exclusively breastfeed for the first six months of life and who continue to breastfeed until two years of age and beyond tend to develop fewer infections and to suffer from less severe illness. The protection concerned has been shown to be higher where the maternal literacy is lower and where the sanitation is worse (United Nations Children's Fund/World Health Organisation, 2009:14).

Food contamination plays an important role in 70% of all cases of diarrhoea in children aged six to 24 months. Contaminated food leads to the increase of diarrhoea after the introduction of complementary food (Usfar, Iswarawanti, Davelyna & Dillon, 2010:33-40). Cooked food can become contaminated if it is stored at room temperature for later use, or

from contact with contaminated containers. Bacteria can multiply many times if food is kept for several hours at room temperature (Osumanu, 2007:59-68). Infant feeding bottles may become contaminated with bacteria if poorly cleaned or, if they are not sterilised when milk is added to them, the milk may become contaminated, leading to the occurrence of bacterial growth (Redmond, Griffith & Riley, 2009:85-94). A study conducted in rural South Africa showed that some mothers rinse infant feeding bottles with water, but do not wash them with soap, while others wash used bottles and teats with soap and water, but do not disinfect the utensils (Dorosko & Rollins, 2003:117-130).

Scott, Curtis, Rabie & Garbrah-Aidoo (2007:225-233) noted that poor hygiene and sanitation is experienced when mothers or caregivers failed to wash hands after defecation of faeces or before handling of food. Poor personal hygiene is aggravated by living in impoverished surroundings, where unhygienic toilet facilities contribute to diarrhoeal episodes. Drinking water might be contaminated with faecal bacteria during storage at home if the container holding the water is not covered, or if a contaminated hand comes into contact with water while collecting it from the container (Etiler, Velispasaoqlu & Aktekin, 2004:62-69).

Increased risks of diarrhoea and persistent diarrhoea are associated with having an uneducated mother and a self-employed father. Uneducated and younger mothers usually have less knowledge of appropriate childrearing practices and less effective problem-solving skills than do more educated and older mothers, which can lead to malnutrition, as the former are unable to support their infants when food supplies are limited (Nahar et al., 2010:476-483).

Mothers who have a basic, secondary or higher education tend to practise good hygiene and better child feeding, supporting a child's resistance to infectious diseases. Such mothers are also more aware of disease-causation factors and preventative measures that can be taken (Boadi & Kuitunen, 2005:2-13).

Malnutrition affects normal development and growth in children (Jacobs, Guthrie, Montes, Jacobs, Mickey-Colman, Wilson & DiGiacomo, 2006:723-732). Malnutrition results from

inadequate nutrition; undernutrition is due to insufficient intake of energy and other nutrients; whereas overnutrition results in an excess of energy and nutrient intake (Ge & Chang, 2001:283-291). Studies have shown that malnourished children have an increased duration and incidence of diarrhoea (Guerrant, Oriά, Moore, Oriά & Lima, 2008:487-505). Malnutrition has been shown to increase the risk of mortality from diarrhoeal disease. However, in a study conducted in Malawi, after controlling for age and HIV infection, severity of disease was not influenced by nutritional status (Cunliffe, Gondwe, Kirkwood, Graham, Nhlawe, Thindwa, Dove, Broadhead, Molyneux & Hart, 2001:550-555). Children who are small due to malnutrition tend to lose a greater proportion of their total body fluid during diarrhoea than do infants of normal size, with the former also tending to have a higher frequency of severe dehydration resulting in death. A mother who is malnourished tends to produce a lower daily volume of milk than does a mother who is well-nourished, which leads to lower provision of antibodies to the infant in the former case (Huppertz, Salman & Giaquinto, 2008:S11-S19).

2.4 INCIDENCE OF DIARRHOEA

Diarrhoeal disease continues to be a major cause of childhood mortality in South Africa, especially in the Western Cape province. In 2006, 2 288 admissions for diarrhoea were reported by the Red Cross War Memorial Children's Hospital. Subsequently the admission figures increased in 2007 and 2008, being reported as 2 930 and as 3 975 children admitted, respectively. For 2006 to 2007, child admissions for diarrhoea increased by 28%, while for the 2007 to 2008 period, a 35% increase was reported. The diarrhoeal mortality rate was 52 children in 2006. For 2007 and 2008, it was 57 children and 46 children respectively. The mortality rate for 2006 was 2.3%, which decreased to 1.9% in 2007, remaining constant at 1.2% in 2008 (Red Cross War Memorial Children's Hospital, 2009).

In 2009, the top three hospitals with high admissions were Red Cross War Memorial Children's Hospital, Tygerberg Hospital and New Somerset Hospital. In 2010, Helderberg Hospital replaced Somerset Hospital with the third highest number of admissions related to diarrhoeal disease in the Western Cape (Western Cape Department of Health, 2009-2010).

The admission of children with diarrhoeal disease in the Western Cape is increasing significantly. The two most significant increases of diarrhoea were at Carnation Ward in Lentegeur Hospital, Mitchell's Plain and at Khayelitsha District Hospital, which, respectively, experienced an increase of 168% and 300% in 2010 (Western Cape Department of Health, 2009-2010). The Western Cape reported an increase of diarrhoea-related admissions for 2006-2007, while South Africa, as a whole, reported a decrease of diarrhoea-related admissions for 2006 and 2007 (Health Systems Trust, 2010).

The determinants of the increase in diarrhoea were reported to be poor water quality and inadequate sanitation, poor food hygiene and hygienic practices, and poor nutrition. The listed determinants led to overcrowding in smoky, poorly ventilated homes, which helped to reduce immunity to infection in the cases of the reported gastroenteritis (South African Human Rights Commission, 2006-2009).

The increases in the number of diarrhoea-related admissions in the City of Cape Town were also related to an increase in the child population and to deterioration of child health in the region (South African Human Rights Commission, 2006-2009). The 2007 Community Survey Analysis for Cape Town found that the population of Cape Town had grown by 20.9% since 2001. The Western Cape was the province with the largest population increase (16.7%), with 80.2% of the population increase occurring in Cape Town. Most children admitted for diarrhoeal disease came from Khayelitsha and other informal settlements areas. The poorer areas affected exhibited the following conditions that tend to make children highly vulnerable to severe illness, including diarrhoea: poverty; lack of adequate clean water and sanitation; and inadequate ventilated and overcrowded housing. The deterioration of health care systems, particularly at community and district level, impacts on the efficacy of child health care. The quality of child health care has been further compromised by the decline in the number of competent health care personnel, and by the crowded, under-resourced public health facilities (South African Human Rights Commission, 2006-2009).

2.5   PREVENTION OF DIARRHOEA

Preventative measures are an important aspect in both developing and developed countries in decreasing morbidity and mortality due to acute gastroenteritis. The South African national government, represented by the Department of Health, is responsible for the implementation and management of preventative measures to decrease the incidence of diarrhoea.

Preventative interventions for diarrhoeal disease include: improved sanitation; access to clean water; good hand-washing practices; the promotion of breastfeeding; vitamin A supplementation; and rotavirus immunisation (Fischer Walker, Friberg, Binkin, Young, Walker, Fontaine, Weissman, Gupta & Black, 2011:1-10).

Improvement in sanitation reduces the transmission of pathogens that cause diarrhoea by preventing human faecal matter from contaminating environments. Improved sanitation facilities have been associated with an estimated median reduction in diarrhoeal incidences of 36% across reviewed studies (United Nations Children's Fund/World Health Organisation, 2009:11-16).

Hand washing has been cited as one of the most cost-effective public health interventions. Accessible and plentiful water has been shown to encourage better hygiene, and hand washing in particular. However, such encouragement depends upon the type of water source available, such as public taps or standpipes, and protected dug wells or boreholes (United Nations Children's Fund/World Health Organisation, 2009:11-16). The washing of hands tends to decontaminate hands and to prevent cross-infection or transmission. A study in hand washing to prevent diarrhoea has shown that there is a reduction in diarrhoea episodes after interventions of promoting hand washing (Ejemot, Ehiri, Meremikwu & Critchley, 2009:893-939). Hand washing with soap could reduce the risk of diarrhoea by between 42% and 47% (Curtis & Cairncross, 2003:275-281). A clustered randomised controlled trial found a 51% reduction in the prevalence of diarrhoea in hand washing with soap (Luby, Agboatwalla, Painter, Altaf, Billhimer, Keswick & Hoekstra 2006:479-489). A pilot study describing infant formula preparation and feeding practices noted that only 27% of mothers washed their hands prior to infant bottle preparation (Herbold & Scott, 2008:451-459).

20

Clean water is important in the prevention of diarrhoea. A study conducted by Cairncross, Hunt, Boisson, Bostoen, Curtis, Fung & Schmidt, (2010:193-205) has shown that, with improved water quality, the incidence of diarrhoea could be reduced by 17%. Interventions to improve water quality at the source, along with treatment of household water and safe storage systems, have been shown to reduce diarrhoeal incidences by 47%. Proven and field-tested household water treatment options that are currently being promoted include chlorination, filtration, combined flocculation and disinfection, boiling and solar disinfection (United Nations Children's Fund/World Health Organisation, 2009:11-16).

Infants who are not breastfed have a sixfold greater risk of dying from infectious disease, including from diarrhoea, in the first two months of life than are those who are breastfed (United Nations Children's Fund/World Health Organisation, 2009:13). A healthy baby who is growing normally should receive only breast milk, with no other fluids or food supplementation (such as water, tea, juices or formula) for the first six months of life (Kent, 2006:8).

Breast milk helps to protect the child against episodes of severe diarrhoea, Alexander, LaRosa, Bader, Garfield & Alexander (2009:158-159) note the following important advantages of breast milk:

- Exclusive breastfeeding during the first four to six months greatly reduces the risk of severe or fatal diarrhoea. The risk of other serious infections is also reduced.
- Breastfeeding is a relatively clean method of feeding, as it does not require the use of bottles, teats, water and formula that are easily contaminated with bacteria that might cause diarrhoea.
- Breast milk has immunological properties (antibodies) that protect the infant from infection, and especially diarrhoea. Said antibodies are present neither in animal milk nor in formula.
- The composition of breast milk is ideal for infants. Formula or cow's milk may be too diluted (reducing its nutritional value) or too concentrated (so that it does not provide sufficient water), and may also provide too much salt and sugar.

- Breast milk is a complete food that provides all the nutrients and water that are needed by a healthy infant during the first four to six months of life.
- Breastfeeding is cheap, leading to the incurring of none of the expenses associated with feeding breast milk substitutes, such as the cost of fuel, utensils and special formulas and of the mother's time in formula preparation.
- Breastfeeding helps with birth spacing. Mothers who breastfeed usually have a longer period of infertility after giving birth than do mothers who do not breastfeed.
- Milk intolerance rarely occurs in infants who take only breast milk.
- Breastfeeding immediately after delivery encourages the bonding of the mother to the infant, which has important emotional benefits for both and which also helps to secure the child's place within the family (Alexander et al., 2009:158-159).

Studies have shown that babies who drink water or other liquids before six months of age tend to drink less breast milk, which can cause malnutrition in the babies. Inappropriate bottle use is associated with tooth decay, anaemia and overweight, and it may adversely affect the dietary patterns of infants. The fact that the death rate among artificially fed babies is much greater in developing countries than among breast-fed babies has led to the development of a major public health problem (Nawaz, ur Rehman, Nawaz & Mohammed, 2009:93-95).

Vitamin A is an important supplement for the reduction of diarrhoea incidences (Mayo-Wilson, Imdad, Herzer, Yakoob, Bhutta & Sheriff, 2011:5094). Studies have shown mortality reductions of 19% to 54% from diarrhoea in children receiving vitamin A supplements. The reduction is associated, in large part, with declines in the number of deaths due to diarrhoeal disease and measles. Vitamin A supplement has been shown to reduce the duration, severity and complications associated with diarrhoea (United Nations Children's Fund/World Health Organisation, 2009:11-16).

In the Millennium Development Goals, WHO recognised the potential of a rotavirus vaccine to reduce mortality rates among children less than five years old, as a result recommending the inclusion of such a vaccine in all infant national immunisation programmes (Pawinski, Debrus, Delem, Smolenov, Surkyakiran & Han, 2010:S80-86). An estimated 16% of all

diarrhoeal deaths in children less than five years of age could be prevented by means of the introduction of an effective vaccine (Steele et al., 2003:354-360).

South Africa has the greatest burden of HIV incidence in the world (Houliham, Mutevedzi, Lessells, Cooke, Tanser & Newell, 2010:23), which has resulted from the explosive growth in the epidemic from a prevalence of less than 1% amongst pregnant women in 1990 to 30.2% in 2005 (Doherty, 2006:11). The prevalence of HIV was the highest amongst women aged 25 to 29 years old in 2005 (National Department of Health, 2006).

