NAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY

THE DISTRIBUTION OF *ESCHERICHIA COLI* IN PREGNANT WOMEN AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF THE ISOLATES IN SELECTED RURAL AREAS IN NAMIBIA

By

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DECLARATION

I, Lea Nangolo hereby declare that the work contained in the thesis entitled The distribution of *E. coli* in pregnant women and antimicrobial susceptibility pattern of the isolates in selected rural areas in Namibia is my own original work and that I have not previously in its entirety or in part submitted it at any university or other higher education institution for the award of a degree.

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ACKNOWLEDGEMENTS

DEDICATION

I gratefully dedicate this work to my daughter

NDINELAGO JONAS

AND

All mothers who participated in the study, without you I would not have been able to accomplish this

study.

ABSTRACT

BACKGROUND: *Escherichia coli* (*E. coli*) is a bacterium that can asymptomatically colonize a woman's vagina and up to 31% of pregnant women are colonized worldwide. Furthermore, vaginal colonization with *E. coli* is associated with adverse pregnancy outcomes, such as preterm birth and stillbirth. It is also associated with neonatal sepsis or meningitis when transmitted to the foetus through amniotic fluid or to the neonate during delivery. To date in Namibia data on the colonization and the antibiotic susceptibility patterns of *E. coli* in pregnant women is sparse. This study aimed at determining the prevalence of *E. coli*, antimicrobial susceptibility patterns and genetic based resistance of the isolates from pregnant women at 35 weeks of gestation and above, who attended antenatal screening at Eenhana, Okongo, Onandjokwe state hospitals.

STUDY DESIGN: The descriptive cross-sectional study targeted pregnant women who were at 35 weeks of gestation and above. A convenience sampling technique was used to recruit 208 pregnant women for the study. Both qualitative and quantitative data were collected using structured questionnaires. A participant consent form was signed prior to recruitment in the study and socio-demographic information was captured in a questionnaire by registered nurses. A lower vaginal swab was collected from each participant.

RESEARCH METHODOLOGY: In total, 208 lower vaginal swabs were collected from participants by registered nurses. Samples were transported to the laboratory using a cold chain system. All swabs collected were cultured on MacConkey agar and the growth was confirmed on Chromogenic Agar. Presumptive *E. coli* isolates were confirmed by molecular techniques using the real time Polymerase Chain Reaction (PCR). Gene confirmed *E. coli* isolates were tested against selected antibiotics for empiric treatment of patients using the Kirby Bauer Disk Diffusion method and results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guideline (2018). All isolates were also screened for the presence of selected β-lactam genes [*blaTEM*, *blaFOX*, *blaCTXM*, *blaSHV* and *blaMOX*] using conventional PCR. Statistical analysis was performed using the Bonferroni's Multiple Comparison Test, and a *P* value < 0.05 was considered statistically significant.

RESULTS: Out of a total of 208 pregnant women screened, thirty-one (14.9%) were colonized by *E. coli*. Maternal *E. coli* colonization was high in pregnant women between the ages of 20-39 (11.5%), those with a low level of education (10.1%), unemployed (13.0%), unmarried (12.5%) and rural dwelling (12.5%). The study also revealed that *E. coli* was largely isolated from pregnant women with no history of miscarriage (12.5%) and stillbirth (13.9%), while *E. coli* was prevalent in women with 1-2

parity (7.7%). Significant association was reported between *E. coli* colonization and maternal age, habitat, marital status, education, employment, parity (*P* values <0.05). Antimicrobial resistance against Ampicillin (72.2%), Ciprofloxacin (2.7%), Gentamicin (13.9%), Nalidixic acid (8.3%) and Trimethoprim /Sulfamethoxazole (61.1%) were seen. Piperacilin/tazobactum (2.7%), Ampicillin (25%), Gentamicin (2.8%) and Nalidixic (2.8%) showed intermediate resistance. No antimicrobial resistance was seen against Cefuroxime, Cefoxitin, Ceftazidime, Amikacin, Imipenem, Meropenem, Ertapenem and Cefepime. The most commonly detected *bla* genes were *blaTEM* and *blaSHV* among *E. coli* isolates

CONCLUSION: *E. coli* prevalence amongst the screened women in the study was 14.9%. Most risk factors showed statistical significance with maternal *E. coli* colonization. A high resistance to Ampicillin was reported which was not surprising, since studies have shown that *E. coli* isolates have a high Ampicillin resistance rate. It may be important to screen and treat pregnant women for genital tract colonization with *E. coli* during prenatal care.

Key words: *E. coli*, pregnant women, antimicrobial susceptibility pattern, gene based resistance, rural Namibia

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LIST OF SYMBOLS

Symbol	Meaning
°C	Degree Celsius
µg	Micro gram
µl	Micro litre
µM	Micro moles
L	Litre
ML	Millilitre
MM	Millimetre
Rpm	Revolutions per minute
µg/ml	Microgram per millilitre
Bp	Base pairs

LIST OF ABBREVIATIONS

AIEC	Adherent Invasive *E. coli*
AMR	antimicrobial resistance
AST	Antimicrobial susceptibility testing
CDC	Centres for Disease Control
CLSI	Clinical Laboratory Standards Institute
DA	diffuse adherence
DAEC	Diffusely Adherent *E. coli*
DDW	Double Distilled Water
DNA	Deoxy Ribonucleic Acid
E. coli	*Escherichia coli*
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EOS	Early-onset sepsis
EPEC	Enteropathogenic *E. coli*
ESBL	Extended Beta-Lactamase
ETEC	Enterotoxigenic *E. coli*
ExPEC	Extraintestinal *E. coli*
GBS	group B *Streptococcus*
HUS	hemolytic uremic syndrome
MDR	multi-drug resistance
MOHSS	Ministry of Health and Social Services
NMEC	Neonatal Meningitis *E. coli*
NUST	Namibia University of Science and Technology
PABA	Para-aminobenzoic acid
PAI	pathogenicity islands
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Disease
PROM	Preterm rapture of the membranes
PTB	Preterm birth
SPSS	Statistical Package for Social Sciences
STEC	Shiga toxin-producing *E. coli*
UPEC	Uropathogenic *E. coli*
UTI	Urinary Tract Infections
VEC	Vaginal *E. coli*

WHO   World Health Organisation

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

E. coli is an enteric gram-negative bacterium that belongs to Enterobacteriaceae family. Naturally, it inhabits the intestinal tracts of humans and animals and is released into the environment through deposition of fecal material. Furthermore, *E. coli* inhabits the female genital tract as a normal flora (Devi *et al.*, 2014; Guiral *et al.*, 2010; Lebea & Davies, 2017; Pathak *et al.*, 2013). According to Saez-Lopez *et al* (2016), *E. coli* is reported as one of the most common organisms found in the genital tract of non-pregnant (9–28%) and more prevalent in pregnant women (24–31%) (Devi *et al.*, 2014; Saez-Lopez *et al.*, 2016). Vaginal *E. coli* (VEC) strains are known to be a reservoir for vaginal and/or endocervical colonization in pregnant women, and individual cause of urinary tract, intra-amniotic and puerperal infections through 'fecal-vaginal-urinary/neonatal' transmission (Saez-Lopez *et al.*, 2017).

There are various virulent components or factors that promote the virulence state of *E. coli* such as papG alleles I, II, and III; *papA*; *papC*; *papE*; *sfaS*; *focG*; *afa-draBC*; *fimH*; *hlyA*; *cnf1*; *iutA*; *irp2*; *iroN*; *kpsMT II*; *kpsMK I*; *traT*; and *malX* that are responsible for pathogenicity of *E. coli* strains. These factors have different properties such as adhesins, capsules and toxins (Devi *et al.*, 2014; Guiral *et al.*, 2010; Kuhnert *et al.*, 2000; Lavigne *et al.*, 2011; Saez-Lopez *et al.*, 2016; Watt *et al.*, 2003). Virulence factors are made in such a way that they work together to transform *E. coli* which is part of the normal flora into invasive *E. coli* strains. A single factor alone cannot change non-invasive *E. coli* into invasive *E. coli* (Kuhnert *et al.*, 2000).

Several factors such as exposure to spermicides, Non-secretor phenotype, high oestrogen level, improper usage of antibiotics and multiple sexual partners promote vaginal *E. coli* colonization in women of child bearing age (Hooton, 1999).

The production of lactic acid by lactobacilli has long been known to play a protective role of normal bacterial flora against vaginal infection (Hawes *et al.*, 1996). However, during pregnancy, lactobacilli decrease leading to vaginal infectious bacteria to take over and cause infections (Anders *et al.*, 1994). A study done by Son *et al.* (2018) reported that *E. coli* colonizing isolates can cause ascending infection or can be transmitted to neonates during birth leading to early and late neonatal sepsis. *E. coli* is reported as the predominant organism responsible for neonatal meningitis which remains a major cause of death and neurological consequences in neonates (Boyer-Mariotte *et al.*, 2008, Moissenet *et al.*, 2010).

Clinically, not all *E. coli* vaginal infections are accompanied by clinical symptoms, in most cases they are asymptomatic and this makes it more difficult for proper laboratory diagnosis and treatment

(Saez-Lopez *et al*., 2016; Lebea & Davies, 2017). Therefore, maternal infection by *E. coli* c can be prevented by proper screening of pregnant mothers and the bacteria can be treated through proper laboratory diagnosis, as well as adequate antibiotic administration. However, inappropriate use of antibiotic is the major burden for antibiotic resistance and helps horizontal transmission of bacterial resistance to neonates (Pathak *et al*., 2013). Several studies worldwide have reported emergence of drug resistant *E. coli* isolates among pregnant women, as well as neonates (Manges *at al*., 2001, Johnson *et al*., 2010, Pathak *et al.,* 2013, Villar *et al*., 2013). There is a rise of Extended Beta-Lactamase (ESBL) bacteria and one of their unique characteristics is that they produce β -lactamase enzymes that promote resistance of *E. coli* against broad spectrum of antibiotics (Hammerum & Heuer, 2009, Al-Mayahie, 2014). Various genes such as *blaTEM*, *blaCTX-M* and *blaSHV* are commonly associated with *E. coli* resistance to β -lactam antibiotics (Moissenet *et al.,* 2010, Boyer-Mariotte *et al*., 2008).

Clinical screening of *E. coli* in antenatal women in this study for antibiotic resistance patterns will provide guidelines for proper diagnosis and factual remedy particularly for sick new-borns. In addition, molecular analysis of the resistant isolates will reveal the genes associated with *E. coli* resistance to antibiotics. To date, the knowledge about the predominance of vaginal *E. coli* colonization and carriage of resistant *E. coli* during pregnancy is lacking in general and especially in Namibia. Therefore, this study aimed to determine the distribution of *E. coli* in pregnant women, as well as to assess the molecular characterization of resistant genes within resistant *E. coli* isolates from selected rural settings in Namibia.

1.2 Literature review

1.2.1 *E. coli* overview

E. coli belongs to the Enterobacteriaceae family that includes other familiar pathogens, such as *Salmonella*, *Yersinia*, *Klebsiella*, *Shigella*, *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter*. These bacteria are gram negative facultative anaerobes that ferment glucose, are oxidase negative and reduce nitrate to nitrites. Furthermore, Enterobacteriaceae are commonly catalase positive except *shigella dysenteriae* type 1. It is a facultative anaerobic that live in the intestinal tracts of warm-blooded animals. Furthermore, *E. coli* is among the most important bacteria and quite a number of genera within the family are human intestinal pathogens (e.g. *Salmonella, Shigella, and Yersinia*). Several others are commensal of the human gastrointestinal tract (e.g. *Escherichia, Enterobacter, and Klebsiella*). However, these bacteria may as well sometimes be associated with human diseases (Sousa, 2006). *E. coli* is non-spore forming bacillus that is about 0.5 µm in diameter and 1.0-3.0 µm in length. Its bacterial membrane has a single layer of peptidoglycan with a typical subunit structure

where the N-acetylmuramic acid is linked by an amide bond to a peptide consisting of L-alanine, D-glutamic acid, meso-diaminopimelic acid and finally D-alanine. In addition, *E. coli* are commonly motile in liquid media by means of flagella. Most *E. coli* strains are capable of growing over a wide range in temperature with the growth rate maximum range of 37-42 °C and can grow within a pH range of about 5.5-8.0 (welch, 2006)

Physiologically, *E. coli* has certain characteristic that helps it to adapt in the environment. It can grow on media with glucose as the only organic component. The bacterium can grow both aerobically (in the presence of oxygen) and anaerobically (without oxygen). Under anaerobic conditions it will grow by means of fermentation, producing characteristic "mixed acids and gas" as end products. In addition, *E. coli* can also grow by means of anaerobic respiration, since it is capable of utilizing nitrate to nitrite. Therefore, this helps *E. coli* to stay in the intestines (anaerobic environment) and extraintestinal (aerobic or anaerobic) (Sousa, 2006).

