DETERMINANTS OF MATERNAL COLONIZATION WITH MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA

by

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Thesis presented in partial fulfilment of the degree of
Doctor of Philosophy (PhD in Public Health)
at the Division of Health Systems and Public Health, Department of Global Health
Faculty of Medicine and Health Sciences, Stellenbosch University
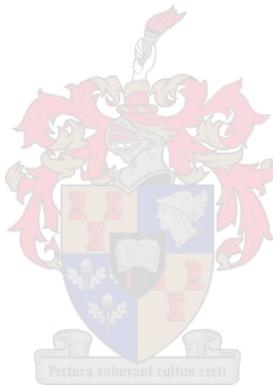
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March 2020

DECLARATION

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

This thesis includes three original papers published/accepted for publication in peer reviewed journals or books and two unpublished publications. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and for each of the cases where this is not the case a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

Date: March 2020

Summary

This doctoral research explored the topic of maternal colonization with multidrug-resistant Gram-negative bacilli (MDR-GNB) in Africa, using analytical cross-sectional studies to generate novel data and systematic reviews and meta-analyses to synthesize existing data. Maternal colonization is an established risk factor for neonatal colonization, which is a precursor to neonatal infection (the third leading cause of neonatal deaths in Africa). Understanding the factors contributing to maternal colonization with MDR-GNB in Africa, will inform the development of preventive interventions and ultimately contribute to reduction of neonatal infection burden.

Through a systematic review and meta-analysis, we underscored the major knowledge gaps regarding the burden of and risk factors for maternal colonization with MDR-GNB in Africa. The meta-analysis allowed us to provide a first estimate of the prevalence of maternal colonization with extended-spectrum beta-lactamases producing Enterobacteriaceae (ESBL-E) among pregnant and postpartum women in Africa (17%), which was 2 to 3-fold higher than that described from high-income countries.

Utilizing an analytical cross-sectional study design, we generated unique data describing the prevalence of maternal colonization with antimicrobial resistance (AMR) genes in a South African cohort of 651 peripartum women. Cefotaxime-M15 (CTX-M15) and New Delhi Metallo-beta-lactamase (NDM) genes were isolated from 12.9% and 2.2% of specimens respectively. Communal taps as the primary water source was the only independent predictor of maternal colonization with CTX-M15. Poverty-related factors (lower educational achievement, low income group and rural residence), and a clinical factor (primiparous status), were independent predictors of maternal colonization with carbapenem-resistant pathogens. Comorbidities like HIV infection and diabetes, or exposures such as recent antibiotic use, did not predict maternal colonization with AMR genes.

The knowledge, attitudes and practices regarding antibiotic use during pregnancy were assessed in a cohort of 301 pregnant South African women. Women with higher mean knowledge score (K-score) had lower reported proportions of antibiotic self-medication compared to women with lower mean K-scores. High monthly household income was an independent predictor of self-medication with antibiotics.

The relationship between the density of hospital environmental contamination with AMR genes and colonization proportions in 180 hospitalized peripartum women and 92 neonates, was prospectively

assessed. The level of hospital environmental contamination with AMR genes was low (5.8%); similarly, the number of colonized patients was small (2.8% of the peripartum women and 9.8% of the neonates), limiting the study's power to determine a clear link between environmental contamination and patient colonization.

Finally, in a second systematic review and meta-analysis, we produced a synthesis of molecular evidence linking MDR-GNB transmission from colonized mothers to their neonates. Although limited by the number and quality of studies, molecular evidence supports an overall 27% transmission proportion for MDR- and/or ESBL *Enterobacteriaceae* from colonized mothers to their infants, resulting in neonatal colonization. Further high-quality research is needed to determine the risk factors that promote mother-to-infant MDR-GNB transmission and evidence to link maternal MDR-GNB colonization and subsequent neonatal infection.

This doctoral thesis has produced new findings on the magnitude of and risk factors for maternal colonization with MDR-GNB in an African context. The novel data will inform the development of interventions to prevent colonization with MDR-GNB in mothers and subsequently reduce neonatal infection.

Opsomming

Hierdie doktorale ondersoek het die onderwerp van moederkolonisasie met multidrugtiewe weerstandige Gram-negatiewe basille (MDR-GNB) in Afrika ondersoek, met behulp van analitiese deursnitstudies om nuwe data en sistematiese oorsigte en meta-analises te genereer om bestaande data te sintetiseer. Moederkolonisasie is 'n gevestigde risikofaktor vir neonatale kolonisasie, wat 'n voorloper is vir neonatale infeksie (die derde grootste oorsaak van neonatale sterftes in Afrika). Deur die faktore wat bydra tot die moederkolonisasie met MDR-GNB in Afrika te verstaan, sal die ontwikkeling van voorkomende ingrypings ingelig word en uiteindelik bydra tot die vermindering van neonatale infeksielas.

Deur 'n stelselmatige oorsig en meta-analise het ons die belangrikste leemtes in die kennis oor die las en risikofaktore vir moederlike kolonisasie met MDR-GNB in Afrika onderstreep. Die metaanalise het ons in staat gestel om 'n eerste skatting te gee van die voorkoms van moederlike kolonisasie met ESBL-E onder swanger en postpartum vroue in Afrika (17%), wat 2 tot 3 keer meer is as wat beskryf is uit lande met 'n hoë inkomste.

Met behulp van 'n analitiese deursnitstudie-ontwerp, het ons unieke data gegenereer wat die voorkoms van moederkolonisasie met AMR-gene in 'n Suid-Afrikaanse groep van 651 peripartumvroue beskryf (CTX-M15- en NDM-gene is onderskeidelik van 12,9% en 2,2% van die monsters geïsoleer). Gemeenskaplike krane as die primêre waterbron was die enigste onafhanklike voorspeller van moederlike kolonisasie met CTX-M15. Armoede-verwante faktore (laer opvoedkundige prestasie, lae-inkomstegroep en landelike woning), en kliniese faktore (primariële status en laat aanbieding vir eerste besoek aan die voorgeboortesorg (ANC)) was onafhanklike voorspellers van moederlike kolonisasie met carbapenem-weerstandige patogene. Komorbiditeite soos MIV-infeksie en diabetes, of blootstellings soos die onlangse gebruik van antibiotika, het nie moederkolonisasie met AMR-gene voorspel nie.

Die kennis, houdings en praktyke rakende antibiotiese gebruik tydens swangerskap is beoordeel in 'n groep van 301 swanger Suid-Afrikaanse vroue. Vroue met 'n hoër gemiddelde kennis (K-telling) het 'n laer gerapporteerde aantal antibiotiese selfmedikasie gehad in vergelyking met vroue met 'n laer gemiddelde K-telling. Die maandelikse huishoudelike inkomste was 'n onafhanklike voorspeller van selfmedikasie met antibiotika.

Die verband tussen die digtheid van besoedeling in die hospitaal met AMR-gene en kolonisasietariewe by 180 vroulike peripartumvroue en 92 pasgeborenes is beoordeel. Die vlak van besoedeling in die hospitaal met AMR-gene was laag (5,8%); Op dieselfde manier was die aantal gekoloniseerde pasiënte klein (2,8% van die vroulike peripartum en 9,8% van die pasgeborenes), wat die studie se vermoë beperk het om 'n duidelike verband tussen die omgewingskontaminasie en pasiëntkolonisasie te bepaal.

Uiteindelik, in 'n tweede sistematiese oorsig en meta-analise, het ons 'n sintese van molekulêre bewyse vervaardig wat MDR-GNB-oordrag van gekoloniseerde moeders na hul pasgeborenes verbind. Alhoewel dit beperk is deur die aantal en kwaliteit van die studies, ondersteun molekulêre bewyse 'n algehele oordragskoers van 27% vir MDR- en / of ESBL Enterobacteriaceae van gekoloniseerde moeders na hul babas, wat lei tot neonatale kolonisasie. Verdere navorsing van hoë gehalte is nodig om die risikofaktore te bepaal wat die oordrag van moeder-tot-baba-MDR-GNB bevorder en bewyse om die MDR-GNB-kolonisasie van die moeder en die daaropvolgende neonatale infeksie te koppel.

Hierdie doktorale proefskrif het nuwe bevindings opgelewer oor die omvang en risikofaktore vir moederlike kolonisasie met MDR-GNB in 'n Afrika-konteks. Die nuwe gegewens sal die ontwikkeling van ingrypings om kolonisasie met MDR-GNB by moeders te voorkom inlig, en neonatale infeksie verminder.

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Chapter 1:

Introduction

Background and rationale

Bacterial colonization is defined as the presence of bacteria on the body surface (like skin, airways, gut, rectum, nose and vaginal canal) without causing disease in the host [1]. These bacteria are found in all humans, regardless of population, and may be susceptible or resistant to antibiotics. Under certain conditions (e.g. immunodeficiency, hospitalization, burns etc.), colonization may be a precursor to infection [2,3].

Multidrug-resistant Gram-negative bacteria (MDR-GNB) are a serious threat to human health, and generally cause greater morbidity and mortality than Gram-positive bacterial infections. The magnitude of colonization varies geographically and between population groups. For example 20% of "elderly residents" at a long-term care facility in Boston (USA) and 26% of inpatients in a Saudi Arabian study [5] were colonized with MDR-GNB [4]. Of note, a lack of studies on risk factors pertaining to colonization with MDR-GNB [3,6–8], including studies of maternal colonization, precludes the development of evidence-based interventions to reduce colonization with MDR-GNB. Maternal colonization with MDR-GNB is of greater concern as peripartum women are vulnerable to infections and their neonates are at increased risk of being colonized and infected with MDR-GNB. In fact, maternal colonization is an established risk factor for neonatal infection [9–11].

Maternal colonization as a major risk factor for neonatal infection

The female genital tract is colonized by a wide range of organisms, both pathogenic and commensal. Depending on the vaginal environment and microbiome, certain bacterial species dominate [12]. Aerobic organisms colonizing the maternal genital tract are more frequently described in the literature as causing neonatal infection. These include Gram-positive cocci (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus species [α-haemolytic, β-haemolytic, non-haemolytic, Group D]*), although Gram-negative bacilli (aerobic and anaerobic) are also maternal genital tract colonizers and known neonatal pathogens (*Escherichia coli, Klebsiella* spp*., Enterobacter* spp*., Proteus* spp*.,* and *Pseudomonas* spp.). Maternal colonization has been shown to be an important risk factor for neonatal colonization and subsequent neonatal infection [11,13,14].

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Maternal risk factors for neonatal infection with antimicrobial susceptible pathogens [11,15–17] include preterm premature rupture of membranes (PPROM) and prolonged rupture of membranes (PROM). However, there is a paucity of research on maternal risk factors or determinants of maternal colonization with MDR-GNB [8]. In Madagascar, Chereau et al [6] determined a prevalence of ESBL-E colonization among pregnant women of 18.5%, which increased to 25% during the rainy season (October to March). The two independent risk factors for maternal colonization with ESBL-E were private indoor access for drinking water supply and individual housing (type of house). The use of antibiotics in the last year was not associated with ESBL-E colonization in pregnant women in that study.

To the best of our knowledge, there is little data on colonization with carbapenemase-producing GNB in pregnant and postpartum women in Africa. A recent study in South Africa, did not find carbapenemase-producing GNB in maternal stools, albeit the sample size was small (90 mother-child pairs) [18].

While it is well described that organisms colonizing the "maternal genital tract", are causative pathogens of neonatal infection, ascending infections may also occur before or during labour through translocation of bacteria from the maternal perineum to the vaginal canal, and amniotic sac/amniotic fluid [11]. Therefore, rectal swabs reflecting maternal colonization with MDR-GNB are an indicator of potential risk for subsequent neonatal infection [19].

The main objectives of this doctoral research were to determine:

(1) the prevalence of and risk factors for maternal colonization with MDR-GNB;
(2) the factors influencing antenatal use of antibiotics during pregnancy;
(3) the prevalence of MDR-GNB hospital environmental contamination with MDR-GNB and its relationship with patient (peripartum women and neonates) colonization.

HIV infection, diabetes and maternal colonization with MDR-GNB

Cutland et al in South Africa reported HIV infection as a risk factor for vaginal colonization with *Escherichia coli* (*E. coli*) but not with Group B *Streptococcus* (GBS) [20]. Maternal genital tract and/or rectal colonization with pathogens that are the predominant cause of neonatal infections in Africa, has not been adequately studied, especially among pregnant women living with HIV.

Pregnant women with diabetes are considered to be at high risk of infections, especially urinary tract infections [21] which can predispose to subsequent neonatal infection. Diabetes is also considered a risk factor for GBS colonization in pregnant women, although a study by Akhlaghi demonstrated that

the proportion of GBS carriage was only increased among rectal swab specimens from pregnant women with diabetes [22].

HIV infection, tuberculosis therapy and diabetes mellitus are locally relevant factors in South Africa which may impact on colonization spectrum and prevalence of MDR-GNB in the maternal genital tract. Further studies are warranted to establish the association of co-morbidities (e.g. HIV and diabetes) and maternal colonization with MDR-GNB.

Infection prevention and control practices

Infection prevention and control (IPC) is the cornerstone of strategies to reduce maternal and neonatal morbidity and mortality, with substantial potential to improve maternal-child health indices. Transmission of MDR pathogens often reflects unsafe water, sanitation and hygiene (WASH) services, inadequate IPC practices in healthcare facilities, including poor adherence to standard precautions including hand hygiene (HH). MDR-GNB may be transmitted by direct and/or indirect contact with colonized and/or infected patients, through health care professionals' contaminated hands and contaminated environmental surfaces [23,24]. Good hand hygiene and environmental cleaning are recognized measures to prevent the transmission of MDR-GNB to patients, including pregnant women [24–26].

Impact of antenatal care on maternal colonization with MDR-GNB

The antenatal care (ANC) and antenatal antimicrobial use are other factors which may impact on the rates of maternal colonization with AMR bacteria. The WHO recommends at least four ANC visits during pregnancy, as an intervention to reduce maternal/neonatal mortality globally including low to middle income countries (LMIC) [27]. Insufficient number of ANC visits is associated with early onset neonatal infection [28], probably owing to fewer antenatal opportunities to screen for, and treat potential risk factors like bacteriuria, HIV infection, and diabetes.

Effective ANC improves neonatal outcomes directly by reducing stillbirths and neonatal deaths [29]. It has been estimated that if 90% of pregnant women received ANC, up to 14% of neonatal deaths could be averted, saving 160 000 newborn lives in Africa annually [29]. In order to generate relevant interventions to reduce maternal colonization with AMR bacteria, more studies assessing the impact of the quantity and quality of ANC are needed in African settings.

Use of antibiotics during pregnancy and caesarean section

Antibiotics are prescribed during pregnancy for different indications, including urinary tract infections, sexually transmitted diseases, premature rupture of membranes, preterm labour, intrapartum fever, prevention of neonatal GBS, and prophylaxis for caesarean section, among others [30]. It has been reported that more than 40% of pregnant women are prescribed antibiotics prior to delivery [31], either for prevention of neonatal GBS disease or caesarean section chemoprophylaxis [32],[33]. In the latter randomized controlled trial, administration of antibiotic prophylaxis at 30 to 60 minutes before skin incision resulted in better maternal outcomes (reduced infectious morbidity and hospital stay) but had no impact on neonatal outcomes. More importantly, a Cochrane systematic review of interventions for preventing postpartum infectious morbidity, reported that administration of intravenous antibiotic prophylaxis pre-operatively significantly decreased maternal infectious morbidity compared with administration after cord clamp, although the effect on neonatal outcomes was unclear [34].

Knowledge, attitudes and practices/perceptions (KAP) regarding antimicrobial use and prescription among pregnant women.

In many LMIC, the practice of self-medication is a frequent occurrence. Antimicrobial access is often less well-regulated compared with most high-income countries, where a medical prescription is required before the purchase of medicines [35]. In addition, the leading reason for antibiotic prescribing in pregnant women, either antenatally or during the peripartum period [36], is urinary tract infection. There is an association between antibiotic resistance in uropathogens and antibiotic prescribing in primary care [37], with bacteria developing resistance to the prescribed antibiotics.

In most cases, clinicians perceive AMR as a complex problem at the national level, but not as relevant to their own institution or practice [38,39], and may fail to adhere to local or national prescription guidelines where these exist [40]. Many factors influence antibiotic prescribing and their use in the community, including patient pressure and concerns about maintaining doctor-patient relationships [41,42].

In South Africa, beta-lactams antibiotics (two thirds represented by penicillins and one quarter by cephalosporins) are the most frequently prescribed antimicrobial classes, followed by anti-viral agents and quinolones [43]. The same study by Truter and co-workers, demonstrated peaks in antibiotic prescriptions during the winter months, when upper respiratory conditions were common [43].

However, it is documented that the vast majority of respiratory infections in winter are of viral origin and self-limiting, therefore the prescribing of antibiotics in these situations was often inappropriate [44,45].

There is little known about the knowledge, attitudes and practices regarding antibiotic use and prescribing among pregnant women in South Africa. In particular, the effect of antenatal and postpartum antibiotic use on maternal colonization with MDR-GNB is unquantified. A better understanding of local drivers of antibiotic use and prescription in pregnant women in South Africa, will inform development of interventions to educate pregnant women and healthcare providers who prescribe antibiotics to this population [46].

Prevalence of Multidrug-resistant Gram-negative bacilli in Africa

Beta-lactamases are most commonly classified according to two general schemes: the Ambler molecular classification scheme and the Bush-Jacoby-Medieros functional system [47]. ESBLs are beta-lactamases that confer bacterial resistance to the penicillins, first-, second- and third-generation cephalosporins, and aztreonam by hydrolysis. They remain susceptible to cephamycins and carbapenems and may be inhibited by certain beta-lactamase inhibitors such as clavulanic acid [47].

Africa is a large continent with variable health delivery and resources; the prevalence of ESBL-producing GNB (ESBL-GNB) varies widely. Malawi reported proportion of ESBL- GNB, based on ceftriaxone-resistant Enterobacteriaceae from blood cultures as 0.7%, in contrast with Egypt where 75.8% of bacterial isolates from patients in intensive care unit (ICU) settings were ESBL-GNB [48]. In South Africa, ESBL-GNB are significant pathogens in both community and hospital settings [48]. ESBL-E have been isolated from various clinical specimens, including blood, urine, stool and pus [48]. Kaba et al [18], reported on ESBL-E faecal carriage in a community-based sample of 90 healthy children and their mothers was 3.5% and 4.4%, respectively. Habte et al [49], documented ESBL production in 42/354 (11.9%) and 76/187 (40.6%) of *E.coli* and *K. pneumoniae* isolates from urine samples, respectively in hospital settings. In South Africa, Perovic and co-workers [50], reported ESBL rate of 68% among *Klebsiella pneumoniae* bloodstream infection isolates at national sentinel South African hospital sites (Gauteng, KwaZulu-Natal, Free State, Limpopo and Western Cape provinces) between 2010 - 2012. Dramowski et al, found even higher proportions of ESBL-producing *E. coli* and *K. pneumoniae* in a South African children's hospital both in community- and hospital-acquired

bloodstream infections (11.7% vs 75.7% and 21.7% vs 78.3%, respectively) between 2008 and 2013 [51]. A recent report by the National Department of Health, South Africa, on AMR and human antibiotic consumption in the country between 2012 – 2017, documented 1 in 4 *E. coli* isolates was an ESBL producer; for the past 6 years the prevalence of ESBL-producing *K. pneumoniae* has remained between 66 – 70% [52].

The most prevalent genotypes among ESBL-E isolates are the CTX-M, SHV and TEM genes. CTX-M has spread worldwide and has been described in patients with community-onset urinary tract infections[6,53–57]. Many isolates contain multiple different ESBL genes simultaneously, e.g. combinations of CTX-M, SHV and TEM [48,58,59].

In South Africa (KwaZulu-Natal), faecal carriage of *E.coli* and *K. pneumoniae* in children from the community, had an overall prevalence of 4.7% (14/300); however, no molecular typing was performed [60]. Peirano characterized 22 non-repeat ESBL-E originating from several private and a state hospitals in Cape Town, and reported 59% (13/22) of isolates produced CTX-M15, seven produced CTX-M14 and one isolate each produced CTX-M3 and SHV2, respectively [58].

A recent study from Mthatha in South Africa, established that the prevalence of ESBL production among 202 Klebsiella species isolates was 57.9% (117/202) [59]. ESBL-genotypic resistance in Mthatha is driven by blaSHV 77.1% (121/202) followed by TEM 66.9% (105/202) and CTX-M 56.7% (89/202), with many bacteria harbouring more than one ESBL gene [59]. The most frequent genotypic combination in this study was TEM + SHV + CTX-M found in 50.3% (79/117).

Carbapenemase-producing GNB

Carbapenemases can be classified, based on amino acid similarity, and are assigned to three of the four classes of beta-lactamases, namely Ambler classes A, B and D [61]. These three classes can also be differentiated using their hydrolytic mechanism at their active sites. Carbapenemases of class A and D are serine carbapenemases, whereas class B have zinc at their active sites and are referred to as metallo-beta-lactamases [61].

Class A carbapenemases may be chromosomal or plasmid-encoded, and they are partially inhibited by clavulanic acid, a beta-lactamase inhibitor [61]. The most frequently identified class A

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carbapenemases are the *Klebsiella pneumoniae* carbapenemases (KPCs) [62].

Metallo-beta-lactamases are plasmid-mediated, or in some cases chromosomal. Among clinical isolates, the most commonly reported enzymes from this group are VIM, IMP and NDM [61]. NDM-1 has been recognized as a serious public health concern because of rapid worldwide spread [63–65]. Class B enzymes can hydrolyse all beta-lactams except for aztreonam, a monobactam, they are not inhibited by clavulanic acid, and their hydrolytic activity is reduced or inhibited by EDTA [61].

Class D enzymes, are referred to as OXA type, are classified in five classes, OXA-23, -24/40, -48 and -58 carbapenemases, which are plasmid-encoded, and the OXA-51 carbapenemase which is chromosomally-encoded and intrinsic in *Acinetobacter baumannii* [66]. Class D enzymes are not inhibited by EDTA or clavulanic acid [61].

In South Africa, between January 2000 and May 2016, 2315 carbapenem-resistant laboratory isolates were identified, with two-thirds of all isolates nationally identified from the Gauteng (n=1220) and KwaZulu-Natal provinces (n=515). The most common bacterial species carrying carbapenemases were *Klebsiella pneumoniae* (n=1138), *Acinetobacter baumannii* (n=332), *Enterobacter cloacae* (n=201), and *Serratia marcescens* (n=108) [67]. However, the abovementioned review by Sekyere reported results based on publications and communiqués from the National Institute of Communicable Diseases and may not be considered representative of South Africa.

Most carbapenem-resistant GNB isolates in South Africa, were obtained from patients with no history of travel outside the country, suggesting local acquisition of infection, possibly driven by increasing use of carbapenems nationally, or poor sanitation [68]. Substantial dissemination of carbapenem resistance is mediated largely by NDM-1 or OXA-48 carbapenemases in *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Serratia marcescens* and other GNB [67].

Carbapenem-resistant infections may result in higher mortality [62,69] with several publications documenting the effect of carbapenem-resistant Enterobacteriaceae (CRE) infections on South African patient outcomes [70–72]. Cases of CRE infection among hospitalized neonates have been reported in KwaZulu-Natal, including a one-day old neonate where maternal colonization with CRE and/or the contamination of the healthcare environment with CREs may have contributed [73,74]. In 2018/19,

the first cases of neonatal bacteraemia and meningitis caused by carbapenem-resistant *Klebsiella pneumoniae* were detected at Tygerberg Hospital. Currently approximately half and one-third of the predominant neonatal pathogen, *Klebsiella pneumoniae* isolates from that neonatal unit produce ESBLs and carbapenemases respectively (personal communication: Angela Dramowski).

It is thus important to identify the determinants of maternal colonization with MDR-GNB, and evaluate the possible contribution of the community and healthcare environments in the acquisition of MDR-GNB, in order to determine interventions to prevent MDR-GNB colonization and potential transmission from mothers and their infants.

Burden of neonatal infection and antimicrobial resistance

Globally, neonatal deaths contribute to almost half of all deaths among children under-five years of age[75,76]. Of the 2.7 million under-five childhood deaths in 2015, 45% occurred in the neonatal period, with half of these deaths recorded in the first week of life [76]. Despite declining under-five mortality rates globally, the proportional contribution from neonatal deaths has increased in all WHO regions over the past 25 years. This phenomenon may be explained by the fact that health interventions needed to address the major causes of neonatal deaths have not been successful, whereas other interventions e.g. vaccination and antiretroviral therapy have reduced under-five deaths substantially [76].

Neonatal infections, prematurity and birth asphyxia are the top three causes of neonatal deaths. Among neonatal infections, serious bacterial infections (including sepsis and meningitis) are the leading cause of death and are increasingly caused by multi-drug resistant (MDR) pathogens [77]. Maternal colonization with MDR pathogens is thought to be an important risk factor for subsequent neonatal colonization and/or infection [13].

Pathogens causing neonatal infection

The role of maternal colonization in vertical transmission and subsequent neonatal infection has been extensively studied for Gram-positive pathogens e.g. Group B *Streptococci* (GBS) [78,79], methicillin-resistant *Staphylococcus aureus* (MRSA) [80–88] and *Streptococcus pneumoniae* [89]. However, there is extremely limited data to support the role of maternal colonization in subsequent neonatal infection with Gram-negative bacilli (GNB) [13,78,90–95].

As resistance to available antimicrobial therapies increased, the spectrum of bacterial pathogens

causing neonatal infection has also changed. GNB are the most prevalent neonatal bloodstream pathogens (especially in Africa and Asia), and are frequently MDR [93,96–98]. The most frequently reported GNB causing neonatal infections are *Klebsiella pneumoniae* and *Escherichia coli. Klebsiella pneumoniae* is the predominant pathogen causing both early- (occurring in the first ≤72 hours of life) and late-onset (>72 hours of life) neonatal infection [93,96], with production of ESBLs [93,97] as the most frequent mechanism of antibiotic resistance. However, GNB-producing carbapenemases have been increasingly isolated and are a greater challenge in terms of clinical management, as they are resistant to most antibiotic classes [99]. Given the changing epidemiology of neonatal infection pathogens and MDR phenotypes, research to better characterize the determinants of maternal colonization with MDR-GNB is needed.

PURPOSE OF THE RESEARCH

This doctoral research study: (1) determined the prevalence of and risk factors for maternal colonization with MDR-GNB in Africa; (2) determined factors influencing antenatal use of antibiotics during pregnancy among women at a South African antenatal clinic; (3) determined the prevalence of MDR-GNB hospital environmental contamination and its relationship with MDR-GNB colonization in unlinked peripartum women and neonates.

An improved understanding of the determinants of maternal colonization with MDR-GNB in an African setting will inform future research and interventions to reduce maternal - and the possible effect on neonatal - colonization and infection with MDR bacterial pathogens.

AIMS, HYPOTHESES, METHODS and PROPOSED MANUSCRIPTS

The central theme of this PhD is maternal colonization with MDR-GNB in pregnancy and the postpartum period. The research approach used analytical cross-sectional studies, a KAP study and systematic review and meta-analyses to assess the association of maternal colonization with different factors, including socio-demographics and relevant co-morbidities such as maternal HIV infection and diabetes. The effect of the quality of antenatal care and use of antibiotics during pregnancy and delivery was also studied as proxies for newly-acquired (in-hospital acquisition of) MDR-GNB colonization. The relationship between hospital environmental contamination and patient (unlinked peripartum women and neonates) colonization was also assessed as a proxy for horizontal transmission of MDR-GNB (Figure 1).