Mother–to–child transmission of HIV can occur during pregnancy, labour and delivery or during breastfeeding (Rupali, Condon, Roberts, Wilkinson, Voss & Thomas, 2007:216-223). Breastfeeding by an infected mother has been found to increase the risk of HIV mother-to-child transmission by 5 to 20% to 20 to 45% (Johri & Ako-Arrey, 2011:3). HIV transmission through breast milk is increased due to a high plasma viral load, a low CD4 count, and breast pathology, such as mastitis and abscesses (Coutsoudis, 2005:185-196). WHO and the United Nations Children's Fund (UNICEF) have developed the Global Strategy for Infant and Young Child Feeding to assist mothers to make appropriate infant-feeding choices (World Health Organisation, 2003). HIV-positive mothers are advised to avoid breastfeeding if replacement feeding is acceptable, feasible, affordable, sustainable, and safe (Department of Health, 2007). The South African Department of Health Prevention of Mother–to–Child Transmission (PMTCT) Protocol, has its own specific recommendations relating to HIV and infant feeding. Recommendations require that, where safe and adequate formula feeding is possible, and where ongoing support for the mother and the monitoring of an infant is available, formula feeding is the recommended method of feeding. The South African protocol makes provision for mothers who choose to formula feed to receive a supply of free commercial formula milk for six months (Doherty, Chopra, Nkonki, Jackson & Greiner, 2006:90-96).

Unhygienic feeding practices and feeding utensils have been found to contribute to diarrhoeal incidences (Usfar et al., 2010:33-40). A study by Ma, Zhang, Swaminathan, Doyle & Bowen (2009:132-139) to assess the efficacy of protocols for cleaning and disinfecting infant feeding bottles in less developed communities was conducted in 2009.

Artificially contaminated infant feeding bottles with low and high inocula of bacterial enteric pathogens were used. Rinsing the bottles with soapy water followed by rinsing with tap water was found to be the most effective cleaning method. Submersing highly contaminated bottles in 50 ppm hypochlorine solution for 30 minutes produced a reduction in bacterial pathogens, resulting in no identifiable pathogens being found in the bottles concerned. The infant bottle cleaning practices evaluated in the current study included no rinsing of bottles; rinsing with tap water one to three times; and rinsing with soapy water with or without brushing the bottles.

World Health Organisation (2006/2010) noted that it is better to feed with a cup than with a bottle, since the former is easier to clean and promotes greater interaction between the mother and her baby. Lanata's (2003:S175-S183) Peruvian-based study found that, when serving tea to children in a cup after a period of time, during which the tea was allowed to cool off, the cup remained uncontaminated; however, 35% of the sample that was served in a baby bottle was found to be contaminated with faecal coliforms.

Redmond, Griffith & Riley (2009:85-94) evaluated organic and microbial contamination of 'in-use' bottles used for feeding infants powdered formula milk in South Wales, United Kingdom. The study showed microbial counts up to 10^5/area and adenosine triphosphate (ATP) levels up to 100 051 relative light units in 'uncleaned' bottles. Enterobacteriaceae and *Staphylococcus aureus* were found in 12% to 15% of 'unclean' bottles (up to 10^2 cfu/area sampled). The contamination was most frequently found to have been from the screw cap and teat interiors. Of the ready-to-use bottles that were cleaned and disinfected, some had aerobic colony counts up to 5.8×10^4 cfu / area sampled. *S. aureus* was found in 4% bottles/components, but no Enterobacteriaceae were found. The infant bottle cleaning practices that were evaluated in the study included cleaning methods involving the use of hot water, the use of detergent, and rinsing. The disinfection methods involved the use of a microwave unit, a steam unit, a cold water hypochlorite solution, and a dishwasher / hand wash.

Bergström (2003) performed a study in South Africa to assess how mothers in an urban/peri-urban PMTCT area prepared and fed commercial infant milk to their infants, as well as to assess the safety of the feeds. Seventy per cent of the mothers received free

formula milk from the clinic. The results showed that the mothers had received good counselling on hygiene and milk preparation as part of the support that they had received from the clinic personnel. The infant bottle cleaning practices that mothers reported using included the boiling of utensils for variable periods, boiling together with soap/salts, and the use of a sterilisation solution and bleach water. *E. coli* were detected in 64% and *Enterococci* in 26% of the prepared milk samples collected from mothers. No contamination of *Shigella* or *Salmonella* was found amongst the samples. Boiling water separately for every feed reduced the risk of contamination, compared to boiling and storing water for several feeds. In contrast, the risk of preparing contaminated milk during the night was higher if only one or two feeds were prepared. Over-dilution of the feed was found in 28% of the milk samples collected at the clinic and in 47% of the samples collected at home.

Sterilisation of feeding utensils prevents infants from the ingestion of bacteria (Tassoni, 2006:167). A steam steriliser allows steam to circulate in the unit and items must reach high temperatures under manufacturer's instructions. Boiling sterilisation is the cheapest method and feeding utensils must be completely immersed and boiled for at least ten minutes. Mothers are advised to add sterilising fluid or tablets to cold water if chemical or cold water sterilisation method are used. Feeding utensils must be completely immersed and must remain in the solution until required. However, the chemical solution must be changed every 24 hours (Tassoni, 2006:167).

Mothers are advised to practise good hand-washing techniques and hygiene when preparing infant formula feeds to reduce diarrhoeal incidences. Infants and young children receiving Vitamin A supplementation have a lower number of diarrhoeal episodes, as their immune system is protected. The ingestion of breast milk has been found to protect children against severe diarrhoeal episodes; however, HIV-positive mothers should avoid breastfeeding if replacement feeding is acceptable, safe, feasible and affordable. The implementation of said recommendations should lower the rate of diarrhoeal incidence in infants and young children.

2.6 PREVENTION AND CONTROL OF DIARRHOEA AMONG CHILDREN IN THE WESTERN CAPE

At the time of the current study, various programmes and measures were available for the control and prevention of diarrhoea in the Western Cape. Hospitals and clinics were in possession of prevention guidelines. The Western Cape Metropole control and prevention programme followed the guidelines of the Baby-Friendly Hospital Initiative and community-based intervention programmes.

2.6.1 The Baby Friendly Hospital Initiative

The Baby-Friendly Hospital Initiative was launched by WHO and UNICEF in 1991, following the Innocenti Declaration of 1990. The initiative is a global effort to implement practices that protect, promote and support breastfeeding (Toma & Rea, 2012:171).

Breast milk is the most valuable food for infants, since it provides all complete nutrients that are needed for growth, including cleanliness and the promotion of a warm relationship between the mother and child. Breastfeeding has been linked to a reduced incidence of diarrhoea-related disease. Therefore, UNICEF and WHO have promoted the Baby-Friendly Hospital Initiative, by suggesting 10 steps to successful breastfeeding. The Initiative states that every facility providing maternal services and care for newborn infants should:

- have a written breastfeeding policy that is routinely communicated to all health care staff;
- train all health care staff in skills necessary to implement the policy;
- inform all pregnant women about the benefits and management of breastfeeding;
- help mothers initiate breastfeeding within half an hour of birth;
- show mothers how to breastfeed and how to maintain lactation, even if they should be separated from their infants;
- give newborn infants no food or drink other than breast milk, unless medically indicated to do elsewise;

26

- practise rooming-in, allowing mothers and infants to remain together for 24 hours a day;
- encourage breastfeeding on demand;
- encourage the giving of no artificial teats or pacifiers (dummies or soothers) to breastfeeding infants; and
- foster the establishment of breastfeeding support groups and refer mothers to them on their discharge from the hospital or clinic (Toma & Rea, 2012:171).

A study was conducted in 2001 in the Western Cape to assess the extent of the implementation of the above-mentioned steps in both public and private maternity facilities in the Western Cape. Poor implementation of specific steps in both sectors was reported and a follow-up study was conducted in 2005 targeting private health care facilities. The 2005 study noted that breastfeeding counselling delivered by trained health professionals and community health workers was an effective intervention to improve exclusive breastfeeding rates. The findings of the two studies highlighted the importance of the establishment and the correct implementation of breastfeeding policies in health care facilities that care for mothers and their infants. Also emphasised was the importance of appropriate and continuous breastfeeding training to ensure the initiation and establishment of early breastfeeding, exclusive breastfeeding practices, and support on an on-going basis to ensure the best start in life for infants (Marais, Koornhof, du Plessis, Naude, Smit, Hertzog, Treurnicht, Alexander, Cruywagen & Kosaber, 2010:40-45).

However, many facilities fall short in promoting exclusive breastfeeding (Abba, De Koninck & Hamelin, 2010:8). The inadequate training of personnel, the existence of misinformed or uninformed mothers, and a lack of ongoing support have been identified as factors contributing to non-promotion of exclusive breastfeeding practices (Abba et al., 2010:8).

To conclude this discussion, breastfeeding during the first 4-6 months reduces the risk of severe diarrhoea, since the child is protected from diarrhoea by maternal immunological antibodies (Alexander et al., 2009:158-159). Health care workers at clinics and maternity facilities educate mothers on the importance and benefits of breastfeeding, the use of cups and diarrhoeal prevention methods. The diarrhoeal prevention methods included are

appropriate feeding bottle hygiene and hand-washing techniques (Western Cape Department of Health, 2009-2010).

2.7   CONCLUSION

The current chapter reviewed the prevalence of diarrhoea amongst infants in South Africa and other countries. The severity, and the methods for prevention, of the disease were discussed in detail. The first two chapters focused on the existence of diarrhoea as a serious global issue that is associated with morbidity and mortality amongst infants and young children. Unhygienic maternal preparing of infant feeding has been found to be one of the main causes of diarrhoea. Therefore, in order to decrease the severity of the disease, increased access to clean water, improved sanitation, effective hand-washing techniques and the promotion of the use of breast milk are of utter importance. The provision of education and health promotion by health care professionals to mothers is important and the evaluation of an effective sterilisation method for infant-feeding utensils is needed, since not all infants are breastfed.

CHAPTER THREE
RESEARCH METHODOLOGY

3.1 INTRODUCTION

The aim of Chapter Three is to provide an in-depth discussion of the research methodology that was implemented in the study. The discussion covers the research design, the population and sampling, and issues of reliability and validity, data collection and data analysis.

3.2 RESEARCH AIM

The aim of the current study was to investigate which out of three particular sterilising methods is the most effective for sterilising feeding bottles and cups.

3.3 OBJECTIVE

The objective of the current study was to determine the efficacy of three different methods of feeding utensil sterilisation that are commonly used in resource-poor areas, namely:

- chemical sterilisation using Milton™;
- dishwashing liquid using Sunlight™ dishwashing liquid; and
- natural sunlight.

3.4 RESEARCH METHODOLOGY

Research methods are a broad plan consisting of the steps, procedures and strategies for gathering and analysing data in a research investigation (De Vos, Strydom, Fouché & Delport, 2005:118).

3.4.1 Research design

Mouton (2001:55) defines a research design as a plan or blueprint of how the researcher intends to conduct the research process. Rubin and Babbie, (as quoted in De Vos et al., 2005:133) state that research design refers to the act of designing a study in its broadest

sense, which refers to all the decisions made in planning a study. The decisions include the overall type of design, sampling, sources and procedures for data collection, measurement issues, and data analysis. For the purpose of the current study, an *in vitro* experimental, quantitative approach with a descriptive design was utilised under controlled laboratory conditions.

3.4.1.1 *Quantitative approach*

Mouton and Marais (as quoted in De Vos et al., 2005:73) state that the quantitative approach is a relatively formalised and explicitly controlled approach to research. The approach is regarded as being more objective and the range of the research more defined than is the qualitative approach. Quantitative research employs such strategies of inquiry as experiments and surveys and the collecting of data on predetermined instruments that yield statistical data (Creswell, 2003:18). The research involves collecting data so that information can be quantified and subjected to statistical treatment in order to support or refute "alternate knowledge claims" (Creswell, 2003:153). Quantitative research can be used in response to relational questions of variables within the research. The intent is to establish, to confirm, or to validate relationships and to develop generalisations that contribute to theory (Leedy & Ormrod, 2001:102). A quantitative approach was used in the study to evaluate different methods of cleaning and sterilising infant-feeding utensils to ensure that the objectives of the study would be reached. In other words, the findings of which sterilisation method is the most effective will be applicable to most mothers who choose to use such methods when feeding their infants.

3.4.1.2 **In vitro** *experimental design*

In vitro refers to studies in experimental biology that are conducted in glass, such as in a test tube. The studies are conducted outside of the living body and are normally undertaken in a laboratory (Blackwells, 2002).

In vitro studies are studies that use micro-organisms or material, which has been isolated from whole organisms or simulations thereof, as test systems (World Health Organisation, 2010:277). *In vitro* studies permit a high level of simplification of the system under study, in order to enable the researcher to focus on a small number of components (Vignais &

30

Vignais, 2010). Such an approach was deemed appropriate for the current study, because the micro-organism *E. coli* was used as a contaminant; the study was conducted in a laboratory; and, by means of using such an approach, the objectives of the study could be reached.

3.4.1.3   Descriptive design

The term 'descriptive research' refers to the type of research question, design, and data analysis that are applied to a given topic. Descriptive research involves gathering data that describe events and then organising, tabulating, depicting and describing the data collection (Knupfer & McLellan, 2001:1196-1213). Descriptive research attempts to describe a situation, phenomenon, service or programme systematically, to provide information about a living condition of a community, or to describe attitudes adopted toward an issue (Kumar, 2005:10). A descriptive approach was used in the current study, as it sought to describe phenomena of real-life situations, consisting of the quest for an appropriate sterilisation method for infant-feeding utensils, existing sterilisation methods, and the innovative use of sunlight as a sterilisation method.