E. coli responds to environmental signals such as chemicals, pH, temperature, osmolality, and other stimulants in a number of very outstanding ways because it is a single-celled organism (Sousa, 2006). This means that, it can detect the presence or absence of chemicals and gases in its environment and swim towards or away from them. Apart from swimming, *E. coli* can also develop fimbriae capable of attaching to the host receptor; possess an outer membrane with different diameters of pores, adapt to accommodate larger molecules and also devise inhibitory substances to respond to changes in temperature and osmolality. Furthermore, *E. coli* does not produce enzymes for degradation of carbon sources except they are available and it does not produce enzymes for synthesis of metabolites if they are available as nutrients which make it survive the chemical components in the environment (Sousa, 2006).

There are various types of *E. coli* species, ranging from very specialized pathogenic strains (that cause worldwide outbreaks of severe diseases), opportunistic pathogens (which possibly cause disease in the immunocompromised human host) and non-virulent isolates (which are part of the normal intestinal flora). These species include intestinal diarrheagenic *E. coli* and extraintestinal pathogens (Sousa, 2006).

1.2.1 *Escherichia coli*: ecology and public health implications

E. coli includes not only commensal strains but also pathogenic ones that cause a variety of human diseases— resulting in more than 2 million deaths each year (Kaper *et al*. 2004). There are six well-studied intestinal pathotypes of *E. coli*, including Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* and enteroinvasive *E. coli*, including Shigella strains. These strains are classified by virulence properties and pathogenicity mechanisms causing gastrointestinal diseases

such as diarrhea (Nataro and Kaper 1998; Kaper *et al*. 2004). Enterohaemorrhagic E. *coli* (EHEC) is one type of STEC that can cause severe enteric diseases, such as haemolytic muraemic syndrome and haemorrhagic colitis (Kaper *et al*. 2004) Pathogenic *E. coli* strains are implicated in many waterborne outbreaks, and STEC and EPEC have been frequently reported to be responsible for waterborne outbreaks worldwide (Chandran and Mazumder 2015).

Pathogenic *E. coli* contamination of the environment may occur through manure and other animal wastes, wastewaters from slaughterhouses and effluent from wastewater treatment plants (Baliere *et al*. 2015). Although extensive studies have been done on the clinical aspects of the pathogenic *E. coli* strains, including mode of pathogenesis, diagnosis and sources (Kaper *et al*. 2004; Croxen *et al*. 2013), their prevalence in the environment has not been extensively examined in greater detail.
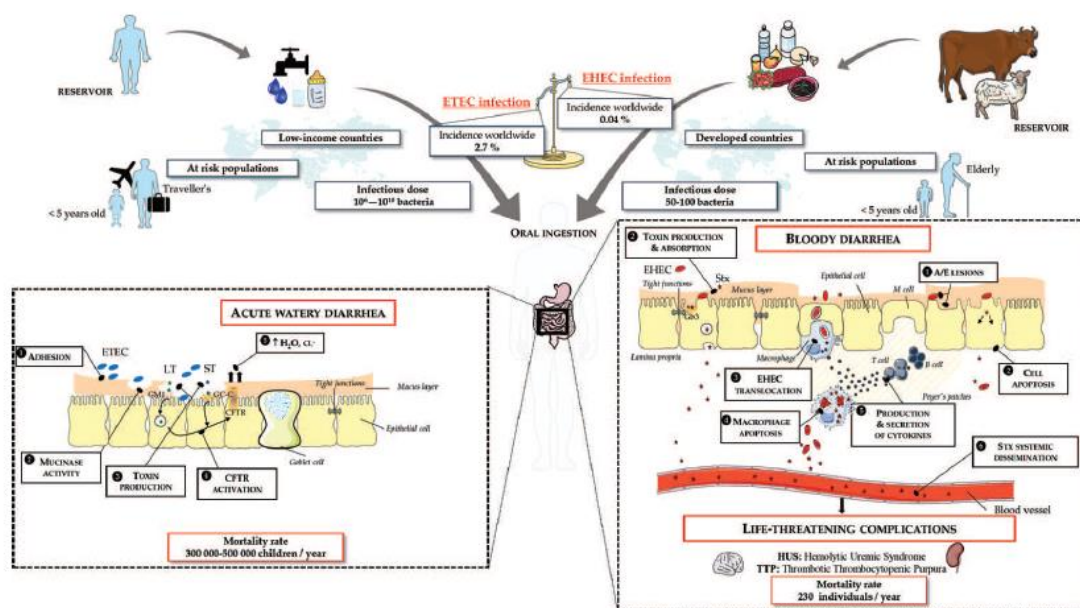
Figure 1.1: *Escherichia coli*: ecology and public health implications **(**Adopted from Roussel *et al., 2017).*

1.2.2   *E. coli* Epidemiology

Bacterial infections during pregnancy remain a significant complication. It is predicted that infections can be accountable for 10–25% of fetal deaths in developed countries and more in developing countries (Sacerdoti *et al*., 2018). Bacterial infections more commonly associated with adverse outcomes such as sepsis, miscarriage, stillbirth etc. (Sacerdoti *et al*., 2018). Bacterial infections are especially feared because they may compromise not only the mother but also her child (Sacerdoti *et al*., 2018).

Sepsis is now the most common cause of direct maternal death (Surgers *et al*., 2014). The incidence of bacteraemia during pregnancy varies from 3 to 7.5 for 1000 pregnancies (Surgers *et al*., 2014). This

is also life-threatening for foetuses with 10% of foetal death in a retrospective study (Surgers *et al*., 2014). *E. coli* is one of the most common organisms involved in bacteraemia recorded in the general population and during pregnancy (Surgers *et al*., 2014). Knowles *et al*. (2015) reported that maternal sepsis has become uncommon in developed countries following the introduction of antibiotic therapy, improvement in social infrastructure and systematic use of infection control measures in healthcare. However, bacterial sepsis still occurs and severe sepsis may result in maternal death. Furthermore, the incidence of maternal sepsis is reported to be 0.1–0.3% (Knowles *et al*., 2015). It has significantly reduced since the 1970s, when the incidence of obstetric bacteraemia was reported to be 0.7–0.75% (Knowles *et al*., 2015). The most common source of bacteraemia during pregnancy is chorioamnionitis or genital tract infection. Bacteraemia during pregnancy is associated with a 10–28% foetal mortality (Knowles *et al*., 2015). In addition, sepsis at any stage of pregnancy can be serious and possibly life-threatening. In the study conducted by Knowles *et al*. (2015) *E. coli* was the main pathogen isolated from pregnant women with sepsis, accounting for overall 37% of all sepsis cases: 55% of antenatal, 22% of intrapartum and 42% of postpartum cases (Knowles *et al*., 2015).

A urinary Tract Infections (UTIs) result from bacterial infection or vaginitis and it is common during pregnancy (Perera *et al*., 2012). These infections could either be symptomatic or asymptomatic. UTIs may manifest as asymptomatic bacteriuria, acute cystitis or pyelonephritis (Perera *et al*., 2012). If bacteriuria during pregnancy is not treated it may be associated with adverse maternal and perinatal outcomes. Worldwide asymptomatic bacteriuria affects 2–10% of all pregnant women (Perera *et al*., 2012). Adverse maternal outcomes include symptomatic cystitis in 30% cases and development of pyelonephritis in 20% of cases and acute respiratory distress syndrome in 2% (Perera *et al*., 2012). The related adverse foetal outcomes include prematurity, low birth weight and increased perinatal mortality. Furthermore, there are increased maternal risks reported for pre-eclampsia; anaemia; chorioamnionitis and post-partum endometritis in patients with significant bacteriuria (Perera *et al*., 2012). Foetal risks include foetal growth retardation, stillbirth, mental retardation and development delay (Perera *et al*., 2012).

In 2003, the incidence of neonatal *E. coli* infection was estimated to be between 0.2 and 5 per 1,000 live births. Thus, vaginal colonization, observed in 3 to 20% of pregnant women was the leading cause of neonatal infection during delivery (Watt *et al*., 2003).

In a year, around 215 million reproductive tract infection cases occur in the world (Mobasheri *et al*., 2014). A study by Tolosa *et al*. (2006) reported 24.4% predominance of reproductive tract infections among pregnant women. In addition, these vaginal infections were linked to significant risk of diseases in pregnant women, such as Pelvic Inflammatory Disease (PID), as well as long-term consequences such as tubal infertility, ectopic pregnancy and reproductive dysfunction (Siu-keung, 2011). Some studies have reported various organisms responsible for vaginal infection and *E. coli* was one of those organisms. According to these studies *E. coli* was either the most or the second most

organism isolated from pregnant women accounting for vaginal colonization of between 17% and 56.3% (Rad *et al*., 2016; Devi *et al*., 2014; Tameliene *et al*., 2012).

1.2.3   Mode of transmission

The mode of transmission of *E. coli* varies with the type of infection caused. Organisms may be endogenous or spread from person to person for non-gastrointestinal infections, particularly in hospital settings. For gastrointestinal infections, it varies with the type of *E. coli* organism although it, involves fecal-oral contamination spreading to human beings through contaminated food or water (Ishii and Sadowsky, 2008). *E. coli* may also inhabit the female genital tract (Dang, 2012; Donnenberg & Whittam, 2001; Ishii and Sadowsky, 2008; Lebea & Davies, 2017; Sáez-López *et al*., 2016;) and it can be acquired from sex partners (Hooton, 1999). Moreover, *E. coli* can be transmitted to the foetus through amniotic fluid or to neonates from *E. coli* colonized birth canal (Ulleryd *et al*., 2015).

1.2.4   Bacterial Pathogenesis

E. coli can be a harmless resident of the gastrointestinal tract. However, it also has the pathogenic capability to cause significant intestinal and extraintestinal diseases such as the urinary tract, bloodstream, and central nervous system. In addition, various pathogenic *E. coli* pathotypes studied in humans, animals, food, as well as the environment are responsible for many diseases and deaths in the world. The pathotypes possess different features that allow them to inhabit the intestinal mucosa and cause diseases in humans (Croxen *et al*., 2013).

Figure 1.3 below illustrates its cellular interaction with the host tissue, highlighting features relevant to its pathogenicity.
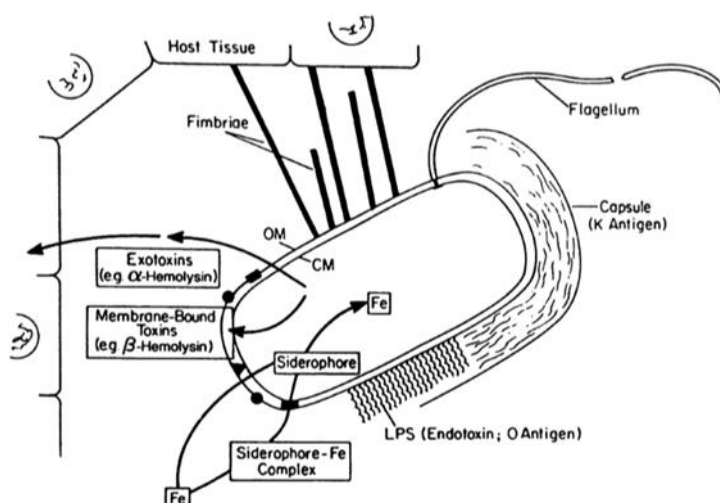
Figure 1.3: Bacterial cellular features (Adapted from Johnson, 1991)

1.2.5   Virulent factors

Though most *E. coli* bacteria are regarded harmless, small proportions are a significant cause of disease worldwide (Fairbrother & Nadeau, 2006). These possibly harmful *E. coli* are classified into categories established according to the production of virulence factors and on the clinical signs that they cause (Fairbrother & Nadeau, 2006).

The pathogenicity is a complex multi-factorial tool involving a large number of virulence factors which differ based on the "pathotypes". The virulent factors include attachment functions, host cell surface modifying factors, invasins, and many different toxins as well as secretion systems which export toxins and other virulence factors to the targeted host cells. The virulent components transform normal *E. coli* strain into a pathogenic strain, meaning that a component alone cannot turn harmless *E. coli* into a harmful strain (Kuhnert *et al*., 2000).