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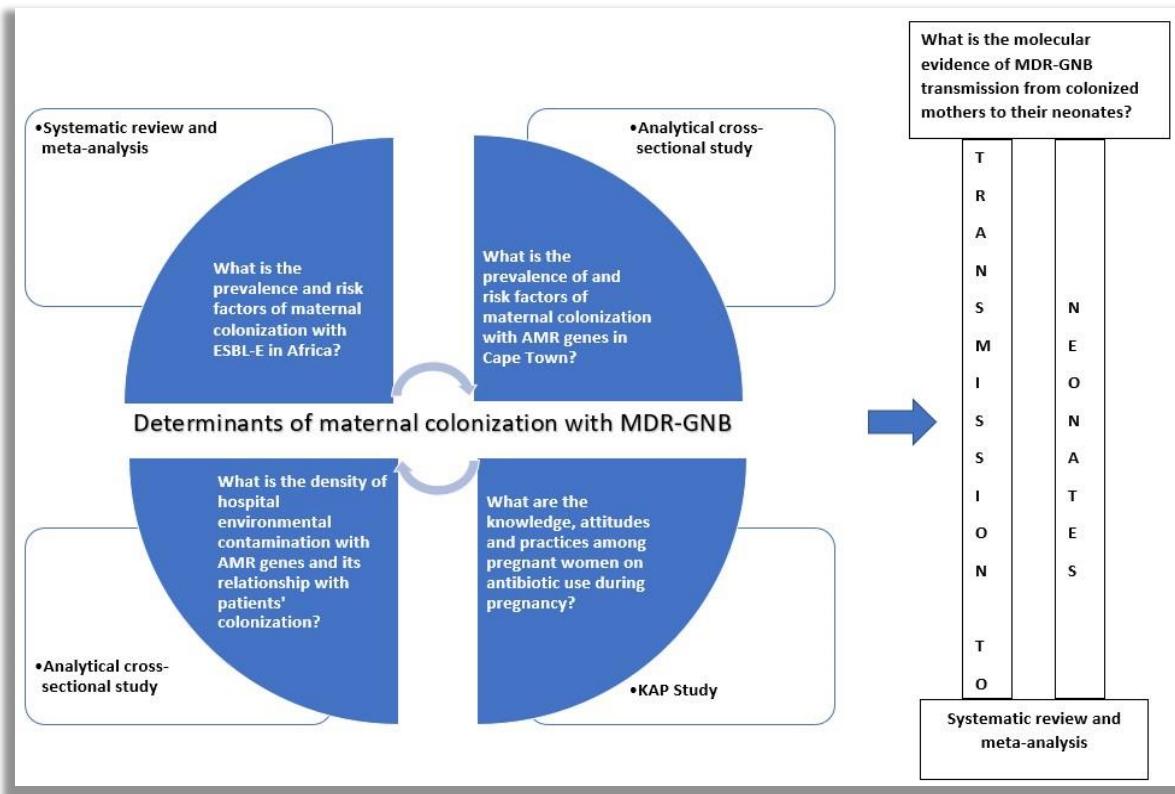
Figure 1: Outline of the studies included in this doctoral research

Objectives

Study 1: Maternal colonization or infection with ESBL-E in Africa: a systematic review and meta-analysis

Aim: To summarize published studies on the prevalence of and risk factors for maternal bacterial colonization and/or infection with ESBL-E in pregnant and/or post-partum women in Africa.

Study 2: Rectal colonization with MDR-GNB: prevalence and risk factors among pregnant women in Cape Town, South Africa

Aim: To determine the prevalence of and risk factors for maternal colonization with MDR-GNB.

Null hypothesis: There is no association between HIV status, diabetes, use of antimicrobials, quality of ANC and maternal colonization with MDR-GNB.

Study 3: Antibiotic use in pregnancy: knowledge, attitudes and practices among pregnant women in Cape Town, South Africa

Aim: To determine factors influencing use of antibiotics during pregnancy among pregnant women.

Null hypothesis: There is no relationship between the KAP of pregnant women on antibiotic use during pregnancy, and risk factor of maternal colonization with MDR-GNB.

Study 4: What is the relationship between hospital environmental contamination and colonization of hospitalized peripartum women and neonates with antimicrobial resistance genes?

Aim: To determine the prevalence of MDR-GNB hospital environmental contamination with MDR-GNB and its relationship with patients' (peripartum women and neonates) colonization.

Null hypothesis: There is no relationship between hospital environmental contamination, hand hygiene compliance rate, environmental cleaning and patients' (peripartum women and neonates) colonization with MDR-GNB.

Study 5: Transmission of multidrug-resistant Gram-negative bacteria from colonized mothers to their infants: a systematic review and meta-analysis

Aim: To review the molecular evidence supporting transmission of MDR-GNB from colonized mothers to their infants and the risk factors for MDR-GNB transmission.

Candidate's Contribution:

I was involved at each stage of the research process of the work included in this thesis. I identified the research problem, framed the research question, chose the appropriate research design, designed the data collection tools, conducted and/or supervised the data collection, performed data cleaning and coding of variables with the data dictionary, conducted statistical analyses, drafted the full manuscript and abstracts, finally reviewed the final version after the comments of my Supervisors. I was the corresponding author for all the publications included in this thesis.

Chapter 2:

Maternal Colonization or Infection with Extended-Spectrum Beta-Lactamase-producing Enterobacteriaceae in Africa: a systematic review and meta-analysis

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Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Maternal colonization or infection with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Africa: A systematic review and meta-analysis

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ARTICLE INFO

ABSTRACT

Objective: To summarize published studies on the prevalence of and risk factors for maternal bacterial colonization and/or infection with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) in pregnant and/or post-partum women in Africa.
Methods: A systematic review was conducted using the PubMed, Scopus, and Google Scholar databases. Bibliographies of included eligible studies were manually searched to identify additional relevant articles. No language restriction was applied. The timeframe of the search included all records from electronic database inception to July 15, 2017. A random-effects meta-analysis was performed to summarize the prevalence and the 95% confidence intervals (CI) of ESBL-E colonization or infection in pregnant or post-partum women in Africa. The meta-analysis was conducted using STATA IC 13.1 software and the metaprop function/plugin.
Results: Ten studies (seven on pregnant women and three on post-partum women) were included, documenting a 17% prevalence of maternal colonization with ESBL-E in Africa (95% CI 10–23%). The prevalence of ESBL-E in community isolates exceeded that in isolates from the hospital setting (22% vs. 14%). The most frequently reported ESBL-encoding gene was CTX-M (cefotaxime hydrolyzing capabilities). Data on risk factors for maternal ESBL-E colonization and infection are very limited.
Conclusions: The prevalence of colonization and/or infection with ESBL-E in pregnant and post-partum women in Africa exceeds that reported from high- and middle-income settings, representing a risk for subsequent neonatal colonization and/or infection with ESBL-E.

Introduction

Antimicrobial resistance (AMR) is a growing threat to human health globally (WHO, 2014; Frieden, 2013). A major mechanism of AMR is the production of extended-spectrum beta-lactamase (ESBL) enzymes, which confer resistance to penicillins, cephalosporins, and monobactams, but not to cephamycins and carbapenems (Paterson and Bonomo, 2005; Pitout et al., 2005), leaving limited therapeutic options for AMR infections. In 2013, the US Centers for Disease Control and Prevention (CDC) identified ESBL-producing *Enterobacteriaceae* (ESBL-E) as a serious threat (Frieden, 2013). In addition, the World Health Organization (WHO) has published a priority pathogens list, and resistant ESBL-E are classified as 'critical', priority number 1 (WHO, 2017).

ESBL-E occur worldwide in both community and hospital settings (Paterson and Bonomo, 2005; Pitout et al., 2005; Sonda et al., 2016; Storberg, 2014; Luvsansharav et al., 2011), and the reported incidence of infections in paediatric and neonatal populations is increasing (Paterson and Bonomo, 2005; Sonda et al., 2016; Storberg, 2014; Flokas et al., 2017; Peirano and Pitout, 2010; Tansarli et al., 2014; Dramowski et al., 2015; Logan et al., 2014). Clinical infections with ESBL-E are associated with increased morbidity (including prolonged hospital stay), increased health-care costs, and higher mortality rates compared to infections with non-ESBL-E (Blomberg et al., 2005; Zaoutis et al., 2005; Kim et al.,

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2002; Ndir et al., 2016). Among neonates, children, and pregnant/ post-partum women, ESBL-E are frequently implicated in urinary tract infections (UTI) (Pitout et al., 2005; Peirano and Pitout, 2010) and bloodstream infections (BSI) (Luvsansharav et al., 2011; Flokas et al., 2017; Dramowski et al., 2015; Zaoutis et al., 2005; Loh and Sivalingam., 2007). Among neonatal ESBL-E infections, *Klebsiella pneumoniae* and *Escherichia coli* are the most frequently isolated species (Flokas et al., 2017; Dramowski et al., 2015; Zaidi et al., 2005).

Well-established risk factors for early-onset neonatal sepsis include maternal infections (e.g. UTIs and chorioamnionitis) and prolonged rupture of the membranes (Chan et al., 2013; Chan et al., 2015). In a well-powered systematic review, additional risk factors identified were maternal bacterial colonization of the vaginal tract (Chan et al., 2013; Chan et al., 2015) and poor antenatal care (defined as less than four antenatal visits per pregnancy) (Mizumoto et al., 2015; Lincetto et al., 2006).

Although ESBL-E are well-documented as important blood-stream pathogens in several African settings (Flokas et al., 2017; Kim et al., 2002; Kang et al., 2004; Schiappa et al., 1996), little is known about the determinants and magnitude of maternal colonization with ESBL-E. Factors contributing to the development of AMR (including ESBL-E) in Africa include socio-economic challenges and health-associated factors (Kariuki and Dougan, 2014) (suboptimal hygiene and sanitation (Toole et al., 1995), weak health systems (Essack et al., 2016), lack of laboratory capacity (Okeke et al., 1999), and misuse of and easy access to antibiotics (Okeke et al., 1999)). A high burden of immunocompromised patients (HIV infection (Emacar et al., 2010) and diabetes

(Ntirenganya et al., 2015)), environmental contamination (Dusé, 2005), and inadequate decontamination of medical devices (Dusé, 2005), are other important factors exacerbating AMR pathogen transmission in Africa. The relationship between these factors and maternal colonization or infection with ESBL-E is unclear and requires investigation.

This systematic review and meta-analysis summarizes the magnitude of colonization or infection with ESBL-E among pregnant and post-partum women in Africa and the associated risk factors. Knowledge of the burden and risk factors of maternal colonization or infection with ESBL-E will assist with clinical care, infection prevention, and antibiotic stewardship, and inform the future development of targeted interventions to reduce both maternal and neonatal ESBL-E-associated morbidity and mortality.

Methods

Literature search

A systematic review was conducted in PubMed, Scopus, and Google Scholar. The search strategy included the following words, medical subject heading (MeSH) terms, and Boolean operators: "(enterobacteriaceae OR bacteria OR resistant OR resistance OR 'non-susceptible' OR "non susceptible" OR "not susceptible") AND (coloni* OR infect* OR carri*) AND ((Africa OR Algeria OR Angola OR Benin OR Botswana OR Burkina Faso OR Burundi OR Cabo Verde OR Cameroon OR Central African Republic OR Chad OR Comoros OR Democratic Republic of the Congo OR Republic of the Congo OR Cote d'Ivoire OR Djibouti OR Egypt OR Equatorial Guinea OR Eritrea

Table 1
Characteristics of studies included in the review.

Number	Author and year of publication	Country	Study design	Clinical samples	Population	Number of participants colonized with ESBL-E/Total participants (% colonized)	ESBL-E species included (isolate) and proportion	Setting
1	Olufunke et al. (2014)	Nigeria	Cross-sectional	Urine	Pregnant women	69/264 (26.1%)	*Escherichia coli* 69/264	Hospital
2	Onwuezobe (2015)	Nigeria	Cross-sectional	Urine	Pregnant women	16/80 (20%)	*Klebsiella pneumoniae* 8/16 *Escherichia coli* 6/16 *Klebsiella oxytoca* 1/16 *Enterobacter cloacae* 1/16	Community
3	Chereau et al. (2015)	Madagascar	Cross-sectional	Stool	Pregnant women	66/356 (18.6%)	*Escherichia coli* 46/66 *Klebsiella pneumoniae* 11/66 *Enterobacter cloacae* 5/66 *Citrobacter freundii* 3/66 *Morganella morganii* 1/66	Community
4	Nelson et al. (2014)	Tanzania	Cross-sectional	Stool	Post-partum women	16/113 (15%)	*Escherichia coli* 6/20 *Enterobacter spp* 3/20 *Klebsiella pneumoniae* 1/20 *Citrobacter spp* 2/20 *Pantoea spp* 3/20 *Proteus spp* 1/20	Hospital
5	Kaba et al. (2016)	South Africa	Cross-sectional	Stool	Post-partum women	4/90 (4.4%)	*Klebsiella pneumoniae* 2/90 *Escherichia coli* 1/90 *Enterobacter cloacae* 1/90	Community
6	Sáez-lópez et al. (2016)	Mozambique	Cross-sectional	Vaginal swabs	Pregnant women	1/51 (1.9%)	*Escherichia coli* 1/51	Hospital
7	Djuikoue (2016)	Cameroon	Cross-sectional	Stool	Pregnant women	15/26 (57.7%)	*Escherichia coli* 15/26	Community
8	Fortini et al. (2015)	Nigeria	Cross-sectional	Stool	Pregnant women	32/101 (31.7%)	*Escherichia coli* 32/101	Hospital
9	Bebell et al. (2017)	Uganda	Prospective cohort	Urine Blood	Post-partum women	8/174 (4.6%)	*Escherichia coli* 5/174 *Klebsiella pneumoniae* 3/174	Hospital
10	Tito et al. (2017)	Tanzania	Cross-sectional	Urine	Pregnant women	4/49 (8.2%)	*Escherichia coli* 4/49	Hospital

ESBL-E, extended-spectrum beta-lactamase-producing *Enterobacteriaceae*.

OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR Guinea-Bissau OR Kenya OR Lesotho OR Liberia OR Libya OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Morocco OR Mozambique OR Namibia OR Niger OR Nigeria OR Rwanda OR "Sao Tome and Principe" OR Senegal OR Seychelles OR Sierra Leone OR Somalia OR South Africa OR South Sudan OR Sudan OR Swaziland OR Tanzania OR Togo OR Tunisia OR Uganda OR Zambia OR Zimbabwe)) AND (ESBL OR extended-spectrum-beta-lactamase OR extended-spectrum-β-lactamase OR extended spectrum beta lactamase) AND ((pregnant OR pregnancy OR gestation OR post-delivery OR postdelivery OR "post delivery" OR post-part* OR postpartum OR "post partum" OR perinatal OR antenatal OR prenatal OR women))". Bibliographies of eligible studies were manually searched to identify additional relevant articles. It was attempted to contact the authors of relevant studies to obtain specific details. Following the screening of publications, a total of 10 articles were included (Table 1).

Study selection and eligibility criteria

All publications describing colonization and/or infection with ESBL-E in pregnant or post-partum African women in both community and hospital settings were selected, irrespective of the study design. Primary study designs reporting the proportion or prevalence of ESBL-E in pregnant or post-partum women, as well as risk factors, were eligible. No language restriction was applied. The timeframe of the search included all records from electronic database inception to July 15, 2017. The article selection and exclusion process is shown in Figure 1.

Data extraction

A data extraction form was designed to capture the following information: first author and year of publication; country; study design (any type); study setting (community or hospital); study population; number and percentage of women colonized or infected with ESBL-E; total number of women recruited into the study; ESBL-E isolates and their proportions; molecular identification of ESBL-E isolates (if performed); factors associated with ESBL-E colonization or infection in women.

Data synthesis

A random-effects meta-analysis was performed to summarize the proportions and the 95% confidence intervals (CI) of ESBL-E in pregnant or post-partum women in Africa. To ensure proportionate weight distribution to studies presenting extreme prevalence (near 0 or 1), the Freeman–Tukey arcsine methodology was applied (Nyaga et al., 2014). The between-study heterogeneity was assessed using the I^2 statistic (which quantifies the percentage of variation across studies due to heterogeneity rather than to chance): I^2 <75% was considered as moderate heterogeneity (Higgins and Thompson, 2002) and >75% reflected high heterogeneity, in which case subset analyses were performed. The random-effects model was chosen based on the anticipated assumption that studies reporting on the magnitude of ESBL-E in pregnant and post-partum women used different laboratory methods, were conducted in different settings (hospital and community), or had other unknown factors influencing the magnitude of ESBL-E in
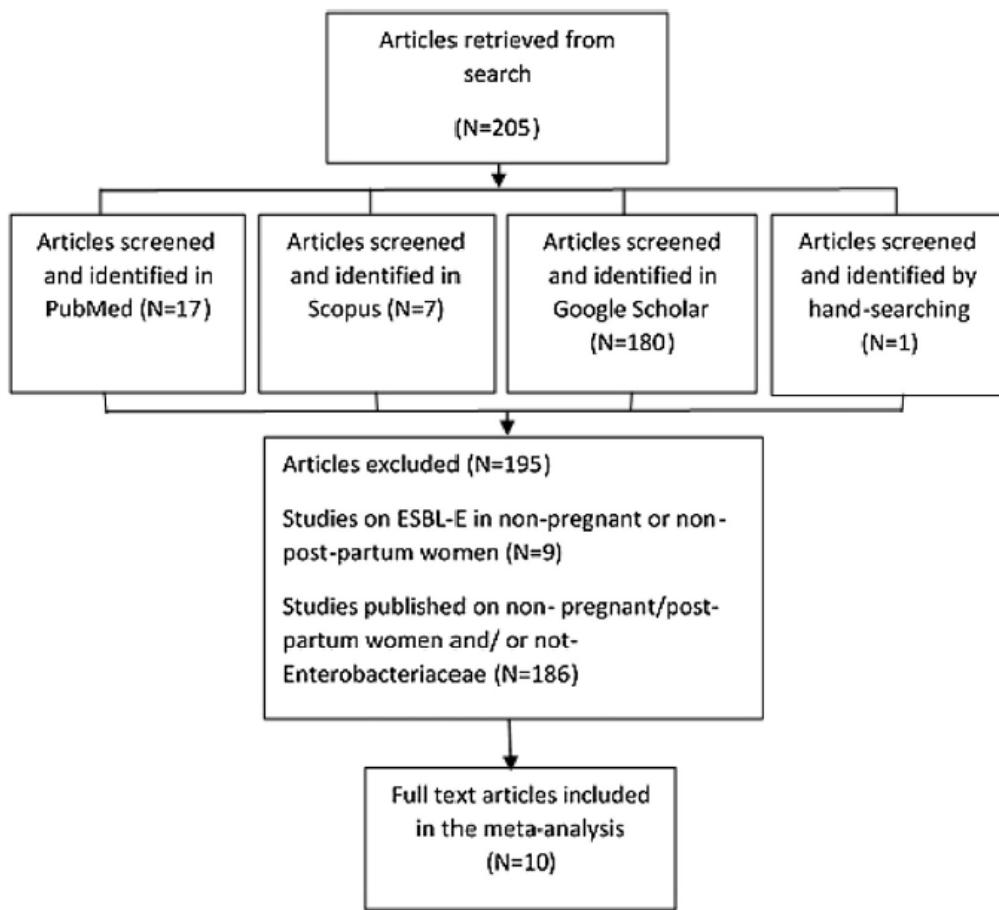

Figure 1. Flow diagram of study selection.

pregnant and post-partum women in Africa. The meta-analysis was performed using STATA IC version 13.1 software and the metaprop function/plugin, which is a specific STATA program designed for the meta-analysis of binomial data, allowing pooling of proportions (Nyaga et al., 2014). A total of 1304 participants were pooled from the studies included (927 pregnant women and 377 post-partum women).

Reporting of the meta-analysis of observational studies

This meta-analysis of observational studies is reported in compliance with the MOOSE statement and checklist (Stroup et al., 2000) (Meta-analysis of Observational Studies in Epidemiology).

Assessment of bias

The Newcastle–Ottawa Scale (Herzog et al., 2013) adapted for cross-sectional studies (see below) was used to assess the risk of bias for each selected study. This scale includes an evaluation of

participant selection, comparability, and outcome; each section has a maximum number of 'stars' that can be awarded as a score (5, 2, and 3, respectively). For the overall quality assessment, the maximum score is 10 stars. Studies with a minimum score of 3 were considered of acceptable methodological quality for inclusion in the meta-analysis (Table 2).

Results

*Geographic distribution of articles describing ESBL-E in women in Africa*

Ten observational studies were included for quantitative analysis. These were from Nigeria ($n = 3$), Tanzania ($n = 2$), Madagascar ($n = 1$), South Africa ($n = 1$), Mozambique ($n = 1$), Uganda ($n = 1$), and Cameroon ($n = 1$). Six studies reported on pregnant women and four on post-partum women. Three studies reported ESBL-E isolates from urine, five others from stool, one from vaginal swabs, and one from both urine and blood.

Table 2
Risk of bias assessment for studies included in the quantitative synthesis (all observational studies).[a]

Author and year of publication	Selection		Comparability	Exposure	Overall quality assessment score (out of a maximum of 10)
	Representativeness of the sample	Ascertainment of exposure	Comparability of the groups on the basis of design or analysis	Assessment of outcome	
Olufunke et al. (2014)	*Truly representative of average pregnant women with ESBL *Enterobacteriaceae*	*Pregnant women diagnosed with clinical isolates producing ESBL – DDST	Study did not control for other factors	*Independent blind assessment	4
Onwuezobe (2015)	*Truly representative of average pregnant women with ESBL *Enterobacteriaceae*	**Pregnant women diagnosed with clinical isolates producing ESBL – DDST	Study did not control for other factors	*Independent blind assessment	4
Kaba et al. (2016)	*Truly representative of post-partum women with ESBL *Enterobacteriaceae* in the community	**ESBL production was confirmed using the combination disc test	Study did not control for other factors	*Independent blind assessment	4
Chereau et al. (2015)	*Truly representative of pregnant women with ESBL *Enterobacteriaceae* in the community	**Production of ESBL in ESC-resistant *Enterobacteriaceae* was confirmed by DDST (CASFM)	*Study controlled for other factors, multivariate analysis	*Independent blind assessment	5
Nelson et al. (2014)	*Truly representative of post-partum women with ESBL *Enterobacteriaceae* in the hospital	*Rectal swabs plated onto MacConkey agar (OXOID, Basingstoke, UK) supplemented with cefotaxime 2 mg/l for preliminary screening of ESBL bacterial isolates	Study did not control for other factors	*Independent blind assessment	3
Sáez-lópez et al. (2016)	No description of participant selection	**ESBL producers were identified by DDST using CTX, AMC and CAZ	Study did not control for other factors	*Independent blind assessment	3
Djuikoue et al. (2016a,b)	*Truly representative of outpatient women consulting for a suspicion of UTI, during the study period	**The presence of ESBL was determined using the double disc diffusion phenotypic method	*Study controlled for other factors, multivariate analysis	*Independent blind assessment	5
Fortini et al. (2015)	*Truly representative of healthy pregnant women on the day of admission to hospital in Ibadan (Nigeria)	**Phenotypic and genotypic characterization of ESBL production	Study did not control for other factors	*Independent blind assessment	4
Tito et al. (2017)	*Truly representative of HIV-positive pregnant women attending the PMTCT clinics; all consenting HIV-positive pregnant women were included during the study period	**ESBL production was concomitantly tested on the same Mueller–Hinton agar plate, using the DDST method	*Study controlled for other factors, multivariate analysis	*Independent blind assessment	5
Bebell et al. (2017)	*Truly representative of febrile, hypothermic, or normothermic post-partum women in the hospital	**ESBL phenotype if synergy was observed between AMC and CAZ or CTX	The study design allowed a 4:1 ratio of normothermic to febrile/hypothermic post-partum women, but did not control for other factors	**Record linkage and the statistical test used clearly described and appropriate; *p*-value presented	5

AMC, amoxicillin–clavulanic acid; CASFM, Committé Antibiogramme – Société Française de Microbiologie; CAZ, ceftazidime; CTX, cefotaxime; DDST, double disc synergy test; ESBL, extended-spectrum beta-lactamase-producing; ESC, extended-spectrum cephalosporin; PMTCT, prevention of mother to child transmission; UTI, urinary tract infection.
[a] Stars (*) represent the number of points awarded for the category; *= 1, **= 2.

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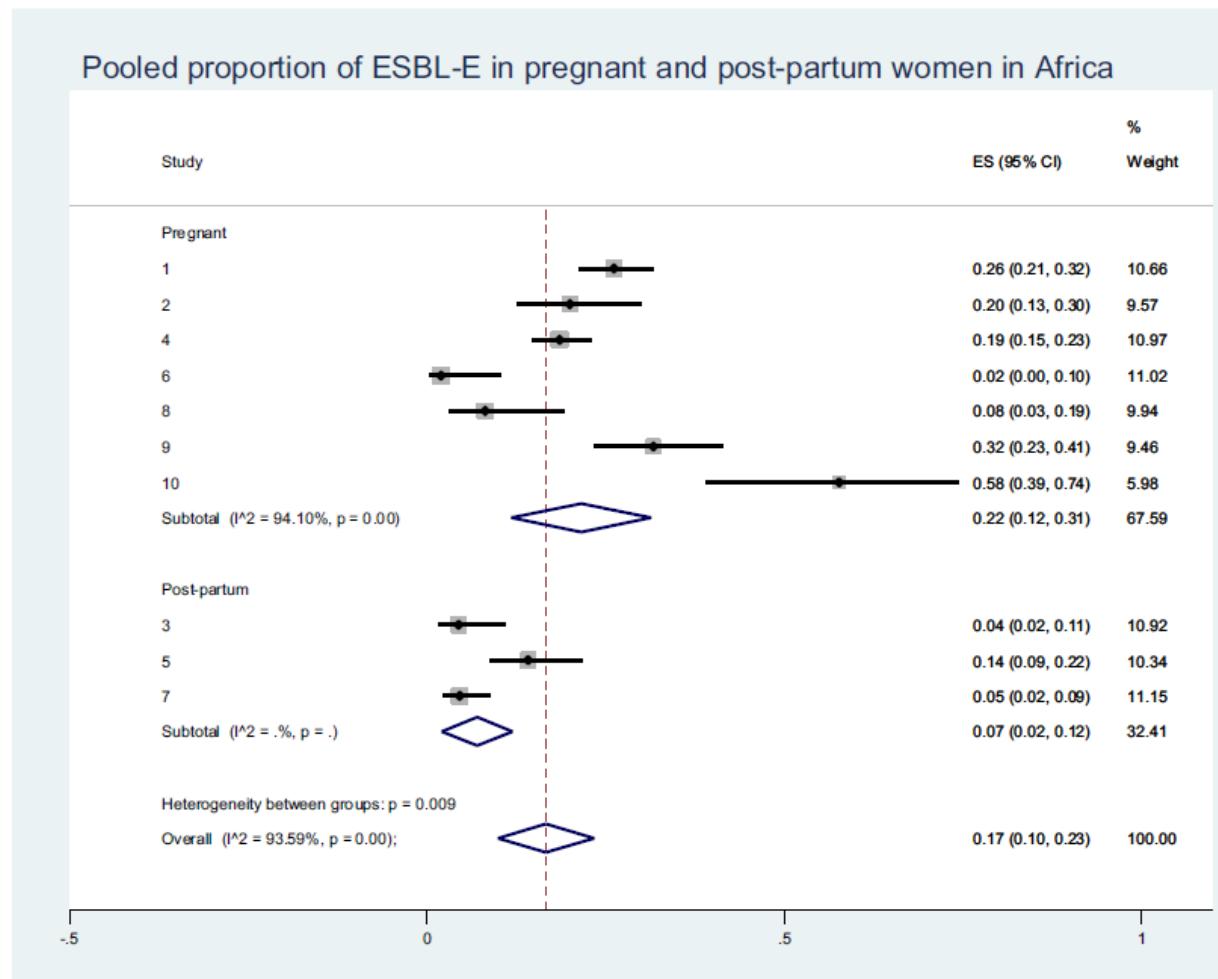
Figure 2. Pooled proportion of ESBL-E in pregnant and post-partum women in Africa.

Proportion estimates of ESBL-E in pregnant and post-partum women in Africa

Through meta-analysis of the eligible studies, the overall pooled estimate of the ESBL-E proportion in pregnant and post-partum women in Africa was determined to be 0.17 (95% CI 0.10–0.23) or 17% (95% CI 10–23%) (Figure 2). The heterogeneity was high ($I^2 = 93.6\%$, $p < 0.001$).