3.4.2   Research setting

The current study took place in the microbiology laboratory at UWC. The setting was chosen due to its accreditation by the Council of Higher Education as a fully-equipped, sterile laboratory that was safe for testing infant-feeding utensils.

3.4.3   Study population and sample

Polit and Beck (2006:259) define a population as the aggregate of cases that meet a specified criteria and that are accessible for a study. The target population is the entire population in which a researcher is interested (Polit & Beck, 2006:260). The target population for the current study comprised infant feeding bottles and feeding cups.

Polit and Beck (2006:260) define sampling as the process of selecting a portion of the population to represent the entire population. The study sample comprised eight infant feeding bottles and eight feeding cups. The bottles were purchased locally at a supermarket, and the cups were provided to the researcher by a local hospital milk kitchen.

31

The bottles were the standard infant feeding bottle, with the same characteristics and of the same make. The cups were Sinapi cups made from polypropylene, and were all the same in make and characteristics. The cups had never before been used and came sealed in a polypropylene bag.

Two samples were used for each method of sterilisation, namely:

- 2 bottles and 2 cups for sunlight;
- 2 bottles and 2 cups for Milton™;
- 2 bottles and 2 cups for Sunlight™ dishwashing liquid; and
- 2 bottles and 2 cups as control utensils.

3.4.4   Data collection and management

Burns and Grove (2007:536) define data collection as the identification of subjects and the precise, systematic gathering of information that is relevant to the research purpose or the specific objectives, questions, or hypotheses of a study. Data were collected by making use of structured observational measurements. Structured observational measurements enabled the researcher to define carefully what is to be observed and how the observations are to be made, recorded and coded (Burns & Grove, 2001:283).

3.4.4.1   Preparation for data collection

The bacterium, *E. coli,* was used as the contaminant, as it is commonly associated with the contamination of infant feeding utensils. One hundred µl of *E. coli* spp. were grown up in 10 ml of TSB and incubated at 37ºC for 24 hours. Doing so allowed *E. coli* spp. to approach the stationary phase of growth at a concentration of approximately 10^{-6} cfu/ml.

All the study feeding bottles and feeding cups were sterilised in boiling water for five minutes. For the experimental utensils per bottle, 100 µl of *E. coli* spp. culture was inoculated (introduced) with a pipette into six infant feeding bottles and six feeding cups, each of which contained 100 ml formula milk (Lactogen). The inoculated utensils were then incubated at 37ºC for 24 hours, to allow *E.coli* to approach growth of 10^{-6} cfu/ml, after which the formula milk was discarded from each infant feeding bottle and cup.

The feeding utensils were sterilised, using one of the three identified methods, namely direct sunlight, Sunlight™ dishwashing liquid or Milton™ solution. The sterilising treatments were used one at a time (with two replicates being used for each combination).

Sixteen samples in total of infant feeding bottles and feeding cups were used. Two samples were taken (two bottles and two cups for sunlight; two bottles and two cups for Milton™; two bottles and two cups for Sunlight™ dishwashing liquid; and controls that consisted of two bottles and two cups), which gave a total of 16 samples.

During the process of utensil sterilisation, any contamination of the items was prevented through working aseptically, by not touching any other areas or equipment than the feeding utensils and the prescribed equipment, by washing hands, as well as by the wearing of gloves, masks and a clean laboratory coat.

3.5 Sterilisation methods

The feeding utensils were sterilised using one of the three identified methods, namely direct sunlight, Sunlight™ dishwashing liquid or Milton™ solution. These methods were used, since it is easy accessible in households. The sterilising treatments were used one at a time (with two replicates being used for each combination).

3.5.1 Sunlight (28°C)

Two infant feeding cups containing 100 ml of formula milk (Lactogen) were inoculated (using a pipette) with 100 µl of *E. coli*, per bottle, and incubated at 37°C for 24 hours. The inoculated milk was discarded after 24 hours. The cups were then rinsed with Sunlight™ dishwashing liquid (5 ml in 5 L cold water) for 15 seconds in a sterilised container and dried upended on a sterilised surface. The feeding cups were placed in direct sunlight on a bench outside the microbiology laboratory for four hours.

Two infant feeding bottles containing 100 ml of formula milk (Lactogen) were inoculated (using a pipette) with 100 µl of *E. coli*, per bottle, and incubated at 37°C for 24 hours. The inoculated milk was discarded after 24 hours. The bottles were then rinsed with Sunlight™ dishwashing liquid (5 ml in 5 L cold water) for 15 seconds in a sterilised container and dried

upended on a sterilised surface. The feeding bottles were placed in direct sunlight on a bench outside the microbiology laboratory for four hours.

3.5.2 Milton™ solution

Two infant feeding cups containing 100 ml of formula milk (Lactogen) were inoculated (using a pipette) with 100 µl of *E. coli*, per bottle, and incubated at 37°C for 24 hours. The inoculated milk was discarded after 24 hours. The cups were then rinsed with Sunlight™ dishwashing liquid (5 ml in 5 L water) for 15 seconds in a sterilised container and dried upended on a sterilised surface. The feeding cups were placed in a Milton™ solution (12.5 ml in 1 L cold water) for 30 minutes. The formula for the Milton™ solution was devised according to the recommended instructions for dilution of Milton™ for use in sterilisation on the Milton™ bottle to follow.

Two infant feeding bottles containing 100 ml of formula milk (Lactogen) were inoculated (using a pipette) with 100 µl of *E. coli*, per bottle, and incubated at 37°C for 24 hours. The inoculated milk was discarded after 24 hours. The bottles were then rinsed with Sunlight™ dishwashing liquid (5 ml in 5 L water) for 15 seconds in a sterilised container and dried upended on a sterilised surface. The feeding bottles were placed in a Milton™ solution (12.5 ml in 1 L cold water) for 30 minutes. The formula of Milton™ solution was diluted as previously described.

3.5.3 Sunlight™ dishwashing liquid

Two infant feeding cups containing 100 ml of formula milk (Lactogen) were inoculated (using a pipette) with 100 µl of *E. coli*, per bottle, and incubated at 37°C for 24 hours. The inoculated milk was discarded after 24 hours. The cups were then rinsed with Sunlight™ dishwashing liquid (5 ml in 5 L cold water) for 15 seconds in a sterilised container and dried upended on a sterilised surface.

Two infant feeding bottles containing 100 ml of formula milk (Lactogen) were inoculated (using a pipette) with 100 µl of *E. coli*, per bottle, and incubated at 37°C for 24 hours. The inoculated milk was discarded after 24 hours. The bottles were then rinsed with Sunlight™

dishwashing liquid (5 ml in 5 L cold water) for 15 seconds in a sterilised container and dried upended on a sterilised surface.

3.5.4 Controls

The two control utensils were inoculated with formula milk containing no *E. coli* spp. Two infant feeding cups, each containing 100 ml of formula milk (Lactogen), were incubated at 37°C for 24 hours. The milk was discarded after 24 hours. The cups were then rinsed with distilled water for five seconds in a sterilised container and dried upended on a sterilised surface.

Two infant feeding bottles containing 100 ml of formula milk (Lactogen) were incubated at 37°C for 24 hours. The milk was discarded after 24 hours. The bottles were then rinsed with distilled water for five seconds in a sterilised container and dried upended on a sterilised surface.

3.5.5 Sampling method and culture preparation

Ringers Lactacte is a neutral medium that does not encourage growth; therefore, it was used as a solvent in which to dilute the milk sample. As the number of organisms that might be found is large, one needs to conduct a dilution in order to obtain a detectable number of such organisms for counting. The functions of serial dilutions are to dilute culture to phase out inhibitors and other micro-organisms in order to develop the micro-organisms to a detectable or countable number (25-250) cfu/ml.

Nine millilitres of quarter-strength Ringers lactate solution were aseptically transferred into each infant feeding bottle and feeding cup. A quarter-strength Ringers lactate solution was made by adding two Ringer tablets to one litre distilled water, of which 9 ml was aseptically transferred, using a pipette, into sterilised tubes. One millilitre from each infant feeding bottle and cup was aseptically transferred into 9 ml of quarter-strength Ringers lactate solution, followed by of serial dilutions up to 10^{-6}.

One millilitre of each serial dilution was plated onto an APC and *E. coli* petrifilm, and incubated at 37ºC for 24 hours.

35

3.5.6 Aerobic plate count and *E. coli* petrifilm

An APC was used as an indicator of bacterial populations on the sample. The test performed was a generic one for organisms that grow aerobically at mesophilic temperatures (Morton, 2001:63-65). All media and materials were sterilised as described.

The recording of micro-organisms required dilutions of the sample to achieve a population that was countable by means of the chosen method. Decimal or tenfold dilutions were used for each calculation of final results. Results are given in cfu/ml. Distilled water was used in preparations of diluents.

Escherichia coli petrifilm count plate allowed the simultaneous enumeration of *E. coli* on a single petrifilm. Plates are incubated at 37ºC for 24 hours. The *E. coli* count plate yielded confirmed results for *E. coli* and is official method[2] for *E. coli* devised by The Scientific Association Dedicated to Analytical Excellence (AOAC) (Kornacki & Johnson, 2001:78).

3.6   DATA RECORDING AND MANAGEMENT

Data were recorded by counting the number of cfu/ml. The average of the cfu was added together, and then divided, with the log cfu/ml being calculated using a calculator. Data were recorded in duplicate to monitor the bias of data. The above was done with the assistance of control samples and precision.

3.6.1 Validity

Validity is the degree to which an instrument measures or reflects the concepts that are being studied (Burns & Grove, 2003:274). According to De Vos et al. (2005:160), validity ensures that the instrument actually measures the concept in question, and that the concept is measured accurately.

Validity of the results was ensured by means of a colony counter, which is an instrument that is used to count colonies of bacteria. The counter count of colonies was counted electronically, by identifying individual areas of dark and light according to automatic threshold and then counting the resulting contrasting spots.

3.6.2 Reliability

Reliability refers to the consistency with which an instrument measures an attribute (Burns & Grove, 2003:270). According to De Vos et al. (2005:162), reliability is the stability or consistency of the measurement concerned. For the purpose of the current study, validity and reliability were ensured by using standard calibrated equipment. Carefully measured numbers of bacteria was used in each of the samples, under controlled laboratory conditions. Bacterial counts in each sample were carefully undertaken under controlled laboratory conditions. All the findings were recorded to ensure that the *in vitro* study was done using exactly the same method. Trained microbiology students assisted the researcher with the study. A statistician was consulted to assist with data analysis. The SAGLP, as well as UWC precaution laboratory policy, was followed to ensure the safety and accuracy of the results. The guidelines of the SAGLP were followed by ensuring that the study was conducted in accordance with the ethical principles, protocol and the regulatory requirements of the Ethical Committee. The study was scientifically sounded and described in a clear, detailed protocol, and the benefits for the society justified the foreseeable risk thereby. No problems were experienced during the data collection or throughout the entire experiment.

3.6.3 Analysis of data

The results of the various sterilisation methods and the liaison of the cups and bottles were laid out in a Microsoft Excel® document after the log cfu/ml calculations had been done. The results was summarised in two sections: the APC and the *E. coli* petrifilm count. The APC and the *E. coli* petrifilm count were found to contain the bacterial counts per cfu of the two infant feeding utensils and the sterilisation methods used in the study.

With the support of a statistician, the data were analysed using the Number Cruncher Statistical System (NCSS) and Excel computer programme. The data were given in frequencies and correlations of the contamination. The analysis was done in cooperation with the SU Department of Statistics and Actuarial Science and a statistician, Dr T Kotze, assisted with the formulation of the data sheet and the analysis and interpretation of the data.

37

Frequency tables and graphs were structured for all variables. Analysis of variance (ANOVA) was used to compare the differences between the different methods and container types simultaneously. A t-test was also conducted on both the APC and the *E. coli* petrifilm to compare the cups versus the bottles to see whether there was a significant difference between the mean bacterial counts.

The level of significance, which was set at $\rho < 0.05$, was applied to the above tests.

3.7   ETHICAL CONSIDERATIONS

The current study was an *in vitro* one, so that no participant consent was needed. Consent to execute the study was obtained from SU (see Annexure 1), although the laboratory work (the study) was conducted in the microbiology laboratory at UWC with the permission of the facility manager, Prof Gouws.

Ethical research requires the consideration of, and precaution measures for, any safety risks. UWC precaution laboratory policy was followed by ensuring that the researcher was aware of any potential biosafety risks in the laboratory, such as the biological risks, including exposure to potential infectious pathogens like *E. coli*. Measures to ensure the safety of the researcher and the laboratory were taken, such as training in activities related to first aid, chemical spills, health and safety precautions and fire safety. In the laboratory, up to three trained microbiology students were available to assist the researcher. The researcher attended updating sessions on laboratory practice to ensure that the most appropriate techniques and processes were implemented. For personal protection, sterile gloves and a clean laboratory coat and closed shoes were worn at all times to prevent contamination by any chemical spills that might occur. Masks were worn when working in the laboratory.