Toxins

Toxins are powerful pathogenicity factors produced by certain bacteria, fungi, animals and plants (Popoff, 2018). Moreover, some toxins show a strong association with specific pathotypes. The Shiga toxins Stx1 and Stx2, encoded by the genes *stx1* and *stx2*, respectively, are prominent toxins and give the name to the Shiga toxin-producing *E. coli* pathotype STEC (Kuhnert *et al*., 2000). Toxins have the ability to damage the extracellular matrix or the plasma membrane of eukaryotic cells. The damage may not only result in the direct lysis of cells but may also enable bacterial spread through tissues. Toxins that facilitate this cellular damage do so by either enzymatic hydrolysis or pore formation (Schmitt *et al*., 1999). Classically, bacterial toxins are divided into exotoxins and endotoxins. Endotoxins are membrane compounds of Gram-negative bacteria which produce an inflammatory response in host, while exotoxins are secreted proteins which act closely and at distance of the bacterial colonization site. Exotoxins are directly injected into the cell where the bacterium is attached by specific secretion types, such as the type III secretion system, and their activity is limited to the attacked cell (Popoff, 2018). CNF1 toxin causes HEp-2 cells in the host to produce large vacuoles for the bacteria to invade. Furthermore, the *cnf1* gene has been identified in *E. coli* K1 strains involved in meningitis (Soltani *et al*., 2018). The CNF1 toxins enter the host cell through receptor-dependent endocytosis (Soltani *et al*., 2018). CNF1 gene, located between the alpha-haemolysin (*hly*) operon and the adhesins encoding genes related Pap (*prs*). Practically all of the Uropathogenic *E. coli* strains to produce CNF1 toxins (about 30% of the *E. coli* strains are involved in urinary tract infections) contain the alpha-haemolysin gene (*hly*) in up-stream of the *cnf1* gene. It is

possible that CNF1, along with *hly* hemolysin are associated in a virulence mechanism that makes I beneficial for the bacteria (Soltani *et al.*, 2018).

Adhesions

Bacterial surface adhesion is a universal occurrence in the natural world, and as the first step in biofilm formation, it allows microbes to survive changing environments, chemical, physical assaults and depletion of resources (Friedlander *et al.*, 2015). Adhesion is the second major class of virulence determinants represents the adhesion factors including mostly fimbriae. However, type I fimbriae are not limited to pathogenic *E. coli* and can be found in most strains including the laboratory strain K-12, other fimbriae, in particular P fimbriae (*pap/prs*), F1C fimbriae (*foc*) and S fimbriae (*sfa*), are typical for UPEC (Kuhnert *et al.*, 2000).

E. coli has been well studied as a laboratory model organism in general, and in adhesion and biofilm formation in particular. It has been shown that specific flagella isoforms can allows attachment of some *E. coli* strains to mammalian antigens. For example, the flagella filament of *E. coli* adheres to porcine mucin and secrets protein, *EtpA* that can mediate the bond between the tips of enterotoxigenic *E. coli* flagella filaments and host cell surface receptors (Friedlander *et al.*, 2015)

Adhesions are encoded by: *afa-dra*, *daaD*, *tsh*, *vat*, *ibeA*, *fyuA*, *mat*, *sfa-foc*, *malX*, *pic*, *irp2*, and *papC* gene cluster but display distinct receptor specificities (Frömmel *et al.*, 2013).

Invasins

Invasion plasmid antigens are naturally found with frequent other invasion-associated factors in the EIEC pathotype and in Shigella. The gene product of *iucC* is involved in biosynthesis of aerobacteria and iucC is therefore an indicator for the presence of the aerobactin-mediated iron transport system in the organism. This gene is usually found in extraintestinal *E. coli* isolates causing septicemia and sometimes in EIEC (Kuhnert *et al.*, 2000).

Invasion is associated with translocation of the bacteria from one host to other. The translocation of bacteria across the intestinal epithelium of immunocompromised patients can lead to bacteremia and life-threatening sepsis. Passive translocation results when there is a physical break in the host cellular wall. In contrast, active translocation occurs when the essential properties of the bacteria allow for their passage through the host barrier without causing damage or trauma to the epithelial wall. In addition, *fimH* encodes a type I pilus tip adhesin protein. These certain *FimH* and allelic variants of this protein are important for the invasion of the urinary epithelium, as well as the formation of intracellular bacterial communities. In the urinary tract, a persistent intracellular

infection that forms a reservoir of UPEC can also act as a source of recurring infections (Poole *et al*., 2017).

1.2.7 *E. coli* genomes

The sizes of *E. coli* genome can differ by a million base pairs between commensals and pathogenic variants and this extra genetic content can contain virulence and fitness genes. Virulence is the severity or harmfulness of a disease, while fitness genes mean the effectiveness of the Deoxy Ribonucleic Acid (DNA) (Croxen *et al*., 2013). The study further described that *E. coli* genomes are split between a shared, conserved set of genes, called the core genome, and a flexible gene pool (Croxen *et al*., 2013).

The genome of *E. coli* is of high flexibility allowing it to gain and lose (virulence) genes at a quite high occurrence (Kuhnert *et al*., 2000). Further, the genome flexibility is because many virulence genes in *E. coli* are located on mobile elements like plasmids, phages or transposons. They are often clustered on large genetic blocks, so-called pathogenicity islands (PAI) (Kuhnert *et al*., 2000).

1.2.8 Host factors which influence vaginal microecology

1.2.8.1 Non-secretor phenotype

The non-secretor is referred to people's secretions that do not contain blood group antigens for example women with a history of recurrent UTI (Hooton, 1999). Further, the infections reoccur, because the vaginal lactobacilli strains on the epithelial cells accepted *E. coli* P and F addesins resulting in vaginal infections (Hooton, 1999).

1.2.8.2 Exposure to spermicide

It has been found that spermicides have antibacterial activity against sexual transmitted infections, but it is less active against uropathogenic and yeast strains (Hooton, 1999). Meanwhile, these spermicides reduce lactobacilli of the vagina allowing to uropathogenic bacterial such as *E. coli* to colonize the genital tract and cause vaginal infections (Hooton, 1999).

1.2.8.3 Exposure to antimicrobial

In animals, exposure to some antimicrobials, mostly β-lactam, can enable vaginal colonization with uropathogens. According to Hooton (1999), certain antimicrobials also facilitate susceptibility to UTIs in human. Moreover, Hooton (1999) also reported that Ampicillin can reduce genital flora if

administered to adult women with acute cystitis and it is associated with increase genital *E. coli* colonization. In addition, the study have also found that women with *E. coli* cystitis who are treated with amoxicillin or cefadroxil are more possible to have persistent vaginal and urethral colonization with *E. coli* as well as frequent relapses of cystitis than women treated with trimethoprim-sulfamethoxazole or fluoroquinolones. This means that trimethoprim-sulfamethoxazole and fluoroquinolones drugs eradicate *E. coli* while maintaining genital tract anaerobic flora which leads to the best outcome (Hooton, 1999).

1.2.8.4 Exposure to oestrogen

Experiments suggested that oestrogens are more likely to increase *E. coli* adherence as compared to progesterones, meaning that increased concentrations of oestrogens can progressively improve attachment of *E. coli*, staphylococci and other bacteria (Hooton, 1999. The study further demonstrated that attachment of *E. coli* and other uropathogens to human vaginal or uro-epithelial cells is always high during the menstrual cycle phase when oestrogen peaks (Hooton, 1999).

1.2.9 *E. coli* and diseases in human

E. coli is part of the normal flora in the intestine in humans and animals. However, it is also pathogenically classified as evident and opportunistic pathogen, meaning that *E. coli* is able to cause diseases in the healthy individual and it can also take advantage of the immune-compromised people (Forbes *et al*., 2002). On the other hand, *E. coli* is the most frequent causative agent of community and hospital acquired urinary tract infections (including infections of the kidney) (Global Report on surveillance 2014). It also causes bloodstream infection at all ages. *E. coli* is associated with intra-abdominal infections such as peritonitis, and with skin and soft tissue infections due to multiple microorganisms (Forbes *et al*., 2002). In addition, it causes neonatal meningitis and is one of the leading contributing agents to foodborne infections in the world. Infections with *E. coli* usually originate from the person affected (self –infection by direct contact with contagious agent). Moreover, the strains with a particular resistance or disease-causing properties can also be transmitted from animals, via the food chain or between individuals (Global Report on surveillance 2014).

1.2.10 *E. coli* vaginal colonization

Pregnant women are more prone to different types of bacterial infections, due to reduction of vaginal lactobacilli during pregnancy (Ayenalem, Yusuf & Ashenafi, 2010; Baisley *et al*., 2009;

Ericksson, 2011; Zodzika, 2014). The pathogenic strain for E. coli cause several asymptomatic and symptomatic infections (Lebea & Davies, 2017) and seems to colonize up to 20% of pregnant women (Devi *et al.*, 2014).   Studies reported that these colonizing isolates can sometimes cause complications during pregnancy and severe maternal outcomes (Devi *et al.*, 2014; Saez-Lopez *et al.*, 2016).

Lactobacilli (vaginal normal flora) produce lactic acid that prevents vaginal infection and some strains of lactobacilli produce hydrogen peroxide, which may inhibit the growth of other genital microorganism (Hawes *et al.*, 1996).

There are several reasons to why lactobacilli may inhibit the growth of *E. coli*; competition for nutritional factors, production of simple bacteriostatic compounds (e.g. lactic acid), competitive co-aggregation to other bacteria and to uroepithelial cells, and production of specific growth inhibitors for other bacteria (Anders *et al.*, 1994).

Vaginal colonization with *E. coli* seems to be an important requirement for the development of UTIs. Whereas, P fimbriated strains colonization of the urinary tract has evidently revealed to influence pyelonephritis. Several host genetic and behavioral factors have been found to be associated with increased *E. coli* colonization and other uropathogens as well as consequent UTIs (Hooton, 1999). UTIs in women develop when uropathogens, from the faecal flora, colonize the vagina, raises into the bladder and, in some cases the kidney (Croxen *et al.*, 2013).

1.2.10.1   *E. coli* and infertility

Infertility is referred to the lack of giving birth after a year of consecutive unprotected sexual intercourse (WHO 2012). It is becoming a health concern worldwide and seen in 13-15% of reproductive couples. *E. coli* can affect both male and female fertility, hence causing Pelvic Inflammatory Disease (Cools, 2017). This is a disease of the reproductive system and leads to infertility in female due to fallopian tubes damage. Bacterial vaginosis-associated bacteria are responsible for up to 85% of PID cases, whereby *E. coli* is part of the remaining 15% of infection agents including enteric and respiratory pathogens (Cools, 2017).

About 15% of male infertility is caused by genital tract infection (Cools, 2017). However, there are no data to elucidate the specific cause of these infertility cases. Furthermore, *E. coli* predominate in urinary tract infection and it is associated in up to 80% of acute or chronic prostatitis cases (Cools, 2017).

1.2.10.2 *E. coli* and miscarriage

The spontaneous loss of pregnancy at 22weeks of gestation is called miscarriage (Cools, 2017). There are two types of miscarriage; early miscarriage that occurs in less than 3 months of gestation and late miscarriage that occurs after 3 months of gestational period. Furthermore, one in five pregnancies ended in early miscarriage, while 1-2% of pregnancies end in late miscarriage (Cools, 2017).

Even though, a little has been done to elucidate the cause of most miscarriage cases, it was estimated that 15% and 66% of early and late miscarriage were caused by preventable bacterial infection (Cools, 2017). In reference to the study conducted by McDonald and coworkers (1991) the foetus losses and the respective placenta were tested for bacteria. The study revealed that *E. coli* was in one of the forth miscarriage case accounting for 8 cases in total (Cools, 2017).

1.2.10.3 *E. coli* and stillbirth

Stillbirth is one of the common causes of pregnancies loss worldwide, accounting for 3.2 million cases annually. It is defined as the loss of pregnancy at 22 weeks of gestational period. Stillbirth can be 3 deaths in 1000 births in some developing countries, but other developing countries the rate can reach up to 45 per 1000 births. In addition, bacterial infection is clearly linked to early stillbirth (22-28 weeks) as compared to late stillbirth (after 28 weeks) (Cools, 2017).

Ascending of the bacterial infection leads to chorioamnionitis (intra-amniotic infection) prior or post membrane raptures. It is commonly known to cause stillbirth and it is the situation where mother and foetus get exposed to the same organism. Even though, there is limited data of the cause of stillbirth, *E. coli* is the most common bacteria associated with cause of stillbirth cases (Cools, 2017).

1.2.10.4 *E. coli* and spontaneous preterm labor

Preterm labor occurs due to the bacteria that ascend into the amniotic cavity causing the release of cytokines, chemokines, proteases and inflammatory mediators that stimulate or induce myometrial contractility, leading to early delivery or premature birth (Cools, 2017).

1.2.10.5 *E. coli* and preterm rapture of the membrane

Preterm rapture of the membranes (PROM) means that the amniotic sac breaks before the onset of labor. This situation the bacteria will ascend into the sac and infect the foetus. Studies reported that *E. coli* was associated with PROM. Moreover, in the study conducted by MacDonald and coworkers revealed that *E. coli* was isolated in 8% of amniotic fluid and placenta materials of PROM and

preterm labor cases. In addition, Cools (Cools, 2017) mentioned of the similar study that was conducted in China, which revealed that *E. coli* presented 5.4% of PROM cases.

1.2.10.6   ***E. coli*, preterm birth and low birth weight**

Preterm birth (PTB) means giving birth before 37 weeks of gestational period. It is known to cause neonatal mortality and morbidity all over the world. PTB accounts for 28% death rate in world and it is estimated to be high in developing countries (Cools, 2017). Several studies reported expressively higher vaginal *E. coli* carriage in women who delivered before the onset as compare to those delivered on term (Holst *et al*., 1994; Krohn *et al*., 1997; Usui *et al*., 2002).