The pooled proportion of ESBL-E in pregnant women was 0.22 (95% CI 0.12–0.31) (Figure 2) and that of ESBL-E in post-partum women was 0.07 (95% CI 0.02–0.12). The pooled proportion of ESBL-E in hospital settings was 0.14 (95% CI 0.05–0.23) compared to 0.22 (95% CI 0.09–0.34) in community settings (Figure 3).

The pooled proportion of ESBL-E infections was 0.13 (95% CI 0.01–0.27) compared to 0.19 (95% CI 0.01–0.27) for ESBL-E colonized pregnant and post-partum women (Figure 4).

Molecular epidemiology of ESBL-E colonization/infection

Chereau et al. (2015) (Madagascar) identified 66 ESBL-producing isolates in pregnant women, including *E. coli* (n = 46), *Klebsiella spp* (n = 11), *Enterobacter cloacae* (n = 5), *Citrobacter freundii* (n = 3), and *Morganella morganii* (n = 1). Forty-five isolates carried a bla_{CTX-M} gene, 15 carried bla_{SHV} and bla_{CTX-M} genes, and two carried a bla_{SHV} gene alone. No bla_{ESBL} gene and no cefoxitin resistance was detected in four ESBL-producing *E. coli* isolates. Sáez-lópez et al.

(2016) identified one ESBL-E isolate and it carried the CTX-M-15 gene. Kaba et al. (2016) reported that one mother–infant pair was ESBL-positive at birth with SHV-5-producing *E. cloacae*. Djuikoue et al. (2016a) reported that among 15 *E. coli* ESBL, all were CTX-M group 1 (n = 15). Fortini et al. (2015) found *E. coli* (n = 32) producing CTX-M-15.

Detection and confirmation of ESBL in the laboratory: what methods were used?

Five studies (Nelson et al., 2014; Olufunke et al., 2014; Aljabri et al., 2010; Bebell et al., 2017; Tito et al., 2017), detected ESBL production using the double disc synergy test (DDST). Chereau et al. (2015), Djuikoue et al. (2016b), and Kaba et al. (2016) utilized both synergy testing and molecular identification of ESBL (PCR sequencing). Sáez-lópez et al. (2016) and Fortini et al. (2015) used molecular identification of ESBL.

Factors associated with ESBL-E in pregnant women in Africa

Three studies included a multivariate analysis of risk factors for ESBL-E colonization/infection among pregnant women. Chereau et al. (2015) identified private indoor access to drinking water (odds ratio (OR) 3.8, 95% CI 1.2–11.6) and living in an individual house (OR 2.2, 95% CI 1.0–4.8) as independent risk factors for ESBL-E colonization among Malagasy women (after adjusting for
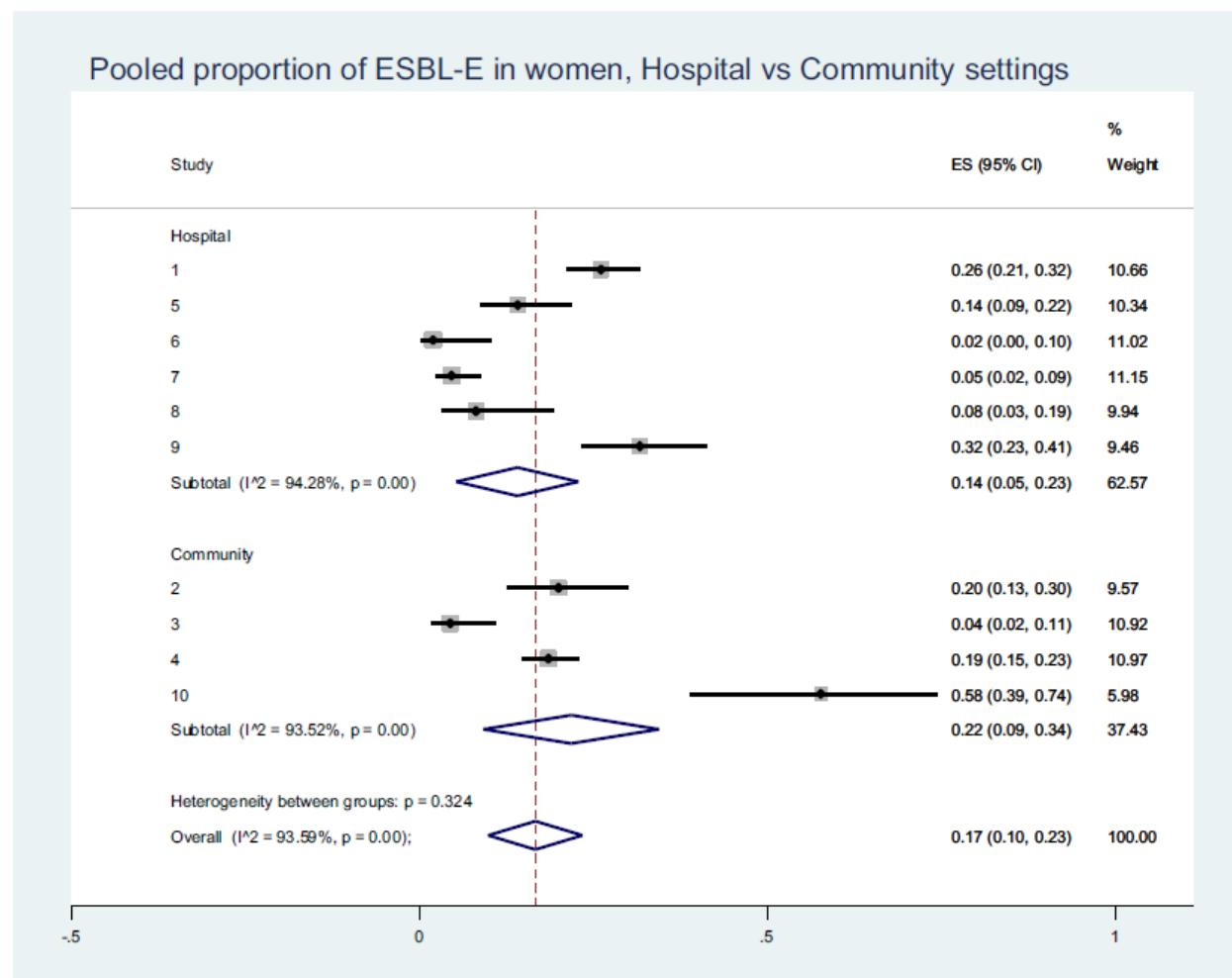
Figure 3. Pooled proportion of ESBL-E in women, Hospital vs Community settings.

delivery period and study area). Djuikoue et al. (2016b)) found that the detection of antimicrobial activity in the stool sample was the only independent risk factor associated with ESBL *E. coli* carriage in Cameroonian women (OR 5.4, 95% CI 2.0–14.7). Bebell et al. (2017)) identified single marital status (OR 2.6, 95% CI 1.1–6.1, *p* = 0.026), low CD4+ count of <200/µl (OR 2.9, 95% CI 1.1–7.7, *p* = 0.031), and current UTI symptoms (OR 2.5, 95% CI 1.1–6.0, *p* = 0.03) as independent predictors of ESBL-E infection.

The following elements were not found to be significantly associated with ESBL-E colonization/infection in these three studies: being employed or being a housewife (Tito et al. 2017), drinking water supply from a spring or a well (Chereau et al., 2015), and hospitalization in the last 3 months (Djuikoue et al. 2016a,b).

Discussion

The close relationship of the mother–infant pair represents a potential risk for cross-transmission of maternal pathogens leading to neonatal colonization or infection. Several studies have confirmed the role of maternal colonization in the subsequent development of neonatal sepsis (particularly for group B Strepto-coccus, but also for ESBL-E) (Chan et al., 2013; Kaba et al., 2016; Denkel et al., 2014; Rettedal et al., 2015).

However, the scarcity of research on determinants of maternal colonization in Africa is worrisome. This meta-analysis of eligible published studies determined an overall pooled prevalence of

colonization or infection with ESBL-E in pregnant and post-partum women in Africa of 17% (95% CI 10–23%). This rate of colonization or infection with ESBL-E is in line with a well-powered systematic review conducted in Africa by Tansarli et al. (2014), who reported proportions varying from 1.5% to 22.8% (pooled from 13 studies with isolates from clinical urine samples) among patients either infected or colonized with *Enterobacteriaceae*. However, these proportions were not for pregnant and post-partum women as specific groups. The pooled ESBL-E rate documented herein is substantially higher than the rates found in high- and middle-income countries, e.g., Norway (2.9%) (Rettedal et al., 2015) and Argentina (5.4%) (Villar et al., 2013). Possible explanations for increased ESBL-E carriage among African populations (both in community (Pitout et al., 2005; Pallecchi et al., 2004) and hospital settings) include poverty, suboptimal hygiene, contamination of drinking water (faeces (Bain et al., 2014), antibiotics), water sewage, communal toilets, easy access to antibiotics among pregnant and post-partum women in Africa, and possibly also increased use of antibiotics in livestock in Africa (Kariuki and Dougan, 2014). Another possible explanation is that the lack of trained healthcare workers (Kimang'a, 2012) and weak laboratory and infection control capacity (Petti et al., 2006) may contribute to healthcare-associated transmission of ESBL-E to pregnant and post-partum women.

Another important finding is that the pooled proportion of ESBL-E in community settings exceeded that in hospital settings
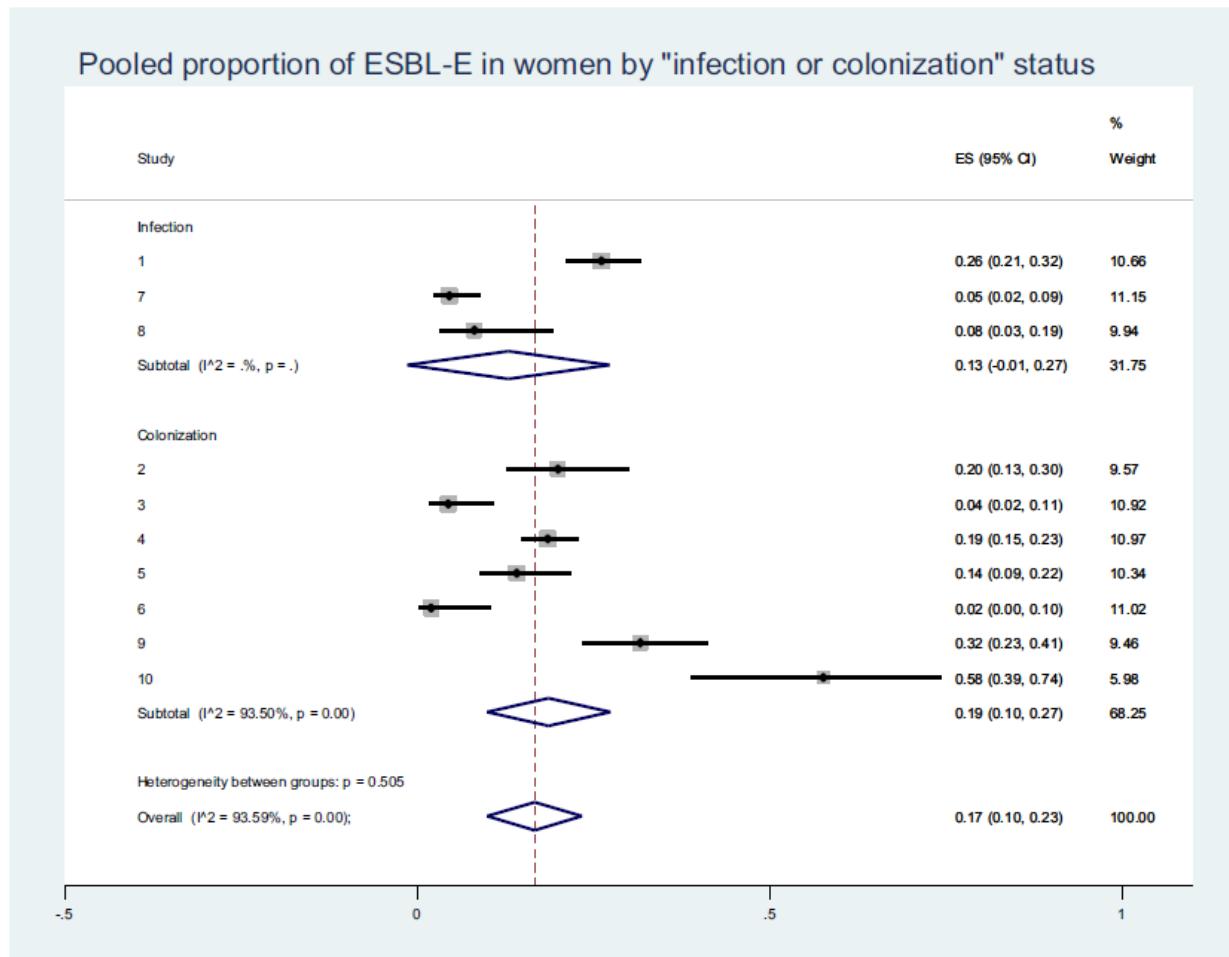
Figure 4. Pooled proportion of ESBL-E in women by "infection or colonization" status.

(22% vs. 14%) (Figure 3). This finding may have been influenced by the high prevalence of ESBL-E in community isolates (58%) in the Cameroonian study. However, another explanation may be the effect of high-level ESBL-E contamination of community water and food sources, lack of sanitation, and possibly overuse of antibiotics. The apparent difference in ESBL-E proportions between community and hospital settings could also be explained by the fact that all included studies from the community only studied ESBL-E colonization. In this meta-analysis, the proportion of ESBL-E colonization was found to be greater than ESBL-E infection.

The detection of ESBL-E in samples from hospitalized pregnant women with short lengths of stay may represent community-acquired colonization and/or infection with ESBL-E. Because ESBL-E stool carriage persists for a variable length of time (Ahmed et al., 2014), the study population may have acquired ESBL-E during pregnancy that then persisted up to delivery and the post-partum period, reflecting community-acquired antimicrobial resistance genes rather than healthcare-associated acquisition.

The pooled proportion of ESBL-E was higher among pregnant women than post-partum women (22% vs. 7%) (Figure 2). This difference may be explained by a greater probability of UTIs with ESBL-E during pregnancy than in the post-partum period (Sáez-lópez et al., 2016). It may also possibly represent differences in asymptomatic bacteriuria during pregnancy, differences in antibiotic prescribing practices, and different decision-making when choosing to send samples from pregnant vs. post-partum women for culture.

The clinical significance of increased ESBL-E-associated UTI during pregnancy is the potential for adverse pregnancy and neonatal outcomes, including intrauterine growth restriction, low birth weight, premature rupture of the membranes, foetal death, and neonatal infections (Loh and Sivalingam., 2007; Romero et al., 1989; Ovalle and Levancini, 2001; Matuszkiewicz-Rowińska et al., 2015).

In this systematic review and meta-analysis, a few studies attempted to report on factors associated with ESBL-E colonization in women in Africa; however, the risk factors analysed varied from one study to another and their pooled estimates could not be produced. The molecular epidemiology of the ESBL-E enzymes identified in the Madagascar (Chereau et al., 2015), Nigeria (Fortini et al., 2015), Cameroon (Djuikoue et al., 2016b), and Mozambique (Sáez-lópez et al., 2016) studies was in keeping with the global predominance of the CTX-M clone, which is also widely reported in community-acquired UTI (Pitout et al., 2005; Peirano and Pitout, 2010; Pallecchi et al., 2004; Livermore et al., 2007).

This systematic review has several strengths. A comprehensive search of several electronic databases was performed, in addition to manual searches and attempts to contact authors of relevant studies to obtain specific details. The authors made an effort to search the 'grey literature' by using Google Scholar, as many African publications are not listed in PubMed or Scopus. This appears to be the first systematic review and meta-analysis on ESBL-E colonization and/or infection focusing on pregnant and post-partum women in Africa. This group is of particular

importance for vertical transmission and subsequent neonatal colonization and/or infection. Each study was thoroughly assessed for risk of bias.

While informative, this systematic review and meta-analysis has a number of limitations. The number of eligible studies was small, demonstrating the apparent lack of studies on ESBL-E colonization and infection in this population group: pregnant and post-partum women in Africa. Given the limited sample size, the findings of this systematic review and meta-analysis may not be generalizable to all pregnant and/or post-partum women in Africa. The publications retrieved and included in the meta-analysis were all observational studies, a study type that is prone to many biases, including selection and information biases (Archer and Horn, 2006).

More robust studies are needed to understand how frequently pregnant and/or post-partum women become colonized or infected with ESBL-E in Africa, as well as the related risk factors in both community and hospital settings, to inform future interventions to reduce their rates. Interventions could include improved sanitation and water supplies, education of mothers on personal hygiene, restriction of antibiotic use during pregnancy, and strengthening of infection prevention in healthcare facilities.

Plain language summary

The level of colonization and/or infection with ESBL-E in pregnant and post-partum women in Africa is higher than that reported from high- and middle -income settings. In African pregnant and post-partum women, the prevalence of ESBL-E in community isolates exceeds that in hospital isolates. Maternal ESBL-E colonization/infection represents a risk for mother to child pathogen transmission with the potential for subsequent neonatal colonization and/or infection.

Key recommendation

Further studies are needed to establish ESBL-E colonization and infection rates amongst pregnant and post-partum women and their determinants in all African regions. Interventions to reduce ESBL-E colonization and carriage in Africa should focus on preventing both community- and healthcare-associated ESBL-E acquisition. Potential interventions could include the provision of safe sanitation and clean water supplies, education of mothers on personal hygiene, restricted use of antibiotics in pregnancy, and strengthening of infection prevention efforts in healthcare facilities (hand hygiene and appropriate disinfection of obstetric equipment and the environment).

Research gaps

It is not well understood how frequently pregnant and post-partum women become colonized and infected with ESBL-E, which risk factors promote ESBL-E colonization/infection, and how this could be prevented or managed, both in community and hospital settings in Africa. In addition, health systems research is needed to increase the understanding of the problem of antimicrobial resistance in maternal and neonatal infections at the macro (leadership or governance), meso (healthcare facilities and programmes), and micro (pregnant women or service consumers) levels.

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Chapter 3:

Rectal colonization with multidrug-resistant Gram-negative bacteria: prevalence and risk factors among pregnant women in Cape Town, South Africa

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Rectal colonization with multidrug-resistant Gram-negative bacteria: prevalence and risk factors among pregnant women in Cape Town, South Africa

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ABSTRACT

OBJECTIVE: To determine the prevalence of and risk factors for maternal colonization with multidrug-resistant Gram-negative bacteria (MDR-GNB).

DESIGN: Analytical cross-sectional study.

SETTING: A tertiary hospital in Cape Town, South Africa.

PARTICIPANTS: A subset of pregnant women admitted for antenatal complications and/or for delivery to Tygerberg Hospital at between 28- and 40-weeks gestation from February to December 2016.

METHODS: Baseline demographic and clinical characteristics were collected using a validated questionnaire. A rectal swab was obtained from consenting pregnant women (n=651); Gram-negative antimicrobial-resistant isolates were phenotypically identified using vancomycin + cefotaxime (VC) and vancomycin + ertapenem (VE) plates. Genotypic identification screening for selected antimicrobial resistance genes (CTX-M-15 and NDM, OXA-48 like and KPC) was conducted using polymerase chain reaction.

RESULTS: Among pregnant women, 319/651 (49.0%) rectal swabs yielded growth on a VC impregnated agar plate and 413/651 (63.4%) on a VE impregnated agar plate. The resistance genes identified were CTX-M-15 (41/319; 12.9% [95% CI, 9.7% - 17.0%]) and NDM (9/413; 2.2% [95% CI, 1.1% - 4.1%]). Four factors were associated with maternal NDM rectal colonization: having either no education or only primary school education, (aOR = 7.9, 95% CI: [1.3 – 48.3], p=0.03), living in a rural area (aOR = 9.0, 95% CI: [1.6 – 50.7], p=0.01), monthly household income ≤ 100 US$ (aOR = 8.5, 95% CI: [1.6 – 45.9], p=0.01), primiparous status (aOR = 11.0, 95% CI: [1.9 – 63.6], p=0.007). Only one factor independently predicted maternal colonization with CTX-M-15: use of communal taps as the primary household water source (aOR = 2.6, 95% CI: [1.02 – 6.8], p=0.046).

CONCLUSIONS: Pregnant women had a high prevalence of CTX-M-15 carriage but lower prevalence of NDM carriage. Poverty-related factors predicted maternal rectal colonization with multidrug-resistant Gram-negative bacteria.

Introduction

Neonatal sepsis remains one of the top three causes of neonatal mortality together with prematurity and birth asphyxia. Both Gram-positive and Gram-negative bacteria are responsible for neonatal sepsis, although Gram-negative pathogens predominate in low-middle income countries (LMIC) and are associated with higher mortality rates [1]. In addition, Gram-negative pathogens are more likely to be multidrug-resistant, which is problematic in LMIC settings with limited access to appropriate antibiotics. Magiorakos et al, in 2012, provided the interim standard definitions for acquired resistance and MDR-GNB: (a) ESBL-producing *Enterobacteriaceae*; (b) microorganisms with intrinsic resistance mechanisms such as *Stenotrophomonas maltophilia, Burkholderia cepacia*, and *Ralstonia pickettii*; and (c) any GNB (e.g. *Acinetobacter spp., Enterobacteriaceae*, and *Pseudomonas spp.*) resistant to three or more of the following drug class: piperacillin/tazobactam, cephalosporins (cefazolin, ceftriaxone, ceftazidime, and cefepime), carbapenems (imipenem), monobactams (aztreonam), aminoglycosides (gentamicin, tobramycin, and amikacin), and fluoroquinolones (ciprofloxacin and levofloxacin).[2] Among the multidrug-resistant Gram-negative bacteria (MDR-GNB), extended-spectrum β-lactamases (ESBL) and carbapenemases are the most frequent resistance mechanisms encountered in clinical practice.

Maternal colonization with bacteria is well-recognized as a major risk factor for neonatal sepsis.[3] In Africa, the overall prevalence of maternal colonization with ESBL-E was 17% reported in a systematic review and meta-analysis, and higher in the community (22%) than in hospital settings (14%).[4] The prevalence of ESBL-E varies widely between countries from 0.7% in Malawi, reporting only ceftriaxone-resistant Enterobacteriaceae, to 75.8 % in Egypt.[5]

In South Africa, ESBL-E are important human pathogens in both community and hospital settings [5] isolated from various clinical specimens, including blood, urine, stool and pus [5]. Kaba et al [6] reported on ESBL-E faecal carriage in 4/116 (3.5%) apparently healthy neonates at birth and their mothers 4/90 (4.4%). Habte et al, [7] documented an ESBL-E rate of 13.6% among uropathogens in hospital settings. Perovic et al [8], reported 68% ESBL prevalence among patients with *Klebsiella pneumoniae* bloodstream infections, samples included in this study came from thirteen academic centres serving the public healthcare sector in South Africa. Dramowski et al, found even higher proportions of ESBL-production among hospitalised South African children with *K. pneumoniae* bacteraemia (75.7% in community-acquired and 78.3% in hospital-acquired isolates) and *E.coli* bacteraemia (11.7% and 21.7% ESBL-prevalence in community-acquired and hospital-acquired isolates respectively).[9]

The most prevalent ESBL-genes among ESBL-E isolates are the CTX-M, SHV and TEM families of genes. CTX-M has spread worldwide and is commonly found in isolates causing community-acquired urinary tract infections (UTI).[10–15] Many isolates contain multiple ESBL genes simultaneously, e.g. combinations of CTX-M, SHV and TEM.[5,16,17]

A recent study from Mthatha in South Africa, established prevalence of ESBL production among 202 Klebsiella species isolates at 57.9%.[17] ESBL-genotypic resistance in Mthatha was driven by SHV (121; 77.1%) followed by TEM (105; 66.9%) and CTX-M at (89; 56.7%).[17] The most common genotypic combination among Klebsiella species in this study was TEM + SHV + CTX-M at (79/117; 50.3%).

In South Africa, the number of studies reporting on carbapenemase-producing GNB is increasing.[18–23] In a recent report by Singh-Moodley et al, which evaluated isolates from a referral diagnostic programme, 2014/2678 (75.2%) of which harbored one or more carbapenemases.[21] The same report indicated OXA-48 and its variants as predominant (978, 36.5%), followed by NDM (904, 33.8%). Two species predominantly drove the carbapenemases production, *Klebsiella pneumoniae* (1413, 52.8%) and *Enterobacter* spp., (212, 7.9%) of which *Enterobacter cloacae* was the major species (170, 6.3%). Most carbapenem-resistant GNB isolates in South Africa, were obtained from patients with no history of travel outside of South Africa, suggesting local acquisition of infection, possibly driven by increasing use of carbapenems nationally and/or environmental contamination and suboptimal water and sanitation services.[24] Carbapenem-resistant infections cause high mortality [25,26] with several publications reporting the effect of carbapenem-resistant Enterobacteriaceae (CRE) infections on South African patient outcomes.[18–20]

This study aimed to assess the determinants of maternal colonization with MDR-GNB (ESBL-producing and carbapenemase-producing). A better understanding of factors underpinning maternal colonization will allow the development of tailored interventions to prevent neonatal colonization with MDR-GNB and subsequent neonatal sepsis.

Methods

Study setting

This study was conducted at Tygerberg Hospital (TBH) in Cape Town, in South Africa. The obstetric department at TBH is a referral centre, managing only complicated pregnancies with 144 beds in six wards and an antenatal clinic. The antenatal HIV prevalence in the Western Cape Province was 18.7% in 2013 and the Cape Metro district where TBH is located, had the heaviest burden of HIV in the

Western Cape, providing care to more than 70% of the HIV-infected pregnant women in the province.[27]

Study design

We conducted an analytic cross-sectional study at a tertiary hospital, Tygerberg Hospital, in Cape Town, as part of the BARNARDS (Burden of Antimicrobial Resistance in Neonates from Developing Societies, opportunity ID number CPT000490) study in South Africa. We performed a sample size calculation using the prevalence of ESBL-E in pregnant women of 20% published by Onwuezobe et al. 2015 [28], N = 646 (N=588, plus 10 to 15% of this number to reduce the impact of participation refusal or non-response proportion, based on the experience in this setting). We used a convenience sampling method which included every consenting pregnant woman admitted to the maternity ward, with antenatal complications or about to deliver, between 28 and 40 weeks of gestation. During the study period between February to December 2016, the first 651 pregnant women enrolled to the BARNARDS study were entered into this sub-analysis. A validated investigator-administered questionnaire was completed, which included data on participant socio-demographics, HIV infection status, presence of diabetes/gestational diabetes, timing and number of antenatal clinic (ANC) visits, services received, and use of antibiotics during pregnancy. In addition, we reviewed the medical records where necessary to reduce the rate of missing data. After obtaining informed consent, a rectal swab was collected from participants to identify colonization with MDR-GNB, defined as the presence of resistance genes, CTX-M-15 (for ESBL production) or NDM (for carbapenemase production). The primary study outcomes were maternal colonization with CTX-M-15 (ESBL) or NDM, KPC and OXA-48 like. (CRE).

Microbiology procedure for analysis of mother rectal samples

Tests were carried out at Cardiff University in the United Kingdom.

1. For bacterial isolation, swabs were streaked in Liofilchem® chromogenic agar (Liofilchem, Italy) supplemented with vancomycin (V, 10mg/L), vancomycin + cefotaxime (VC, 10+2mg/L) and vancomycin + ertapenem (VE, 10+1mg/L). The plates were then incubated overnight at 30°C – 37°C.

2. The presence of β-lactamases (CTX-M-15, NDM, OXA-48 like and KPC) was determined in the total bacterial culture (and in isolated bacteria) by polymerase chain reaction (PCR). Primers and conditions used are described in Table A.

3. The bacterial growth obtained from the VC plate underwent PCR to identify CTX-M-15; bacterial growth from the VE plate was screened by a multiplex-PCR for the presence of the following carbapenemases: NDM, KPC and OXA-48 like.