3.8   LIMITATIONS

A limitation of the study was that the sample size was small. Only a small sample was used, since it is common practice in microbiology laboratories to perform only a few

replicates, because even two replicates are labour-intensive, and the researcher had a limited amount of time available in which to conduct the experiments.

Furthermore, not using soap and water and a brush to clean the utensils could also be regarded as a limitation. On Ma et al.*'s* (2009:132-139) rinsing infant feeding utensils with soapy water (containing Sunlight™ dishwashing liquid), a great (2-$\log_{10}$) reduction of contaminating bacterial pathogens was observed.

3.9   CONCLUSION

In the current chapter, detailed information relating to the research methodology, as implemented in the study, was described, including the research design, population and sampling, data collection and data analysis, and validity and reliability. Validity of the results was ensured by means of a colony counter. Bacterial counts in each sample were carefully undertaken in controlled laboratory conditions. All the findings were recorded to ensure that the entire *in vitro* study was conducted using exactly the same method. The research methodology implemented was aligned to the proposal that was submitted for ethical approval.

CHAPTER FOUR
DATA ANALYSIS AND DISCUSSION

4.1   INTRODUCTION

In the current chapter, the results obtained from the three sterilisation methods are analysed and discussed. The discussion includes the findings of the study and the analysis of the results. Analysis is the categorising, ordering, manipulating and summarising of data to obtain answers to research questions. The purpose of analysis is to reduce data to an intelligible and interpretable form, so that relations of research problems can be studied and tested, enabling conclusions to be drawn (De Vos et al., 2005:218).

4.2 DATA ANALYSIS

The data for the study were obtained by counting the cfu/ml of the *E. coli* that was left after emptying each cup and bottle per sterilisation method. A cfu is the number of bacterial cells, or clumps of cells, that can be developed into a colony when grown under laboratory conditions (Stamell, 2001:138). In each sample, the average of the cfu/ml was calculated. The log cfu/ml was determined using a calculator. The log phase of growth is the phase during which bacteria multiply logarithmically. Bacterial data are presented as $\log^{10}$, since the bacteria grow logarithmically and it is impractical to plot between 1 000 and 100 000 000 on a linear scale on a normal piece of paper (Food Safety First, 2004:2). A colony counter was used to count the results. A colony counter is an instrument that is used to count the number of bacterial colonies in a petrifilm plate by placing each plate individually on the platform of a colony counter (Aneja, 2003:71). After counting data with a colony counter, the data obtained were written down in duplicate in order to monitor the bias of data. The above was done precisely, with the assistance of control samples. Continuous monitoring and checking of the produced data was done to decide whether the data obtained were reliable enough to be released.

Data were recorded on a datasheet using a Microsoft Excel computer programme. The data were analysed using an NCSS computer programme, an Excel computer programme,

ANOVA and a t-test. The NCSS computer programme is a data analysis, statistical analysis and statistical graphic analysis programme. Microsoft Excel is a computer programme that simulates a graph paper sheet consisting of cells that are organised into rows and columns to make data entry, calculations and analysis of data easy and quick using formulas, functions and what-if tools. Both Excel and NCSS were used, with the former being used to capture the raw determinations (counts) and the latter to do the actual statistical analysis and data representation. The purpose of the ANOVA test is to test for a significant difference between the mean values of sample datasets, while the t-test is used to compare the mean values between two sample datasets.

The data were presented in frequency tables, box plots and graphs. Tables are easier to read and scan, providing an overview of the data. Box plots indicate the lower and upper quartiles, as well as median values, which allows the researcher to quickly examine one or more sets of data graphically. Graphs display more data than can be shown in a table, allowing for greater detailed data representation, and are most suited to the handling of data that cannot be easily displayed in a table.

4.3 PRESENTATION OF TABLES

Tables 4.1a and 4.1b below provide the results for the APC and the *E. coli* petrifilm experiments, indicating the cfus obtained at different dilutions for all the treatment groups in the sample.

An APC, which is an all-bacterial count, was used as an indicator of the presence of bacterial populations in the sample. The test is a generic one for organisms that grow aerobically at mesophilic temperatures (Morton, 2001:63-65).

The *E. coli* petrifilm plate count allowed the enumeration of *E. coli* on a single petrifilm. The plates concerned were incubated at 37⁰C for 24 hours. The *E. coli* count plate that yielded confirmed results for *E. coli* was the official Method[2] devised by the AOAC for *E. coli* (Kornacki & Johnson, 2001:78).

4.3.1 Results of raw data

Table 4.1a below represents the results of the APC of *E. coli* in baby formula incubated at 37ºC for 24 hours. The infant feeding bottles and cups were emptied and sterilised using different methods, as reported in the table, with two replicates being done for each method. A replicate is an exact copy of the material, which was, in the current study, the treatment group. In the first column of the table, the treatment groups are listed, with the two adjacent rows representing the replicate determinations done to check the counts. The second column represents the cfu/ml counted for each treatment group.

In the following instances, the replicate counts differed considerably:
- Sun cup: The cfu/ml for the sun cup was 7 800 cfu/ml and <10 cfu/ml respectively.
- Milton™ cup: The Milton™ cup obtained a cfu/ml count of >300 and 410 cfu/ml respectively.
- Milton™ bottle: The Milton™ bottle obtained a count of 1 230 000 cfu/ml and 2 100 cfu/ml, respectively.
- Sunlight™ dishwashing liquid cup: For the sunlight dishwashing cup, the cfu/ml was >300 and 28 900 000 cfu/ml, respectively.
- Sunlight™ dishwashing liquid bottle: The cfu/ml was 10 600 000 and >300 cfu/ml respectively.

The difference in replicate count meant that the concentration of micro-organisms was not homogeneous in the sample (with the micro-organisms not being similar). Within the different treatment groups, the dissimilarity between the two replicates was influenced by the treatments.

Table 4.1a: Laboratory results of APCs of *E. coli* in baby formula

Sample: treatment groups	Cfu/ml
Sun (cup)	7 800
Sun (cup)	<10
Sun (bottle)	830 000
Sun (bottle)	850 000
Milton™ (cup)	>300
Milton™ (cup)	410
Milton™ (bottle)	1 230 000
Milton™ (bottle)	2 100

Sunlight™ dishwashing liquid (cup)	>300
Sunlight™ dishwashing liquid (cup)	28 900 000
Sunlight™ dishwashing liquid (bottle)	10 600 000
Sunlight™ dishwashing liquid (bottle)	>300
Control (cup)	<10
Control (cup)	<10
Control (bottle)	<10
Control (bottle)	<10

Table 4.1b below, represents the results of *E. coli* by using *E. coli* petrifilm in baby formula incubated at 37⁰C for 24 hours. The infant feeding bottles and cups were emptied and sterilised using different methods, as reported in the table, with two replicates being done for each method. A replicate is an exact copy of the material, which was, in the case of the current study, the treatment group. In the first column of the table, the treatment groups are listed, with the two adjacent rows representing the replicate determinations done to check the counts. The second column represents the cfu/ml counted for each treatment group.

In the following instances, the replicate counts differed considerably:

- Sun cup: The cfu/ml for the sun cup was 2 600 cfu/ml and <10 cfu/ml, respectively.
- Milton™ cup: The Milton™ cup obtained a cfu/ml count of 21 100 000 and <10 cfu/ml, respectively.
- Milton™ bottle: The Milton™ bottle obtained a count of 2 800 cfu/ml and 20 cfu/ml, respectively.
- Sunlight™ dishwashing liquid cup: For the sunlight dishwashing cup, the cfu/ml was >300 and 15 500 000 cfu/ml, respectively.
- Sunlight™ dishwashing liquid bottle: The cfu/ml was 2 500 000 and >300 cfu/ml, respectively.

The difference in replicate count means that the concentration of micro-organisms was not homogeneous in the sample (with the micro-organisms not being similar). Within the different treatment groups, the dissimilarity between the two replicates was influenced by the treatments.

Table 4.1b: Laboratory results of culturing of *E. coli* by using *E. coli* petrifilm in baby formula

Sample: treatment groups	Cfu/ml
Sun (cup)	2 600
Sun (cup)	<10
Sun (bottle)	269 000
Sun (bottle)	291 000
Milton™ (cup)	21 100 000
Milton™ (cup)	<10
Milton™ (bottle)	2 800
Milton™ (bottle)	20
Sunlight™ dishwashing liquid (cup)	>300
Sunlight™ dishwashing liquid (cup)	15 500 000
Sunlight™ dishwashing liquid (bottle)	2 500 000
Sunlight™ dishwashing liquid (bottle)	>300
Control (cup)	<10
Control (cup)	<10
Control (bottle)	<10
Control (bottle)	<10

Table 4.2a below, represents the results of APC of *E. coli* in infant formula incubated at 37ºC for 24 hours. The two replicates (from sun to controls) were compared by means of the development of ratios and were separated into worst and best scenarios with respect to the *E. coli*. With respect to the sterilisation, excessive growth indicated that the sterilisation was unsuccessful (worst scenario). The lower *E. coli* growth indicated a more successful sterilisation method (best scenario).

For the APC samples listed in Table 4.2a below, four out of eight determinations were excessively different for the two replicates (highlighted in gold). One way of comparing the unusual discrepancies found was to calculate the logarithm of the ratio of the larger to the smaller counts, which would logically be larger than one (1.0). The ratio (larger than one) concerned would increase as the disparity between the two quantities escalated. Four pairs of replicates were found that had small differences between the smaller and larger

44

determinations. Four pairs of replicates, of which the log ratio was more than 1.0, were 6.4, 7.4, 10.4 and 11.4. The differences in the log ratios could be classified as large.

The difference in replicate count meant that the concentration of micro-organisms was not homogeneous in the sample (with the micro-organisms not being similar). Within the different treatment groups, the dissimilarity between the two replicates was influenced by the treatments concerned. With reference to table 4.1.a, cfu/ml of >300 was obtained for the Milton™cup, Sunlight™ dishwashing liquid cup and bottle. Since the cfu/ml was >300, the statistician round the total off to 310 to allow practical analysis.

Table 4.2a: Laboratory results of APCs of *E. coli* in baby formula

Sample	Derived concentration of *E. coli*	Ratio large/ small	Log ratio	Worst scenario Count concentration	Best scenario Count concentration
Sun (cup) Sun (cup)	7 800 5	1 560.0	7.4	7 800	5
Sun (bottle) Sun (bottle)	830 000 850 000	1.0	0.0	840 000	840 000
Milton™ (cup) Milton™ (cup)	310 410	1.3	0.3	360	360
Milton™ (bottle) Milton™ (bottle)	1 230 000 2 100	585.7	6.4	1 230 000	2 100
Sunlight™ dishwashing liquid (cup) Sunlight™ dishwashing liquid (cup)	310 28 900 000	93 225.8	11.4	28 900 000	310
Sunlight™ dishwashing liquid (bottle) Sunlight™ dishwashing liquid (bottle)	10 600 000 310	34 193.5	10.4	10 600 000	310
Control (cup) Control (cup)	5 5	1.0	0.0	5	5
Control (bottle) Control (bottle)	5 5	1.0	0.0	5	5

Table 4.2.1 below, explains the stem-and-leaf diagram of the log ratio in Table 4.2a. The figure was devised after discussion with Prof P. Gouws (2012) on the quality of the determinations for validity.

To find more than half of the determinations to be discrepant was unusual. To explain the occurrence of such a strange pattern, one could study the stem-and-leaf diagram. In this diagram, there were four similar pairs of replicates. Four sets of replicates that were extremely dissimilar can be seen from the log ratio calculated for the four pairs (6.4, 7.4, 10.4 and 11.4).

Table 4.2.1: Stem-and-leaf diagram of log ratio

Log ratio	Comment on log ratio
0.0003	Four differences (Control bottle, Control cup, Milton™ Cup and Sun bottle)
1	No observations
2	No observations
3	No observations
4	No observations
5	No observations
6.4	Large differences
7.4	Large differences
8	No observations
9	No observations
10.4	Large differences
11.4	Large differences

Dilution results were summarised in Table 4.2b below, with the two replicates being compared by means of ratios, and being separated into worst and best scenarios with respect to the *E. coli* found.

47

Five out of eight determinations were different for the two replicates (highlighted in gold). One way of comparing the unusual discrepancies found was to calculate the logarithm of the ratio of the larger to the smaller counts, which would logically be larger than one (1.0). The ratio (larger than one) concerned would increase as the disparity between the two quantities escalated. Three pairs of replicates were found that had small differences between the smaller and larger determinations. Five pairs of replicates, of which the log ratio was more than 1.0, were 4.9, 6.3, 9.0, 10.8 and 15.3. The differences in the log ratios could be classified as large.