1.2.10.7   ***E. coli* and neonatal sepsis**

Bacteria can spread and contaminate the foetus or neonate in many ways such as through mother's blood flow (maternal bacteremia) (Rad *et al*., 2016). Bacteria can also spread through vagina; cervix or fecal contamination of the birth canal (both ruptured and intact amniotic sac cases) (Rad *et al*., 2016). Therefore, these cases lead to adverse birth outcomes such as prematurity, low birth weight, birth defects, stillbirth and sepsis (Adane *et al*., 2014). These outcomes represent significant problems in both developing and developed countries (Adane *et al*., 2014; Patnaik *et al*., 2017).

Neonates, both term and preterm, are vulnerable to septicemia and meningitis (Makvana & Krilov, 2015). Appearance in the first week after birth (early onset) and mostly in the first 2 days after birth indicates maternal *E. coli* vertical transmission to neonates during delivery, where late-onset infection indicates nosocomial or community acquisition. Different organisms are responsible for each onset such as group B *Streptococcus* (GBS), *E coli*, and *Listeria monocytogenes* causes early-onset meningitis. Moreover, late-onset meningitis may be caused by other gram-negative organisms and staphylococcal species (Makvana & Krilov, 2015).

Early-onset sepsis (EOS) remains a common and severe problem for neonates (Simonsen *et al*., 2014). Among various organisms reported to be leading cause of EOS was *E. coli*, the frequent bacterium involved in neonatal meningitis (45-64%), preterm birth (27%) and low birth weight (LBW) (33.4%) (Adane *et al*., 2014; Rad *et al*., 2016; Simonsen *et al*., 2014; Saez-Lopez *et al*., 2016). According to studies finding, the occurrence of perinatal transmission of *E. coli* during birth ranges between 21% and 50% globally (Akbarian *et al*., 2016; Rad *et al*., 2016; Saez-Lopez *et al*., 2016; Tameliene *et al*., 2012). Although, several studies revealed that *E. coli* is one of the leading causes of early-onset neonatal sepsis in many developing countries (Tameliene *et al*., 2012) including Namibia, there is a lack of studies on *E. coli* colonization in women and neonates, as well as its transmission rate before or during delivery in developing countries. Furthermore, some studies reported neonatal

sepsis the leading cause of neonatal death including the study conducted in Namibia by Hatupopi (2013), but there is no data to elucidate the organism responsible for all the cases.

The influencing factors in neonatal gram-negative bacterial infections include maternal intrapartum infection, gestation of less than 37 weeks, low birth weight, and prolonged rupture of membranes. Neonates with defects in the integrity of their skin, mucosa, and abnormalities of gastrointestinal or genitourinary tracts are at increased risk of *E. coli* infection (Makvana & Krilov, 2015).

1.2.10.8   Morbidity and mortality outcomes

Most morbidity and mortality cases are caused by various bacterial agents (Cools, 2017). However, *E. coli* was found to be highest causing agent of EOS compared to GBS mostly due to meningitidis (Cools, 2017). In addition, in developing countries, *E. coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* are regarded as major pathogens.

In US, population surveillance reported that the rate of fatality was 24.5% with *E. coli* alone causing 44% of EOS cases. Furthermore, the neonates with *E. coli* EOS suffer a lot of morbidity as compared to those with GBS cases; this is because of conflicting difference between morbidity and mortality caused by ampicillin-sensitive and ampicillin-resistance *E. coli* infections (Global Report on surveillance 2014).

1.2.10.9   *E. coli* and maternal sepsis

Cools (Cools, 2017) described that women death during birth is called maternal mortality due to directly or indirectly causes. This cases rate has increased to half a million in 2005 and more than 10% of these cases were caused by sepsis. According to Cools, several studies were conducted to determine the causative agents of maternal sepsis. In one of the study conducted in the UK by Acosta *et al*. (Acosta *et al*., 2014), *E. coli* was commonly found to be responsible for maternal sepsis, present in one-fifth of 365 cases of maternal sepsis and urogenital tract. The different study conducted in Ireland reported that the laboratory confirmed the blood stream infection, in which *E. coli* was isolated from 103 making it the most single isolated bacteria causing infection (Knowles *et al*., 2015) Another two separate studies conducted in Nigeria and Papua New Guinea reported that 19.8% and 7.9% of *E. coli* was isolated from cultured specimens of maternal cases, separately (Dare *et al*., 1998; Vacca & Henderson, 1980).

1.2.11 Global prevalence and risk factors in cervicovaginal carriage of *E. coli*

Several studies (Anderson *et al.*, 2011; Cools *et al.*, 2016; Cutland *et al.*, 2012; Ghartey *et al.*, 2012; Guiral *et al.*, 2011; Glakoumelou *et al.*, 2016; Karou *et al.*, 2012; Kazi *et al.*, 2012; Kim *et al.*, 2016; Ocak *et al.*, 2007; Razzak *et al.*, 2011; Tameliene *et al.*, 2012; Sagna *et al.*, 2010; Villar *et al.*, 2013) reported different rates of cervicovaginal carriage of *E. coli* in women of childbearing age (Cools, 2017). Women from Africa had the highest *E. coli* carriage (35.6%), women from South America, Europe and North America had the middle and comparable *E. coli* carriage rate (19.7%, 13.3% and 12.0%, respectively). Asian women were found to have the lowest *E. coli* carriage rate (6.3%) (Cools, 2017)

In the same study, Cools (2017) argued that women who were first half of their menstrual cycles were more likely to have high rate of *E. coli* carriage. The author further claimed that the women who self-acknowledged being sex workers were found to have high rate of *E. coli* carriage. Furthermore, the researcher underlined that women who use contraceptive barriers like cervical caps had increased *E. coli* carriage and urinary tract infections. Moreover, Cools reported that the use of spermicide and absence of lactobacilli in the vagina were also independent risk factors that promote *E. coli* cervicovaginal carriage (Cools, 2017).

Cools (2017) reported that the world overall pooled prevalence of cervicovaginal *E. coli* carriage rates in women of childbearing age was 35.6% in Africa. In Burkina Faso two separate studies were conducted in 2010 and 2012 and the prevalence reported was 28.4% and 16.7% respectively. In Kenya three studies were also conducted, two in the same year 2016 and one in 2011. The prevalence was 14.3%, 25.7% and 40.1% respectively (Cools, 2017). In Rwanda the prevalence reported by two studies conducted 2016 was 20.0% and 70.0% (Cools, 2017). In South Africa two studies were conducted in 2012 and the rate reported was 42.3% and 46.5%, respectively (Cools, 2017). In addition, other two studies were carried out in 2016 and the prevalence reported was 33.3% and 25.3% (Cools, 2017).

UTIs are outcomes of *E. coli* genital tract colonization. Last year 2018, a study conducted in Onitsha, Nigeria investigating the prevalence of UTIs in pregnant women, concluded that out of 200 pregnant women recruited 52(26%) were colonized (Nwachukwu *et al.*, 2018). Similar study was carried out in Ghana and the overall prevalence of bacteriuria was 42.8% (n = 171) of 400 participants. In that study *E. coli* was the most predominant isolate (47.95%) (Forson *et al.*, 2018).

1.2.12 Diagnosis and management

1.2.12.1 Clinical diagnosis

The pathogenic strains of *E. coli* cause several asymptomatic (no clinical signs) and symptomatic (clinical signs) infections (Lebea & Davies, 2017; Saez-Lopez *et al*., 2016). Bacterial vaginosis shows clinical signs such as vaginal discharge, foul-smelling vaginal odor, vaginal itching and burning sensation (Forbes *et al*., 2002). The diagnosis of pathogenic *E. coli* is problematic since the harmless and pathogenic strains of this species cannot be distinguished by applying phenotypic criteria (metabolic performance, morphology, culturing on differential media). Accordingly, this constitutes a fundamental difference to the diagnosis of obligate pathogens such as Salmonella or Shigella. Since *E. coli* are de facto present in all samples, suitable diagnostic instruments must be made available to distinguish pathogenic from harmless strains of this species as part of a rapid, reliable process.

The clinical signs of septicemia include fevers, temperature instability, heart rate abnormalities, respiratory distress, apnea, cyanosis, lethargy, irritability, jaundice, vomiting, diarrhea and abdominal distention (Makvana & Krilov, 2015).

1.2.12.2 Laboratory diagnosis

Culture

Most Enterobacteriaceae grows on routine laboratory culture media such as Blood Agar 5 %, Chocolate and MacConkey Agar (Akmal *et al*., 2014). Most *E. coli* species are lactose fermenter. The change in pH due to lactose fermentation can be used to differentiate between lactose-fermenting and non-lactose-fermenting strains. The lactose-positive *E. coli* colonies will appear red or pink on media such as MacConkey agar (Croxen *et al*.2013; Forbes *et al*., 2002) *E. coli* is able to grow both aerobically and anaerobically, preferably at 37°C. Although it has peritrichous flagella it can either be non- motile or motile.

E. coli can frequently be isolated from various clinical specimens (Hadadi *et al*., 2017), such as vaginal swabs, rectal swabs, urine and blood. However, *E. coli* bacterium was first isolated from the feces of a newborn in 1885 by Theodor Escherich (Muinck, 2013). Since 1990, a wide range of chromogenic culture media has been made commercially available providing useful tools for clinical microbiological organism's diagnosis. The traditional approach to the detection of pathogenic bacteria in pathological specimens is still essential, but is time consuming and can be costly in terms of serological or biochemical reagents compared to Chromogenic culture media usage that have high specificity (Perry and Freydiere, 2007). According to Perry, (2017) chromogenic media have been

shown to provide a greater differentiation of mixed cultures due to the fact that different species may grow with different colony colours which may not be easily distinguished on conventional agars. In 1976, Kilian and Bu low were the first to use a selective chromogenic medium for the direct identification of *E. coli* in primary culture of urine (Akter *et al*., 2014) and they suggested that a b-glucuronidase assay would be a useful identification test, based on their findings that the association of b-glucuronidase with the genus Escherichia was 97% positive (Rice *et al*., 1990).

Gram stain

Gram staining is a test used to classify pathogens by their gram reaction and morphology. The gram reaction depends on the difference in permeability of bacterial cell walls during the staining process. Furthermore, staining with crystal violet and iodine, the crystal violet–iodine complex is formed in the bacterial cell walls. The complex is easily removed from the more permeable cell wall of gram negative bacteria but not from the less permeable cell wall of gram positive bacteria (Winn *et al*., 2006).

Gram negative bacteria therefore de-stain with acid alcohol and counterstain with calbol fuchsin stain, whereas gram positive bacteria do not de-stain with acid alcohol, but retain the crystal violet-iodine complex. Gram negative bacteria result in pink color after counterstaining, whereas gram positive bacteria remain dark blue. *E. coli* is a gram-negative bacilli organism (Winn *et al*., 2006).

1.2.12.3   Antimicrobial susceptibility testing (AST)

Disk diffusion method

AST for Enterobacteriaceae can be done using disc diffusion method on Mueller Hinton agar. several studies used the method to test for antimicrobial susceptibility patterns. One of the study conduted by Devi *et al*. (2014) also used the same method. The following antimicrobial agents were used: ampicillin (10 μg), amikacin (10 μg), doxycycline (30 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), cefotaxime (30 μg), gentamycin (10 μg), ciprofloxacin (5 μg), gatifloxacin (5 μg), trimethoprim-sulfamethoxazole. Interpretation as sensitive, intermediate or resistant was done as per CLSI, 2018 interpretive standards for Enterobactereriaceae and *E. coli* American Type Culture Collection (ATCC) 25922 was used as the control strain (Devi *et al*., 2014).

In the study conducted by Sangare *et al*. (2017) *E. coli* was prevalently isolated (37.8%) compared to other organisms such as *K. pneumoniae*, (31.7%), *E. cloacae* (18.3%), *Salmonella enterica serotype Enteritidis* (6.1%), *Morganella morganii* (3.6%),   (1.2%)*Proteus mirabilis* and   (1.2%) *Leclercia adecarboxylata* . The study also reported antibiotics resistance rates of 98.8% for amoxicillin, 75.6% for cotrimoxazole, 62.2% for third generation cephalosporin (48 ESBL and 3 non ESBL), 61.0% for

amoxicillin + clavulanic acid, 53.7% for fluroquinolones, 52.4% for gentamicin, 11.0% for piperacillin + tazobactam, 2.4% for amikacin, and 1.2% for carbapenem. The rate of ESBL producing strains were 76.9% in *K. pneumoniae*, 64.5% in *E. coli* and 53.3% in *E. cloacae*. One *E. coli* produced an ESBL and a carbapenemase. Furthermore, the co-associated antibiotic resistance rates were higher in ESBL than in non ESBL ones, 89.6 and 55.9% for cotrimoxazole, 85.4 and 5.9% for gentamicin, 72.9 and 26.5% for fluoroquinolones, respectively. All ESBLs strains carried *blaCTX-M-1* group genes (89.6%) (Sangare *et al*., 2017).