Table A.: Primers and conditions

Primers pair	Target	Sequence (5'-3')	Tan °C	Amplicon size (bp)	Reference
CTXM15- F CTXM15- R	$bla_{CTX-M-15}$	ATGCGCAAACGGCGGACGTA CCCGTTGGCTGTCGCCCAAT	55	~600	Walsh group (Carvalho M.)
NDM-M-F NDM-M-R	bla_{NDM}	AGCTGAGCACCGCATT CTCAGTGTCGGCATCAC	52-58	648	Walsh group (Hassan B.)
KPC-M-F KPC-M-R	bla_{KPC}	TAGTTCTGCTGTCTTGTCTC CCGTCATGCCTGTTGTC	52-58	333	Walsh group (Hassan B.)
OXA-48-M-F OXA-48-M-R	bla_{OXA-48} (and OXA-48-like genes: $bla_{OXA-162,}$ -163, -181, and possibly -204 & -232)	GGCGTAGTTGTGCTCTG AAGACTTGGTGTTCATCCTT	52-58	155	Walsh group (Hassan B.)

Operational definitions of resistance

The following criteria were used to define MDR-GNB:

a) The presence of CTX-M-15 gene from VC plates (ESBL-producing GNB)

b) The presence of NDM, OXA-48 like or KPC gene from VE plates (Carbapenemase – producing GNB)

Data analysis

We performed descriptive and analytical analyses of data. For descriptive analysis, we summarized data using the mean (and standard deviation, SD) or median (interquartile range, IQR) as appropriate for continuous variables, and proportions for categorical variables, with 95% confidence intervals. We compared demographic and clinical variables between MDR-GNB colonized and non-colonized mothers analyzing for ESBL+ and carbapenemase+ groups separately, using chi-square test for categorical variables, and Student's t-tests or Wilcoxon ranksum tests, for continuous variables. In addition, we built logistic regression models to identify factors independently associated with maternal MDR-GNB colonization. The cut-off for inclusion to the multivariable logistic regression model was p-value <0.1 in the univariate model. The denominators varied for some variables due to missing data. Stata software version 13.1 (Stata, College Station, TX, USA) was used for all statistical analyses. P-value<0.05 was considered statistically significant.

Ethical approval

The study was reviewed and approved by the Health Research Ethics Committee of Stellenbosch

University (Reference #: S17/10/200) and permission to conduct the study was granted by Tygerberg Hospital management.

RESULTS

Participant demographics:

Of the 651 study participants, mean age at the time of delivery was 29 years (SD, 6). Most women had completed high school (547/651; 84.0%) and resided in an urban area (486 /651; 74.7%). Approximately two-thirds (405/651; 62.2%) lived in freestanding houses, with the majority having indoor access to municipal water supplies (578/651; 88.8%) and flush toilets (606/ 651; 93.1%). Twenty four percent (159/651) of the cohort were HIV-infected and 13.3% (79/596) had diabetes/gestational diabetes. The median number of ANC visits was 5 (IQR, 3 – 7) with 59.6% women (350/587) attending their first ANC visit after 20 weeks of gestation (i.e. late bookers). A quarter of women, (143/594; 24.1%) had received antibiotics during pregnancy for urinary tract infection (UTI). Nearly one-fifth of the cohort (119/650; 18.3%) had been hospitalized in the preceding 12 months, and more than half (66/ 119, 55.5%) of these women were HIV-infected (Table 1).

Prevalence of maternal colonization with genotypic AMR (CTX-M-15):

Of the 651 participants' rectal swabs that were phenotypically screened for potential production of ESBLs, 319 (49.0%) specimens showed growth on the VC plate. Genotypic screening was performed and identified CTX-M-15 in 41/319, 12.9% (95% CI, 9.6% - 17.0%). Of these 41 pregnant women with a positive CTX-M-15 on screening, 22 (53.7%) had been swabbed within 48 hours of admission in hospital, while 19 (46.3%) specimens were obtained after 48 hours of hospitalization (P=0.01).

Comparative analyses of the subsets of participants with CTX-M-15 carriage (multivariable analysis):

One factor was found to independently predict the presence of CTX-M-15 in our participants, the use of communal taps as their primary water source (aOR = 2.6, 95% CI: [1.02 – 6.8], p=0.046), after adjusting for the primary water source and the number of ANC visits. An increasing number of ANC visits showed a protective trend without reaching statistical significance (aOR = 0.9, 95% CI: [0.7 – 1.01], p=0.06) (Table 3)**.** HIV infection, diabetes and the use of antibiotics during pregnancy were not associated with the carriage of MDR-GNB defined by the presence of CTX-M-15.

Prevalence of maternal colonization with genotypic AMR (NDM):

Of the 651 participants' rectal swabs that were phenotypically screened for potential production of carbapenemases, 413 (63.4%) specimens showed growth on the VE plate. Genotypic screening (for NDM, KPC and OXA-48 like genes) was performed on the 413 samples exhibiting growth on the VE

plate. Resistance genes for NDM were identified in 9/413 [2.2% (95% CI, 1.1% - 4.1%)]; no KPC or OXA-48 like genes were identified. Of the nine genotypic positive specimens, six NDM had been swabbed within 48 hours versus three after 48 hours of hospitalization (P=0.38)

Comparative analyses of the subsets of participants with NDM carriage (multivariable analysis):

After adjusting for monthly income, education status, residential area, parity and gestational age at the first ANC visit, we had the following predictors of NDM carriage: Having had either no education or having completed primary school education only, independently predicted (aOR = 7.9, 95% CI: [1.3 – 48.3], p=0.03) carbapenemase resistance gene NDM colonization among pregnant women. Other factors independently associated with NDM carriage were: living in a rural area (aOR = 9.0, 95% CI: [1.6 – 50.7], p=0.01), monthly household income ≤ 100 US$ (aOR = 8.5, 95% CI: [1.6 – 45.9], p=0.01), primiparous status (aOR = 11.0, 95% CI: [1.9 – 63.6], p=0.007) (Table 5). HIV infection, diabetes, the use of antibiotics during pregnancy and delayed attendance of the first ANC visit were not associated with the carriage of MDR-GNB defined by the presence of NDM.

Discussion

We observed a prevalence of 12.9% of CTX-M-15 (for ESBL) and 2.2% of NDM (for carbapenemases) among pregnant women at Tygerberg Hospital, Cape Town who had a positive rectal swab culture screening result. Our findings are in line with a recent meta-analysis reporting a pooled prevalence of 17% of maternal colonization/infection with ESBL-producing Enterobacteriaceae in seven African countries.[4] In contrast, Kaba et al, found a much lower prevalence of maternal colonization with ESBL-E in the Drakenstein region, 4/90 (4.4%) and found no carbapenemase-producing bacteria.

In this study, maternal colonization with carbapenem-resistant Enterobacteriaceae (CRE) carrying the NDM gene was predicted by lower education levels (primary school or no education), low household monthly income (≤100 US$), residence in a rural area and primiparous status. Overall, these predictors are mostly poverty-related. In other settings for instance in Israel, Henig et al found that individuals with low socio-economic status had twice the risk of carbapenem-resistant *Acinetobacter baumannii* colonization and bacteraemia compared to counterparts in higher socio-economic strata.[29] The relationship between low income and antimicrobial resistance remains unclear; however, the effects of other poverty dimensions (housing and living conditions, water and sanitation) may further contribute to an individual's risk of becoming colonized by AMR Gram-negative pathogens.[30] Nomamiukor et al, in the United Kingdom found that low education level was positively associated with colonization or infection with cefuroxime- and nitrofurantoin-resistant *E. coli,* in addition to poor living conditions.[30]

In our study, residence in a rural area predicted maternal colonization with NDM, possibly indicating inadequate purification of potable water sources and poor sanitation conditions, known to be drivers of resistant pathogen transmission in community settings [31]. In this study, 3/9 of participants colonized with NDM had travelled outside of the Western Cape Province (study site) and all 3 had travelled to the Eastern Cape Province. Studies conducted in the Eastern Cape Province on wastewater quality after treatment, found micro-organisms, including MDR-GNB such as *E.coli* in the final treated effluent from wastewater treatment plants [32,33]. Travel to endemic areas for MDR-GNB is a risk factor for colonization or infection with resistant micro-organisms[34,35]. In addition, the majority of NDM colonized pregnant women 6/9 had a positive swab for NDM within the first 48 hours of admission, suggesting the acquisition of NDM in the community, although this did not reach statistical significance.

We also found that primiparous status was associated with maternal colonization with CRE, however, the explanation of this finding is unclear and needs further research. On the other hand, late presentation (first ANC visit after 20 weeks gestation) showed a trend of association with carriage of CRE without reaching statistical significance. Late utilization of antenatal services is an established risk factor for poor maternal and neonatal outcomes.[36] To the best of our knowledge this is the first report exploring the association between late first ANC visit and CRE colonization in pregnant women. In our context, the explanation for the trend we got is unclear and needs further research, however, reportedly, drivers of late first ANC visit are: low income, long distance from the ANC clinic, low education level, unemployment, living in rural areas and unintended pregnancy.[37–40] Some of the abovementioned drivers (low income, low education level and living in rural area) of late first ANC visit were found to predict maternal colonization with NDM in our study.

The use of communal taps as the primary source of water was the only predictor of maternal colonization with CTX-M-15. Drinking water distribution channels can be contaminated by AMR bacteria and/or their genes, and water contamination rates may increase with the increasing number of users, as in the case of a communal tap. Both private and communal water taps were found contaminated with resistant bacteria.[41] In Madagascar, Chereau et al, found that private water tap was associated with maternal colonization with ESBL-producing Enterobacteriaceae.[10] We believe it is likely that the majority of NDM (CRE+) or CTX-M-15 (ESBL) colonized pregnant women in this study acquired CRE or ESBL in the community rather than in hospital, since in many cases the swabs taken within 48 hours of hospitalization were positive (for either CTX-M-15 or NDM).

Our study had a number of strengths; we provide the first report in Africa with a large sample of pregnant women assessed for maternal colonization with CRE and ESBL-E, in a high HIV prevalence setting; we explored a wide range of demographic characteristics, produced adjusted analyses to control for confounders and in spite of the wide confidence intervals, many adjusted estimates showed strong association and reached statistical significance. To the best of our knowledge this is the first report assessing the association between ANC visits, parity, poverty and maternal colonization with MDR-GNB. Limitations included the small number of colonized pregnant women which produced less precise estimates as evidenced by wide confidence intervals. A further limitation is the use of molecular genotyping as a proxy for carbapenemase and ESBL production, without species identification–therefore there is no link between identification to genus or species level and susceptibility result. Finally, the study was based at a single institution in Cape Town and the findings may not be generalized to the rest of South Africa.

Conclusion

Pregnant women had a high prevalence of CTX-M-15 and a low prevalence of NDM rectal carriage. Predictors of maternal colonization with NDM (CRE) were low education level, low income, rural residence, and primiparous status. The use of communal taps was associated with maternal colonization with CTX-M-15 (ESBL). Maternal colonization with MDR-GNB in this study was largely community-acquired. Poverty-related factors were most closely correlated with increased risk of MDR-GNB carriage.

Acknowledgments

Financial Support:

Conflict of interests:

All authors report no conflict of interest relevant to this article.

Figure 1.: Study flow diagram

N= 651 pregnant women selected

N= 651 rectal swabs performed and analvsed

Phenotypic identification of MDR-GNB, N=651

Growth on VC plate, N=319 (49.0%)

Growth on VE plate, N=413 (63.4%)

Single PCR, N=319

Multiplex PCR, N= 413

CTX-M-15, N= 41 (12.9%)

NDM, N = 9, (2.2%)

KPC, N= 0

OXA-48, N=0
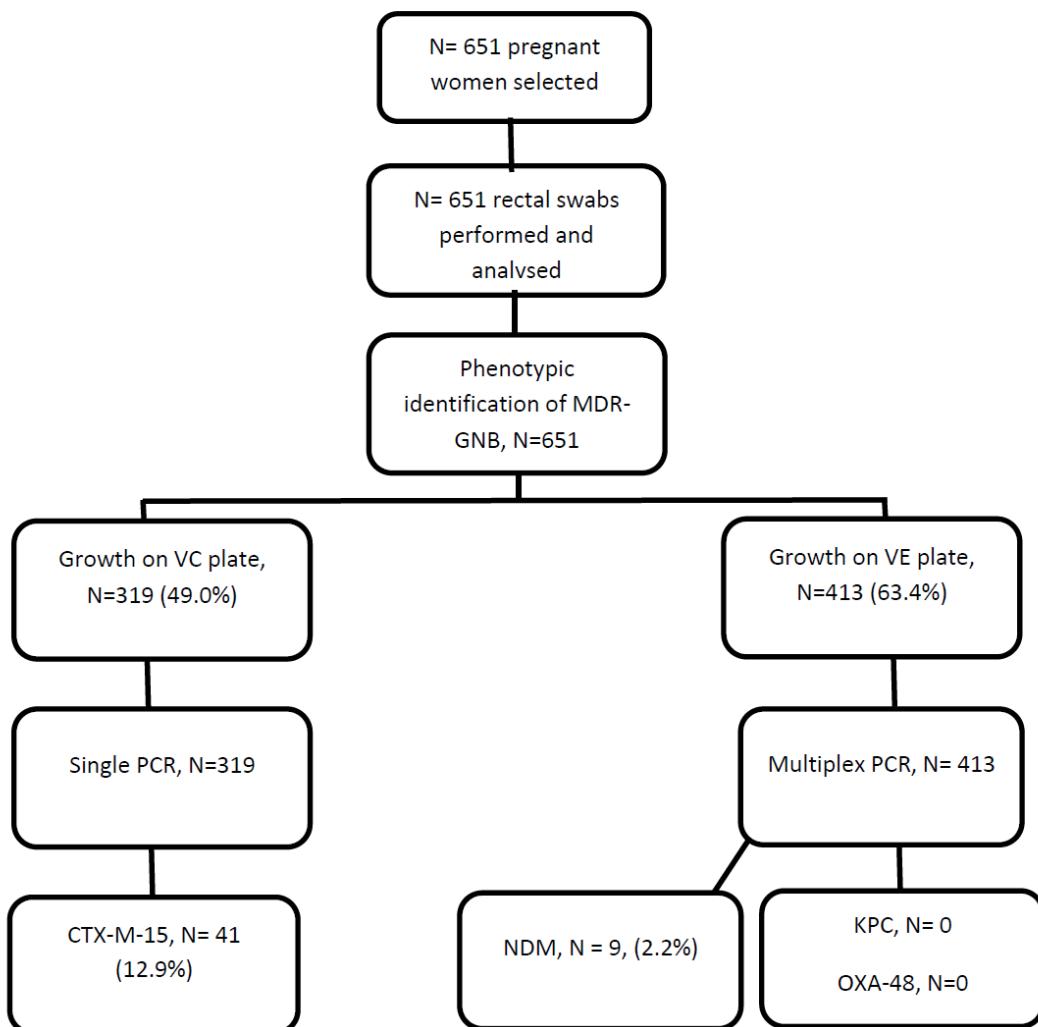
VC: vancomycin + cefotaxime; VE: vancomycin + ertapenem; MDR-GNB: multidrug-resistant Gram-negative bacteria

Table 1: Baseline demographic and household characteristics of mothers screened for colonization with MDR-GNB

Variable	Total cohort (n = 651)
Age in years, mean (SD)	29±6
First pregnancy, n/N (%)	107/651 (16.4)
Gestational age (in weeks ± SD)	
At the first visit	19.8±6.8
At the last visit	35.1±4.2
First ANC visit before or at 20 weeks of gestation, n/N (%)	237/587 (40.4)
Antenatal visits, median (IQR)	5 (3 -7)
HIV infection, n/N (%)	159/651 (24.4)
Urinary tract infection during current pregnancy, n/N (%)	121/594 (20.4)
Use of antibiotics during pregnancy, n/N (%)	143/594 (24.1)
Diabetes, n/N (%)	79/596 (13.3)
Mother hospitalized in the preceding 12 months, n/N (%)	119/650 (18.3)
Length of stay in hospital (days), median (IQR)	3 (1 - 5)
Mother travelled outside of the Western Cape Province in the preceding 12 months, n/N (%)	277/651 (42.6)
Travelled to the Eastern Cape Province in the preceding 12 months, n/N (%)	217/277 (78.3)
Monthly income (in US$), n/N (%)	
<100	134/645 (20.8)
100 - 499	402/645 (62.3)
500 - 999	83/645 (12.9)
≥1000	26/645 (4.0)
Education status, n/N (%)	
Completed High School	547/651 (84.0)

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Table 1: Baseline demographic and household characteristics of mothers screened for colonization with MDR-GNB (continued)

Residential area, n/N (%)	
Rural	165/651 (25.3)
Urban	486/651 (74.7)
Dwelling type, n/N (%)	
Apartment	51/651 (7.8)
Freestanding house	405/651 (62.2)
Shack	190/651 (29.2)
Other	5/651 (0.8)
Primary water source, n/N (%)	
Municipal network	578/651 (88.8)
Communal taps	67/651 (10.3)
Private well	6/651 (0.9)
Number of people in the house, median (IQR)	4 (3 - 5)
Type of toilet, n/N (%)	
Sit down with flush	606/651 (93.1)

ANC: antenatal care, HIV: Human Immunodeficiency Virus, MDR-GNB: Multidrug-resistant Gram-negative bacteria, IQR: Interquartile range

Table 2: Baseline demographic and household characteristics of mothers colonized with ESBL (CTX-M-15)-producing Gram-negative pathogens versus non-colonized mothers

Variable	Total cohort (n = 319)	CTX-M-15+ (n = 41)	CTX-M-15- (n = 278)	p-value
Age in years, mean (SD)	29.7 ± 6.1	29.7 ± 5.2	29.7 ± 6.2	0.96
First pregnancy (current), n/N (%)	53/319 (16.6)	4/41 (9.8)	49/278 (17.6)	0.21
Gestational age (in weeks ± SD)				
At the first visit	20.7 ± 7.0	22.3 ± 7.3	20.4 ± 6.9	0.13
At the last visit	35.3 ± 3.6	35 ± 3.6	35.4 ± 3.7	0.6
First ANC visit before or at 20 weeks of gestation, n/N (%)	53/319 (16.6)	4/41 (9.8)	49/278 (17.6)	0.2
Antenatal visits, median (IQR)	5 (3 - 7)	4.5 (3 - 6)	5 (3 - 7)	0.09
HIV infection, n/N (%)	80/319 (25.1)	10/41 (24.4)	70/278 (25.2)	0.9
Urinary tract infection during current pregnancy, n/N (%)	58/294 (19.7)	7/36 (19.4)	51/258 (19.8)	0.9
Use of antibiotics during pregnancy, n/N (%)	65/294 (22.1)	7/36 (19.4)	58/258 (22.5)	0.7
Diabetes, n/N (%)	32/294 (10.9)	2/36 (5.6)	30/258 (11.6)	0.3
Mother hospitalized in the preceding 12 months, n/N (%)	55/319 (17.2)	8/41 (19.5)	47/278 (16.9)	0.7
Length of stay in hospital (days), median (IQR)	3 (1 - 6)	2.5 (1.5 - 7)	3 (1 - 5)	0.8
Mother travelled outside of the Western Cape Province in the preceding 12 months, n/N (%)	146/319 (45.8)	15/41 (36.6)	131/278 (47.1)	0.21
Travelled to the Eastern Cape Province in the preceding 12 months, n/N (%)	121/146 (82.9)	14/15 (93.3)	107/131 (81.7)	0.88

Table 2: Baseline demographic and household characteristics of mothers colonized with ESBL (CTX-M-15)-producing Gram-negative pathogens versus non-colonized mothers (continued)

Monthly income (in US$), n/N (%)				
≤100	62/319 (19.4)	7/41 (17.1)	55/278 (19.8)	0.68
>100	257/319 (80.6)	34/41 (82.9)	220/278 (80.2)	
Education status, n/N (%)				
Completed High School	265/319 (83.1)	31/41 (75.6)	234/278 (84.2)	0.13
Residential area, n/N (%)				
Rural	77/319 (24.1)	7/41 (17.1)	70/278 (25.2)	0.26
Urban	242/319 (75.9)	34/41 (82.9)	208/278 (74.8)	
Dwelling type, n/N (%)				
Apartment	26/319 (8.2)	5/41 (12.2)	21/278 (7.6)	0.65
Freestanding house	193/319 (60.5)	23/41 (56.1)	170/278 (61.2)	
Shack	96/319 (30.1)	12/41 (29.3)	84/278 (30.2)	
Primary water source, n/N (%)				
Municipal network	283/319 (88.7)	33/41 (80.5)	250/278 (89.9)	0.09
Communal taps	33/319 (10.3)	8/41 (19.5)	25/278 (8.9)	
Private well	3/319 (0.9)	0/41 (0.0)	3/278 (1.1)	
Number of people in the house, median (IQR)	4 (4 - 5)	4 (4 – 5)	4 (3 – 5)	0.2
Type of toilet, n/N (%)				
Sit down with flush	301/319 (94.4)	39/41 (95.1)	262/278 (94.2)	0.78

Table 3: Factors predicting maternal colonization with ESBL (CTX-M-15) - producing Gram-negative bacteria

Variable	Unadjusted		Adjusted	
	Odds Ratio (95%)	p-value	Odds Ratio (95%)	p-value
Age	1.0 (0.9 – 1.1)	0.9		
Mother ill in the last 3 months	1.2 (0.6 – 2.4)	0.5		
HIV infection	0.9 (0.4 – 2.0)	0.8		
Mother travelled outside the Western Cape Province in the last 12 months	0.6 (0.3 – 1.3)	0.2		
Mother hospitalized in the last 12 months	1.2 (0.5 – 2.8)	0.7		
Length of stay in hospital				
<3 days	Reference			
≥3days	0.7 (0.2 – 3.3)	0.7		
Monthly income (in US$)				
>100	Reference			
≤100	0.8 (0.3 – 1.9)	0.7		
Education status				
None	Reference			
High school matric or above	1.1 (0.2 – 5.1)	0.8		
Residential area				
Urban	Reference			
Rural	1.6 (0.7 – 3.8)	0.3		
Type of house				
Apartment	Reference			
Freestanding house	0.6 (0.2 – 1.7)	0.3		
Shack	0.6 (0.2 – 1.9)	0.4		

Table 3: Factors predicting maternal colonization with ESBL (CTX-M-15) - producing Gram-negative bacteria (continued)

Number of people in the house	1.1 (0.9 – 1.3)	0.4		
Primary source of water				
Municipality network	Reference			
Communal taps	2.4 (1.0 – 5.8)	0.05	2.6 (1.02 – 6.8)	0.046
Type of toilet				
Sit down with flush	Reference			
No access to formal toilet or use of a "bucket toilet" system	1.2 (0.3 – 5.4)	0.8		
Gestational age at ANC first visit				
Before or at 20 weeks	Reference			
After 20 weeks	0.9 (0.5 – 1.9)	0.9		
Number of ANC visits	0.9 (0.7 – 1.009)	0.06	0.9 (0.7 – 1.01)	0.069
Use of antibiotics during pregnancy	0.8 (0.3 – 2.0)	0.7		
Urinary tract infection during current pregnancy	0.9 (0.4 – 2.4)	0.9		
Diabetes	0.4 (0.1 – 1.9)	0.3		
Mother visited a private clinic in the last 3 months	1.5 (0.5 – 4.5)	0.5		
Mother visited a traditional healer in the last 3 months	-			

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Table 4: Baseline demographic and household characteristics of mothers colonized with carbapenemase (NDM) - producing Gram-negative pathogens versus non-colonized mothers

Variable	Total cohort (n = 413)	NDM+ (n = 9)	NDM- (n = 404)	p-value
Age in years, mean (SD)	29.5 ± 6.1	30.6 ±6.4	29.5 ± 6.1	0.6
First pregnancy (current), n/N (%)	73/413 (17.7)	4/9 (44.4)	69/404 (17.1)	0.03
Gestational age (in weeks ± SD)				
At the first visit	20.4 ± 7.2	24.2 ± 6.9	20.3 ± 7.2	0.1
At the last visit	35.0 ± 4.3	37.4 ± 1.8	34.9 ± 4.4	0.1
First ANC visit before or at 20 weeks of gestation, n/N (%)	211/379 (55.8)	2/9 (22.2)	209/370 (56.5)	0.04
Antenatal visits, median (IQR)	5 (3 - 7)	6 (3 - 6)	5 (3 - 7)	0.87
HIV infection, n/N (%)	104/413 (25.2)	2/9 (22.2)	102/404 (25.3)	0.84
Urinary tract infection during current pregnancy, n/N (%)	79/382 (20.7)	2/9 (22.2)	77/373 (20.6)	0.9
Use of antibiotics during pregnancy, n/N (%)	93/382 (24.4)	2/9 (22.2)	91/373 (24.3)	0.88
Diabetes, n/N (%)	53/383 (13.8)	2/9 (22.2)	51/374 (13.6)	0.46
Mother hospitalized in the preceding 12 months, n/N (%)	67/413 (16.2)	2/9 (22.2)	65/404 (16.1)	0.62
Length of stay in hospital (days), median (IQR)	3 (1 - 5)	11 (1 - 21)	3 (1 - 5)	0.76
Mother travelled outside of the Western Cape Province in the preceding 12 months, n/N (%)	191/413 (46.2)	3/9 (33.3)	188/404 (46.5)	0.15
Travelled to the Eastern Cape Province in the preceding 12 months, n/N (%)	149/191 (78.0)	3/3 (100)	146/188 (77.7)	0.9

Table 4: Baseline demographic and household characteristics of mothers colonized with carbapenemase (NDM) - producing Gram-negative pathogens versus non-colonized mothers (continued)

Monthly income of the household (in US$), n/N (%)				
≤100	91/413 (22.0)	6/9 (66.7)	85/404 (21.0)	0.001
>100	322/413 (77.9)	3/9 (33.3)	319/404 (78.9)	
Education status, n/N (%)				
Completed High School	347/413 (84.0)	6/9 (66.7)	341/404 (84.4)	0.008
Residential area, n/N (%)				
Rural	112/413 (27.1)	7/9 (77.8)	105/404 (25.9)	0.001
Urban	301/413 (72.9)	2/9 (22.2)	299/404 (74.0)	
Dwelling type, n/N (%)				
Apartment	31/413 (7.5)	1/9 (11.1)	30/404 (7.4)	0.9
Freestanding house	252/413 (61.0)	5/9 (55.6)	247/404 (61.1)	
Shack	127/413 (30.8)	3/9 (33.3)	124/404 (30.7)	
Primary water source, n/N (%)				
Municipal network	368/413 (89.1)	7/9 (77.8)	361/404 (89.4)	0.47
Communal taps	42/413 (10.2)	2/9 (22.2)	40/404 (9.9)	
Private well	3/413 (0.7)	0/9 (0.0)	3/404 (0.7)	
Number of people in the house, median (IQR)	4 (3 - 5)	5 (4 - 6)	4 (3 - 5)	0.23
Type of toilet, n/N (%)				
Sit down with flush	385/413 (93.2)	9/9 (100)	376/404 (93.1)	0.99

ANC: antenatal care, IQR: Interquartile range, SD: Standard deviation

Table 5: Factors predicting maternal colonization with Carbapenemases (NDM) – producing Gram-negative bacteria

Variable	Unadjusted		Adjusted	
	Odds Ratio (95%)	p-value	Odds Ratio (95%)	p-value
Age	1.03 (0.9 – 1.1)	0.57		
Mother ill in the last 3 months	1.3 (0.3 – 5.4)	0.69		
HIV infection	0.85 (0.2 – 4.1)	0.84		
Mother travelled outside the Western Cape Province in the last 12 months	0.33 (0.07 – 1.6)	0.17		
Mother hospitalized in the last 12 months	1.5 (0.3 – 7.3)	0.62		
Length of stay in hospital				
<3 days	Reference			
≥3 days	1.2 (0.07 – 19.5)	0.92		
Monthly income (in US$)				
>100	Reference			
≤100	7.5 (1.8 – 30.6)	0.005	8.5 (1.6 – 45.9)	0.01
Education status				
High school matric or above	Reference			
Completed primary school or no education received	10.7 (2.5 – 46.4)	0.001	7.9 (1.3 – 48.3)	0.03
Residential area				
Urban	Reference			
Rural	7.7 (1.9 – 30.9)	0.004	9.0 (1.6 – 50.7)	0.01
Type of house				
Apartment	Reference			

Table 5: Factors predicting maternal colonization with Carbapenemases (NDM) – producing Gram-negative bacteria (continued)

Freestanding house	0.6 (0.07 – 5.4)	0.65		
Shack	0.7 (0.07 – 7.2)	0.79		
Number of people in the house	1.2 (0.9 – 1.6)	0.27		
Primary source of water				
Municipality network	Reference			
Communal taps	2.6 (0.5 – 12.8)	0.25		
Type of toilet				
Sit down with flush	Reference			
No access to formal toilet or use of a "bucket toilet" system	-			
Parity				
Multipara	Reference			
Primipara	3.9 (1.01 – 14.8)	0.047	11.0 (1.9 – 63.6)	0.007
Gestational age at ANC first visit				
Before or at 20 weeks	Reference			
After 20 weeks	4.5 (0.9 – 22.2)	0.06	5.8 (0.9 – 34.3)	0.05
Number of ANC visits	0.9 (0.7 – 1.3)	0.96		
Use of antibiotics during pregnancy	0.9 (0.2 – 4.3)	0.88		
Urinary tract infection during current pregnancy	1.1 (0.2 – 5.4)	0.91		
Diabetes	1.8 (0.4 – 8.9)	0.47		
Mother visited a private clinic in the last 3 months	-			
Mother visited a traditional healer in the last 3 months	-			

ANC: antenatal care

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Chapter 4:

Antibiotic use in pregnancy: knowledge, attitudes and practices among pregnant women in Cape Town, South Africa

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Journal of Antimicrobial Chemotherapy

Antibiotic use in pregnancy: knowledge, attitudes and practices among pregnant women in Cape Town, South Africa

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Objectives: To establish the knowledge, attitudes and practices (KAP) regarding antibiotic use and self-medication among pregnant women.