Table 4.2b: Laboratory results of APCs of *E. coli* by using *E. coli* petrifilm in baby formula

Sample	Derived concentration of *E. coli*	Ratio large / small	Log ratio	Worst scenario Count concentration	Best scenario Count concentration
Sun (cup) Sun (cup)	2 600 5	520.0	6.3	2 600	5
Sun (bottle) Sun (bottle)	269 000 291 000	1.1	0.1	280 000	280 000
Milton™ (cup) Milton™ (cup)	21 100 000 5	4 220 000.0	15.3	21 100 000	5
Milton™ (bottle) Milton™ (bottle)	2 800 20	140.0	4.9	2 800	20
Sunlight™ dishwashing liquid (cup) Sunlight™ dishwashing liquid (cup)	310 15 500 000	50 000.0	10.8	15 500 000	310
Sunlight™ dishwashing liquid	2 500 000				

(bottle) Sunlight™ dishwashing liquid (bottle)	310	8 064.5	9.0	2 500 000	310
Control (cup) Control (cup)	5 5	1.0	0.0	5	5
Control (bottle) Control (bottle)	5 5	1.0	0.0	5	5

In the following tables, the APC method was directly compared to the *E. coli* petrifilm method. In Table 4.3a, the scale of measurement was the number of CFUs and in Table 4.3b the scale of measurement was the logarithm of the CFUs. The larger of the two counts was called the 'worst scenario', which implies that there was a higher concentration of *E. coli* growth compared to the average of the two counts obtained. The smaller of the two counts was called the 'best scenario', because the *E. coli* growth concentration was less. According to Prof Gouws (2010), the researcher needed to concentrate on the specimens (replicates) which had a more abundant growth of *E. coli*. Furthermore, Prof Gouws viewed 'any growth' as an indication of the presence of *E. coli* and 'no growth' as an indication of absence of *E. coli*, such as, for example, in the case of the controls.

Table 4.3a below represents the results of the comparison between the APC method samples and the *E. coli* petrifilm method. The worst scenario and the best scenario on the CFU scale were compared. In the following instances, the replicate counts were similar:

- Sun bottle (APC method): The cfu/ml for the sun bottle was 840 000 cfu/ml for both the worst and the best scenario.
- Sun bottle (*E. coli* petrifilm method): The cfu/ml for the sun bottle was 280 000 cfu/ml for both the worst and the best scenario.
- Milton™ cup (APC method): The cfu/ml for the Milton™ cup was 360 cfu/ml for both the worst and the best scenario.

Table 4.3a: APCs (on the CFU scale) of *E. coli,* dilution samples summarised, comparing the

APC method samples to the *E. coli* petrifilm method

Sample	APC method		*E. coli p*etrifilm method	
	Worst scenario	Best scenario	Worst scenario	Best scenario
	CFU Scale	CFU Scale	CFU Scale	CFU Scale
Sun (cup) Sun (cup)	7 800	5	2 600	5
Sun (bottle) Sun (bottle)	840 000	840 000	280 000	280 000
Milton™ (cup) Milton™ (cup)	360	360	21 100 000	5
Milton™ (bottle) Milton™ (bottle)	1 230 000	2 100	2 800	20
Sunlight™ dishwashing liquid (cup) Sunlight™ dishwashing liquid (cup)	28 900 000	310	15 500 000	310
Sunlight™ dishwashing liquid (bottle) Sunlight™ dishwashing liquid (bottle)	10 600 000	310	2 500 000	310
Control (cup) Control (cup)	5	5	5	5
Control (bottle) Control (bottle)	5	5	5	5

Table 4.3b represents the results of the comparison between the APC method and the *E. coli* petrifilm method. The worst and the best scenario on the natural logarithmic scale were compared.

50

In the following instances, the replicate counts differed considerably:

- Milton™ cup (APC method): The Milton™ cup obtained a cfu/ml count of 5.89 for the worst scenario.
- Milton™ cup (*E. coli* petrifilm method): The Milton™ cup obtained a cfu/ml count of 16.86 for the worst scenario.
- Milton™ bottle (APC method): The Milton™ bottle obtained a cfu/ml count of 14.02 cfu/ml for the worst scenario.
- Milton™ bottle (*E. coli* petrifilm method): The Milton™ bottle obtained a cfu/ml count of 7.94 for the worst scenario.

An excessive difference was found in the cfu counts between the worst scenario of the APC method and the *E. coli* petrifilm method. Such a finding implies that there was a higher concentration of *E. coli* growth for the Milton™ cup using the *E. coli* petrifilm method and a higher concentration of *E. coli* growth for the Milton™ bottle using the APC method.

Table 4.3b: APCs (on the natural logarithmic scale) of *E. coli,* with dilution samples summarised, comparing the APC method to the *E. coli* petrifilm method

Sample	APC method	APC count method	*E. coli* petrifilm method	*E. coli* petrifilm method
	Worst scenario	Best scenario	Worst scenario	Best scenario
	Concentrations transformed to the nat log	Concentrations transformed to the nat log	Concentrations transformed to the nat log	Concentrations transformed to the nat log
Sun (cup) Sun (cup)	8.96	1.61	7.86	1.61
Sun (bottle) Sun (bottle)	13.64	13.64	12.54	12.54
Milton™ (cup) Milton™ (cup)	5.89	5.89	16.86	1.61
Milton™ (bottle) Milton™ (bottle)	14.02	7.65	7.94	3.00
Sunlight™ dishwashing liquid (cup) Sunlight™ dishwashing liquid (cup)	17.18	5.74	16.56	5.74
Sunlight™ dishwashing liquid (bottle) Sunlight™ dishwashing liquid (bottle)	16.18	5.74	14.73	5.74
Control (cup) Control (cup)	1.61	1.61	1.61	1.61
Control (bottle) Control (bottle)	1.61	1.61	1.61	1.61

4.4 INTRODUCTION TO GRAPHICAL REPRESENTATION

Figure 4.1 and 4.2 below provide the results for the APC method versus the *E. coli* petrifilm method (the worst scenario and the best scenario) in terms of the Ln scale. The graphs show a more detailed data representation of the different treatment groups than was previously shown.

Figure 4.1 below shows the APC vs. the *E. coli* petrifilm results obtained for the different sterilisation methods (worst scenario). A natural logarithmic scale was used to analyse the data obtained. The graphs represent an experiment indicating the differences between infant feeding cups and infant feeding bottles. Both infant feeding cups and bottles were exposed to different sterilisation methods. Infant feeding cups and bottles were exposed to (1) direct sunlight for a certain timeframe, (2) Sunlight™ dishwashing liquid, and (3) Milton™.

The graphs show how the cups and bottles performed under different sterilisation methods. The line of equality shown divides the rectangle into two parts, with the vertical scale representing the *E. coli* petrifilm and the horizontal scale representing the APC. Two small counts coincided for the two methods of growing *E. coli*. These counts are the controls of the cups and bottles. In the lower half, five observations are reflected, indicating which APC method provided a larger count than did the *E. coli* method. The five observations are the sun cups, sun bottles, Milton™ bottles, Sunlight™ dishwashing liquid bottles and Sunlight™ dishwashing liquid cups. In the upper half, reflecting the *E. coli* petrifilm method, a single observation was larger than those obtained using the APC method. This single observation is the Milton™ cups. The bivariate points displayed in the graphs represent the different sterilisation methods used with both the cups and the bottles.
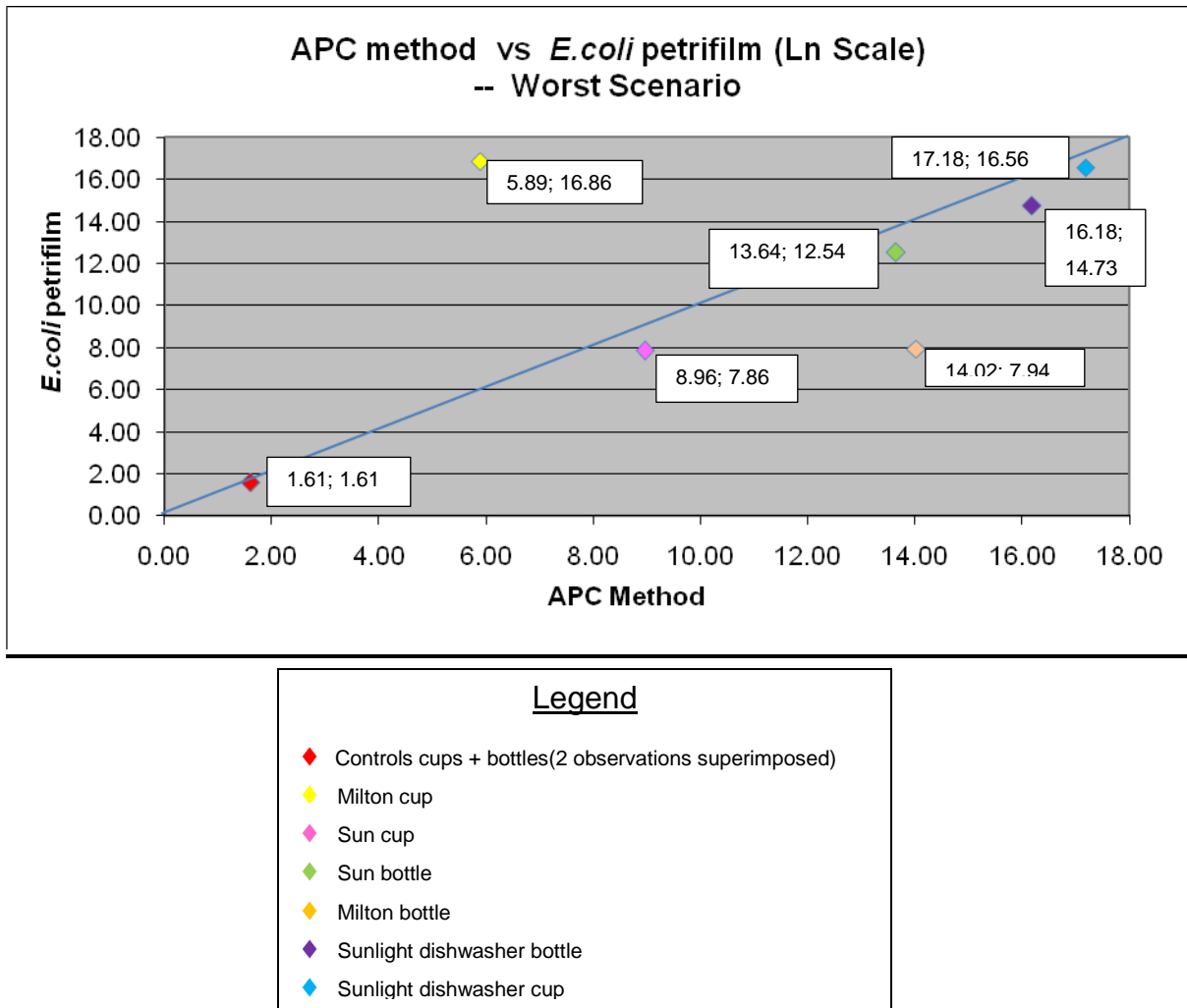
APC method vs *E.coli* petrifilm (Ln Scale)
-- Worst Scenario

Legend

♦ Controls cups + bottles(2 observations superimposed)
♦ Milton cup
♦ Sun cup
♦ Sun bottle
♦ Milton bottle
♦ Sunlight dishwasher bottle
♦ Sunlight dishwasher cup

Figure 4.1: Comparison, on the log scale, of the measurements of the APC method in contrast to those of the *E. coli* petrifilm