In Ghana 2018, high levels of antimicrobial resistance were observed to ampicillin (79.3%), tetracycline (70.7%) and cotri- moxazole (59.8%), except for cefuroxime (32.9%). Resistance genes analyses revealed 58.5% were positive for *BlaTEM* (Forson *et al*., 2018).

Vitek-2 identification and sensitivities method

Principle

The vitek-2 (bioMerieux) system is an automated microbiology system which utilizes a growth-based method for identification and sensitivity testing of bacteria. This system uses colorimetric reagent cards that are incubated and interpreted automatically (bioMerieux, 2014). Sensitivity testing is based on a growth-based method at antimicrobial intervals to determine the minimum inhibitory concentration that completely inhibits bacterial growth.

1.2.13   Bacterial infection Management

1.2.13.1   Treatment of *E. coli*

In general, Enterobacteriaceae are treated with broad spectrum antibiotics. However, the resistance mechanism of the therapy varies from species to species. For gastro intestinal infection inclusion of antimicrobial agents as part of treatment is controversial (Forbes *et al*., 2002).

Intravenous antibiotic should be given to Infants suspected of having a systemic infection while waiting for isolation of the organism from cultures. About 50% of *E. coli* are resistant to amoxicillin or ampicillin, so while the patient is waiting for sensitivity results, an aminoglycoside or a third-generation cephalosporin is recommended as empiric therapy. The specific antibiotic to infection can then be selected once susceptibility results become available from the laboratory. The clinical response of the patient and the site of the infection will determine the duration of therapy. The normal duration of therapy is 10 to 14 days for uncomplicated bacteremia, 7 to 14 days for UTIs, and a minimum of 21 days for meningitis (Makvana & Krilov, 2015).

1.2.13.2 Mechanisms of Resistance

Globally, antimicrobial resistance is a major and increasing healthcare problem. The antimicrobial resistance (AMR) is currently estimated to account for more than 700,000 deaths per year worldwide (Tadesse *et al*., 2017). Most bacteria became resistant to antibiotics ever since the introduction of penicillin and they developed capabilities to transmit the drug resistance to other organism's species. In addition, too much exposure of human to antibiotics and their inappropriate use are some of the factors that promote drugs resistance. Other factors that can contribute to spread of multidrug resistant strains are continuous movement of people from country to country, international tourism and business travel (Allocati *et al*., 2013).

Multidrug resistance is defined as resistance to more than 3 antimicrobial classes to which bacteria do not show intrinsic resistance. In addition, multi-resistant strains results from combination of plasmids, integrons and transposons (Magiorakos *et al*., 2012).

In animals, there is also increased resistance to antibiotics. These resistant bacteria from animals can directly infect humans through direct contact as well as via food products of animal origin (Allocati *et al*., 2013).

E. coli resistance develops voluntarily either through mutation, which is often the case for fluoroquinolone resistance, or by gaining of mobile genetic elements, which has been the case for broad-spectrum penicillins (e.g. ampicillin or amoxicillin) and resistance to third-generation cephalosporins. Ampicillin was reported by several researchers all over the world with the resistance ranging between 14% and 95% (Mumtaz *et al*., 2008; Adegoke, &Okoh, 2011; Tameliene *et al*., 2012; Villar *et al*., 2013; Ogunlesi, 2013; Shatalov, 2015; Hadadi *et al*., 2016; Lebea & Davies, 2017).

1.2.13.3 *E. coli* Resistance to third-generation cephalosporins and fluoroquinolones

According the World Health Organisation (WHO) global surveillance report (2014), *E. coli* resistance to third-generation cephalosporins was between 2-70% in African regions, in Americas regions it was 0-48%, in Eastern Mediterranean Regions it was 22-63%, in European Regions it was 3-82%, in South-East in Asia it was 16-68% and in Western Pacific it was 0-77%. Furthermore, *E. coli* resistance to third-generation cephalosporins was between 14-71% in African regions, in Americas regions it was 8-58%, in Eastern Mediterranean Regions it was 21-62%, in European Regions it was 8-48%, in South-East in Asia it was 32-64% and in Western Pacific it was 3-96%.

1.2.13.4   Epidemiology of Resistance

Antimicrobial resistance in Gram-negative bacteria is escalating, predominantly in *E. coli*, which constitutes a majority of invasive Gram-negative isolates in worldwide. Enterobacteriaceae, mainly *E. coli*, are the most common cause of hospital- and community-acquired infections and this complicating the treatment of several serious infections in human (Allocati *et al*., 2013).

Multidrug-resistant *E. coli* strains are also emerges from animals and human can get it from animal food products. *E. coli* is able to survive and adapt in various extraintestinal environments and it can spread resistances between humans, animals, their products and the environment through several transmission routes. Environment is the mode of multi drug resistance genes transmission. This means that, *E. coli* may acquire other drug resistance traits from other environmental bacteria and on the other hand spread its resistance genes to potential pathogens in different surroundings. Apart from environment and animals, hospitals sewages can also be the source of multi-resistant *E. coli* transmission (Allocati *et al*., 2013).

 The overview of *E. coli* in Europe demonstrated that the percentage of isolates that express resistance to third-generation of cephalosporins was the lowest in Sweden (3.0%), Norway (3.6%) and Finland (5.1%) and highest was in Bulgaria (22.9%), Slovakia (31%) and Cyprus (36.2%). *E. coli* strains resistant to fluoroquinolones were present in low numbers in Sweden (7.9%), Norway (9.0%) and Estonia (9.9%) while they were leading in Italy (40.5%), Slovakia (41.9%) and Cyprus (47.4%). Moreover, the prevalence of isolates resistant to aminoglycosides ranged from 3.7% (Sweden) to 23.9% (Cyprus). The percentage was also higher in Romania (19.6%), Slovakia (17.9%) and Greece (16.8%). Finally, the prevalence of isolates with multi-resistance ranged from about 1% in Estonia, Iceland and Sweden to more than 10% in Romania, Slovakia and Cyprus. *E. coli* strains resistant to broad-spectrum penicillins were reported in 28 countries, falling in the range of 34.8% (Sweden) to 77.6% (Cyprus). Despite, only 0.04% isolates of *E. coli* were found to be resistant to carbapenems (Allocati *et al*., 2013).

Today, antimicrobial resistance is a severe public health concern in Africa. Particularly Ampicillin, which was reported by several researchers with the resistance ranging between 14% and 95% (Mumtaz *et al*., 2008; Adegoke, &Okoh, 2011; Tameliene *et al*., 2012; Villar *et al*., 2013; Ogunlesi, 2013; Shatalov, 2015; Hadadi *et al*., 2016; Lebea & Davies, 2017) Antibiotic resistance varies from one environment to another (Lebea & Davies, 2017). Yet, the high resistance of empiric antibiotic therapy for different diseases is making increasingly difficult to provide effective antibiotic treatment in Africa. In addition, there is an increase in number of multidrug-resistant strains which is a matter of abundant worry (Saez-Lopez *et al*., 2016; Habibi & Khameneie, 2016; Hadadi *et al*., 2016; Devi *et al*., 2014; Tameliene *et al*., 2012).

To date, the position of multi-drug resistance (MDR) among pathogens currently circulating in most countries of sub-Saharan Africa are practically unknown. This is due to that the drug resistance is increasing day by day. In the study conducted by Leski *et al*. (2016), antimicrobial susceptibility testing demonstrated that 85.7 % of these isolates were MDR while 64.3 % produced an ESBL. The most notable observations included widespread resistance to sulphonamides (91.4 %), chloramphenicol (72.9 %), gentamycin (72.9 %), ampicillin with sulbactam (51.4 %) and ciprofloxacin (47.1 %) with C. freundii exhibiting the highest and *E. coli* the lowest prevalence of multidrug resistance (Leski *et al*., 2016).

In Africa, recent study conducted by Tadesse and coworkers (2017) reported that *E. coli* bacterium was commonly isolated (60.4%) with the most numerous susceptibility data for gentamicin (53.5%), ciprofloxacin (49.3%) and sulfamethoxazole/trimethoprim (47.2%). In total, 13 Gram-negative opportunistic pathogens were tested against 37 antibiotics. Furthermore, resistance to commonly used drugs like amoxicillin (72.9%) and trimethoprim/sulfamethoxazole (75.0%) was high, whereas low to moderate resistance was found to gentamicin (22.1%), ciprofloxacin (16.7%) and ceftriaxone (17.2%) (Tadesse *et al*., 2017).

1.2.13.5 β-lactam resistance

The main characteristic of β-lactam resistance in *E. coli* and other gram-negative bacteria is the production of b-lactamases enzymes, which are encoded on chromosomes or by plasmids. There are two spectrum-b-lactamases; narrow-spectrum b-lactamases known as penicillinases or cephalosporinases and broad-spectrum, such as ESBLs that can hydrolyze many different blactams. Most of the ESBLs belong to the ambler class A as well as the Bush group 2 and they are mainly composed of the *TEM*, *SHV*, *OXA*, and *CTX-M* enzymes (Hammerum & Heuer, 2009, Al-Mayahie, 2014).

The first ESBL was identified in 1982, during a hospital outbreak of *K. pneumoniae* infections in Germany. Ever since then, more than 200 ESBL variants have been identified, some of which have spread rapidly worldwide. Moreover, many ESBL variants primarily identified in *K. pneumoniae* have successively conveyed to *E. coli*. These ESBL-positive strains are resistant to all extended beta-lactam antibacterial drugs such as cephalosporins. For these resistant strains, the carbapenems are the main drug of choice treatment (World Health Organisation, 2014).

Studies have demonstrated the presence of ESBL-producing *E. coli* in animals and meat, most likely caused by the use of the third generation cephalosporin ceftiofur in food animals. Since the late 1990s, ESBL-producing *E. coli* have been detected in retail meat and production animals in Europe, Asia, Africa, and the United States. In addition, *CTX-M-14*, *CMY-2*, *SHV-2*, and *TEM-52* producing *E. coli* isolates in animals were detected in in Spain (Hammerum & Heuer, 2009).

In *E. coli*, β-lactamase production is the most important mediator of resistance to broad spectrum of β-lactams antibiotics. Generally, β-lactamase enzymes are produced by Enterobacteriaceae and by *E. coli* in particular. β-lactamase cause resistance to penicillins and cephalosporins and develop multidrug resistance in Gram-negative bacteria. The *CTX-M*-1 cluster is now the most prevalent type all over the world with the *CTX-M-15* being the most identified variant. In Europe, *CTX-M*-14 and *CTX-M*-15 types are widely distributed among humans, while, *CTX-M*-1 variant is the most widespread among animals (Allocati *et al.*, 2013).

To date the new Carbapenem resistance in Enterobacteriaceae emerged caused by plasmid-encoded carbapenemases. Moreover, these enzymes are mainly found in nosocomial isolates of *Klebsiella pneumoniae* and *E. coli* (Allocati *et al.*, 2013).

1.2.13.6   Types of ESBLs

TEM-Type ESBLs

The native *TEM*-1 β-lactamase convenes resistance to ampicillin, penicillin and first-generation cephalosporins such as cephalothin. This enzyme, which is accountable for 90% of ampicillin-resistance in *E. coli* isolates. Furthermore, is also accountable for penicillin resistance in *H. influenzae* and *Neisseria gonorrhoeae*. The gene also expands its hydrolysis capabilities to particular extended spectrum cephalosporins and aztreonam (Rupp & Fey, 2003).

SHV-Type ESBLs

The native *SHV*-1β-lactamase, found primarily in *K. pneumoniae*, is a plasmid or chromosomally encoded-enzyme that confers resistance to penicillins and first-generation cephalosporins. Same as with *TEM*-1, this gene also expand the hydrolysis capabilities of *SHV*-1 to extended-spectrum cephalosporins and monobactams (Rupp & Fey, 2003).

Other ESBLs

Other ESBLs are genes that are different from *TEM*-1-or *SHV*-1-derived enzymes. These β-lactamases, are found in a variety of different species within the family Enterobacteriaceae and *P. aeruginosa*, include *OXA* type, *CTX-M*-type and *PER*-type β-lactamases. The preferred substrate of these ESBLs differs significantly ranging from cefotaxime (*CTX-M*-type) to ceftazidime (*PER*type). Although not firmly defined as an ESBL, another group of β-lactamases, called the inhibitor resistantβ-lactamases, have been isolated with increasing frequency (Rupp & Fey, 2003).

1.2.13.7   Laboratory detection of ESBLs

Double Disk Approximation Test

In this test, the organism to be tested is spread onto a Mueller-Hinton Agar plate. After that, two antimicrobial disks are placed 30mm apart (centre to centre) on the plate. One of the disks contains amoxicillin/ clavulanic acid, while the other contains an expanded-spectrum cephalosporin (i.e. ceftriaxone, cefotaxime or ceftazidime).The plate is incubated for 24hrs at 37◦C, the zone of inhibition in between the disks is enhanced. The enhancement is due to the inhibition of the ESBL by clavulanic acid (provided by the amoxicillin/clavulanic acid disk) and the subsequent action of the expanded-spectrum cephalosporin. This test remains a reliable method to detect ESBLs in the clinical laboratory though false results can occur if the disks are placed both 30mm and 20mm apart (Rupp & Fey, 2003).