Methods: We conducted a KAP survey of 301 pregnant women hospitalized at a tertiary hospital obstetric service in Cape Town, South Africa in November and December 2017, using an interviewer-administered 12 item questionnaire. We stratified analysis of attitudes and practices by participants' mean knowledge score (K-score) group (<6 versus ≥6 out of 7 questions). Multivariate models were built to identify independent predictors of antibiotic self-medication and K-score.

Results: The mean age of pregnant women was 29 (SD 6.1) years, 44/247 (17.8%) were nulliparous, 69/247 (27.9%) were HIV-infected, 228/247 (92.3%) had completed secondary school and 78/247 (31.6%) reported a monthly household income in the lowest category of ≤50–100 US dollars (USD). The mean K-score was 6.1 (SD 1.02) out of 7 questions. Sixteen percent of the cohort reported antibiotic self-medication, with higher rates among pregnant women with K-score <6 [18/48 (37.5%) versus 32/253 (12.6%); *P*<0.001]. The monthly household income category of >500 USD (the highest category) was the only predictor of antibiotic self-medication behaviour [adjusted OR=6.4 (95% CI 1.2–35.2), *P*=0.03].

Conclusions: Higher antibiotic knowledge scores are associated with lower rates of antibiotic self-medication, whereas higher household income is correlated with increasing self-medication behaviours. Education of pregnant women regarding the potential dangers of antibiotic self-medication and stricter enforcement of existing South African antibiotic prescribing and dispensing regulations are needed.

Introduction

The use of antibiotics during pregnancy is prevalent worldwide, raising concerns about increasing antenatal antibiotic exposure and antimicrobial resistance (AMR) in this population group.[1–5] The threat of AMR is of global concern and is increasingly reported from both hospital and community settings.[6–10] In these settings, the transmission of MDR Gram-negative bacteria (MDR-GNB) is a major cause of morbidity and mortality, especially in countries with sub-optimal health systems and inadequate management of water, sanitation and hygiene (WASH).[11,12] Transmission of MDR-GNB occurs in every human population group, including pregnant women. In a systematic review and meta-analysis, the prevalence of maternal (pregnant and post-partum women) colonization with ESBL-producing Enterobacteriaceae (ESBL-E) in Africa was 17.6%.[13]

Despite the concerns of pregnant women regarding the potential teratogenic effects of medications used during pregnancy,[14–16] there is less awareness of the potential harm associated with antibiotic self-medication.[1,17]Self-medication is defined as the acquisition, and use of, one or more medicines without a physician's opinion or diagnosis, as well as without prescription or therapeutic monitoring, including the use of herbal medicines.[18,19] In South Africa, the Medicines and Related Substances Act [Act 101, 1965,[20] section 22A (4)(b)] and the Regulations Relating to the Practice of Pharmacy made in terms of the Pharmacy Act,[21] 1974, stipulate that a medical prescription must be presented before a medicine can be dispensed to an individual patient. Studies from Africa have reported the practice of self-medication in adult populations, including pregnant women.[15,22–26] The prevalence of self-medication among pregnant women varies between countries; for example, a Nigerian study reported a 63.8% prevalence (of which 9.6% self-medicated with antibiotics)[25] and 46.2% has been reported in Tanzania (1.9%

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with antibiotics).[27] In South Africa, Abrahams *et al.*[15] studied self-medication among pregnant women and reported that self-medication with non-prescribed drugs, such as herbs and Dutch remedies was common practice amongst Afrikaans-speaking women for both themselves and their babies. The authors also reported that Xhosa-speaking women followed indigenous healing practices for both themselves and their babies because of the need to 'strengthen' their womb against sorcery, to prevent childhood illnesses and to treat symptoms they perceived biomedical services would not be able to treat.[15]

Five elements are considered as pillars of safe motherhood: choice of contraception, antenatal care (ANC), clean and safe delivery, essential obstetric care and choice on termination of pregnancy.[28] It is well recognized that ANC visits represent an important opportunity to identify risk factors and perform early diagnosis of pregnancy complications and appropriate management, as well as provide health education. In South Africa, the basic antenatal care (BANC) approach is applied and during the first ANC visit pregnant women receive advice (including advice to avoid self-medicating) and health education about pregnancy danger signs (bleeding and reduced fetal movements).[29] However, there is little or no evidence of efforts to provide education on antibiotic use or antibiotic self-medication during pregnancy at ANC visits.[29] Noncungu,[30] in his work in Cape Town, found that the health education needs of pregnant women might be addressed through the individualized tailoring of the health information provided based on the pregnant woman's demographics.

There are limited data on the effect of pregnant women's antibiotic knowledge on their attitudes and practices regarding antibiotic use. Understanding potential misconceptions and knowledge gaps regarding antibiotic use in pregnancy and associated attitudes and practices will inform the development of appropriate interventions to encourage prudent use of antibiotics in pregnancy.

We conducted a knowledge, attitudes and practices (KAP) study among pregnant women at a tertiary obstetric service in Cape Town, South Africa to establish their KAP regarding antibiotics and self-medication practices.

Methods

Study setting

This study was conducted at the Tygerberg Hospital (TBH) Department of Obstetrics in Cape Town, South Africa. This public-sector tertiary, referral obstetric centre manages women with complicated pregnancies, with 144 inpatient beds in six wards and an antenatal outpatient clinic.

Study population

Three hundred and one hospitalized pregnant women were interviewed between 28 and 40 weeks of gestation after obtaining their written consent. The study was conducted between November and December 2017.

Study design

We conducted a study to explore the KAP of pregnant women regarding antibiotic use during pregnancy. Convenience sampling was used to select available and eligible participants. We approached all available pregnant women (to improve the representativeness of the sample) in each obstetric ward and clinic room to obtain their consent prior to their inclusion in the study. The study sample was solely made of consenting pregnant women from the available groups in the Department of Obstetrics at TBH. No prior power or sample size estimations were made. Two trained research nurses (B.Z. and S.E.) who are fluent in three locally spoken languages conducted the patient interviews and data collection during the early morning and at lunch time, to avoid disrupting clinical activities.

The KAP study used an interviewer-administered 12-item questionnaire including seven knowledge questions (true/false questions, each correct response scored 1). For the knowledge score (K-score, defined as the number of correct answers out of 7 questions), we considered as satisfactory a K-score of equal to or above the mean K-score. For analysis purposes, we further subdivided the participants into two groups based on K-score, using the mean K-score as the cut-off value (<mean K-score and ≥mean K-score).

The questionnaire also included three attitude questions with responses graded on a Likert scale (1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree) and there were two practice questions, with either 'yes' or 'no' answers. We worked out the attitude score (A-score) for attitudes (each desired response was scored 1, maximum A-score was 3) and practice score (P-score) for practices (each desired response was also scored 1, maximum P-score was 2). Participants were verbally informed that the questions would cover antibiotic use during the current pregnancy.

Questionnaire development

The literature search did not identify any validated questionnaire measuring KAP regarding antibiotic use among pregnant women. The study team designed a questionnaire to assess KAP regarding antibiotic use in pregnant women attending a tertiary hospital obstetric service in Cape Town, South Africa. The setting included a multicultural patient population, with high levels of socioeconomic deprivation and mixed educational backgrounds. Given these challenges and uncertainty regarding the mean educational level of the participants, the research team designed a short questionnaire with brief, uncomplicated statements regarding concepts of antibiotic use. The questionnaire was provided in English and, where needed, was verbally translated by the research nurse into the patient's language of preference. The internal consistency (the degree of the inter-relatedness among the items/questions in a multi-item questionnaire measure) of items for each section (i.e. knowledge, attitudes and practices) was assessed by computing the Cronbach's alpha value. Each section was evaluated separately: the knowledge section had a reliability of 0.64; attitudes, 0.45; and practices, 0.21. No pilot study was conducted but the questionnaire was assessed for content and face validity by two senior professionals (A.D. and S.M.) in the field.

Data analysis and result reporting

Data analysis of the KAP study included descriptive and analytic components. For descriptive analysis we calculated frequencies, proportions, mean and SD for the K-score after checking for its normal distribution. For the section on attitudes, we combined 'strongly agree' and 'agree' answers to define 'agree'; we also performed the same combination for 'strongly disagree' and 'disagree' to define 'disagree'. We reported proportions of correct answers in the knowledge section and desired answers in the attitude and practice sections.

The analytic component compared proportions of baseline characteristics, desired attitudes and practices, between K-score <6 and K-score ≥6 by using the chi-squared test; proportions of desired attitude and practice between K-score <6 and K-score ≥6 levels were compared. In addition, we built univariate and multivariate logistic regression models (K-score and attitudes) to identify predictors of antibiotic self-medication (practice). Moreover, we performed univariate and multivariate linear regression analyses to assess the correlation between K-score and the following baseline characteristics variables: age, education, monthly household income (MHI),

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residential area (rural and urban), number of previous pregnancies, HIV status and A-score.

In addition, we performed the *t*-test to compare means and the Wilcoxon rank sum test to compare medians, where appropriate.

A *P* value <0.05 was considered statistically significant. Stata software version 13.1 (Stata, College Station, TX, USA) was used for all statistical analyses.

Ethics approval

Ethics approval was obtained from the Health Research Ethics Committee of Stellenbosch University (HREC Reference #: S17/10/200).

Results

Baseline characteristics of participants

Of 301 participants, 247 (82.1%) provided demographics and clinical data (HIV status and parity). The mean (SD) age of pregnant women in this study was 29 (6.1) years, 44/247 (17.8%) were nulliparous, the median (IQR) number of previous pregnancies for the multiparous women was 2 (1–3) and 69/247 (27.9%) women were HIV infected. The MHI groups were as follows: 78/247 (31.6%) earned ≤50–99 US dollars (USD), 42/247 (17%) 100–249 USD, 93/247 (37.7%) 250–500 USD and 34/247 (13.8%) >500 USD. Of 247 participants with demographic data, 14 (5.7%) had a primary school education, 228 (92.3%) had completed secondary school and 5 (2%) had a university-level educational qualification. One hundred and thirty-eight (55.9%) participants described their place of residence as a rural setting.

Knowledge regarding antibiotic use and AMR among pregnant women

A total of 301 KAP survey questionnaires were completed between November and December 2017. Pregnant women had an overall mean correct score of 6.1/7 (SD 1.02) for the knowledge questions, which assessed the participants' understanding of the role of antibiotics in pregnancy, AMR, medical prescriptions and access to antibiotics (Table 1). Ninety-two per cent (278/301) of participants defined AMR as the resistance of 'germs' to antibiotics. A similar number, 277/301 (92.0%), knew that AMR is a serious problem to health. Over half of the participants, 163/301 (54.2%) knew that antibiotics are ineffective for the treatment of influenza. The univariate linear regression analysis did not find a significant association between the K-score and the number of previous pregnancies; however, a trend of positive correlation was found between the two variables (Pearson's *r*=0.011, *P*=0.76). No association was found between K-score and age, education level, MHI, residential area (rural and urban), number of previous pregnancies or HIV status. (Table 2) However, a significant difference in proportions of antibiotic self-medication was found between K-score categories: pregnant women with a lower K-score were more likely to self-medicate; 18/48 (37.5%) among pregnant women with K-score <6 and 32/253 (12.6%) for the K-score ≥6 group, *P*<0.001.

Attitudes to antibiotic use in pregnant women

Ninety-five per cent (286/301) of participants agreed that 'pregnant and lactating women need to see a doctor before

Table 1. Overall KAP of pregnant women regarding antibiotic use and AMR (*N*=301)

Question	Correct/desired responses, *n* (%)
Knowledge (correct response)	
An antibiotic kills germs (true)	291 (96.7)
Antibiotics may be used to treat infections like urinary tract infections (UTIs) (true)	296 (98.3)
'Antimicrobial resistance' is the failure of an antibiotic to kill germs (true)	278 (92.4)
Resistance to antibiotics is a serious health issue (true)	277 (92.0)
Misuse of antibiotics is the major cause of resistance (true)	276 (91.7)
Is it important to have a medical prescription before you buy an antibiotic? (yes)	284 (94.4)
One can use an antibiotic to cure flu (no)	163 (54.2)
Attitudes (desired responses)	
Pregnant and lactating women need to see a doctor before taking antibiotics (agree)	286 (95.0)
Infection with resistant germs during pregnancy can be life-threatening (agree)	159 (52.8)
Pregnant women should not buy antibiotics over the counter (agree)	132 (43.4)
Practices (desired responses)	
Do you buy antibiotics over the counter/without a medical prescription/self-medicate? (no)	251 (83.4)
Do you take antibiotics to treat influenza ('flu')? (no)	191 (63.5)

taking antibiotics'. Approximately 52.8% (159/301) agreed that infections with resistant 'germs' may be life-threatening (Table 1).

Practices regarding antibiotic use among pregnant women

Seventeen per cent (50/301) of pregnant women reported that they had purchased antibiotics over the counter or without medical prescription during pregnancy and approximately 36% (110/301) used antibiotics to treat 'flu' (Table 1).

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Table 2. Baseline characteristics, attitudes to and practices of antibiotic use among pregnant women analysed by mean K-score level

	K-score <6, n (%)	K-score ≥6, n (%)	P
Baseline characteristics, N=247	n=21	n=226	
age, years, mean±SD	29.6±7.1	29.9±5.9	0.5
education level			
primary school	3 (14.3)	11 (4.9)	0.2
secondary school	18 (85.7)	210 (92.9)	0.2
university	0 (0.0)	5 (2.2)	0.2
MHI			
≤250 USD	12 (57.1)	108 (47.8)	0.4
>250 USD	9 (42.9)	118 (52.2)	0.4
residential area			
rural	12 (57.1)	126 (55.8)	0.9
urban	9 (42.9)	100 (44.2)	0.9
number of previous pregnancies, median (IQR)	2 (1–3)	2 (1–2)	0.6
HIV positive	3 (14.3)	66 (29.2)	0.1
Antibiotic self-medication	n=48	n=253	
	18 (37.5)	32 (12.6)	<0.001
Attitudes (desired responses), N=301	n=48	n=253	
Pregnant and lactating women need to see a doctor before taking antibiotics (agree)	43 (89.6)	243 (96.0)	0.1
Infection with resistant germs during pregnancy can be life-threatening (agree)	17 (35.4)	142 (56.1)	0.01
Pregnant women should not buy antibiotics over the counter (agree)	17 (35.4)	115 (45.5)	0.09
Practices (desired responses), N=301	n=48	n=253	
Do you take antibiotics to treat flu? (no)	30 (62.5)	161 (63.6)	0.9
Do you buy antibiotics over the counter/without a medical prescription/self-medicate? (no)	30 (62.5)	221 (87.4)	<0.001

K-score <6 and K-score ≥6 correct answers out of 7 knowledge questions; mean K-score=6.

Correlation of attitudes and practices with K-scores regarding antibiotic use (K-score <6 and K-score ≥6)

There was a statistically significant difference in proportions of participants providing desired responses with regards to one attitude question ('Infection with resistant germs during pregnancy can be life-threatening') between the K-score <6 and K-score ≥6 groups: 17/48 (35.4%) agreeing for K-score <6 versus 142/253 (56.1%) for K-score ≥6, P=0.01.

With regards to the correlation of practices and K-score levels, a higher proportion of participants with a K-score ≥6 reportedly did not buy over-the-counter (OTC) antibiotics or get antibiotics without a medical prescription: 221/253 (87.4%) versus 30/48 (62.5%) for K-score <6, P<0.001 (Table 2).

Predictors of antibiotic OTC/self-medication in pregnant women

From the univariate model, the following factors showed statistically significant ORs: two factors for reduced likelihood of self-medication behaviour, K-score ≥6 [OR=0.24 (95% CI 0.12–0.48, P<0.001)] and increasing A-score [OR=0.7 (95% CI 0.4–0.9, P=0.03)], each increase of A-score by one mark was associated with a 30% decrease in the odds of antibiotic OTC/self medication; and one factor for increased likelihood of self-medication, the MHI category of >500 USD [OR=6.6 (95% CI 1.2–35.7, P=0.03)]. Higher K- and A-scores were both protective factors against self-medication with antibiotics, associated with a decrease of 76% and 30% in the odds of self-medication, respectively. The MHI category of >500 USD was associated with 7-fold higher odds of self-medication with antibiotics than the category of ≤50–99 USD. The three significant variables (the MHI of >500 USD, K-score ≥6 and increasing A-score) from the univariate logistic regression model were then included in a multivariate logistic regression model; only one factor remained independently significant, the MHI category of >500 USD, adjusted OR=6.4 (95% CI 1.2–35.2, P=0.03). Participants with an MHI >500 USD had about 6-fold higher odds of self-medicating than participants with MHI ≤50–99 USD (Table 3).

Predictors of knowledge regarding antibiotic use in pregnant women

In a univariate linear regression model, two variables were significantly correlated with the K-score: MHI category of 100–249 USD [r = −0.14 (95% CI −0.24 to −0.04, P=0.009)] and the A-score [r=0.08 (95% CI 0.03–0.13, P=0.002)]. The two variables were

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Table 3. Univariate and multivariate logistic regression of predictors for antibiotic self-medication/OTC among pregnant women

Variable	Total, N	Self-medication, n (%)	Univariate crude OR (95% CI)	P	Multivariate adjusted OR (95% CI)	P
Overall	301	50 (16.6)				
Baseline characteristics						
K-score out of 7, mean 6 (SD 1.02)						
<6			1			
≥6			0.24 (0.12–0.48)	<0.001[a]	0.8 (0.2–3.6)	0.7
age			0.98 (0.91–1.1)	0.7		
education level						
primary school			1			
secondary school			1.25 (0.15–10.1)	0.8		
university			1	—		
MHI			1.6 (1.03–2.55)	0.04[a]	**1.6 (1.02–2.55)**	**0.04**
≤50–99 USD			1			
100–249 USD			5.1 (0.9–27.7)	0.06	5.4 (0.9–29.9)	0.053
250–500 USD			4.1 (0.9–19.4)	0.08	4.1 (0.8–19.4)	0.080
>500 USD			6.6 (1.2–35.7)	0.03[a]	**6.4 (1.2–35.2)**	**0.032**
residential area						
rural			1			
urban			0.6 (0.2–1.6)	0.3		
number of previous pregnancies			0.9 (0.6–1.2)	0.4		
HIV status (reference group=negative)			0.6 (0.2–1.8)	0.3		
Attitudes, A-score out of 3			0.7 (0.4–0.9)	0.03[a]	1.3 (0.7–2.4)	0.4
Overall	301	50 (16.6)				
K-score out of 7, mean 6 (SD 1.02)						
K-score<mean K-score, n (%)	48 (15.9)	18 (37.5)	1.00			
K-score ≥ mean K-score, n (%)	253 (84.4)	32 (12.6)	0.2 (0.1–0.5)	<0.001[a]	0.3 (0.1–0.5)	<0.001
Attitudes						
Pregnant and lactating women need to see a doctor before taking antibiotics						
disagree	8 (2.7)	2 (25)	1.00			
agree	286 (95.0)	43 (15)	0.5 (0.1–2.6)	0.4	—	—
neutral	7 (2.3)	5 (71.4)	7.5 (0.8–74)	0.09[a]	10.6 (1.8–59)	0.009
Infection with resistant germs during pregnancy can be life-threatening						
disagree	14 (4.7)	4 (28.6)	1.00			
agree	159 (52.8)	19 (11.9)	0.3 (0.09–1.1)	0.08[a]	0.6 (0.3–1.2)	0.1
neutral	128 (42.5)	27 (21.1)	0.7 (0.2–2.3)	0.5	—	—
Pregnant women should not buy antibiotics over the counter						
disagree	94 (31.2)	8 (18)	1.00			
agree	132 (43.9)	23 (17.4)	0.4 (0.2–1.01)	0.05[a]	1.3 (0.7–2.6)	0.4
neutral	75 (24.9)	19 (25.3)	1.7 (0.8–3.3)	0.15	—	—

Statistically significant adjusted ORs are shown in bold.
[a]Variables with P value <0.1.

included in a multivariate linear regression model; only the MHI category of 100–249 USD remained independently and negatively correlated with K-score, $r=-0.13$ (95% CI −0.22 to 0.034, $P=0.008$), i.e. K-score was lower in the group with MHI of 100–249 USD as compared with ≤50–99 USD (Table 4).

The comparison of means of K-score between pregnant women who reported self-medicating with antibiotics [mean K-score 5.5 (SD 1.4)] and those who reportedly did not [mean K-score 6.2 (SD 0.9)], showed a statistically significant difference, $P<0.001$, suggesting that self-medication occurred in the lower mean K-score group.

Discussion

Overall mean K-score on antibiotic use and AMR awareness was satisfactory based on the questionnaire used (mean K-score was 6 correct answers out of 7 knowledge questions) in a South African cohort of pregnant women; about 80% had a K-score equal to or above the mean K-score, in contrast to KAP studies of the general population on antibiotic use conducted in other African countries or the Middle East, where the knowledge of the participants was reported as poor.[17,31–35] Despite the fact that the majority of participants had completed secondary school as their highest

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Table 4. Univariate and multiple linear regression of predictors of knowledge regarding use of antibiotics in pregnant women

Variable	Total, N	Self-medication, n (%)	Univariate		Multivariate	
			crude coefficient (95% CI)	P	adjusted coefficient (95% CI)	P
Overall	301	50 (16.6)				
Baseline characteristics						
age			−0.002 (−0.02 to 0.015)	0.8		
education level						
primary school			0			
secondary school			0.25 (−0.18 to 0.68)	0.3		
university			0.52 (−0.28 to 1.34)	0.2		
MHI						
≤50–99 USD			0			
100–249 USD			−0.14 (−0.24 to −0.034)	0.009[a]	**−0.13 (−0.22 to −0.034)**	**0.008**
250–500 USD			−0.045 (−0.13 to 0.038)	0.28		
>500 USD			0.05 (−0.06 to 0.16)	0.34		
residential area						
rural			0			
urban			0.15 (−0.05 to 0.35)	0.14		
number of previous pregnancies			0.01 (−0.06 to 0.08)	0.8		
HIV status (reference group=HIV negative)			0.07 (−0.2 to 0.29)	0.5		
Attitudes, A-score out of 3			0.16 (0.008 to 0.3)	0.04[a]	−0.04 (−0.18 to 0.09)	0.54

Statistically significant adjusted ORs are shown in bold.
[a]Variables with *P* value <0.1.

education level at the time of this study, participants had an overall satisfactory mean K-score, suggesting that there are means through which they acquire information on antibiotic use, which may include the media or health bodies.[36] In South Africa, one of the objectives of the South African Antibiotic Stewardship Program (SAASP) is to promote rational use of antibiotics and antibiotic education for healthcare workers and the public.[36] In addition, the Antimicrobial Resistance National Framework from the Department of Health in South Africa encourages communication with the public to create antibiotic awareness and patient education on the dangers associated with inappropriate use of antibiotics, including self-medication.[37]

Accessing antibiotics without prescription, mainly identified as self-medication, is common in many parts of the world, including Africa and the Middle East.[17,27,38–41] A recent systematic review and meta-analysis has studied the prevalence of self-medication among pregnant women; it reported a pooled prevalence of 32% (13 studies, from 5 countries: Tanzania, Nigeria, Ethiopia, Iran and China) and about 20% of self-medication with an antibiotic.[1] In our study, 50/301 (16.6%) participants reported self-medicating with antibiotics [a higher proportion was in the group with a K-score <6, 18/48 (37.5%) versus 32/253 (12.7%) in the group with K-score ≥6] despite the fact that they know it is not recommended, especially in pregnancy. In South Africa, Abrahams *et al.*[15] reported self-medication with non-prescribed drugs and herbs among Afrikaans-speaking pregnant women, and indigenous healing practices among Xhosa-speaking women; no details on the nature of the non-prescribed drugs were provided.

It is reported in the literature that factors associated with antibiotic self-medication may be grouped into three categories:

sociocultural factors (past successful use, the idea of self-care, good knowledge of antibiotics, advice or influence of a relative or friend and health-seeking behaviour); health system-related factors (long delays at clinics/hospitals, lack of trust in the health facilities and workers, non-compliance with prescribing and dispensing regulations and easy access to antibiotics); and economic factors (individual and family income, time and money saving).[22,42–45] In our study, education level, knowledge (K-score) and attitudes (A-score) regarding antibiotic use during pregnancy, HIV status, residential area (rural and urban), number of previous pregnancies and age were not independently associated with antibiotic self-medication practices. We found only one factor that independently predicted self-medication with antibiotics among pregnant women, which was an MHI of >500 USD. In addition, we found a statistically significant negative correlation between MHI and K-score; on the other hand, pregnant women who self-medicated with antibiotics had a lower mean K-score compared with those who did not self-medicate.

Although K-score was not an independent predictor of self-medication with antibiotics in our cohort of pregnant women, our findings suggest that pregnant women with lower K-scores and power of purchase (higher MHI) do self-medicate with antibiotics. There is additional evidence that income predicts self-medication practices in low- and middle-income countries. Studies conducted in Guatemala, Nigeria, Ethiopia and Eritrea have documented an association between self-medication (including with antibiotics) and monthly income; the authors of these studies explained this association by the role of medication-purchasing power of the participants.[46–49] Knowledge regarding antibiotics may affect a pregnant woman's decision to self-medicate. In Lebanon, Jamhour *et al.*[50] reported that self-medication with antibiotics

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was highly correlated with both knowledge of antibiotics and lower educational level; people with lower antibiotic knowledge scores were more likely to exhibit bad practices, such as stopping a course of antibiotics prematurely.

A recent KAP study of patients on antibiotic use at a regional hospital in KwaZulu-Natal, South Africa, reported that patients with higher knowledge scores were six times more likely to report desirable antibiotic practices.[51] Similar findings have been reported in a Palestinian study on the relationship between knowledge score and attitudes and practices.[52] Theoretical models conceptualize that greater knowledge predictably leads to an enhanced attitude–practice consistency.[53] Thus educational interventions targeting pregnant women might have a positive impact on their attitudes and practices regarding antibiotic use.