Figure 4.2 below, compares the measurements obtained using the APC method to those obtained using the *E. coli* petrifilm method (best scenario). On the line of equality, five of the bivariate points (observations) coincided for the two methods. These five observations are the controls of the cups and bottles, sun cups and Sunlight™ dishwashing liquid cups and bottles. In the lower half of the rectangle, three pairs of replicates occurred. The three pairs are the Milton™ cups and bottles and the sun bottles. The APC method resulted in larger counts for the three pairs concerned.
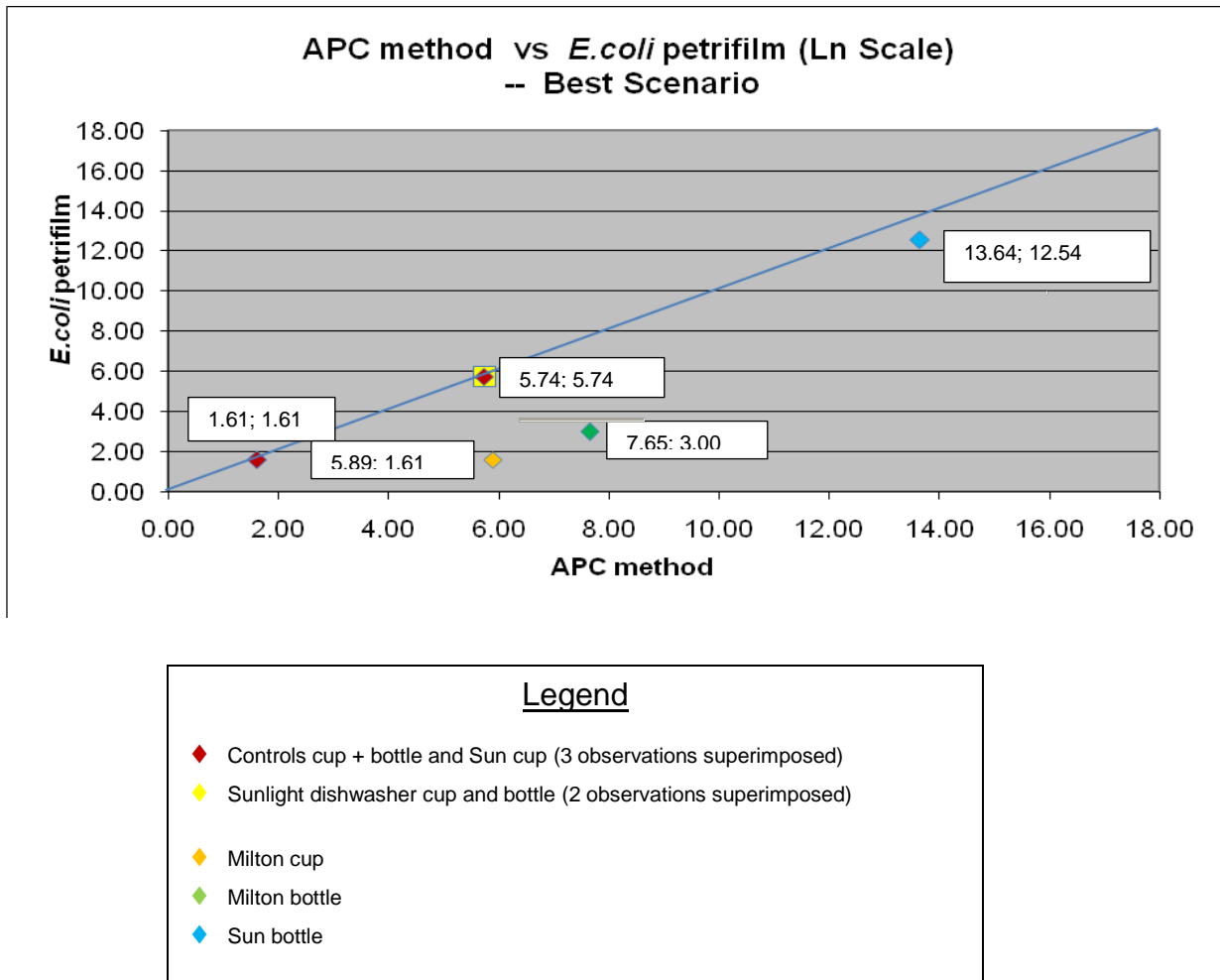
Figure 4.2: Comparison, on the log scale, of the measurements of the APC samples in comparison to those of the *E. coli* petrifilm samples

4.5 INTRODUCTION TO BOX PLOTS

The graphical representations below are box plots that were devised to determine the average number of cfu/ml for the APC method and for the *E. coli* petrifilm method. The averages between the different methods and containers were analysed. The use of box plots allowed the researcher to quickly examine one or more sets of data graphically.

The graph in Figure 4.3 below, shows the averages per cfu/ml of the APC for the different methods (i.e. sun, Milton™, Sunlight™ dishwashing liquid, and control) employed. The box in the middle of the above figure shows the spread/variation of 50% of the data (i.e. the data between the 25th and 75th percentiles). The line inside the box indicates the median

55

that is the middle value when the data are sorted from smallest to largest. 'Whiskers' extending above the box to the farthest non-outlying values represent the maximum values.
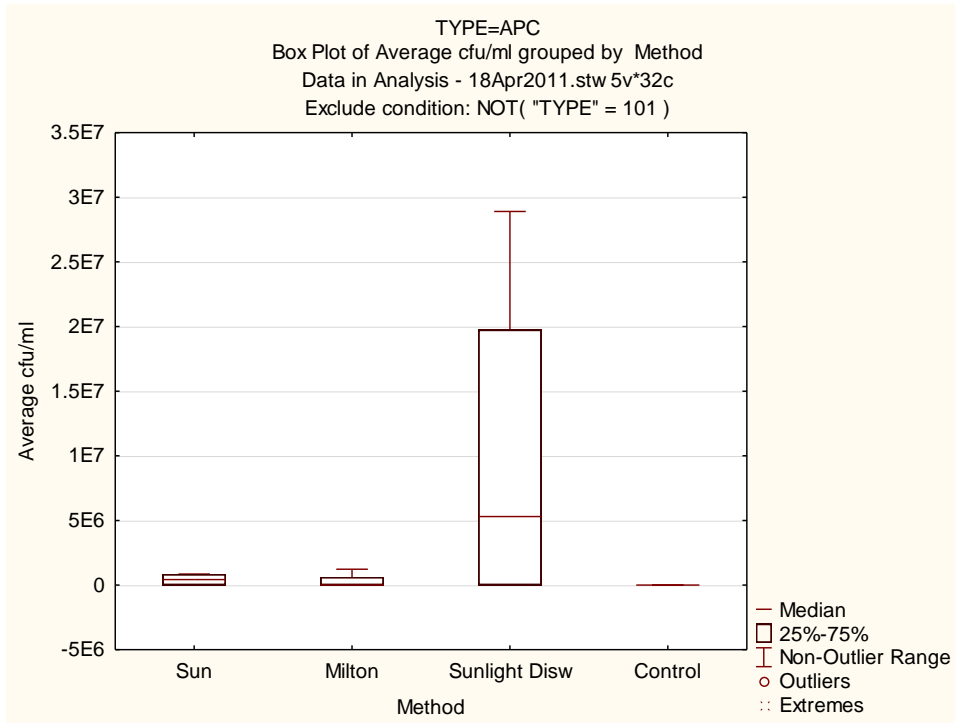

Figure 4.3: APC: box plot of average cfu/ml, grouped by method

The graph in Figure 4.4 below, shows the *E. coli* averages per colony-forming unit per millilitre for the different methods (i.e. sun, Milton™, Sunlight™ dishwashing liquid, and control) used.

The box in the middle of the above figure shows the spread/variation of 50% of the data (i.e. the data between the 25th and 75th percentiles). The line inside the box indicates the median that is the middle value when the data are sorted from the smallest to the largest. 'Whiskers' extending above the box to the farthest non-outlying values represent the maximum values.
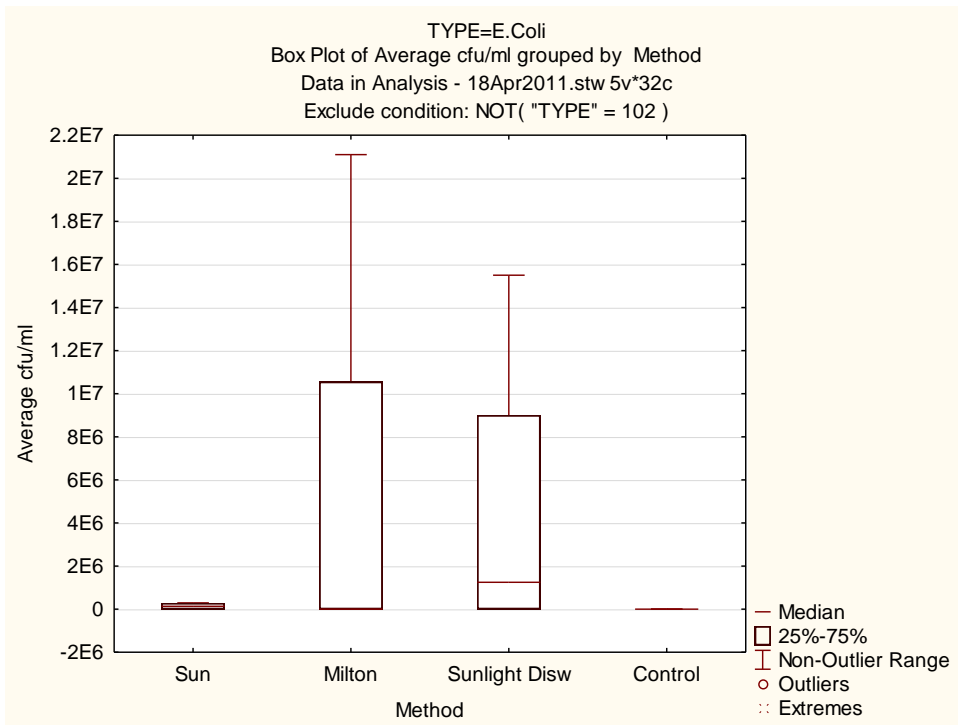
Figure 4.4: *E. coli*: **box plot of average cfu/ml, grouped by method**

The graph in Figure 4.5 below, shows the average per cfu/ml of the APC method for the cups vs. bottles tested. The graph contains outliers, which are data points with extreme values (i.e. values that lie far from other plotted points on the graph). The average bacteria count (on the ln scale) is 6 cfu/ml and 9 cfu/ml for cups and bottles, respectively. The value of $\rho = 0.184150$, indicating that there is no significant difference between the cups and bottles.
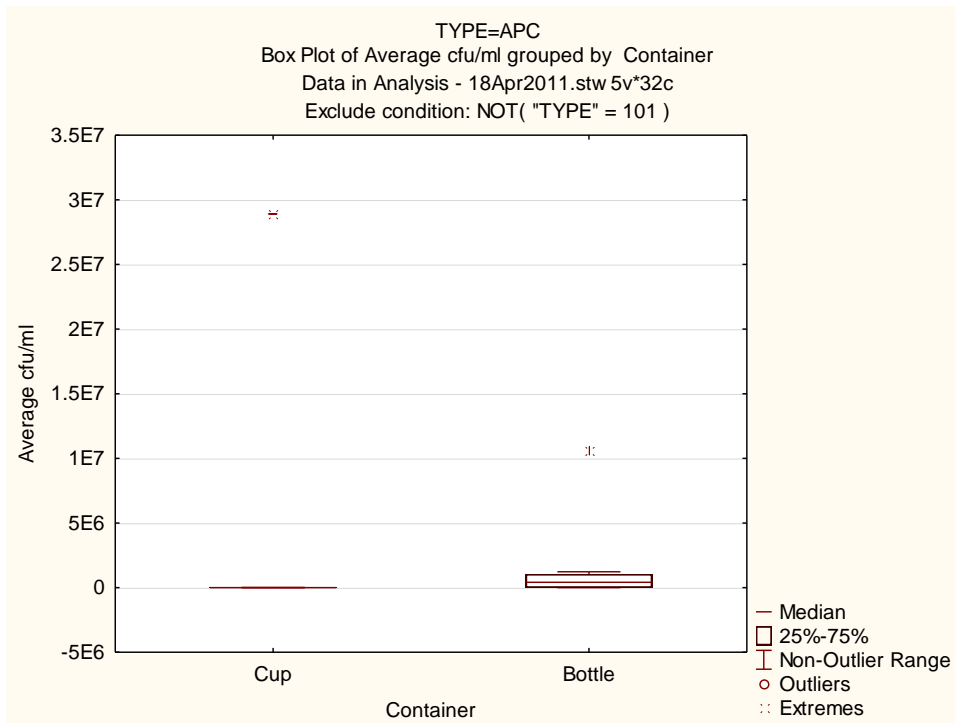
Figure 4.5: APC: box plot of average cfu/ml, grouped by container

The graph in Figure 4.6 below, shows the average per cfu/ml of the *E. coli* method for the cups vs. bottles tested**.** The box in the middle shows the spread/variation of 50% of the data (i.e. the data between the 25th and 75th percentiles).The graph contains 'whiskers' extending above the box to the farthest non-outlying values, which are the maximum values and outliers, being data points with extreme values (i.e. values that lie far from other plotted points on the graph).

The average bacteria count is 7 cfu/ml and 7.6 cfu/ml for cups and bottles, respectively. The ρ value is 0.821874, indicating that there is no significant difference between the cups and bottles.
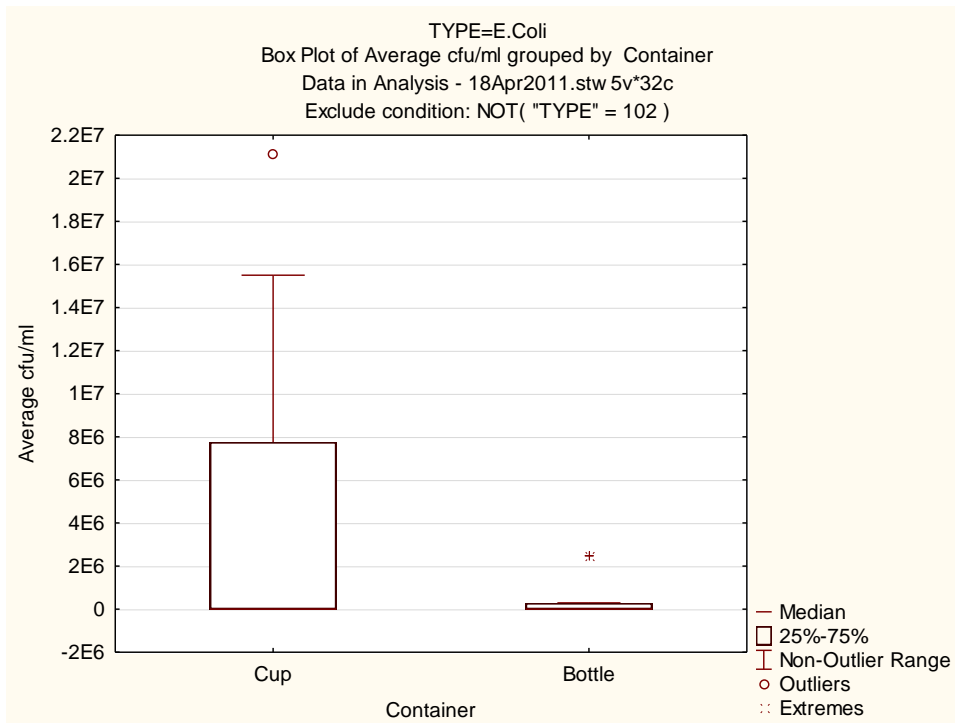
Figure 4.6: *E. coli*: box plot of average cfu/ml, grouped by container

4.6 INTRODUCTION TO T-TEST AND ANOVA ANALYSIS

Tables 4.4 to 4.7 below, provide the results of the t-values and the ANOVA for the APC method and the *E. coli* petrifilm method used on the cups and bottles surveyed. The mean value between the two sample datasets was compared with the t-test and the significant differences between the mean values of the sample datasets was compared with the ANOVA test.