Three-Dimensional Test

In this test, the organism to be tested is spread onto a Mueller-Hinton agar plate and a split is cut into the agar the length of the plate. The test organism is then inoculated into the split. Further, an expanded-spectrum cephalosporin is placed 3mm from the slit. A partial zone on the side of the split is considered a positive test. The test was shown to be as sensitive at detecting ESBLs as the double disk approximation test but is more technically challenging (Rupp & Fey, 2003).

E-Test

The two-sided ESBL E-test strip contains either a combination of ceftazidime and ceftazidime/ clavulanic acid or cefotaxime and cefotaxime/ clavulanic acid. Both strips have a decreasing gradient of ceftazidime or cefotaxime alone on one end and a decreasing gradient of ceftazidime or cefotaxime plus a fixed gradient of clavulanic acid on the other end. A >3log reduction in the MIC of cefotaxime or ceftazidime in the presence of clavulanic acid is interpreted as positive test.

Vitek

The Vitek automated susceptibility system (Biomerieux, Hazlewood, Missouri, USA) has presented an ESBL test on their system whereby ceftazidime and cefotaxime are tested alone and in combination with clavulanic acid. Logarithmic reduction in growth within the well containing clavulanic acid compared to the well not containing clavulanic acid indicates expression of an ESBL. The ESBL test in combination with the Vitek 2 Advanced Expert System software represents a very sensitive methodology to detect ESBLs in clinical isolates. If an ESBL is detected, all penicillins, aztreonam and

cephalosporins including cefepime (but excluding cefoxitin and cefotetan as ESBLs typically do not hydrolyze cephamycins as well as the cabarpenems) are to be reported as resistant notwithstanding of the original susceptibility report. For infection control purposes, a comment should be added to the final susceptibility report stating that the particular isolate is an ESBL producer so that suitable isolation safety measures can be employed (Rupp & Fey, 2003).

1.2.13.8   Treatment of ESBLs

Currently, carbapenems are generally regarded as the preferred agent for treatment of infections due to ESBL-producing organisms. Carbapenems antibiotics are resistant to ESBL-mediated hydrolysis, hence they exhibit excellent in vitro activity against strains of Enterobacteriaceae expressing ESBLs. Carbapenems are generally considered the drug of choice for the treatment of ESBL -E coli infections.(Makvana & Krilov, 2015; Rupp & Fey, 2003).

1.2.14 Prevention and Control of *E. coli* Infections

In general, approaches for the prevention and control of the spread of *E. coli* should include access to safe water, good handling practices to reduce the risk of food contamination, sanitation measures, public education and vaccination. Access to safe water should be a priority for the prevention of *E. coli* infections. Improper storage and cooking of food is a mode of infection transmission. However, proper measures to prevent infections from food products such as appropriate storage and cooking temperatures should be implemented (Allocati *et al.*, 2013).

Cross-contamination of multi-resistant pathogens in the hospitals should be avoided by implementing strict hygienic standard protocols as well as control over the use of antimicrobial drugs. Antibiotics are essential for the control and treatment of *E. coli* infections in humans and animals. Yet, it is generally accepted that antimicrobial resistance is associated with the quantity of antibiotic consumption. Too much exposure of patients to antibiotics increases the resistance in pathogens as well as in normal human bacterial flora. In addition, animal reservoir is also an important source for resistance strains. The effects of environmental release of the resistance genes are poorly studied. Antibiotic pollution promotes the fixation and mobilization of resistance genes between natural and clinical environments with world-wide spreading of resistance traits. For this reason, a rational and responsible use of antibiotics should be a requirement for the prevention of the emergence and transmission of resistant bacteria (Allocati *et al.*, 2013).

Finally, Probiotics could be an approach to the prophylaxis of several *E. coli* infections probiotics are viable and safe microorganisms, principally belonging to the genera Lactobacillus and

Bifidobacterium, which are able to colonize intestinal tract and thereby compete with pathogenic bacteria (Allocati *et al.*, 2013).

The use of Lactobacillus, which is part of the microbiota in healthy humans, in the form of probiotics reduced the risk of UTI and vaginal infections. Moreover, about 80% of uncomplicated UTI are caused by UPEC, and the annual economic impact of this type of illness is very high, principally due to the costs associated with medical care and loss of productivity. All these considerations stimulate an ongoing search for the effective UPEC vaccine (Allocati *et al.*, 2013).

1.3 Problem statement

Every year, about 4.7 million mothers, new-borns, and children die in Sub-Saharan Africa due to pregnancy and childbirth complications, new-born illnesses and childhood infections (Kinney *et al.*, 2010). In Africa, around 65 000 000 cases of genital tract infections occur annually (Mombasheri *et al.*, 2014). In addition, studies revealed various organisms responsible for genital tract infections during pregnancy (Tolosa *et al.*, 2006; Abdelaziz *et al.*, 2014; Ayenalem, Yusuf and Ashenafi, 2010) and *E. coli* is one of the organisms that can cause genital tract infections in pregnant women. According to literature, *E. coli* has the ability to ascend into the uterus, leading to intra-amniotic infection and adverse maternal complications such as preterm birth, pre-mature rupture of the membranes, miscarriages, stillbirths and neonatal sepsis (Son *et al.*, 2018). Early work by Cools *et al.* (2016) reported that the vaginal carriage rates with reference to *E. coli* from Kenya and South Africa were 25.0% and 27.1% respectively. In general, maternal infection due to *E. coli* is preventable and treatable. However there is a serious emergence of multi-drug resistant *E. coli* infections worldwide (Johnson *et al.*, 2010).

Despite all shocking statistics, the epidemiological data of *E. coli* carriage and treatment, which are vital for the development and implementation of strategies, are very limited in Sub-Saharan Africa. Even though *E. coli* is reported as a Non-Pathogenic Organism when clinically isolated from pregnant women, the current study would like to open doors for further studies to be conducted to prove this bacteria significance. To date there are no studies in Namibia to elucidate the predominance and antibiotic susceptibility of vaginal *E. coli* carriage with reference to severe maternal outcomes. Therefore the current study seeks to identify the distribution of *E. coli* bacterium among pregnant women, the antibiotic susceptibility of the isolates, as well as molecular characterization of the resistant genes within the isolates.

1.4 Research objectives

Main objective: To determine the distribution and antimicrobial susceptibility patterns of *E. coli* among pregnant women of rural Namibia.

Specific objectives

1. To determine the colonization rate of *E. coli* among pregnant women in selected rural settings of Namibia.

2. To evaluate the antibiotic sensitivity patterns of *E. coli* isolates.

3. To identify genetic basis of resistance of *E. coli* isolates.

CHAPTER TWO: METHODOLOGY

1.5 Study design

The study was descriptive cross-sectional study and it targeted pregnant women who were at 35 weeks of gestation and above. A convenience sampling technique was used to recruit 208 pregnant women for the study. Both qualitative and quantitative data were collected using structured questionnaires. A participant consent form was signed prior to recruitment in the study and socio-demographic information was captured in a questionnaire by registered nurses. A lower vaginal swab was collected from each participant.

2.2 Study population

The study targeted pregnant women at 35 weeks of gestation and onwards, attended antenatal screening at selected hospital facilities in Ohangwena and Oshikoto region. A population of approximately 1683 pregnant women attended community health clinics in Ohangwena and Oshikoto region (MOHSS, 2012).

2.3 Inclusion criteria

Pregnant women from 35 weeks of gestation and onwards who attended antenatal care at the selected Hospitals were included in the study.

2.4 Exclusion criteria

The study excluded pregnant women on their first and second trimester, on antibiotic prophylaxis or who has received antibiotic treatment 2 weeks prior to recruitment.

2.5 Study setting

The study was conducted at Eenhana, Okongo and Onandjokwe state hospitals, situated in Oshikoto and Ohangwena region of Namibia. The specimens were collected from women who attended antenatal screening at the selected hospitals. These areas were selected based on ease of accessibility by the researcher. Laboratory analysis was carried out at the Namibia University of

Science and Technology (NUST), Department of Medical Laboratory Sciences, Medical Microbiology Laboratory.

2.6 Sample size

$$n = [\frac{(z)2 \times P(1-P)}{\sqrt{d}}]$$

The above formula was used to calculate the number of participants who were included in the study, whereby **n** is number of participants, **z** is confidence interval 95% (1.96), **p** is proportion of *E. coli* colonization among pregnant women (Saez-Lopez *et al*., 2016), and **d** was margin of error (5%) = 0.05. Ten percent for non-response rate will be added. Therefore, the total of 408 participants was the calculated sample size for the study. However, 208 participants were recruited due to unavailability of funds.

2.7 Sampling technique

A convenience sampling technique was used to recruit participants in the study. This was a non-probability sampling technique which included selection of participants based on their availability. The sampling technique was suitable for this study because it allowed inclusion of participants based on their availability and voluntary participation. In addition, consecutive employment of participants was used for the study.

2.8 Materials and methods

2.8.1 Specimen collection and transportation

On sampling, a registered nurse administered the participant questionnaires (Appendix A) which included questions relating to socio-economic status, previous and current obstetric history, parity, and current health status. The questionnaire used in this current study was adapted from Moyo *et al*., 2000. All participants needed to give signed written consent to participate in the study (Appendix B). According to the Centres for Disease Control and Prevention (CDC) morbidity and mortality weekly report of May 1996, vaginal swab from pregnant women should be collected between the 35th and the 37th week of gestation (CDC, 1996), hence the current study adopted the same sampling method In this study, a lower vaginal swab was collected by a registered nurse from each of the 208 participants. A sterile swab was inserted about 2 centimetre (cm) into the vagina and the peri-urethra area and the 23 medial aspects of the labia were swabbed. The guideline on collection of vaginal screening was provided by CDC in 2010. The swabs were transported in Amie's transport media at 2-8°C on Ice. Amie's transport medium preserves microbiological specimens and maintains viability of microorganisms without causing significant increase in the growth or compromising the

quality of the specimens. *E. coli* isolates can remain viable in the transport medium for several days at room temperature (CDC, 2010). The specimens were transported overnight and received the next day at the designated laboratory for further processing. Specimens were kept in 2-8°C fridges prior to culture.

2.8.2   Preparation of culture media

To ensure high quality medium which supported the growth of bacteria, the manufacturer instructions were followed during preparation of all the media used in the study.

Quality control and sterility check was done on a representative 5% of each batch of media prepared. The quality control was done by inoculating *E. coli* ATCC 25922 on prepared Media plates followed by incubation at 37°C for 18-24 hours. The sterility check was done by incubating selected un-inoculated Media plates from the each batch at 37°C for 18-24 hours. The prepared media plates were stored at 2-8°C prior to use. After incubation of the quality control plates, plates were acceptable for use when inoculated plates supported the growth of *E. coli*. ATCC 25922 control was also used for quality control of the broth which was then incubated at 37°C for 18-24 hours. The sterility was done by incubating un-inoculated tubes with broth at 37°C for 18-24 hours. The quality of the broth was acceptable if the inoculated broth was turbid after incubation. Furthermore, the organism in the broth was confirmed by streaking it on MacConkey agar, incubating at 37°C foe 18-24 hours. The control was accepted only if the growth of *E. coli* was supported. The un-inoculated remained clear yellow to gold with no turbidity if the media was not contaminated. The rest of the broths were stored at 2-8°C prior to use (Engelbrecht *et al*., 2015).

2.8.3   Preparation of MacConkey agar

MacConkey agar (Oxfoid Ltd, United Kingdom, Basingstoke) was the first solid differential media to be developed in the 20th century by Alfred Theodore MacConkey. It is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae. Pancreatic digest of gelatin and peptones (meat and casein) provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Sodium chloride maintains the osmotic balance in the medium. Neutral red is a pH indicator that turns red at a pH below 6.8 and is colourless at any pH greater than 6.8 while agar is the solidifying agent (http://www.Neogen.com)

The 49.53 g of dehydrated medium was suspended in 1liter of Double Distilled Water. The suspension was then autoclaved at 121°C for 15 minutes. After autoclaving the solution was cooled to 45-50°C. Before it solidified, it was poured into sterile petri dishes that were clearly marked with the media name, preparation date and expiry date as per manufacturer's instructions.

2.8.4   Preparation of chromogenic agar

CHROMagar Orientation Medium (Becton Dickinson GmbH, Germany, Heidelberg) in a non-selective medium for the isolation, direct identification, differentiation and enumeration of pathogens. It allows for the differentiation and identification of *E. coli* and Enterococcus without confirmatory testing. The medium contains peptones that supply the nutrients. The chromogen mix consists of artificial substances (Chromogens) which release differently coloured compounds upon degradation by specific microbial enzymes, thus assuring the direct differentiation of certain species or the detection of certain groups of organisms, with only a minimum of confirmatory tests (Singh & Bhunia, 2016; Perry & Freydiere, 2007).