There have been attempts to educate the public on appropriate antibiotic use; clinical trials at community level have been conducted in high-income countries, mostly in the USA, and have indicated moderate benefits of patient education on antibiotic use.[54–58] Public awareness on appropriate antibiotic use has also been achieved through public campaigns.[54]

On one hand the fact that pregnant women self-medicate with antibiotics may also suggest that pharmacies fail to comply with the current regulations for dispensing of antimicrobial agents in South Africa.[20,21] On the other hand, increasing knowledge regarding antibiotics has the potential to lead to improved antibiotic-use practices in pregnant women. Our data have the potential to inform the development of tailored interventions such as educational programmes for both pregnant women (during ANC visits) and health professionals (antibiotic prescribers and dispensers) on antibiotic use during pregnancy (as currently these are not included in health education information packages).[28–30] The educational programmes may focus on the danger of antibiotic self-medication for the foetus and the mother and the importance of medical prescription and its legal value in South Africa. In addition, stricter enforcement of the existing regulations on prescribing and dispensing of medicines in South Africa is needed to curb the practice of antibiotic self-medication. The educational programmes need to take into account the demographics of the individual pregnant woman and antibiotic prescribers/dispensers need to have more impact.[30]

The strengths of this study were the assessment of KAP regarding antibiotic use in a particular study population (pregnant women) and the multivariate analysis of KAP, clinical and demographic data giving a clearer picture of the risk factors involved in self-medication with antibiotics.

The limitations were, firstly, that this was a single-centre study based at a tertiary referral hospital receiving only women with pregnancy-related complications, thus interpretation of the findings needs some caution and may not be generalizable to all pregnant women in the country. Secondly, the use of convenience sampling included only pregnant women who were available and willing to take part in the study. Thirdly, recall bias regarding antibiotic use is possible as all the responses are based on memory of past events, thus the accuracy and volume of information might have been influenced by past events. Lastly, the small sample size in a few variables included in the subanalysis may result in non-generalizable findings.

Conclusions

In pregnant women, higher MHI predicted antibiotic self-medication and lower mean K-scores were found in women who self-medicated with antibiotics. These findings may serve as the basis for the development of tailored interventions addressing the danger of antibiotic self-medication during pregnancy both to mother and child, the role of antibiotics and the legal framework around antibiotic prescribing and dispensing in South Africa, all by taking into account the demographics of participants. The ANC visits may be a good opportunity to implement the educational programmes on antibiotic use among pregnant women.

Acknowledgements

Funding

Transparency declarations

None to declare.

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Chapter 5:

What is the relationship between hospital environmental contamination and colonization of hospitalized unlinked peripartum women and neonates with antimicrobial resistance genes?

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What is the relationship between hospital environmental contamination and colonization of hospitalized unlinked peripartum women and neonates with antimicrobial resistance genes?

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Abstract

Introduction: Contamination of the hospital environment with bacteria carrying antimicrobial resistance genes, could contribute to pathogen transmission to patients. Inadequate environmental cleaning methods and poor hand hygiene compliance may transfer AMR pathogens resulting in colonization and/or infection.

Methods: A cross-sectional study was conducted in the obstetric and neonatal wards at Tygerberg Hospital in Cape Town, South Africa between February and December 2016. To establish the density of hospital environmental contamination with bacteria carrying AMR genes, we sampled 378 high- and low-touch surfaces for the presence of the following resistance genes: CTX-M15, NDM, OXA-48 and KPC. To establish the prevalence of patient rectal colonization with bacteria carrying AMR genes, we screened unlinked 180 peripartum women and 92 hospitalized neonates. We assessed the adequacy of environmental cleaning and hand hygiene compliance rates in the neonatal and obstetric wards using the ICAT tool. Univariate analysis models were built to identify associations between environmental contamination and patient colonization.

Results: Overall prevalence of environmental contamination with AMR genes was 5.8% (22/378) including 19 (86.4%) samples with CTX-M15 and 3 (13.6%) with OXA-48. Low-touch surfaces had significantly higher levels of contamination with CTX-M15 than high-touch surfaces (8/72 [11.1%] versus 11/306 [3.6%]; P=0.002). The environmental cleaning score was similar in neonatal (80%) and obstetric (81%) wards; the mean hand hygiene compliance was 63% and 50% in neonatal and obstetric wards respectively. Five percent (14/272 patients) had CTX-M15 rectal colonization, with higher rates among neonates than peripartum women (9/92 [9.8%]vs 5/180 [2.8%]; p = 0.013). CTX-M15 hospital environment contamination was not a predictor of patient CTX-M15 colonization status [positive swab versus negative OR=1.8 (95% CI 0.2 – 22), P=0.6; undetermined result versus negative OR= 1.2 (95% CI 0.3 – 5.5), P=0.8.

Conclusions: Levels of hospital environmental contamination with AMR genes were low, and did not support a link between hospital contamination and maternal colonization with CTX-M15.

Keywords: hospital environment contamination, antibiotic resistance genes, maternal colonization, neonatal colonization, hand hygiene, environmental cleaning.

Introduction

The hospital environment plays an important role in the transmission of healthcare associated infections (HAIs), during both endemic and epidemic situations.[1] Gram-positive and Gram-negative bacteria colonizing and/or infecting patient skin and gut may contaminate hospital environmental surfaces, often in sufficient concentrations to allow for transmission or transfer to healthcare workers' (HCWs) hands.[2] Colonization with AMR pathogens, particularly in the case of rectal colonization, is likely to result in contamination of patient surroundings and clinical surfaces, with pathogen persistence in the absence of cleaning.[3] The presence of ESBL-producing pathogens in the patient surroundings including the environment is well described, with up to 20% of high-touch surfaces reportedly contaminated in intensive care unit (ICU) settings.[4]

The contamination of inanimate surfaces and medical equipment, may play a role in cross- transmission of pathogens and subsequent colonization and/or infection of hospitalized patients.[5] Transfer of bacteria from the contaminated surface to the patient may occur through direct contact, or more commonly, indirectly on HCWs' hands or contact with contaminated equipment. Although it is well established that hand hygiene is a key strategy to prevent cross-transmission of pathogens, in practice compliance rates are reportedly low in many settings.[6,7] Moreover in the absence of adequate cleaning, pathogens may survive in the hospital environment for extended periods,[8] with some organisms persisting despite attempts to clean and disinfect the contaminated surfaces or equipment.[1] Pathogens like *Clostridium difficile*, methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE) and multidrug-resistant Gram-negative bacilli (MDR-GNB) including *Klebsiella pneumoniae* and *Acinetobacter baumannii* have the ability to survive on both wet and dry surfaces for several months.[2,8]

The underlying mechanism of pathogen survival on dry surfaces is still unclear although biofilms have been implicated.[9,10] A biofilm is a community of microorganisms adhering to a surface and surrounded by a complex matrix of extrapolymeric substances.[11-13] In addition to the environment, wet areas such as sinks and drains are particularly likely to develop biofilms. These can also form on the internal surfaces of indwelling medical devices and medical equipment tubing.[14] The presence of biofilm increases the risk of colonization or infection for patients and HCWs and it has implication for the types of cleaning and disinfection methods used.

This study aimed to assess the burden of hospital environmental contamination with AMR genes and its relationship with subsequent maternal and neonatal colonization or vice versa. We also measured hospital cleaning practices and hand hygiene compliance rates, as a proxy for the likelihood of horizontal

transmission of AMR genes to patients from contaminated hospital surfaces. A better understanding of the relationship between the hospital environmental contamination, patient colonization rates, adequacy of hospital cleaning and hand hygiene compliance will inform infection prevention and control programs to combat cross-transmission of AMR pathogens.

Methods

Study design and setting

We conducted a cross-sectional study between February and December 2016 in the obstetric and neonatal wards at Tygerberg Hospital, a public sector, university-affiliated institution in Cape Town, South Africa. Tygerberg Hospital provides 1384 beds for tertiary medical and surgical services for neonates, children and adults in Cape Town's Metro East. The obstetric service at Tygerberg Hospital has 144 beds in six wards, with approximately 8000 deliveries per annum an 18% antenatal HIV prevalence [15] and a 38% low birth weight rate (2015-2017). Sick and preterm neonates born at Tygerberg Hospital and those referred in from surrounding hospitals, are cared for in a 124-bed unit, including neonatal ICU, high-care, high-dependency, low-dependency and kangaroo mother care wards. There were very limited resources for hospital cleaning, with only one cleaner per ward per shift. There were no dedicated staff for cleaning of equipment (including incubators) which was included as part of the nurses' clinical duties. There was only one infection prevention and control (IPC) nurse practitioner for the obstetric, neonatal and paediatric service, looking after a total of 485 beds. Terminal environmental cleaning (after discharge of a patient with an AMR pathogen or known colonization) was performed on the advice of the IPC nurse practitioner, using 70% alcohol for most indications other than *C. difficile* for which sodium hypochlorite disinfection was recommended.

Environmental sampling

To establish the density of hospital environmental contamination with bacteria carrying AMR genes, we sampled high- and low-touch surfaces for the presence of the following resistance genes: CTX- M15, NDM, OXA-48 and KPC. A total of 378 environmental samples were taken from surfaces that we categorized as either "high-touch" or "low-touch" surfaces. We defined "high-touch" surfaces as the ones subject to frequent contact by patients and HCWs, and are typically considered to include bed rails, over-bed tables, call and control buttons, and other surfaces near the patient; and "low-touch" surfaces (curtain, chair, ceilings, between mattress and bedframe and floors) are subject to infrequent contact by patients and HCWs.

The 378 environmental swabs were collected from the immediate environment of the 272 patients who

had been enrolled in the study. Surface swabs from moist and/or visibly soiled areas were taken from the immediate environment of the consenting patients' wards. Sterile charcoal swabs moistened with sterile saline were rubbed over the selected area, rotating the swab tip several times along multiple planes to obtain a satisfactory inoculum. Swabs were placed in transportation medium, labelled and sent to Cardiff University, United Kingdom for processing as part of another study (the BARNARDS Project – Burden of Antimicrobial Resistance in Neonates of Developing Societies) where they were screened for AMR genes.

Maternal and neonatal screening for rectal carriage of AMR genes

To establish the prevalence of maternal and neonatal rectal colonization with bacteria carrying AMR genes, we screened 272 patients concomitantly with the environmental sampling. The population included unlinked 180 peripartum women (from the last month of gestation until the first few weeks after delivery) and 92 neonates (with clinical diagnosis of sepsis) were sampled for carriage of the genes CTX-M15, NDM, OXA-48 and KPC. All participating peripartum women provided informed consent, and informed consent was sought from the mother or guardian before the investigation of possible sepsis in the admitted neonate. We included neonates born at Tygerberg Hospital as a proxy for measuring the role of hospital environmental contamination in patient colonization, comparing rectal colonization between unlinked peripartum women and neonates. In this study we did not assess mother to neonate transmission of CTX-M15. We only included available and consenting participants, by using convenience sampling, no prior sample size calculation was performed.

Microbiological analysis of rectal and environmental samples for identification of AMR genes

Phenotyping and genotyping of both environmental and rectal swabs were carried out at Cardiff University (United Kingdom). Both environmental and rectal swabs were streaked in Liofilchem® chromogenic agar (Liofilchem, Italy) supplemented with vancomycin (V, 10mg/L), vancomycin + cefotaxime (VC, 10+2mg/L) and vancomycin + ertapenem (VE, 10+1mg/L). The plates were incubated overnight at 30°C – 37°C. The presence of β-lactamases (CTX-M-15, NDM, OXA-48 like and KPC) was determined in the total bacterial culture (and in isolated bacteria) by polymerase chain reaction (PCR). Primers and conditions used are described in Table A. Bacterial growth obtained from the VC plate underwent PCR to identify CTX-M-15; bacterial growth from the VE plate was screened by a multiplex-PCR for the presence of carbapenemases NDM, KPC and OXA- 48 like.

Table A.: Primers and conditions

Primers pair	Target	Sequence (5'-3')	Tan °C	Amplicon size (bp)	Reference
CTXM15- F CTXM15- R	$bla_{CTX-M-15}$	ATGCGCAAACGGCGGACGTA CCCGTTGGCTGTCGCCCAAT	55	~600	Walsh group (Carvalho M.)
NDM-M-F NDM-M-R	bla_{NDM}	AGCTGAGCACCGCATT CTCAGTGTCGGCATCAC	52-58	648	Walsh group (Hassan B.)
KPC-M-F KPC-M-R	bla_{KPC}	TAGTTCTGCTGTCTTGTCTC CCGTCATGCCTGTTGTC	52-58	333	Walsh group (Hassan B.)
OXA-48-M-F OXA-48-M-R	bla_{OXA-48} (and OXA-48-like genes: $bla_{OXA-162,}$ $_{-163,}$ $_{-181,}$ and possibly $_{-204}$ & $_{-232}$)	GGCGTAGTTGTGCTCTG AAGACTTGGTGTTCATCCTT	52-58	155	Walsh group (Hassan B.)

Assessment of environmental cleaning and hand hygiene compliance

Concurrent with the environmental, maternal and neonatal specimen collection, we conducted a once-off audit of hand hygiene compliance in the obstetric and neonatal wards, using the infection control assessment tool (ICAT) from the Centers for Disease Control and Prevention (CDC, ICAT 2015) adapted by the National Department of Health of South Africa. The ICAT assessed three domains: 1. Provisions for hand hygiene, i.e. the availability of hand hygiene facilities and supplies; 2. Hand hygiene knowledge about correct hand hygiene practices amongst HCW; 3. Hand hygiene practice i.e. measuring actual hand washing/use of alcohol handrub during clinical care. For the purpose of this study only hand hygiene practices (i.e. compliance rates) were included in the analysis. For the environmental assessment, the ICAT looked at the following elements using visual inspection: if all areas were dust free, neat and clean, the furniture (examination beds, tables, chairs) was clean and intact, curtains and blinds were free from stains, dust, and cobwebs, ventilation inlets/outlets were free from dust, patient toilets and surrounding area (floor, walls and surfaces) were clean, staff toilets and surrounding area (floor, walls and surfaces) were clean and if there was no evidence of insect and/or rodent infestation.

Data analysis

In the descriptive component we calculated the frequencies and proportion of environmental contamination with resistance genes (CTX-M15, NDM, KPC and OXA-48) from isolates obtained from the obstetric and neonatal wards and the prevalence of maternal and neonatal rectal colonization with the above resistance genes. Hand hygiene compliance rate and environmental cleaning score were reported as percentages.

The analytical component compared the proportion of environmental contamination observed between

obstetric and neonatal wards, using the chi square test. We also compared the rectal colonization proportions between peripartum women and neonates, using the chi- square test. We built univariate logistic regression model to assess the association of hand hygiene compliance rate (ICAT score) and environmental cleaning score (ICAT) with environmental contamination, and patient' colonization status (maternal and neonatal colonization with AMR genes, as the main outcome). We used the rule of at least 10 outcome events per predictor variable (OPV) to decide on building logistic regression models [16,17 in case of less than 10 OPV we built only univariate logistic regression models. We reported odds ratios, their 95% confidence intervals (CI) and p-value.

Ethical approval

Ethical approval was obtained from the Health Research Ethics Committee of Stellenbosch University (HREC Reference #: S17/10/200) and approval was obtained from Tygerberg Hospital management. In addition, the IPC Nurse personal communication provided data on hand hygiene compliance and environmental cleaning.

Results

Prevalence of hospital environment contamination

A total of 378 environmental samples were collected, 218 (57.7%) from obstetric and 160 (42.3%) from neonatal wards. Of the samples collected, 306 (80.9%) and 72 (19.1%) were obtained from high-touch and low-touch surfaces respectively [Figures 1 and 2].

The combined yield of environmental samples from both wards with pathogens harbouring genes producing extended-spectrum beta-lactamases (ESBLs) (CTX-M15) and carbapenemases (OXA-48) was 22/378 (5.8%). Of these 22, 19(86.4%) harboured CTX-M15 and 3 (13.6%) harboured OXA-48. No NDM or KPC genes were detected. Low-touch surfaces had significantly higher levels of contamination with CTX-M15 than high-touch surfaces (8/72 [11.1%] versus 11/306 [3.6%]; P=0.002) suggesting less frequent or rigorous cleaning of low touch surfaces or possible transmission of CTX-M15 contamination via cleaning cloths/equipment.

There was no difference in hospital environment contamination with CTX-M15 between the obstetric and neonatal wards (11/218 [5.1%] versus 8/160 [5.0%]; P=0.4). The prenatal obstetric wards had a higher rate of CTX-M15 hospital environment contamination compared to postnatal obstetric wards (7/63 [11.1%] versus 4/155 [2.6%]; P=0.001).

Low-touch compared to high-touch surfaces were the most contaminated in the obstetric wards, 8/60 (13.3%) and 3/158 (1.9%), (P=0.001), respectively. On the other hand, in neonatal wards, 8/148 (5.4%) were high-touch and 0/12 low-touch surfaces, P=0.3.

Patient colonization with CTX-M15

Of 272 patients, 92 (33.8%) were neonates and 180 (66.2%) peripartum women [Figure 1]. Overall patient rectal colonization with CTX-M15 was 14/272 (5.2%); with higher rectal colonization rate among neonates than peripartum women (9/92 [9.8%] versus 5/180 [2.8%]), P=0.013. For the 14 CTX-M15 colonized patients, only one [1/14, 7.1%], a neonate had a confirmed CTX-M15 contaminated environmental sample. Of 92 neonates included in this analysis, 86 (93.5%) had clinical sepsis. Of these 86, 10/86 (11.6%) had a positive blood culture, 60/86 (69.8%) were on antibiotics from the time of admission and prior to blood culture being. Premature neonates were predominant 76/86 (88.4%), 51/86 (59.3%) males, 21/86 (24.4%) were HIV-exposed, 46/86 (53.5%) were born by C- section, mean (SD) birthweight was 1822 grams (819) and the median (IQR) length of stay was 13 (7 − 33) days. Mother's mean (SD) age was 30.3 (7.1) years old, 156/180 (86.7%) completed secondary school, 129/180 (71.7%) lived in urban areas, 29/180 (16.1%) were primipara, 105/180 (58.3%) delivered by C-section, 51/180 (28.3%) took an antibiotic in the last three months and 41/180 (22.8%) had HIV infection.

Hand hygiene compliance and environmental cleaning assessment

During the study period (2016) the hand hygiene compliance rate averaged 50% (range 34% to 52%) in the obstetric wards and 63% (range 38% to 78%) in the neonatal wards. The Annual 2016 IPC audit report for Tygerberg Hospital reported an environmental cleaning ICAT score of 81% and 80% in the obstetric and neonatal wards respectively [Figure 3].

Correlation between patient colonization (peripartum women or neonates), hospital environment contamination, hand hygiene and environmental cleaning

In the univariate logistic regression model, neonates were more likely to be colonized with CTX-M15 compared to peripartum women, OR = 3.8 (95% CI 1.2 − 11.7), P=0.02; each increase of 1% in the score of environmental cleaning was associated with a 70% decrease in the odds of patient colonization with CTX-M15 OR = 0.3 (95% CI 0.1 − 0.9), P=0.025, CTX-M15 hospital environment contamination was not associated with patient colonization with CTX-M15 [positive versus negative swab OR=1.8 (95% CI 0.2 − 22), P=0.6; undetermined result versus negative swab OR= 1.2 (95% CI 0.3 − 5.6), P=0.8. Being hospitalized in an obstetric ward was associated with a 70% decrease in the odds of patient colonization compared to being hospitalized in a neonatal ward OR = 0.3 (95% CI 0.1 − 0.9), P= 0.028. We did not find an association between HH compliance rate and patient colonization with CTX-M15 OR = 1.04 (95% CI

1.01 – 1.2) P = 0.1. No multivariable logistic regression was built due to insufficient number of outcome events per predictor variable.

Discussion

The overall CTX-M15 environmental contamination in obstetric wards was 5.1% similar to reports from gynaecology and obstetric wards in Ethiopia, of 5.3% (3/52) of ESBL-E.[18] In contrast, a study conducted in France, by Dubois et al, did not find environmental contamination with ESBL-E in the maternity wards (especially in echographic and urine sampling rooms, where all the pregnant women were received for tests).[19] In our neonatal wards, 5.0% (8/160) of environmental samples were positive for CTX-M15, which was lower than that reported from a children's hospital in France, of 19% (18/94) of ESBL-E in the environment.[3] The difference in these environmental contamination rates may be explained by different methodologies used, sampled areas or surfaces and different cleaning methods. In our study, we are only reporting the presence of AMR genes in the environment, without the identification of their specific producers (Gram-negative bacteria), which might underestimate the proportion of contaminated areas by providing an overall prevalence rather than a rate of specific ESBL producer.

In this work, rectal colonization with CTX-M15 among peripartum women (2.8%) was lower than in a recent systematic review and meta-analysis reporting a prevalence of 17% of rectal colonization/infection with ESBL-E in pregnant/post-partum women in Africa.[20] A study in Argentina, documented a prevalence of 5.4% of perianal colonization with ESBL producing - *Escherichia coli* among pregnant women.[21] Although we performed environmental sampling at the same time as patient screening, not every peripartum woman had a concomitant environmental sample. We therefore included peripartum women with a concomitant environmental sample, which reduced the sample size of participants and might explain the difference with other studies colonization rates. Moreover, the nature of samples studied was variable from one study to the other, although perianal or rectal samples were also included. On the other hand, rectal colonization with CTX-M15 in neonates was 9.8% (9/92), this prevalence was in line with the finding in Kenya which reported 10% (59/569) of ESBL-E carriage in neonates at admission.[22] However, our neonatal colonization rate was lower compared to the prevalence found inSpain among infants born to non-colonized mothers, 14% (7/50) colonized with ESBL producing Enterobacteriaceae.[23] Of note, in our setting, this study (2016) predated the emergence of carbapenem-resistant Enterobacteriaceae (CRE) as endemic pathogens.[24]

In the present work, neonates had significantly higher CTX-M15 carriage proportion than the peripartum women, this difference may be due to the following factors which characterized our neonatal cohort:

prematurity, low birthweight, higher rates of antibiotic exposure among neonates, longer stay in hospital, delivery by C-section and HIV exposure.

The hand hygiene compliance rate was 63% and 50% on average in neonatal and obstetric wards, respectively was lower than hand hygiene compliance rates of 76% and 80% reported from another tertiary hospital in Cape Town, South Africa. [25] A study in India, including 49 newborn units and 35 labour rooms, reported an overall hand hygiene compliance rate of 21%.[26] Our compliance rates were above those reported from other hospitals in sub-Saharan Africa where hand hygiene compliance rate varied between 8 – 39%. [27,28]

Three factors were associated with patient colonization with CTX-M15 in univariate analysis: being a neonate had an increased odds of colonization, environmental cleaning score was associated with a 70% decrease in the odds of patient colonization per each increase by 1% in the cleaning score, and ward type (being admitted in neonatal ward had a higher odd of colonization than in obstetric ward). Environmental contamination with CTX-M15 was not a predictor of CTX-M15 patient colonization, or vice versa. However, these results need to be interpreted with caution, as we had a small number of outcome events per predictor variable and this precluded multivariable analysis. Therefore, a major limitation, as we could not study the effects of complexities like prematurity, low birthweight, antibiotic treatment, length of stay in hospital, delivery by C-section, HIV- exposure status and environmental contamination, all together on neonatal colonization and other specific characteristics on peripartum women colonization.

This study had a number of strengths, firstly it assessed hospital environment contamination by swabbing wet and dry surfaces including a large sample size, in two different departments. Secondly, a large sample size of patients swabbed was paired to at least one surface swabbed from their environment. Thirdly, we investigated the relationship between environment contamination, maternal colonization, hand hygiene compliance and environment cleaning score at a tertiary hospital. There are also limitations, firstly we did not perform molecular testing (e.g.: pulsed-field gel electrophoresis or whole genome sequencing) to link isolates from patients to that found in their environment. Secondly, we had a very small number of positive environmental samples around colonized patients, which precluded the multivariable analysis. Thirdly, hand hygiene compliance rates and environmental cleaning scores were assessed during a once-off audit, at one point in the year.

Conclusions

Hospital environmental surface contamination with AMR genes was low (5.8%). No link between hospital environmental contamination and patient colonization could be determined. Neonates were more likely to be colonized with CTX-M15 than peripartum women, suggesting horizontal AMR genes acquisition either from contaminated hands of healthcare workers, environmental surfaces and/or equipment.

Acknowledgments

We thank Srs Zoleka Bojana and Eveline Swanepoel for the data collection and Mrs Fiki Peter for data capturing. Our gratitude to Sr Marina Aucamp for data on hand hygiene and environmental cleaning. We thank the TBH management team for granting permission to conduct our study in their setting.

Funding

Transparency declarations

None to declare.