The analysis in Table 4.4 below, which is for the APC cultures, compares cups and bottles to see whether there is a significant difference between the mean bacteria counts. This shows that the average bacteria count was 6 cfu/ml and 9 cfu/ml for cups and bottles, respectively. The t-value was -1.17524. As the ρ-value, which was 0.2595, was not less than 0.05, it cannot be said that there is a significant difference between the cups and bottles.

59

Table 4.4: T-test for independent samples (groups): APC: t-tests of containers

Variable	TYPE=APC T-tests; Grouping: Container (Spreadsheet in Analysis - 18Apr2011.stw) Group 1: Cup Group 2: Bottle										
	Mean Cup	Mean Bottle	t-value	df	p	Valid N Cup	Valid N Bottle	Std.Dev. Cup	Std.Dev. Bottle	F-ratio Variances	p Variances
Average cfu/ml	361360!	168905:	0.5019(	14	0.62349'	8	8	1021724	363438:	7.90326'	0.014088
Ln Ave cfu/ml	6	9	-1.17524	14	0.25950(	8	8	5	6	1.267260	0.762619

Table 4.5 below, shows the analysis for the *E. coli* cultures and compares cups to bottles to see whether there is a significant difference between the mean bacteria counts. The figure shows that the average bacteria count was 7 cfu/ml and 7.6 cfu/ml for cups and bottles, respectively. The t-value was -0.211902. The ρ-value was 0.835237, and since this is not less than 0.05, it cannot be said that there is a significant difference between the cups and bottles.

Table 4.5: *E. coli*: t-tests of containers

Variable	TYPE=E.Coli T-tests; Grouping: Container (Spreadsheet in Analysis - 18Apr2011.stw) Group 1: Cup Group 2: Bottle										
	Mean Cup	Mean Bottle	t-value	df	p	Valid N Cup	Valid N Bottle	Std.Dev. Cup	Std.Dev. Bottle	F-ratio Variances	p Variances
Average cfu/ml	457536!	382892.!	1.37158'	14	0.19177'	8	8	860222(	864721.(	98.9622!	0.00000
Ln Ave cfu/ml	7	7.6	-0.21190:	14	0.83523'	8	8	6	5.1	1.54549	0.579805

Table 4.6 below, reflects an ANOVA. The ANOVA test is aimed at determining whether there is a significant difference between the mean values of sample datasets. The value of ρ = 0.070298 shows that there was no significant difference between the methods of sterilisation, and ρ = 0.184150 indicates that there is no significant difference between the cups and bottles.

60

Table 4.6: APC method for cups and bottles and sterilising methods:

Effect	TYPE=APC Univariate Tests of Significance for Ln Ave cfu/ml (Spr Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	990.871(	1	990.871(	51.0743:	0.000001!
Method	181.494:	3	60.498'	3.1183'	0.070298
Container	38.9594	1	38.9594	2.00816	0.184156
Error	213.406:	11	19.4006		

The analysis reflected in Table 4.7 below, was undertaken to determine, in regards to *E. coli*, whether there was a simultaneous difference between the different methods and the container types. The above is an ANOVA analysis. The value of $\rho = 0.194987$, showing that there was no significant difference between the methods of sterilisation used, and $\rho = 0.821874$ indicates that there was no significant difference between the cups and bottles employed.

Table 4.7: *E. coli* method for cups and bottles and sterilising methods:

Effect	TYPE=E.Coli Univariate Tests of Significance for Ln Ave cfu/ml (Spr Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	859.309(	1	859.309(	30.9624(	0.000169
Method	154.768'	3	51.5896	1.85887	0.194987
Container	1.4755	1	1.4755	0.05317	0.821874
Error	305.285!	11	27.753:		

4.7 INTRODUCTION TO THE LEAST SQUARES (LS) METHOD

Figures 4.7 to 4.10 below provide the results of the APC method and the *E. coli* method for the different treatment groups and for the cups versus the bottles. The LS method defines a line that fits through a set of points on a graph (where the cumulative sum of the squared distances between the points and the line is minimised). The goal of the LS method is to explain as much of the variance in the dependent variable as possible. In the current study, the LS method was used for deriving the slope and the intercept that relied on a numerical algorithm that was based on statistical theory (Burns & Grove, 2007:426).

Figure 4.7 below, shows the LS means and 95% confidence intervals for the different treatments for APC. The graph shows that there is no significant difference between the averages of the *E. coli* distribution between the sun, Milton™ and Sunlight™ dishwashing liquid methods, since the value of ρ was found to be .07030.
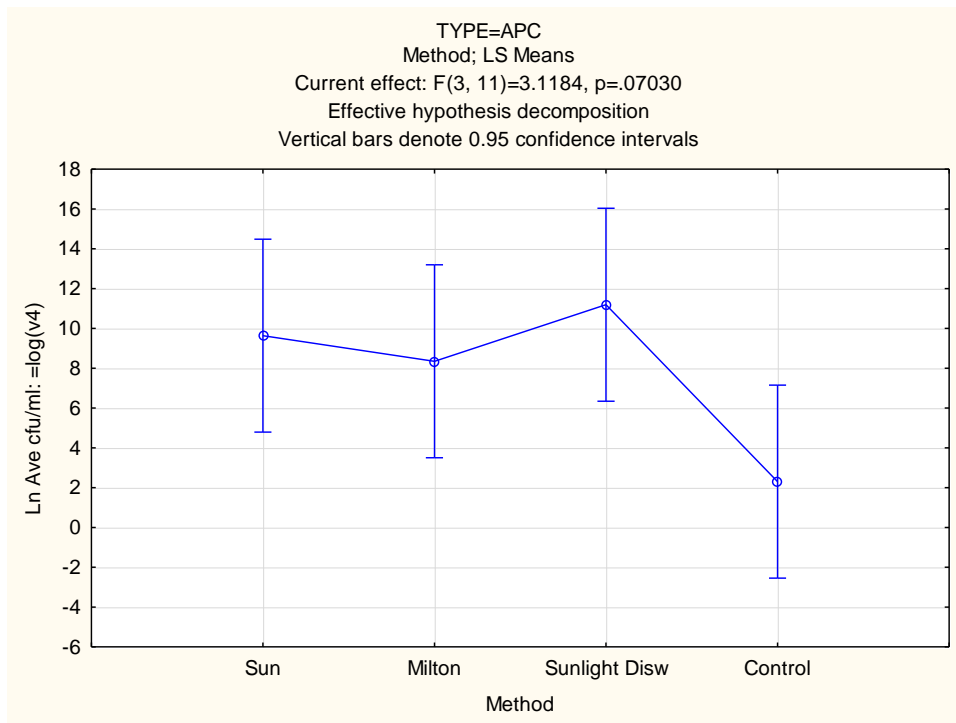

TYPE=APC
Method; LS Means
Current effect: F(3, 11)=3.1184, p=.07030
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals

Figure 4.7: APC method of treatment methods on the Least Squares method

The LS means and 95% confidence intervals for the different treatments for *E. coli* reflect in Figure 4.8 below. With a ρ value of .19499, the graph shows that there is no significant difference between the averages of the *E. coli* distribution between the sun, Milton™ and Sunlight™ dishwashing liquid methods.
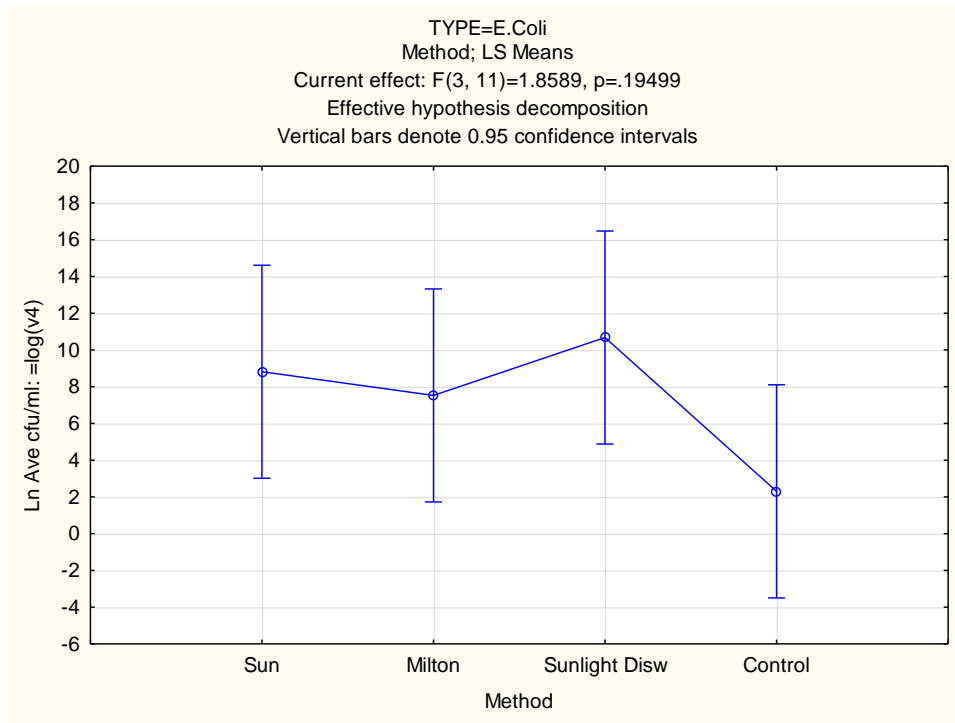

Figure 4.8: *E. coli* **method of treatment methods on the Least Squares method**

Figure 4.9 below, indicates the LS means and 95% confidence intervals for the different containers used for the APC. The graph shows no significant difference between the averages of the cups and bottles, since the ρ value was found to be .18415.
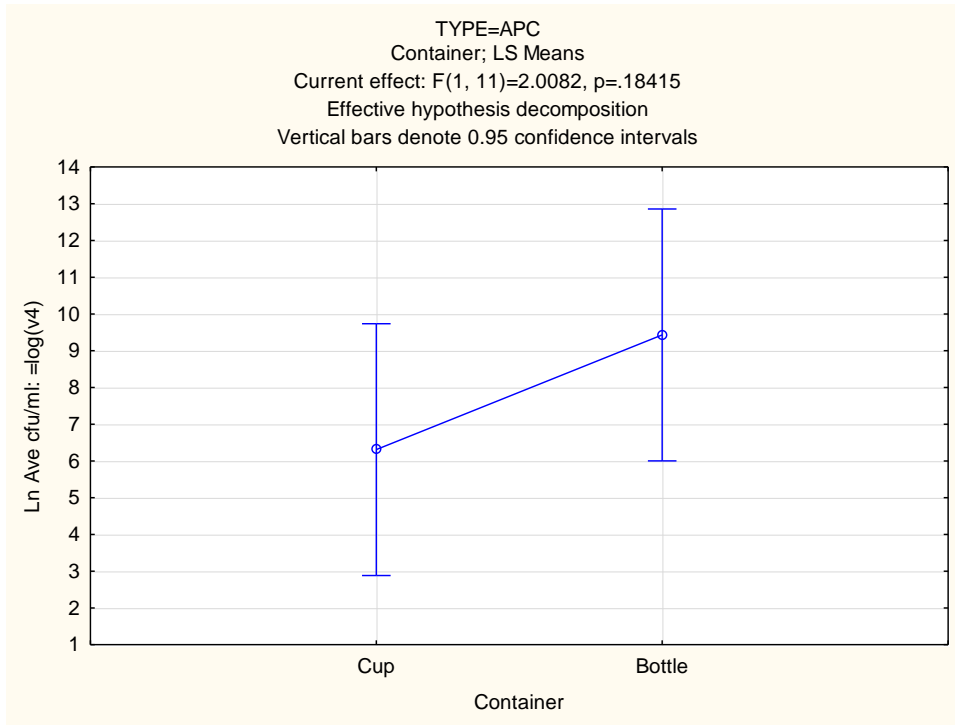