The media was prepared by suspending 33.0 g of powder into 1liter of Double Distilled Water (DDW) using a 2L Erlenmeyer flask. The prepared media was autoclaved at 121°C for 15 minutes, cooled down to 45-50 °C. The final pH was checked using the pH indicator which was supposed to be 7.4±0.2. Before solidifying, the cooled Chromogenic Agar was dispensed into sterile petri dishes on a level, horizontal surface to give uniform depth. After the medium solidified, the plates were labelled with preparation and expiry date. Prepared Chromogenic agar had a shelf life of 1 month when stored correctly at 2-8°C.

2.8.5   Preparation of nutrient agar (NA)

Nutrient Agar (NA) (Oxfoid Ltd, United Kingdom, Basingstoke) is used to support growth of a wide variety of microorganisms. The medium contains gelatin and beef extract which provide nitrogen, carbon, vitamins, and amino acids in Nutrient Agar. Agar is the solidifying agent REF

Twenty-eight grams of agar was mixed with 1litre of DDW according to manufacturer's instructions. The solution was then autoclaved at 121°C for 15 minutes, cooled and the final pH 7.4±0.2 was checked. Before solidifying, the cooled NA was poured into sterile petri dishes on a level, horizontal surface to give uniform depth. The plates were then labelled after they solidified. Prepared Nutrient agar had a shelf life of 2 months when stored at 2-8°C.

2.8.6 Preparation of nutrient broth

Nutrient Broth (Oxfoid Ltd, United Kingdom, Basingstoke) is the regular media used to support the growth of a wide variety of microorganisms. In addition, it is frequently used for isolation and purification of cultures. For this study, Nutrient broth was used for preservation of isolates. In this broth, nitrogen, carbon, vitamins, and amino acids are provided by enzymatic digest of gelatin and beef extract (http://www.Neogen.com).

Thirteen grams and agar as dissolved in 1L of DDW to prepare the broth which was autoclaved in the Erlenmeyer flask first at 121°C for 15 minutes and poured into the sterilized 500ml bottle. The broth had 12 months' shelf life when stored at 2-8°C

2.8.7 Preparation of Mueller Hinton agar

Mueller Hinton Agar (T Mask group, Liverpool, Merseyside, United Kingdom) is used to support growth of bacteria during antimicrobial susceptibility testing by the Kirby Bauer disk diffusion method. It contains beef extract and acid hydrolysate of casein that provide nitrogen, vitamins, carbon and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced while agar is the solidifying agent (http://www.Neogen.com). A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogues. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth, is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim (http://www.Neogen.com).

Mueller Hinton agar was also autoclaved at 121°C for 15 minutes. The agar was cooled, checked for final pH 7.3±0.1 and poured into petri dishes before it solidified. Prepared Mueller Hinton agar had a shelf life of 2 months when stored at 2-8°C.

2.8.8 Isolation and identification of presumptive *E. coli*

All 208 vaginal swabs collected in this study were cultured on MacConkey agar and incubated for 18-24hours at 37°C. After incubation, the plates were inspected for lactose fermenting colonies. *E. coli* ferments lactose and its colonies turn pink due to the presence of neutral red indicator that turns red at a pH below 6.8. All the lactose fermenting isolates on MacConkey agar were further inoculated on Chromogenic agar according to Leski *et al*., (2016) for presumptive identification of *E. coli* isolates. *E. coli* presented as dark pink, medium to large colonies on Chromogenic agar. The ATCC 25922 was

used as a positive control during confirmation of the presumptive *E. coli* isolates. The isolates were sub-cultured on Nutrient agar prior to preservation. Nutrient agar was used to purify the cultures and to avoid other media components which could potentially interfere with further analysis.

2.8.9   Preservation of presumptive *E. coli* isolates

The presumptive isolates detected in this study were preserved in a 50% glycerol solution. The pure growth on NA was emulsified in 1000uL of Nutrient broth. This 1000uL suspension was then added to 1000uL of 50% glycerol in the 3ml cryovial tube. The addition of glycerol stabilizes the frozen bacteria, preventing damage to the cell membranes and keeping the cells alive. A glycerol stock of bacteria can be stored stably at -80°C for many years (Engelbrecht, 2015). All presumptive *E. coli* isolates that were isolated from the participants were labelled and stored at -80°C for further processing.

2.8.10   Molecular confirmation of presumptive isolates

2.8.10.1   Preparation of fresh isolates prior to molecular analysis

Isolates preserved in 50% glycerol were resuscitated by thawing them and inoculating on nutrient agar followed by incubation at 37 degrees Celcius for 18-24 hours.

2.8.10.2   DNA extraction

DNA was extracted from all thirty six *E. coli* isolates obtained in this study. The ATCC 25922 control strain was included in the study. A sterile growth of each isolate on Nutrient agar was picked up with a sterile loop and emulsified into 2ml Eppendorf tubes with 200uL of nuclease free water. The tubes were loaded onto a heating block and the suspension was boiled at 100°C for 15 minutes. After boiling, the suspensions were cooled and centrifuged for 10 minutes at 8000 revolutions per minute (rpm). The supernatant containing DNA was transferred to a sterile labeled Eppendorf tube and preserved at -20 degrees Celcius prior to use.

2.8.10.3   Preparation of primers

The stock solution for the study was prepared based on the manufacturer instructions (Ingaba, South Africa, Pretoria). Table 2.1 below presents the oligonucleotide primers used for confirmation of *E. coli*.

Table 2.1 Oligonucleotide primers used for molecular confirmation of the presumptive *E. coli* isolates

Primer	Sequence (5'-3')	Fragment size (bp)	References	
uspA-F	CCGATACGCTGCCAATCAGT	884	(Anastasi *et al.*, 2010)	**2.8.10.4 Confirmation of *E. coli* using real time PCR**
uspA-R	ACGCAGACCGTAGGCCAGAT			
uidA-F	TATGGAATTTCGCCGATTTT	166	(Rhodes, Saunders & Pickup, 2000)	
uidA-R	TGTTTGCCTCCCTGCTGCGG			

Twelve microliters of One Taq[R] Master Mix with standard buffer (Ingaba Biotechnical Industries , South Africa, Pretoria) containing: 10mM Tris-HCl, 1.5mM MgCl2, 50mM KCl, 0.2mM dATP, 0.2mM dCTP, 0.2mM dGTP, 0.2mM dTTP, 5% glycerol, 0.08% IGEPAL[R] CA-630, 0.05% Tween[R] 20, 0.024% Orange G, 0.003% Xylene Cyanol and 33units/mL One Taq DNA polymerase, was mixed with 10.5µL of nucleic acid free water, 1uL each of 10 pMol of reverse and forward primers for the respective capsular types, 5uL of DNA template to make a final reaction volume of 28.5uL.

The cycling conditions were as follows: 94˚C for 4 minutes as an initial denaturation followed by 35 cycles of denaturation at 93˚C for 1 min adapt in all, annealing at the respective annealing temperature for 1 minute and extension at 72˚C for 1 minute with a final elongation step of 72˚C for 7 minutes followed by a hold at 4˚C. Amplification was verified in a 2% agarose gel electrophoresis at 110 volts for 45 minutes in a 1X TBE buffer and thereafter viewed in a transilluminator and photographed. *E. coli* ATCC 25922 was used a positive control (Elbaradie *et al.*, 2009).

The cycling conditions were as follows: 94˚C for 4 minutes as an initial denaturation followed by 35 cycles of denaturation at 93˚C for 1 min adapt in all, annealing at the respective annealing temperature for 1 minute and extension at 72˚C for 1 minute with a final elongation step of 72˚C for 7 minutes followed by a hold at 4˚C. Amplification was verified in a 2% agarose gel electrophoresis at 110 volts for 45 minutes in a 1X TBE buffer and thereafter viewed in a transilluminator and photographed. *E. coli* ATCC 25922 was used a positive control.

2.8.11   DensiCheck PLUS turbidity meter

The DensiCheck plus (bioMerieux, South Africa, Johannesburg) is a McFarland turbidity meter which was used in measurement of turbidity of bacterial suspensions for manual sensitivity testing. This turbidity meter was initially controlled prior to use, by using controls of known turbidity i.e. 0.00, 0.50, 0.20 and 0.30 McFarland. The turbidity meter was then used for preparation of bacterial suspensions after controls were considered acceptable.

2.8.12 Antimicrobial susceptibility testing

All thirty six isolates were tested for antimicrobial susceptibility using Kirby Bauer disk Diffusion method. This method was performed as described in the CLSI guidelines (CLSI, 2018). The inoculum for each confirmed *E. coli* isolate was prepared using a direct colony suspension to prepare an inoculum equivalent to a 0.5 McFarland standard (CLSI, 2018). A sterile cotton swab was dipped into the broth culture of each isolate and streaked evenly all over the surface of Mueller Hinton Agar for a lawn growth of the organism. Antibiotic sensitivity testing disks were placed on the inoculated plates using sterile needle. The plates were then incubated at 37 ˚C for 18-24 hours. The zones of inhibition were measured using a sliding Vernier calliper and were considered as sensitive, intermediate or resistant to an antibiotic based on CLSI guidelines, 2018. The *E. coli* isolates were tested against the following antibiotics: Ampicillin, Augmentin, PTZ (Piperacillin tazobactam), SXT (Sulphamexazole trimethoprim), Cefuroxime, Cefoxitin, Ceftazidime, Ciprofloxacin, Gentamycin, Amikacin, Imipenem, Meropenem, Ertapenem, Nalidixic acid, Cefepime.

2.8.13 Screening for resistant determinants

List of primers used in this study

Table 2.2 below is a list of oligonucleotide primers used for screening of resistant determinants.

Table 2.2 List of specific primers used for screening of resistant determinants

Primers	Sequence (5' – 3')	Size (bp)	References
blaTEM-F	GCTCACCCAGAAACGCTGGT	686	
blaTEM-R	CCATCTGGCCCCAGTGCTGC		(Ojdana *et al*., 2014)
blaSHV-F	CCC GCAGCCGCTTGAGCAAA	733	
blaSHV-R	CATGCTCGCCGGCGTATCCC		(Ojdana *et al*., 2014)
blaCTXM-F	GTGCAGTACCAGTAAAGTTATGG	278	(Ayodele & Adeniyi,
*bla*CTX-M-R	CGCAATATCATTGGTGGTGCC		2016)
bla_{MOX}-F	GCTGCTCAAGGAGCACAGGAT	520	(Perez-perez & Hanson,
bla_{MOX}-R	CACATTGACATAGGTGTGGTGC		2002)
bla_{FOX}-F	AACATGGGGTATCAGGGAGATG	190	(Perez-perez & Hanson,
bla_{FOX}-R	CAAAGCGCGTAACCGGATTGG		2002)

Multiplex PCR for gene based resistance testing

Twelve microliters of One Taq[R] Master Mix with standard buffer (Ingaba Biotechnical Industries , South Africa, Pretoria) containing: 10mM Tris-HCl, 1.5mM MgCl2, 50mM KCl, 0.2mM dATP, 0.2mM dCTP, 0.2mM dGTP, 0.2mM dTTP, 5% glycerol, 0.08% IGEPAL[R] CA-630, 0.05% Tween[R] 20, 0.024% Orange G, 0.003% Xylene Cyanol and 33units/mL One Taq DNA polymerase, was mixed with 10.5µL of nucleic acid free water, 1uL each of 10 pMol of reverse and forward primers for the respective capsular types, 5uL of DNA template to make a final reaction volume of 28.5uL.

The cycling conditions were as follows: 94˚C for 4 minutes as an initial denaturation followed by 35 cycles of denaturation at 93˚C for 1 min adapt in all, annealing at the respective annealing temperature for 1 minute and extension at 72˚C for 1 minute with a final elongation step of 72˚C for 7 minutes followed by a hold at 4˚C. Amplification was verified in a 2% agarose gel electrophoresis at 110 volts for 45 minutes in a 1X TBE buffer and thereafter viewed in a transilluminator and photographed. *E. coli* ATCC 25922 was used a positive control (Desjardins *et al*., 2004).

2.9 Methods of data analyses

Data obtained from the study questionnaires and experimental results was imported from excel into the Statistical Package for Social Sciences (SPSS) version 24 for data analysis. SPSS is the statistical software available for descriptive statistical data analysis and for generation of graphs and tables. . The statistical analysis aimed to deduce the following: overall prevalence of *E. coli* colonization in pregnant women included in the study, association between *E. coli* colonization and socio-demographic factors, frequency of antibiotic sensitivity tested against *E. coli* isolates and frequency of β-lactam genes within the *E. coli* isolates.

2.10   Ethical considerations

Approval to carry out the study was obtained from by Namibia University of Sciences and Technology (NUST) research committee and the Ministry of Health and Social Services. Written informed consent in relevant local languages was sought from the participants who took part in the study. All information was treated with confidentiality.