Figure 1.: Flow chart of patients, number of environmental swabs generated and surface types
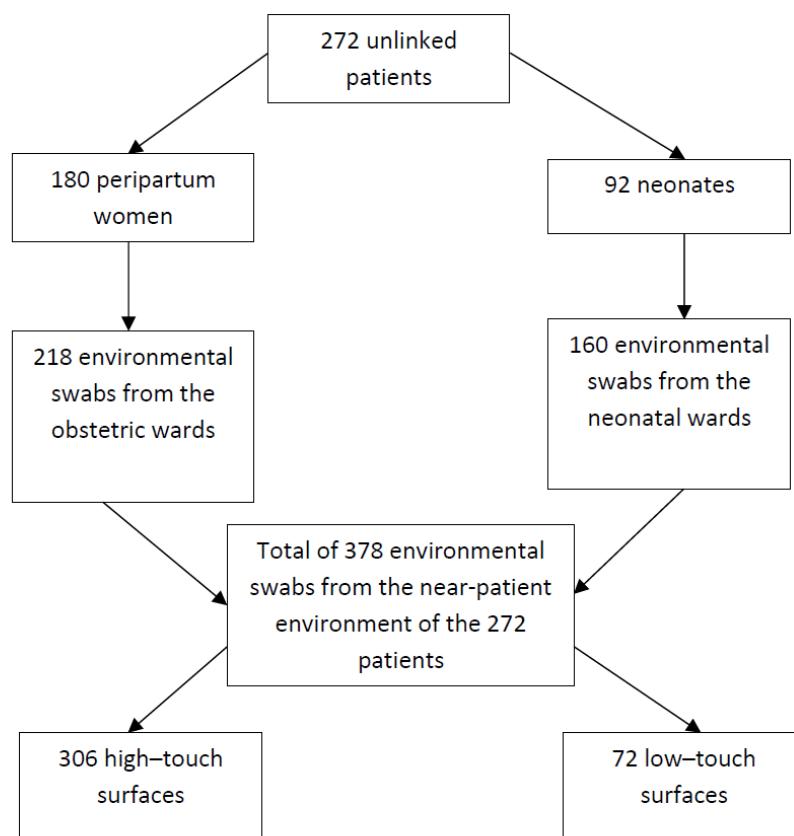
Figure 2.: Distribution of environmental surfaces testing positive and negative for presence of the CTX-M15 gene
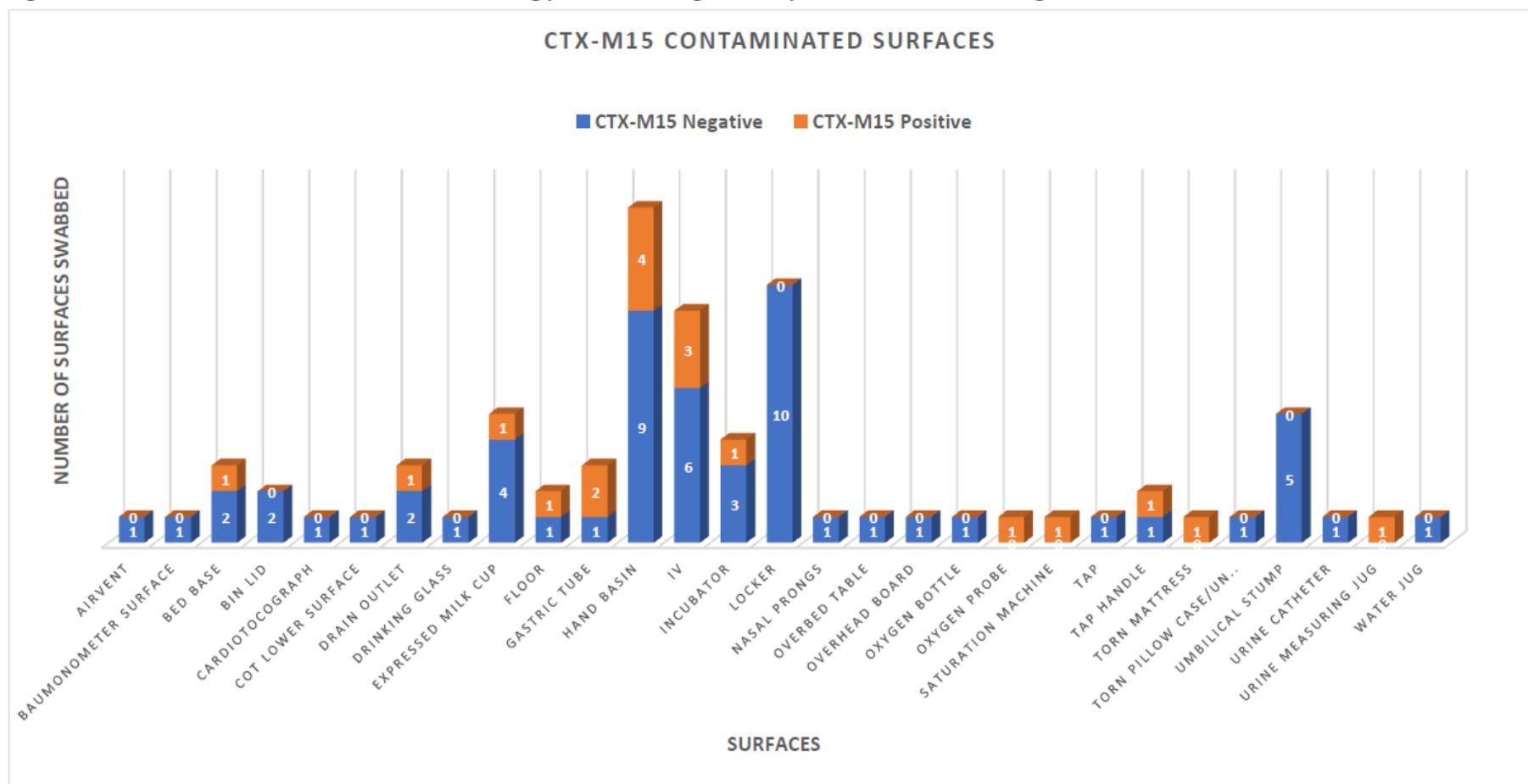

Figure showing the type, number, and frequency of contaminated surfaces with CTX-M15

Figure 3.: Comparison of hand hygiene compliance rates per ward type
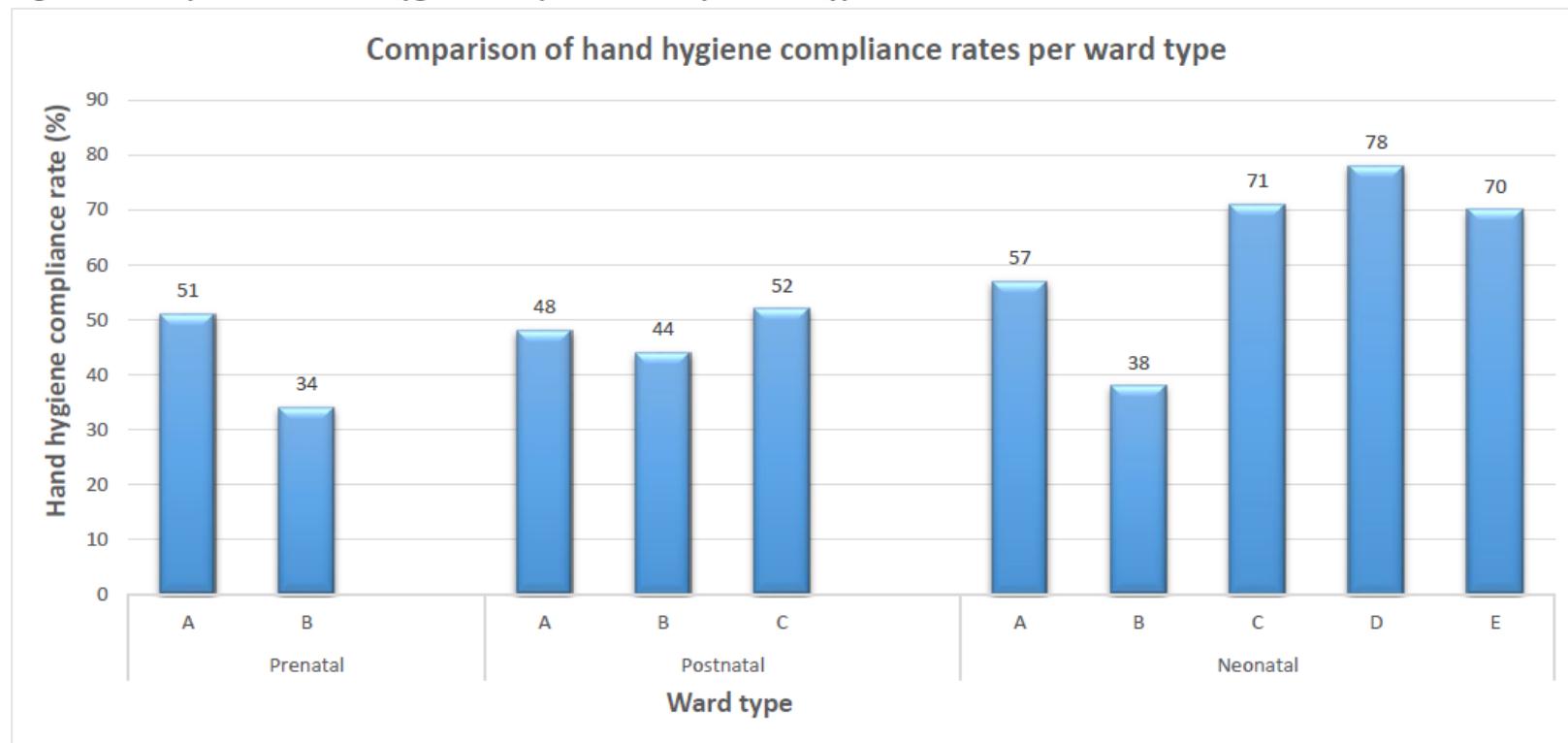
Graph showing HH hygiene compliance rates per ward type as determined by the infection control assessment tool – ICAT

*Letters A – E: were chosen to name different clinical units in a ward (prenatal and postnatal obstetrics, and neonatal wards)

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Chapter 6:

Transmission of multidrug-resistant Gram-negative bacteria from colonized mothers to their infants: a systematic review and meta-analysis

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Systematic review

Transmission of multidrug-resistant Gram-negative bacteria from colonized mothers to their infants: a systematic review and meta-analysis

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S U M M A R Y

Background: Neonatal sepsis remains a leading cause of neonatal mortality. Maternal bacterial colonization plays a major role in transmission to the infant, with potential for subsequent development of neonatal sepsis with maternally derived strains.
Aim: To review the molecular evidence supporting transmission of multidrug-resistant Gram-negative bacteria (MDR-GNB) from colonized mothers to their infants and the risk factors for MDR-GNB transmission.
Methods: PubMed and Scopus were searched for studies investigating the mechanisms, risk factors for and/or scale of transmission of MDR-GNB from colonized mothers to their infants. Random effects meta-analyses were performed to determine pooled proportions of MDR-GNB transmission and the neonatal outcomes of transmission.
Findings: Eight studies were included in the narrative description and six in the meta-analysis. Five studies used pulsed-field gel electrophoresis to assess relatedness of isolates from colonized mothers and their infants. Pooled proportion of MDR-GNB transmission from colonized mothers to their infants was 27% (95% confidence interval (CI): 8—47%). Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae were the most frequently studied MDR-GNB pathogens transmitted between mother—infant pairs. Following mother-to-infant transmission of an MDR-GNB pathogen, the pooled proportion for the outcome of neonatal colonization was 19% (95% CI: 3—35%).
Conclusion: This systematic review strongly supports MDR and/or ESBL Enterobacteriaceae transmission from colonized mothers to their infants, with subsequent infant colonization. The risk factors contributing to transmission of MDR-GNB between colonized mothers and their infants warrants further research.
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Introduction

Neonatal sepsis remains a leading cause of neonatal mortality, along with prematurity and birth asphyxia [1,2]. A recent study of the economic burden of neonatal sepsis in sub-Saharan

Africa reported between 177,500 and 302,870 deaths annually due to neonatal sepsis [3].

Maternal bacterial colonization plays a major role in transmission to the infant, with potential for subsequent development of neonatal sepsis with maternally derived strains [4,5]. Maternal colonization is the presence of bacteria on a mother's body surface (e.g. skin, mouth, vagina, intestines, rectum, perianal region, or airway) which does not evoke an immune response or disease in the pregnant host. However, transmission of bacteria may occur, most notably to her infant, with whom the mother is in direct and constant contact [6,7].

Maternal colonization is usually identified by bacterial culture of swabs from bodily sites such as the nares, rectum, or vagina for the detection and identification of potential pathogens, their antimicrobial susceptibility and occasionally resistance genes [7,8]. Although often inconsequential for the mother, maternal colonization with pathogens, particularly healthcare-associated or multidrug-resistant Gram-negative bacteria (MDR-GNB), may result in serious consequences if pathogens are transmitted to the infant [9–17].

Maternal colonization rates (in pregnant/post-partum women) with extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) are substantially higher in Africa (17.6%) than those reported from countries such as Norway (2.9%) and Argentina (5.4%) [10,18,19]. In African neonates, Gram-negative bacterial (GNB) infections are the leading cause of severe bacterial infections (accounting for two-thirds of bloodstream infection pathogens) and are associated with high mortality [20–27]. Despite increasing evidence of the high burden of MDR-GNB maternal colonization and neonatal infection in Africa, data linking MDR-GNB transmission from colonized mothers to their infants is scant. This is of concern since up to 50% of neonates colonized by MDR-GNB, especially ESBL-E, may develop bloodstream infections [28,29].

Molecular methods have been used to establish the relatedness of isolates in different situations to confirm transmission of pathogens from one source to another such as patient to patient, from healthcare workers' hands or environment [30–32]. Molecular methods provide convincing evidence of pathogen transmission. Frequently used methods include pulsed-field gel electrophoresis (PFGE), random amplification of polymorphic DNA, polymerase chain reaction (PCR), repetitive sequence-based PCR (rep-PCR), multi-locus sequence typing, and whole-genome sequencing [32–35]. There is abundant evidence for transmission of Gram-positive bacteria from mother to infant [36–40]; however, studies with robust evidence of MDR-GNB transmission are limited [10,17].

The aim of this systematic review and meta-analysis was to review the molecular evidence for transmission of MDR-GNB from colonized mothers to their infants, and to summarize factors contributing to transmission of MDR-GNB between mother and infant.

Methods

Search and selection criteria

A systematic review and meta-analysis were conducted by searching the electronic databases PubMed and Scopus, the bibliographies of selected publications, and grey literature (articles not formally published by commercial publishers) [41]. We searched for primary epidemiological studies of any design (observational and experimental) reporting molecular evidence of transmission of MDR-GNB from colonized (carriage of MDR-GNB in the gastrointestinal tract, birth canal, and on skin and hands) mothers to their infants and/or contributing factors to MDR-GNB transmission to the infants. No language or date restrictions were applied, and the electronic databases were searched from their inception to March 31st, 2019. Studies (observational and experimental) reporting transmission of MDR-GNB from colonized mothers to their infants without molecular evidence or transmission of susceptible GNB were excluded, as were studies reporting breast milk contamination, outbreaks in neonatal wards, or infected mothers.

Two separate search strategies were used to answer two research questions: (i) what molecular evidence supports a link and/or relationship between maternal MDR-GNB colonization and neonatal colonization and/or infection; (ii) which factors (community, obstetric, and infection prevention-related) contribute to the risk for transmission of MDR-GNB between mother–infant pairs.

For PubMed, a combination of keywords, Boolean operators and MeSH terms was used, including: (i) (((((maternal colonization OR maternal colonisation) AND (neonatal infection OR neonatal sepsis OR vertical transmission OR mother to neonate transmission OR mother to child transmission OR neonatal colonization OR neonatal colonisation)) AND (whole genome sequencing OR pulsed field gel electrophoresis OR pulsed-field gel electrophoresis OR molecular methods)) AND (gram negative bacteria)), (ii) (multi-drug resistant gram negative bacteria OR multidrug resistant gram negative bacteria OR drug resistant gram negative bacteria OR antimicrobial resistant gram negative bacteria OR antibiotic resistant gram negative bacteria) AND (transmission) AND (risk OR risk factor OR determinant OR contributing factor) AND (neonate OR infant OR baby OR newborn) AND (adjusted analysis OR multivariate analysis OR multivariable analysis).

Risk factors of maternal colonization with MDR-GNB were not studied.

Operational definitions of multidrug resistance in Gram-negative bacteria

(1) As defined by Magiorakos *et al.*, in the interim standard definitions for acquired resistance and multidrug-resistant Gram-negative bacteria (MDR-GNB): (i) ESBL-producing Enterobacteriaceae; (ii) micro-organisms with intrinsic resistance mechanisms such as *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, and *Ralstonia pickettii*; and (iii) any GNB (e.g. *Acinetobacter* spp., Enterobacteriaceae, and *Pseudomonas* spp.) resistant to three or more of the following drug classes: piperacillin/tazobactam, cephalosporins (cefazolin, ceftriaxone, ceftazidime, and cefepime), carbapenems (imipenem), monobactams (aztreonam), aminoglycosides (gentamicin, tobramycin, and amikacin), and fluoroquinolones (ciprofloxacin and levofloxacin) [42].

(2) Any GNB resistant to any two classes of antibiotics after antibiotic susceptibility testing were also considered as MDR-GNB.
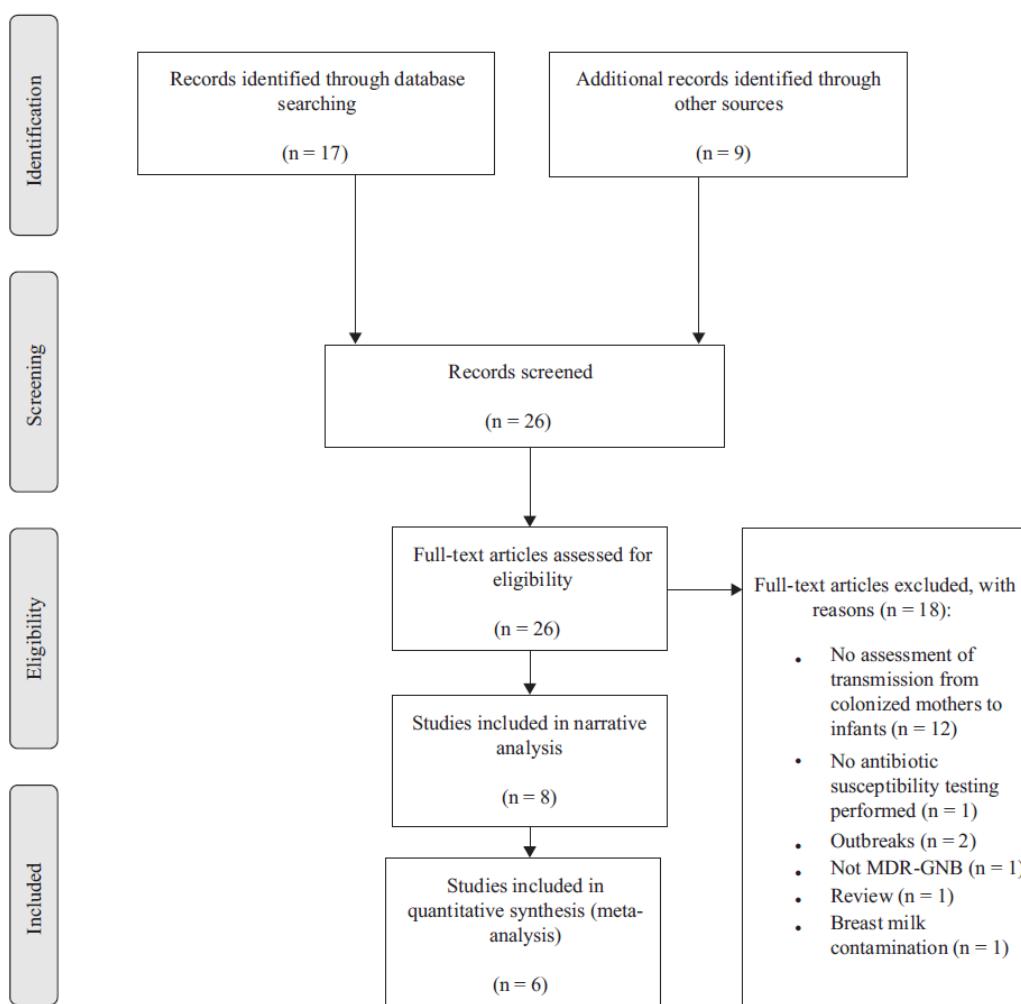
Figure 1. Study flow diagram (PRISMA). MDR-GNB, multidrug-resistant Gram-negative bacteria. Studies included in quantitative synthesis (meta-analysis).

Data analysis

The first author (A.B.) did the searches, two authors (A.B. and A.D.) did data extraction using a pre-designed data extraction form including name of the author and year of publication, country (the geographic distribution was reported as per the World Bank list of economies, June 2019), participants and study design, isolates and clinical specimen, relatedness of isolates, genotyping methods used and risk factors for MDR-GNB transmission [43]. All data were extracted from published studies only; no individual patient-level data were requested.

Two authors (A.B. and A.D.) assessed the quality of studies by using the Newcastle—Ottawa Scale (NOS) for observational studies [44,45]. This scale provides an evaluation of three main domains: participant selection, exposure/outcome ascertainment and comparability between groups. NOS appropriate for each included study design (cohort, case—control, and cross-sectional studies) was used. The NOS score was classified as follows: NOS ≤ 5 for low, 6—7 for moderate, and 8—10 for high quality. All studies with moderate quality and above were included in the meta-analysis.

Three authors (A.B., A.D., and S.M.) performed narrative analysis and A.B. ran quantitative syntheses. A random effects meta-analysis was used for quantitative synthesis (accounting for heterogeneity of studies) of MDR-GNB transmission proportion from colonized mothers to their infants. By heterogeneity, it is assumed that each study included in the analysis reports a specific proportion of MDR-GNB transmission, and the average proportion of transmission is arrived at by a random effects model [46]. The meta-analysis in this work reported the overall proportion of MDR-GNB transmission from colonized mother to child, plus the resulting proportions of neonatal colonization and/or neonatal sepsis.

This systematic review and meta-analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [47].

Data analysis was performed on Stata 13.1 (StataCorp LP, College Station, TX, USA).

Results

A total of 26 titles, abstracts, and full texts was screened, selecting eight eligible studies for quality assessment

Table I
Linking maternal colonization/infection to neonatal colonization/sepsis/infection as studied by molecular methods

No.	Publication	No. of participants (mother–infant pairs)	Study design and period	Isolates and clinical specimen	Relatedness of isolates	Methods of identification and AST	Genotyping methods used
1	Gbaguidi-Haore et al. [14] France	Neonates and their mothers. 125 mother–child pairs (a total of 147 children) were studied for E. cloacae transmission. 16/125 (12.8%) mothers tested positive. 3 neonates born to 16 (18.8%) mothers tested positive.	Cohort study. Dec 2002 to Dec 2004.	MDR Enterobacter cloacae. From rectal swabs.	3/16 pairs colonized with similar E. cloacae.	Biochemical identification of isolates. Antibiotic susceptibility was determined by the disc diffusion method.	PFGE
2	Danino et al. [13] Israel	313/409 (76.5%) mothers and all 478 children were screened for ESBL Enterobacteriaceae. 25 mother–infant pairs were colonized with the same bacterial strain. 10/25 (40%) of mother –infant colonized pairs underwent PFGE analysis.	Cohort study. Jan 2015 to Jan 2016.	ESBL Enterobacteriaceae. Rectal swabs from mothers and their neonates.	A sub-group of 10 isolates underwent PFGE and 70% (7/10) displayed an identical PFGE fingerprint.	Biochemical identification of isolates. AST by disc diffusion method.	PFGE
3	Dubois et al. [48] France	4 mothers were colonized with CTX-M-producing Escherichia coli. 2 (50%) neonates born to these colonized mothers were also colonized.	Cross-sectional study. Nov 2007 to Apr 2008.	CTX-M-producing E. coli. From genital or urine samples in mothers, gastric fluid of neonates.	The strains recovered from two mothers and their respective babies were identical (2/4).	Biochemical identification of isolates. AST by disc diffusion method.	PFGE phylogenetic group determination
4	Prelog et al. [49] Austria	21 mother–infant pairs were investigated. 13/21 (61.9%) mothers were colonized. 3 neonates born to 13 colonized mothers were also colonized (23.1%).	Cohort study. Year 2006.	MDR E. coli. From faecal samples.	3/13 infants had resistant E. coli stool carriage identical to the mothers'.	Biochemical identification of isolates. AST by disc diffusion method.	PFGE

5	Rettedal *et al.* [50] Norway	14 mother—infant pairs were investigated. 5 infants born to 14 colonized mothers were also colonized (35.7%).	Cross-sectional study. 6 months in year 2012.	ESBL *E. coli.* From rectal swabs.	ESBL-E strains indistinguishable from the strains isolated from their respective mothers were detected in 5/14 mother—infant pairs ESBL-E colonized.	Biochemical identification of isolates. AST by disc diffusion method.	PFGE
6	Nanayakkara *et al.* [51] Sri Lanka	159 mother—infant pairs were investigated. 11/159 (6.9%) pairs were involved in the transfer of isolates. 1/11 (9.1%) had a similar isolate.	Cohort study. Oct 1st, 2015 to Jan 6th, 2016	Only results of Enterobacteriaceae (from mothers' vaginal swabs and peri-rectal swabs from neonates) are reported in this work.	1/11 pair was colonized with a similar ESBL-producing Enterobacteriaceae.	Biochemical identification of isolates. AST by disc diffusion method.	Random amplification of polymorphic DNA.
7	Denkel *et al.* [5] Germany	18 mother—infant pairs were ESBL-E colonized. 5 pairs indicated similarity (27.8%).	Cohort study. May 2012 to Jun 2013.	ESBL Enterobacteriaceae. From rectal swabs.	5/18 mother-infant pairs were colonized with similar ESBL *E. coli.*	Biochemical identification of isolates. AST by disc diffusion method.	Repetitive-sequence-based PCR and subsequent microfluidics electrophoresis.
8	Kaba *et al.* [52] South Africa	90 mothers investigated for ESBL-producing Enterobacteriaceae. 116 neonates born to 90 mothers and also screened for ESBL-producing Enterobacteriaceae. 1/90 (1.1%) mother—infant pair was colonized.	Cohort study. Year 2014.	ESBL-producing *Enterobacter cloacae.* From rectal swabs.	In a mother—infant pair (1/90), clonal relations were observed among ESBL-producing *E. cloacae* isolates.	Automated biochemical identification and AST using Vitek 2 system.	PFGE

AST, antibiotic susceptibility test; MDR, multidrug resistant; PFGE, pulsed-field gel electrophoresis; ESBL, extended-spectrum β-lactamase; PCR, polymerase chain reaction.

Table II
Assessment of bias

Study type	Selection		Comparability of the groups on the basis of the design or analysis	Ascertainment of outcome	Overall quality assessment score (out of a maximum of 10)
	Representativeness of the sample	Ascertainment of exposure			
Cohort studies					
Gbaguidi-Haore et al. [14]	Truly representative of neonates admitted to neonatal units plus their mothers. Non-exposed cohort drawn from the same community as the exposed cohort.	Screening for Enterobacter cloacae intestinal colonization of all infants at admission and weekly thereafter was involved.	Infection control measures put in place before the study start.	Independent blind assessment. Follow-up long enough for outcome to occur.	6
Prelog et al. [49]	Somewhat representative of the average mother —child pairs. Non-exposed cohort drawn from the same community as the exposed cohort.	Faecal samples were obtained from 46 neonates and their parents within the first 48 h after delivery.	Many neonatal and maternal risk factors were controlled using specific exclusion criteria.	Independent blind assessment. Follow-up long enough for outcome to occur.	6
Denkel et al. [14]	Truly representative of VLBW neonates and their mothers. Non-exposed cohort drawn from the same community as the exposed cohort.	VLBW infants and their mothers were screened for colonization with ESBL-E.	Multivariate analysis was performed.	Independent blind assessment, follow-up long enough for outcome to occur.	6
Danino et al. [12]	Truly representative of neonates admitted at the NICU and their mothers. Non-exposed cohort drawn from the same community as the exposed cohort.	Surveillance was performed similarly for infants in the NICU at admission and twice weekly until discharge.	Stillbirth deliveries were excluded. Infants of unscreened mothers were excluded from the comparison of infants colonized by ESBL with positive- vs negative-screened mothers.	Independent blind assessment, follow-up long enough for outcome to occur.	6
Nanayakkara et al. [51]	Subjects (mothers and their babies) were selected according to convenience sampling.	A low-vaginal swab from mother collected at the time of admission to antenatal ward; a low-vaginal swab	Admission of the baby to special baby care unit, heavy bleeding per vagina and emergency lower segment caesarean	Independent blind assessment, follow-up long enough for outcome to occur.	5

	Method: non-exposed cohort drawn from the same community as the exposed cohort.	from mother collected at the time of discharge from postnatal ward, a peri-rectal swab from the baby of the mother collected on discharge. Structured interviews.	section were considered as exclusion criteria.		
Kaba *et al.* [52]	Truly representative of post-partum women with ESBL Enterobacteriaceae in the community. Non-exposed cohort drawn from the same community as the exposed cohort.	Rectal swabs. Structured interviews.	—	Independent blind assessment. Follow-up long enough for outcome to occur.	6
Cross-sectional studies					
Dubois *et al.* [48]	Somewhat representative of the average mother —child pairs.	Validated measurement tools/ microbiological methods for antimicrobial resistance detection.	—	Independent blind assessment.	5
Rettedal *et al.* [50]	Truly representative of ≥36 weeks pregnant women admitted.	Women were screened for rectal ESBL-E colonization at 36 weeks of pregnancy and delivery.	Possible risk factors for colonization were studied by logistic regression.	Independent blind assessment.	7

VLBW, very low birth weight; ESBL-E, extended-spectrum β-lactamase-producing Enterobacteriaceae.
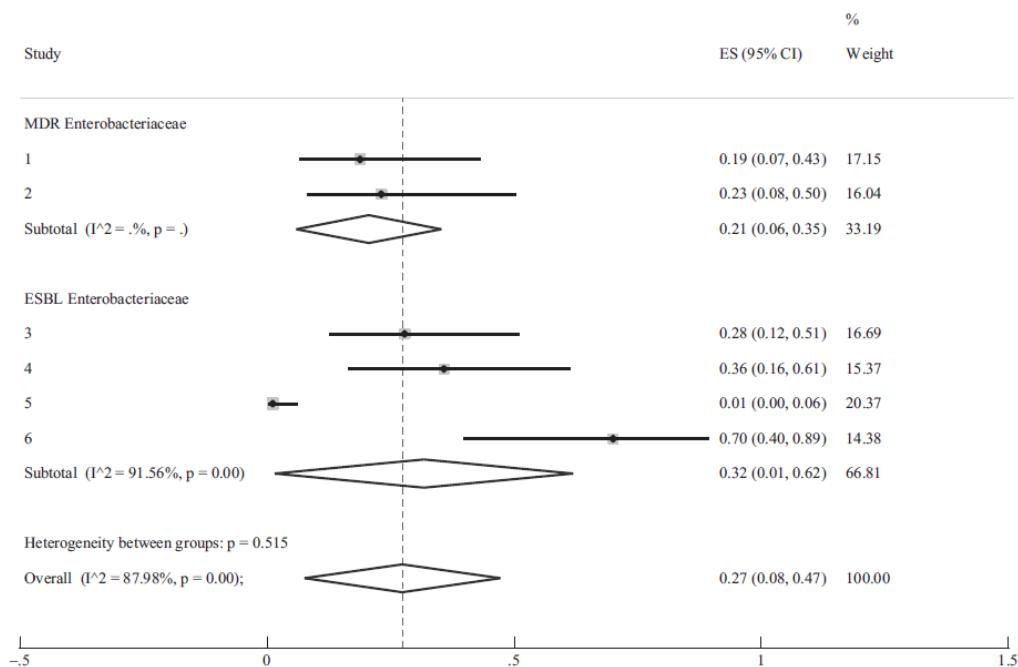
Figure 2. Pooled proportion of multidrug-resistant Gram-negative bacteria transmitted from colonized mothers to their infants. ES, effect size; ESBL, extended-spectrum β-lactamase.