Figure 4.9: APC method of cups and bottles on the Least Squares method

Figure 4.10 below, shows the LS means and 95% confidence intervals for the different containers for *E. coli*. The value of ρ was found to be .82187, which shows that there was no significant difference between the composition of the *E. coli* in the cups and in the bottles.
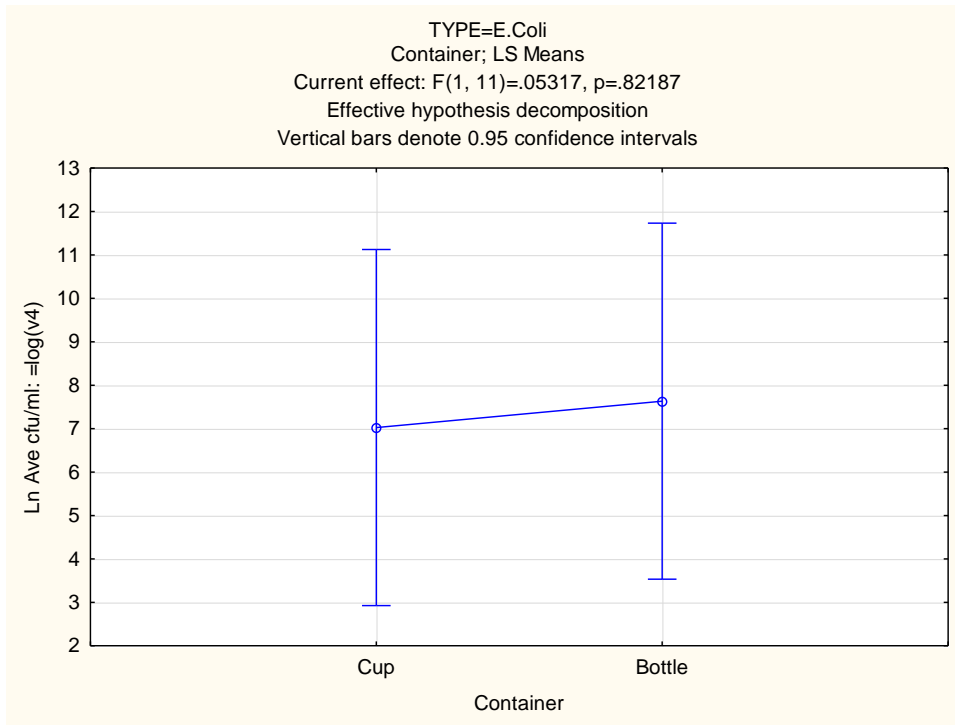

Figure 4.10: *E. coli* **method of cups and bottles on the Least Squares method**

4.8 CONCLUSION

65

The data that were collected in the current study were analysed to reduce them to an interpretable form to study, test and conclude the relations of the research problem. The data were analysed by means of tables, graphs, t-test and ANOVA analysis to determine the significant differences between the mean values of the sample datasets and to compare the mean values between the infant feeding cups and bottles.

The data analyses of the t-test and ANOVA analysis concluded that, with a ρ-value higher than 0.05, no statistical significant differences between the infant feeding cups and bottles and between the three different treatment groups were found.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1   INTRODUCTION

As was discussed in Chapter Two, the variety of sterilisation methods that had previously been studied consisted of boiling, steam and chemical methods, such as the use of Milton™. Other methods that can potentially be used are sunlight and Sunlight™ dishwashing liquid. Infant feeding bottle and cup sterilisation practices that were evaluated in the research were sunlight, and two chemical sterilisation methods, namely the use of Milton™ solution and Sunlight™ dishwashing liquid.

5.2   STUDY PURPOSE AND OBJECTIVES

The aim of the current study was to investigate which out of three particular sterilising methods was the most effective for sterilising feeding bottles and cups. The objective of the study was to determine the efficacy of three different methods of feeding utensil sterilisation commonly used in resource-poor areas, namely:

- chemical sterilisation using Milton™;
- dishwashing liquid using Sunlight™ dishwashing liquid; and
- natural sunlight.

The feeding utensils used were sterilised using one of the three identified methods, namely direct sunlight, Sunlight™ dishwashing liquid and Milton™ solution. These three methods were used, since it is easy accessible in households. Said sterilising treatments were used one at a time (with two replicates being used for each combination).

5.3   CONCLUSIONS

The study objective is used below to guide the discussions of the conclusions and the recommendations made in connection with the current study.

5.3.1 Objective

The objective of the current study was to determine the efficacy of three different methods of feeding utensil sterilisation commonly used in resource-poor areas, namely:

- chemical sterilisation using Milton™;
- dishwashing liquid using Sunlight™ dishwashing liquid; and
- natural sunlight.

From the two replicates (values) for each experiment, 'best' and 'worst' scenarios were heuristically defined. Heuristic defining involves learning from logical deductions made from the results obtained in a specific environment (i.e. a study). From the two replicates, the consistency of the cfu could be determined (which also describes the variability in the colony-forming counts). Comparing the 'best' and 'worst' scenarios resulted in highly varied observations (cfu).

The variability (e.g. standard deviation) of a single colony-forming determination could only be established with great difficulty. Such an error measurement would be very unstable compared to a small sample of replicates, for example for more than 10 repeat determinations. According to Prof Gouws (2011), microbiologists generally are of the opinion that the colony-forming method of determination only indicates 'growth' (the presence of *E. coli*) and 'no growth' (absence of *E. coli*), with nothing being indicated in between. In the current study, the statistical analyst indicated that the cfu determinations provided much more information (on an ordinal to interval scale) than did the binary outcome ('growth'/'no growth'). The statistician made the statement in such regard based on 35 years of experience in statistical analysis (Kotze, 2011).

The two replicates were, consequently, renamed 'worst' scenario (higher growth of the organism) and 'best' scenario (elemental ranking). Graphical methods were used during the analysis phase to compare the various sterilising treatments of the bottles and cups, as they provided a clear picture of the results obtained in the experiments.

A comparison of findings between the current study and the study of Ma et al. (2009:132-139) was made. Ma et al. (2009:132-139) rinsed artificially contaminated infant feeding bottles with low and high inocula of bacterial enteric pathogens with soapy water, followed by rinsing with tap water. The procedure was found to be the most effective cleaning

68

method and reduced pathogen load by 3.7 and 3.1 $\log_{10}$s at the low and high inoculum levels, respectively. Submersing highly contaminated bottles in 50 ppm hypochlorine solution for 30 minutes produced a 3.7 $\log_{10}$ reduction in bacterial pathogens, resulting in no identifiable pathogens being found in the bottles. The infant bottle cleaning practices that were evaluated included no rinse, rinsing with tap water one to three times, and rinsing with soapy water, with or without brushing the bottles.

However, in the current study, the cups and bottles were rinsed with Sunlight™ dishwashing liquid (5 ml in 5 L water) for 15 seconds in a sterilised container, after which they were dried upended on a sterilised surface. The second treatment group was the Milton™ sterilisation method, during which both cups and bottles were placed in a Milton™ solution (12.5 ml in 1 L cold water) for 30 minutes. The last treatment group was the sunlight sterilisation method, during which both cups and bottles were placed in direct sunlight for four hours on a bench outside the microbiology laboratory. The APC test method showed that the average bacteria count was 6 cfu/ml and 9 cfu/ml for cups and bottles, respectively. The *E. coli* test method showed that the average bacteria count was 7 cfu/ml and 7.6 cfu/ml for cups and bottles, respectively. The data obtained showed that there was no significant difference of bacterial contamination between cups and bottles. The value of $\rho = 0.070298$ showed that there was no significant difference between the methods of sterilisation used.

A comparison of findings between the current study and the study of Redmond et al. (2009: 85-94) was undertaken. In their study, microbial counts up to 10^5/area and ATP levels up to 100 051 relative light units were found in 'uncleaned' bottles. Enterobacteriaceae and *S. aureus* were found in 12% to 15% of 'unclean' bottles (up to 10^2 cfu/area sampled). Of the ready–to–use bottles that were cleaned and disinfected, some had aerobic colony counts up to 5.8×10^4 cfu/area sampled. *S. aureus* was found in 4% of the bottles/components, but no Enterobacteriaceae were found. The infant bottle cleaning practices that were evaluated in the study included cleaning methods using hot water alone, using detergent, and rinsing. The disinfection methods studied used a microwave unit, a steam unit, a cold water hypochlorite solution and a dishwasher / hand wash.

However, in the current study, the average cfu/ml for the methods used in the *E. coli* test method was 154.7687 cfu/ml, whereas, for the cups and bottles, the average was 1.4755 cfu/ml. The average cfu/ml for the methods used in the APC test method was 181.4942 cfu/ml, whereas, for the cups and bottles, the average was 38.9594 cfu/ml. Therefore, the data obtained indicated that there was no difference found between the sterilisation methods used and no difference was found between the cups and bottles.

The reason for deviation from previous study findings might be due to the particular research design used in the present study. No participants were involved in the study, since it was an *in vitro* study and the bottles and cups were artificially inoculated with *E. coli*, whereas, in the other study referred to above, participants were involved.

A further reason for the deviation might be due to the small sample size. For the current study, a sample size of 16 infant feeding bottles and cups was used, in comparison with the number that was used in the study conducted by Redmond et al. (2009:85-94). The total sample size in the mentioned study was 225 infant feeding bottles. Using such a large sample size enabled the real differences to be large enough to be significant.

5.4   STUDY LIMITATIONS

A limitation of the current study was that the sample size was too small. Only a small sample was used, since it is common practice in microbiology laboratories to perform only a few replicates, because even two replicates are labour intensive, and because the researcher had limited time available in which to conduct the experiments.

When using a smaller sample size, more observed differences are classified as being not statistical than is the case with larger samples. The observed differences tend not to be large enough to be grouped correctly in the case of small sample sizes, due to unstable (poor) estimates of the true dispersion.

Furthermore, not using soap and water and a brush to clean the utensils can also be ruled as a limitation of the present study. Ma et al. (2009:132-139) rinsed infant feeding utensils with soapy water (containing Sunlight™ dishwashing liquid) and a 2-$\log_{10}$ reduction of contaminating bacterial pathogens was observed.

5.5   RECOMMENDATIONS

Moore and Hesp (as quoted in De Vos et al., 2005:253) state that recommendations are simply suggestions to someone to do something. In the light of such a definition, the recommendations made in the following subsections are suggested, based on the findings of the study.

5.5.1   Study design recommendations

Regarding study design, the recommendation is made to conduct broader studies, using a larger sample size relating to the topic. Studies with a larger sample size enabled the real differences to be large enough to be significant.

5.5.2   Practice recommendations

Regarding practice, the following recommendations are made:

- Nurses and community health care workers, on receipt of the information that is contained in the current thesis, should disseminate it among their colleagues.
- Infant feeding utensils should be cleaned with soap and water and a brush before sterilisation, as the use of soapy water and a brush is proven to reduce contaminating bacterial pathogens.
- After three methods tested, Sunlight is not less than the other two and should be used as a sterilisation method for infant feeding utensils, as it is an effective and cost-effective method. As the use of sunlight is an inexpensive and readily available method of sterilisation, it can be used in poor-resourced socio-economic communities.
- The effectiveness of the sunlight sterilisation method under summer and winter conditions should be studied.

5.6   CONCLUSION

In conclusion, infant feeding bottles and cup sterilisation practices that were evaluated in the research included the use of direct sunlight, chemical sterilisation using Milton™ and

71

the use of Sunlight™ dishwashing liquid. The current study proved that direct sunlight can be used as an inexpensive and readily available sterilisation method for infant feeding bottles and cups. Therefore, sunlight can be used in under-resourced socio-economic communities to improve the microbiological safety of infant formula when using infant feeding utensils. However, the method is likely to be most effective during the summer months, since the amount of accessible sunlight is often limited during the cold winter months.

 The current study showed no significant difference between the sterilising methods or between the use of bottles and cups. Therefore, a study of a larger sample size is recommended for any further research that is undertaken.

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15 July 2010   **MAILED**

Ms N Maloy
Rochester House
Browning Rd
Obsevatory
7925

Dear Ms Maloy

An in-vitro study to assess three different sterilising methods for infant feeding cups, bottles and teats.

ETHICS REFERENCE NO: N10/06/211

RE : MODIFICATIONS REQUIRED

A review panel considered the application for interim approval and registration of the abovementioned project on behalf of the Health Research Ethics Committee.

In principle the panel is in agreement with the project, but requested that you should attend to the following matter(s) before the project could be approved:

  1. Your methodology is not clear. Sections 6-8 should be elaborated in more detail

  2. Describe the any bio-safety risks that your research involves and the measures taken in the laboratory to
     ensure the safety of the researchers and laboratory workers.

On receipt of the additional information/corrected document(s) the application will be reconsidered.

Please provide a letter of response to all the points raised IN ADDITION to HIGHLIGHTING or using the TRACK CHANGES function to indicate ALL the corrections/amendments of ALL DOCUMNENTS, clearly in order to allow rapid scrutiny and appraisal.

Please note that the application for the approval and registration of this project would be cancelled automatically if no feedback is received from you within 6 (six) months of the date of this letter.

Please quote the abovementioned project number in ALL correspondence henceforth.

For standard HREC forms and documents please visit: www.sun.ac.za/rds

15 July 2010 15:00   Page 1 of 2

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84