(Figure 1). The geographic distribution of these studies was as follows: Europe (*N* = 5), Middle East (*N* = 1), Asia (*N* = 1), and Africa (*N* = 1). Of these eight studies, six were from high-income and two from upper-middle-income countries. Included studies were either cohort studies (*N* = 6) or cross-sectional studies (*N* = 2) (Table I).

To provide molecular evidence of mother-to-infant bacterial transmission, the relatedness of isolates from mother–infant pairs was studied predominantly using PFGE alone (five studies), but other methods were also applied in one study each using PFGE plus phylogenetic group determination and rep-PCR with subsequent microfluidics electrophoresis (Table I).
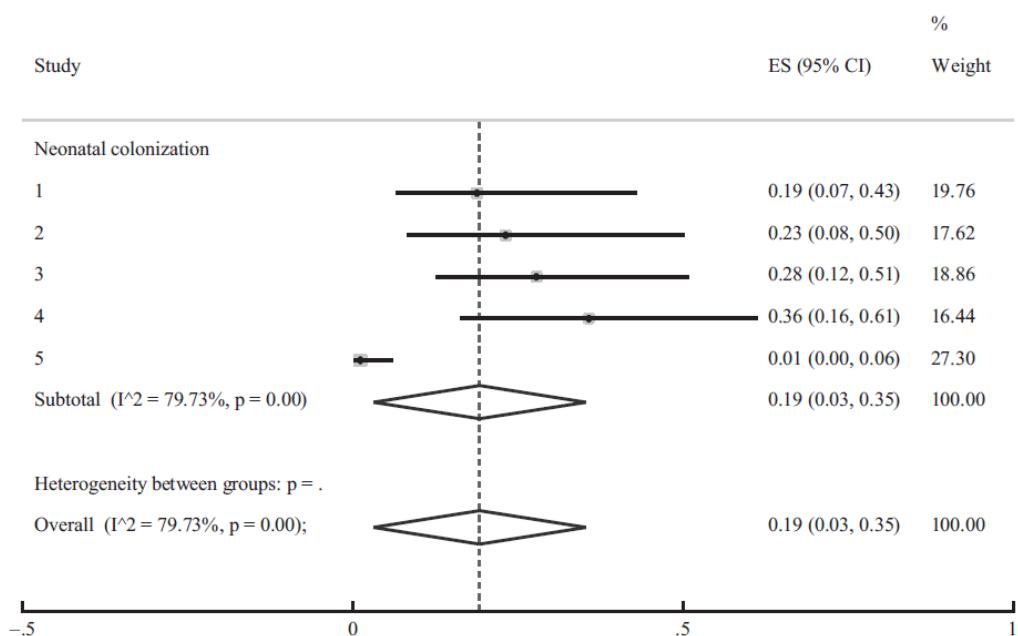

Figure 3. Pooled proportion of multidrug-resistant Gram-negative bacteria transmission's neonatal outcomes. ES, effect size.

The studied isolates involved in transmission were extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (N = 2) [18,41,42], ESBL-producing Enterobacteriaceae (N = 2) [12,14,43], and two studies on MDR *Enterobacter cloacae* [13,44] (Table I).

The overall NOS score was 5.9 ± 0.6 (mean ± SD) from a total score of 10 (Table II).

From six studies the overall pooled proportion of MDR-GNB transmission from colonized mothers to their infants was 27% (95% confidence interval (CI): 8—47%). There was a pooled proportion of 32% (95% CI: 1—62%) reporting transmission of ESBL Enterobacteriaceae; however, the overall heterogeneity was high ($I^2 = 87.8\%$, $P = 0.00$). (Figure 2). The pooled proportions of the neonatal outcome of maternal colonization with MDR-GNB, from five studies with adequate information on neonatal outcomes, was: transmission resulting in neonatal colonization with MDR-GNB 19% (95% CI: 3—35%) (Figure 3).

From the eight studies included in this review, only one study reported on risk factors: Denkel *et al.* found maternal colonization to be a risk factor for neonatal colonization in very-low-birthweight infants [15].

Discussion

This systematic review and meta-analysis included eight studies, reporting molecular evidence of MDR-GNB transmission from colonized mothers to their infants. The included studies were all observational, and the overall quality was moderate, assessed by the mean NOS score. Overall the pooled proportion of MDR-GNB transmission from colonized mothers to their infants was 27%. Most of the studies used PFGE to demonstrate transmission of MDR-GNB from colonized mothers to their infants. The literature reports that PFGE is the current reference standard for studying genetic relatedness of pathogenic bacteria, especially to demonstrate the transmission of bacteria among patients [53].

The current study raises concerns about the scarcity of robust research on maternal colonization with MDR-GNB, the transmission to the infants verified by molecular pairing genotypic methods, and subsequent neonatal outcomes (colonization or infection). Compared to maternal colonization with Gram-positive bacteria, such as Group B streptococcus (GBS), for which there is a large body of evidence (85 studies identified in a recent meta-analysis), there are very limited data on MDR-GNB transmission (we identified only eight studies of poor to moderate quality), with no studies conducted in low-income countries [54].

Our study highlights the existing evidence of correlation between maternal colonization status and neonatal colonization. Most published studies reported only on transmission of susceptible pathogens and very few reported on resistant ones. For instance the Generation R Study demonstrated the correlation of maternal colonization with *Staphylococcus aureus* and *Haemophilus influenzae* and the respective neonatal colonization using molecular methods [55]. Other studies using multivariate analyses have reported maternal colonization status as a key factor in neonatal colonization [15]. On the other hand, a well-powered meta-analysis demonstrated that neonatal infection is associated with maternal colonization and infection [56]; however, in that review, only two studies reported mother—infant pair colonization with *Escherichia*

coli. No molecular evidence was provided for MDR *E. coli* transmission from colonized mothers to their infants.

In the current meta-analysis, 19% (pooled from five studies) of neonatal colonization followed transmission from MDR-GNB-colonized mothers; Chan *et al.* reported 34.3% of colonization with *E. coli* in neonates born to colonized mothers, but no molecular evidence or antibiotic resistance patterns were mentioned [4].

Even less is known about the most important or relevant risk factors for transmission of MDR-GNB from mother to infant. Important routes or factors are likely to be obstetric-related and infection-prevention-related (e.g. suboptimal hand hygiene among mothers and healthcare workers, ineffective environmental and equipment cleaning) particularly in low-resource settings [4,9,50]. Notably, a well-powered systematic review and meta-analysis, by Chan *et al.*, reported that neonatal infection was more likely among newborns of mothers with obstetric risk factors for infection (defined as premature rupture of membranes, preterm premature rupture of membranes, prolonged rupture of membranes) [4]. Our study has a number of strengths. To the best of our knowledge this is the first synthesis of molecular evidence for MDR-GNB transmission from colonized mothers to their infants, providing both narrative and meta-analyses. Second, our study has provided the pooled proportions of neonatal outcomes of MDR-GNB transmission from colonized mothers. Third, although observational studies are considered low-quality studies, we only included studies with moderate or high quality based on NOS score. Finally, we used random effects meta-analyses to account for heterogeneity of the included studies. Our study has highlighted the need for further research on the determinants of maternal colonization with MDR-GNB.

This study's limitations include the small number of studies due to the paucity of research on this subject, preventing general extrapolation of the findings. When analysing the proportion of neonatal sepsis resulting from MDR-GNB transmission from colonized mothers, a wide 95% CI lends itself to an imprecise estimate. The nature of samples was variable — including genital, urinary and faecal/rectal — and this might have overestimated the colonization and/or transmission rates. None of the included studies reported on risk factors for MDR-GNB transmission from colonized mothers to infants.

In conclusion, although limited by the number and quality of studies, molecular evidence supports transmission of MDR and/or ESBL Enterobacteriaceae from colonized mothers to their infants, resulting in neonatal colonization. Further high-quality research is needed to determine the true burden and to identify predictors of mother-to-infant MDR-GNB transmission.

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Chapter 7:

Conclusion & Future directions

The aim of this doctoral thesis was to establish the burden and determinants of maternal colonization with MDR-GNB in a South African cohort of peripartum women. First, existing evidence was systematically reviewed to report the prevalence of maternal colonization with MDR-GNB and associated risk factors in Africa. Then, the prevalence of and risk factors for maternal colonization with MDR-GNB was investigated in a South African cohort of peripartum women. Next, we assessed the knowledge, attitudes and practices of pregnant women regarding antibiotic use during pregnancy. The role of the hospital environment and its effect on colonization and transmission of AMR genes to hospitalized peripartum women and neonates was determined. We measured the density of hospital environmental contamination with AMR genes and correlated this with the AMR gene carriage rate in women and neonates during their admission. Lastly, we reviewed the molecular evidence supporting MDR-GNB transmission from colonized mothers to their neonates.

This thesis revealed new information on the magnitude of and risk factors for maternal colonization with MDR-GNB in an African context. A systematic review and meta-analysis, determined that the prevalence of maternal colonization with ESBL-E in Africa was 17%, which is 2 to 3-fold higher than that reported in high-income countries. A major knowledge gap regarding the risk factors for colonization with resistant pathogens in pregnant/post-partum women in Africa was demonstrated.

An analytical cross-sectional study at a tertiary obstetric unit in Cape Town, assessed rectal carriage of AMR genes among peripartum women and established risk factors for acquisition of these genes. The prevalence of maternal colonization with ESBL genes (CTX-M15) was 12.9% and carbapenemase genes (NDM) was 2.2%. The rate of maternal colonization with CTX-M15 genes in our South African cohort was 2-fold higher than in high-income countries but lower than that reported from Madagascar and Tanzania (18.5% [6] and 15% [223] respectively). Maternal colonization with carbapenemase genes in our cohort was predicted by poverty-related factors like low educational achievement, low income and rural residence, possibly related to inadequate hygiene and unsafe water. Primiparous status was also identified as a risk factor. Maternal colonization with ESBL genes was linked to the use of communal taps as the primary water source reflecting multiple usage and potential contamination of water sources at community level. Recent hospitalization and antibiotic use did not predict maternal colonization with AMR genes, nor did comorbidities such as HIV and diabetes.

In this cohort of pregnant women, many risk factors for acquisition of AMR genes were related to household and demographic characteristics, suggesting that community-acquisition is the predominant route of MDR-GNB

transmission in pregnant women in South Africa. Urgent interventions are needed to address challenges in basic hygiene, access to safe water and sanitation and to enhance public awareness of antimicrobial resistance and its threat to human health. These interventions should be contextually grounded, taking into account the local demographics of the pregnant population and their community.

Building on the abovementioned study findings regarding risk factors for maternal rectal colonization with MDR-GNB producing AMR genes, we conducted a study on knowledge, attitudes and practices of pregnant women regarding antibiotic use in pregnancy, at the same tertiary hospital. Pregnant women with low knowledge scores were more likely to self-medicate with antibiotics compared to those with higher knowledge scores. Higher monthly household income was independently correlated with increased self-medication behaviours in pregnant women, possibly due to greater discretionary purchasing. Of interest was that despite national regulations relating to antibiotic prescribing and dispensing, self-medication was possible because some dispensers were prepared to supply antibiotics without prescription. Public health interventions should aim to improve the use of antibiotics in pregnant women through education regarding the dangers of self-medication generally and during pregnancy, the role of antibiotics, and the legal framework for antibiotic prescribing and dispensing in South Africa. Antenatal clinic visits are an opportune time to implement educational programmes on antibiotic use among pregnant women.

An analytical cross-sectional study to measure the levels of hospital environmental contamination with AMR genes and colonization of hospitalized patients revealed interesting findings. First, the level of environmental contamination with AMR genes was 5.8% (low) particularly for the high-touch surfaces (3.6%), reflecting satisfactory environmental cleaning for high-touch surfaces but less so for low-touch surfaces (11.1%). High touch surfaces were more contaminated in the neonatal wards compared to the obstetric wards despite obtaining similar environmental cleaning scores (80%), possibly indicating greater intensity of clinical activity. The mothers by and large were healthy, and this was reflected in their lower rates of rectal carriage of AMR genes compared with the less immune competent neonates (2.8% vs 9.8% respectively). The neonates were clinically septic, on antibiotics, premature, of low birthweight and had medical devices in situ. Finally, the number of outcome events were too few to clearly establish a link between patients and the hospital environment however, more frequent cleaning of the neonatal wards is recommended, given the higher rates of patient colonization, which may lead to greater environmental contamination.

It has been established that maternal colonization is a risk factor for neonatal colonization and/or sepsis, particularly for Gram-positive pathogens such as Group B *Streptococcus*. However, there is a paucity of data linking maternal colonization with MDR-GNB neonatal infection, the latter being predominant neonatal pathogens in Africa. We conducted a systematic review and meta-analysis including studies utilizing molecular methods to confirm MDR-GNB transmission from colonized mothers to their neonates. The pooled proportion of MDR-GNB transmission resulting in neonatal colonization was high (27%), but a link between maternal colonization and neonatal infection could not be established. Although the evidence supports the transmission of MDR– and/or ESBL Enterobacteriaceae from

colonized mothers to their infants, albeit in small numbers, further high-quality research is needed to determine the true burden and identify predictors of mother-to-infant MDR-GNB transmission (which may differ from Gram-positive bacterial transmission).

The dearth of studies on risk factors for maternal colonization with MDR-GNB has clearly led to the underappreciation of the potential to prevent it through feasible interventions. Based on our findings and publications, this is an opportunity to strengthen the antenatal services to educate both services providers and consumers on maternal colonization with MDR-GNB. The content of these educational sessions (ideally held during ANC visits), may include simple messages relating to the effect of antibiotic resistance and threat to human health, especially for neonates. Mothers-to-be should be made aware of the risks of inappropriate use of antibiotics during pregnancy, particularly the risk of self-medication and the effect it has on the selection of antimicrobial resistance. Such education programmes for both service providers and mothers will increase their understanding and possibly reduce the unnecessary use of antibiotics. A national antimicrobial stewardship programme to include prescribers and dispensers exists and includes an education programme but the effect thereof has yet to be assessed.

An important finding on the role of inadequate access to safe water, and its relation to carriage of antibiotic resistance is supported by the WHO water, sanitation and hygiene (WASH) programme, which clearly links inadequate WASH conditions to antibiotic resistance and transmission. Communities and governing structures should be made aware of the role of unsafe water, suboptimal hygiene and other poverty-related factors in the spread of antibiotic resistance and subsequent maternal (and neonatal) colonization with MDR-GNB.

Based on this PhD thesis, future studies evaluating the risk factors for maternal colonization with MDR-GNB in Africa are needed. Robust evidence of MDR-GNB transmission from colonized mothers to their neonates using genotypic methods (pulsed-field gel electrophoresis, whole genome sequencing, etc.) demonstrating the transmission of MDR-GNB should be considered. Further, the results of MDR-GNB transmission from either the mother, the hospital environment or both, to the neonate resulting in severe consequences requires in-depth scrutiny. Such studies could include follow up, before and after or interrupted time series studies.

In summary, this doctoral thesis has determined the magnitude of maternal colonization with MDR-GNB in Africa and identified several risk factors for maternal colonization with MDR-GNB in a cohort of pregnant women in Cape Town. The influence of knowledge, attitude and practices regarding antibiotic self-medication behaviour shed light on certain community practices. The association between contamination of the hospital environment with AMR genes and rates of AMR gene rectal carriage in peripartum women and hospitalized neonates was a pertinent exercise to demonstrate the potential transmission links between the hospital environment, mothers and their neonates. However, in our study, the hospital environment played a minor role, if any, in MDR-GNB transmission in this group of patients. Lastly, we reviewed evidence from studies using molecular assays, demonstrating strong evidence for mother-to-child transmission of MDR-GNB resulting in neonatal colonization. Further studies are warranted to determine risk factors

for neonatal colonization with MDR-GNB particularly from mothers. Intensified efforts to reduce the prevalence of maternal MDR-GNB carriage may improve neonatal outcomes, through reduced neonatal colonization as the precursor to neonatal infection.

Many of the actions needed to reduce maternal colonization with MDR-GNB, should be at the community level (where transmission of antibiotic resistant pathogens is established). This would include improved access to safe water and sanitation, as well as public education on antibiotic resistance. Furthermore, the benefits of early antenatal care visits for the wellbeing of both the mother and her child should be emphasized to pregnant women, clinicians and policymakers. Similarly, at healthcare facility level, the potential for transmission of pathogenic and antibiotic-resistant bacteria should be highlighted to staff, and efforts to reduce spread should be intensified through enhanced hand hygiene compliance and environmental cleaning.

Appendices

Publications related to infection prevention and control, antimicrobial resistance and public health (2016 – 2019)

[1]   **Bulabula ANH**, Dramowski A, Mehtar S. Maternal colonization or infection with extended- spectrum beta-lactamase-producing Enterobacteriaceae in Africa: A systematic review and meta-analysis. Int J Infect Dis 2017. doi:10.1016/j.ijid.2017.08.015.

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Presentation related to PhD research (2016-2019) at international conferences

1. Bulabula ANH, Dramowski A., Mehtar S, Maternal colonization or infection with extended-spectrum beta-lactamase-producing Enterobacteriaceae in Africa: A systematic review and meta-analysis, 7th ICAN Congress, Cape Town, South Africa 8 - 11 July 2018 – Oral presentation

Participation as a peer reviewer of conference abstracts, journal articles and educational resources

1. Infection Control Africa Network – ICAN Conference 2016
2. Infection Control Africa Network – ICAN Conference 2018
3. 18th ICID Trainee Track Organizing Committee, the 18th International Congress on Infectious Diseases (18th ICID) Trainee Track in collaboration with the Sociedad Argentina de Infectiologia (SADI) in Buenos Aires, Argentina, from March 1-4, 2018
4. Journal of Antimicrobial Chemotherapy – Antimicrobial Resistance (JAC-AMR), Associate Editor

Acknowledgments

I dedicate this PhD thesis to my parents, Prof Dr Ali Bulabula and Mme Esperance Aziza Amisi for their love and the seeds they had planted in me: working hard towards achieving excellence for a better humanity.

My special gratitude and thanks to my Supervisor, Prof Shaheen Mehtar for her willingness to supervise me and her permanent support and encouragement towards starting and completing my doctoral studies. Her unique mentorship, scientific rigour and her humanity have built my scientific authority.

My special thanks also to my Co-Supervisor, Prof Angela Dramowski for her unique guidance and meticulous review of every single detail, her support and encouragement kept me motivated. I still have to learn how she has developed such amazing presentation skills and writing.

My special thanks and appreciation go to my wife, Rosalie Lunjwire and our daughter, Rosandy-Sarah Bulabula for their love, support and patience, without which I would have not reached this level. Many thanks to my family (Juni, Victor, Frank, Marie-Arnold and Charles, Bulabula) friends and colleagues for their support, you are special to me!

Many thanks to the Infection Control Africa Network – ICAN colleagues, to research nurses, Srs Mary Frans, Bojana Zoleka, Swanepoel and Marvina Johson for data collection, to Mrs Fiki Peter the data capturer, their support made this work possible. My thanks to Sr Marina Aucamp and Hewitt de Jager for their support.

Many thanks to Tygerberg Hospital Management, the Unit for Infection Prevention and Control, the BARNARDS Team Cape town.

Funding

List of abbreviations

AMR: Antimicrobial Resistance

AMS: Antimicrobial stewardship

ANC: Antenatal Care

A-score: Attitudes score

bla: β-lactamase

CDC: Centers for Disease Control and Prevention

CI: Confidence Intervals

CTX-M: Cefotaxime hydrolyzing capabilities

DDST: Double Disk Synergy Test

ESBL: Extended-spectrum beta-lactamases

ESBL-E: Extended-spectrum beta-lactamase producing Enterobacteriaceae

GNB: Gram-negative bacteria

HIV: Human Immunodeficiency Virus

KAP: Knowledge, Attitudes and Practices

K-score: Knowledge score

MDR: Multidrug-resistant

MDR-GNB: Multidrug-resistant Gram-negative bacteria

MeSH: Medical Subject Headings

NDM: New Delhi Metallo-beta-lactamase

OTC: Over-the-counter

OR: Odds Ratio

PCR: Polymerase Chain Reaction

PFGE: Pulsed-field gel electrophoresis

P-score: Practices score

SD: Standard deviation

SHV: Sulfhydryl variable

TEM: Temoneira

TBH: Tygerberg Hospital

WASH: Water, Sanitation and Hygiene

WHO: World Health Organization

Modified BARNARDS Questionnaire (for Chapter 3)

1. General information

    1.1 Name of the person collecting data:

    1.2 Mother's study code number:

2. Hospital information

    2.1 Ward number/name:

    2.2 Bed number/name:

    2.3 Type of ward:

☐ Accident and emergency (A&E)	☐ Maternity departments
☐ Cardiology	☐ Neonatal unit
☐ Critical care	☐ Obstetrics and gynaecology units
☐ General surgery	☐ Sexual health (genitourinary medicine)
☐ Gynaecology	☐ Other:

    2.4 Number of beds in this ward:

    2.5 How many bathrooms are in this ward?

        ☐ 0
        ☐ 1-2
        ☐ 3-4
        ☐ >5

    2.6 Where is the mother situated on the ward?

        ☐ First 1/3 (closest to door)
        ☐ Middle 1/3
        ☐ Last 1/3 (furthest from door)
        ☐ Side room – How many side rooms:
        ☐ Other:

3. Information on the mother

    3.1 Age of mother in years (write unknown if estimation):

    3.2 Is this the mothers first pregnancy:

    3.3 Number of previous pregnancies (1 – 10 or 10+):

    3.4 Number of living female children (1 – 12):

    3.5 Ages of living female children:

    3.6 Number of living male children (1 – 10, 10+):

    3.7 Ages of living male children:

    3.8 Has the mother had multiple births:

        (Detail age and genders)

    3.9 Number of miscarriages (0 – 10+, would rather not answer):

    3.10 Number of abortions (0 – 10+, would rather not answer):

    3.11 Number of stillbirths (0 – 10+, would rather not answer):

    3.12 Number of deceased children (0 – 10+, would rather not answer):

        Please provide detail of decreased children:

            Ages

            Gender

            Cause of death

    3.12 In the past three months has the mother suffered with any of these illnesses:

☐ Diabetes
☐ Hypertensions or cardiovascular disease

- Immune compromised
  - Cancer
  - HIV
  - Chronic liver diseases
  - Use of steroids
- TB
  - Has she received TB therapy
    - Yes
    - No
    - Don't know
- Malaria
- None
- Other

3.13 Has the mother attended a private healthcare clinic in the last three months?

3.14 Has the mother seen a traditional healer in the last three months?

3.15 Has the mother travelled outside the following in the past 12 months? (If yes where)

- City:
- Province:
- Country:

3.16 Has a household member travelled outside the following in the past 12 months? (If yes where)

- City:
- Province:
- Country:
- No
- Don't know

3.17 Has the mother been hospitalised in the past 12 months

- Yes
  - Name of hospital:
  - Length of stay:
  - Reason for stay:
- No
- Unsure

3. 18 Has the mother used antibiotics in the last three months (oral or IV)

- No
- Don't know
- Yes

- o Amikacin
- o Amoxicillin
- o Ampicillin
- o Aztreonam
- o Azithromycin
- o Carbecillin
- o Cefaclor
- o Cefadroxil (cefadroxyl)
- o Cefalxin (cephalexin)
- o Cefaloridine (cephaloridine)
- o Cefamandole
- o Cefazolin (cephazolin)
- o Cefditoren
- o Cefepime
- o Cefixime
- o Cefotaxime
- o Cefotetan
- o Cefoperzone
- o Cefoxitin
- o Cefopodoxime
- o Cefradine (cephradine)
- o Ceftaroline
- o Ceftazidime
- o Ceftibuten
- o Ceftiolene
- o Ceftizoxime
- o Ceftobiprole
- o Ceftriaxone
- o Cefuroxime
- o Chloramphenicol
- o Ciprofloxacin
- o Clarithromycin
- o Clindamycin
- o Cycloserine
- o Doripenem
- o Doxycycline
- o Ertapenem
- o Erythromycin
- o Flucloxacillin
- o Fosfomycin
- o Gentamicin
- o IImipenem
- o Kanamycin
- o Levofloxacin

- o Lincomycin
- o Linezolid
- o Meropenem
- o Metronidazole
- o Minocycline
- o Moxifloxacin
- o Nalidixic acid
- o Neomycin
- o Nitrofurantoin
- o Norfloxacin
- o Ofloxacin
- o Oxacillin
- o Oxytetracycline
- o Penicillin G
- o Piperacillin
- o Polymyxin B
- o Pristinamycin
- o Quinupristin/ dalfopristin
- o Rifabutin
- o Rifampin
- o Streptomycin
- o Sulfamethoxazole
- o Telithromycin
- o Teicoplanin
- o Tetracycline
- o Ticarcillin
- o Tigecycline
- o Tobramycin
- o Trimethoprim-Sulfamethoxazole
- o Vancomycin
- o Unknown
- o Other:

4. Sociodemographics of the mother

4. 1 Overall household income per mother:

- ☐ $20
- ☐ $20 - $30
- ☐ $30 - $40
- ☐ $40 - $50
- ☐ $50 - $100
- ☐ $100 - $250
- ☐ $250 - $500
- ☐ $500 - $1000
- ☐ $1000 - $2000

4.2 What is the education status of the mother?

- ☐ None
- ☐ Can read and write
- ☐ Primary school
- ☐ Secondary school
- ☐ College / A-levels
- ☐ Undergraduate
- ☐ Graduate
- ☐ Postgraduate

4.3 How would the mother describe the residential area she lives in?

- ☐ Rural
- ☐ Urban
- ☐ Semi-rural
- ☐ Other:

4.4 What type of residence does the mother live in:

- ☐ Apartment
- ☐ Separate house
- ☐ Shack
- ☐ Homeless
- ☐ Other:

4.5 Number of rooms in entire residence (1 – 10+):

4.6 Number of people residing there (1 – 10+):

4.7 What is the primary source of drinking water for the household

- ☐ Municipal network
- ☐ Water vendor (tanker)
- ☐ Private well
- ☐ Communal taps
- ☐ Other:

4.8 Is the drinking water

- ☐ Boiled
- ☐ Filtered
- ☐ Neither

4.9 What is the primary water source used for

- ☐ Domestic water
- ☐ Drinking water
- ☐ Both

4.10 How any hours a day does the household have running water

- ☐ No supply
- ☐ Less than 4

- ☐ 5 – 12
- ☐ More than 12
- ☐ Access via communal taps
- ☐ Other:

4.11 How many days per week does the household have running water

- ☐ No supply
- ☐ Less than 1
- ☐ 1
- ☐ 2 – 3
- ☐ 4 – 6
- ☐ Continuous supply

4.12 Is there a solid waste pile near the mother's home (proximity of 100m)

- ☐ No
- ☐ Yes
- ☐ How frequently is the solid waste pile collected
  - o Once a week or more
  - o Every two weeks
  - o Every 2 months
  - o We deal with it ourselves
  - o Other:

4.13 What sort of toilet does the mother have within her home

- ☐ Sit down with flush
- ☐ Squat with flush
- ☐ Pit latrine
- ☐ Other:

4.14 ANTENATAL CARE

Number of ANC visits during this pregnancy: _____
Gestational Age at: _____First visit _____ Last visit _____
Dates of ANC visits: _____
Services received at ANC visits: _____
HIV test (VCT) result: _____ (Positive / Negative), if positive, on ART? _____
Diabetes / high blood sugar: _____ (Yes/ No), if yes, are you on chronic medication: _____
Urine tested: _____ (Yes/No)   Urine test result: _____ infection (Yes/No)
Use of antibiotics during pregnancy: _____ (Yes/No) _____
Malnutrition (was the mother malnourished): _____ (Yes /No)

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