CHAPTER THREE: RESULTS

3.1 Prevalence of *E. coli* carriage among pregnant women seen in this study

A total of 208 pregnant women at 35+ weeks of gestation who attended antenatal screening at Eenhana, Onandjokwe and Okongo state hospitals between May and September 2018 were screened for vaginal colonization with *E. coli*. Out of the 208 screened pregnant women, 31 (14.9 %) were colonized by *E. coli*. The prevalence of vaginal *E. coli* in pregnant women was therefore 14.9 % with a median age of 29, ±6.9SD. The prevalence of *E. coli* colonization in pregnant women who were included in the study is shown in **Table 3.1** below.

Table 3.1 Prevalence of *E. coli* among pregnant woman recruited in the study

		Frequency (n)	Percentage (%)
	Not colonized	177	85.1
Culture results	Colonized	31	14.9
	Total	**208**	**100**

Table 3.2 Association of *E. coli* with demographic characteristics and obstetric risk factors, comparing women vaginally colonized to those not colonized

Factors	Colonized n±SD	Percentage (%)	Not colonized n±SD	Percentage (%)
Age(years)				
≤20	1±0.1[a]	0.48	11± 2.24	5.3
>20-≤39	24±1*[a]	11.5	149±3*	71.6
≥40	6±1*[a]	2.9	17±2.24*	8.2
Educational level				
below matric	21±1[a]	10.1	133±2	64.0
Matric	10±1*[a]	4.8	41±2*	19.7
Tertiary	0	0	3±1*	1.4
Employment				
Not employed	27±1[a]	13.0	139±1	66.8
Employed	4±2*[a]	2.0	38±2*	18.2
Geographical location				
Rural	26±1[a]	12.5	146±1	70.1
Semi-rural	3±1*[a]	1.4	6±1*	2.9
Urban	2±1[a]	1.0	25±1*	12.0
Marital status				
Not married	26	12.5	133	63.9
Married	5	2.4	44	21.2
Miscarriage				
0 count	26	12.5	160	76.9
1-2 count	5	2.4	17	8.2
Parity				
0 count	4±1.58[a]	1.9	41±2.16	19.7
1-2 count	16±2.28*[a]	7.7	70±2.82*	33.7
3 and above	11±1.58*[a]	5.3	66±1.41*	31.7
Stillbirth				
0 count	29±1[a]	13.9	164±1.92	78.8
1-2 count	2±1*[a]	1.0	11±1.58*	5.3
3 and above	0	0	2±1.14*	1.0

*= P <0.05 vs any category without a star ; a= P< 0.05 vs Colonized

From **Table 3.2** above there is statistical difference between factors (demographic and obstetric) and *E. coli* colonization in pregnant women who were included in the study except for some factors such as below matric compared to tertiary. In addition there is also statistical significance between the overall colonized and not colonized pregnant women.

3.2 Presumptive identification and confirmation of *E. coli*

Figure 3.1 below represents a sample of amplicons of the *uspA* gene of *E. coli* visualised under ultra-violet light.
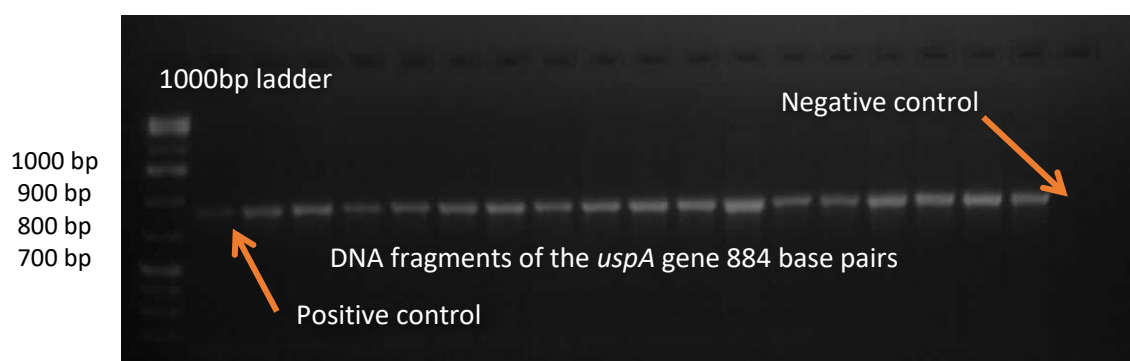
Figure 3.1 DNA fragments of *uspA* visualised under the SYN GENE BIO IMAGING ultra violet system

All 36 presumptive *E. coli* isolates presented with an amplicon size of 884 base pairs. The amplicon size represent *uspA* gene which confirmed all the study isolates as *E. coli*.

3.3 Antimicrobial susceptibility patterns

The susceptibility patterns of the *E. coli* isolates obtained from the study is shown in **Table 3.3** below

Table 3.3 Frequency distribution of antimicrobial susceptibility of *E. coli* isolated from pregnant women

Antimicrobial agent	Percentage of GBS antibiotics sensitive, intermediate and resistant					
	Sensitive (S)		Intermediate (I)		Resistant (R)	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
Ampicillin	1	2.8	9	25.0	26	72.2
Augmentin*	16	80.0	3	15.0	1	5.0
PTZ	35	97.2	1	2.7	0	0
Cefuroxime	36	100	0	0	0	0
Cefoxitin	36	100	0	0	0	0
Ceftazidime	36	100	0	0	0	0
Ciprofloxacin	35	97.2	0	0	1	2.7
Gentamycin	30	83.3	1	2.8	5	13.9
Amikacin	36	100	0	0	0	0
Imipenem	36	100	0	0	0	0
Meropenem	36	100	0	0	0	0
Ertapenem	36	100	0	0	0	0
Nalidixic acid	32	88.9	1	2.8	3	8.3
Cefepime	36	100	0	0	0	0
SXT	14	38.9	0	0	22	61.1

Abbreviations: PTZ – Piperacillin tazobactam, SXT – Sulphamethoxazole trimethoprim (n = 36, n* = 20)

A total number of 36 *E. coli* isolates were tested antimicrobial agents. Five of 31 pregnant women who tested positive were found to have 2 different *E. coli* isolates based on morphology, colour and

size, thus adding up to 36 numbers of isolates. *E. coli* was highly resistant to Ampicillin and Sulphamethoxazole trimethoprim.

3.4 Gene based resistance results

Table 3.4 below shows the resistance genes associated with *E. coli* isolates

Table 3.4 Frequency distribution of resistance genes among *E. coli* isolates

Resistance Gene		Frequency(n)	Percentage (%)
blaTEM	Present	6	16.7
	Absent	30	83.3
blaSHV	Present	9	25.0
	Absent	27	75.0
blaCTXM	Present	0	0
	Absent	36	100
blaMOX	Present	0	0
	Absent	36	100
blaFOX	Present	0	0
	Absent	36	100

(n= 36)

From **Table 3.4** above , Sixteen *E. coli* isolates had the two of the β-lactam genes respectively. The resistance gene *blaTEM* was found in 6 (16.7%) isolates, and *blaSHV* was found 9 (25.0 %). None of the isolates contained *blaCTXM*, *blaMOX* and *blaFOX.*

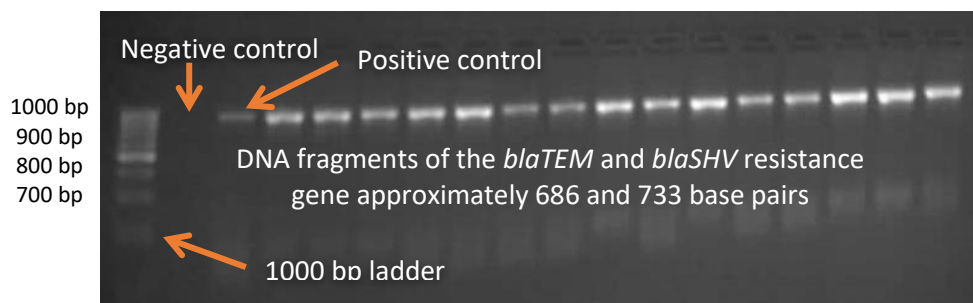Figure 3.2 below shows a sample of amplicons of the *blaTEM* and *blaSHV* gene of *E. coli* isola

Figure 3.2 DNA fragments for *blaTEM* and *blaSHV* resistance genes visualised under the SYN GENE BIO IMAGING ultra violet system

CHAPTER FOUR: DISCUSSION AND CONCLUSION

The current study aimed at determining the prevalence of *E. coli* colonization in pregnant women at 35 weeks of gestation and above, from hospitals in Ohangwena and Oshikoto regions of Namibia, the antimicrobial susceptibility patterns and gene based resistance of the isolates. Therefore, the prevalence of *E. coli* in pregnant was 14.9%.

To our knowledge, this work is the first to study the prevalence of *E. coli* colonization among pregnant women in Namibia; therefore, the comparison of our findings with previous studies in Namibia is limited.

It was reported in the recent study conducted in 2017 by Cools and co-workers that women from Africa had the highest *E. coli* carriage (35.6%) compared to women from South America, Europe and North America (19.7%, 13.3% and 12,0%), while Asian women had the lowest *E. coli* carriage rate (6.3%). Even though prevalence in the current study (14.9%) cannot be compared to the overall prevalence of vaginal carriage rate in Africa (35.6%), it elucidated that *E. coli* was prevalent in Namibia, particularly in the researched areas.

The prevalence found in the current study was lower compared to what was reported in a similar several studies conducted in different countries, whereby the prevalence of *E. coli* colonization was reported between 16.2-56.3% (Devi *et al*. 2014; Mobasheri *et al*. 2014; Tameliene *et al*. 2012; Rad *et al*. 2016). We could not find sufficient evidence to establish reasons to which the lower prevalence rate found in this study may be attributed to. However, it could have been influenced by the small number of participants recruited in our study.

In Kenya and South Africa Cools *et al* reported *E. coli* colonisation of 20.2% and 23.1% respectively (Cools *et al*. 2016). Although the prevalence of vaginal colonization among pregnant women detected in our study was lower than reported in other studies, it could be lowered if considering vaginal screening during pregnancy in selected rural areas in Namibia.

In the current study, the total of 31 presumptive *E. coli* isolates on MacConkey plates were deemed positive on Chromogenic agar. This suggests that Chromogenic Agar alone can be used as a selective media for *E. coli*. The study did not encounter any false results.

The current study evaluated the risk factors for *E. coli* colonization among pregnant women in Northern Namibia. Though number of participants differs per category, we found that women between the ages of ≥20-39≤ were more colonized by *E. coli* 24(11.5%) compared to those who were ≤20 years 1(0.48%) and those who were ≥40 years 6(2.9%). However, as shown in **Table 3.2**, there

was significance difference across all age groups with reference to positive *E. coli* colonization and negative *E. coli* colonization (P<0.05); therefore the study concluded that age could have influence on *E. coli* colonization in pregnant women.

In the current study maternal *E. coli* colonization was high among participants with low level of education 21(10.1%) compared to those with high level of education 10(4.8%). Furthermore **Table 3.2** illustrated that there was statistical significance between educational levels and *E. coli* colonisation (P<0.05). Our study results were not in agreement with what was reported by Tameliene and coworkers that educational levels did not show any statistical significance associated with *E. coli* colonisation (Tameliene *et al*. 2012), but were in agreement with what Mobasheri and coworkers findings that pregnant women with higher level of education had fewer vaginal infections as compared to uneducated (Mobasheri *et al*., 2014). It would increase their awareness of vaginal infections and their consequences for pregnancy, henceforth improving prevention and early diagnosis of bacterial infections.

As shown in **Table 3.2**, it was observed that majority of *E. coli* colonized women were not employed 27(13.6%), compared to employed women 4(2.0%). There was however significant difference association between *E. coli* colonization and employment (*P*< 0.05). In addition, this results could probably also be linked to level of education, by finding out if the participants with low level of education were also unemployment.

Furthermore, more rural dwelling women were more colonized 26(12.5%) by *E. coli* compared to urban dwelling individuals 2(1.0%) as illustrated in **Table 3.2**. Moreover, the study found statistical significance between Geographical location and *E. coli* location (P< 0.05), this means that there is association between maternal *E. coli* colonization and residential areas.

Unmarried women, including divorced, single and widowed were more colonized by *E. coli* 26(12.5%) compared to married women 5 (2.4%) as shown in **Table 3.2**. In addition, the ratio between positive and negative in the unmarried (0.2) showed that 2 in 100 women are colonized with *E. coli* and 1 in 100 married pregnant women is colonized with *E. coli*. Multiple sexual partners can be a mode of *E. coli* transmission from partner to partner (Ulleryd *et al*., 2015) and this could be the reason *E. coli* was more prevalent among unmarried pregnant women than in married women who are subjected to one partner.

Maternal *E. coli* in this study could not be linked to miscarriage, as women with no history of miscarriage were largely colonised 26(12.5%) compare to pregnant women with history of miscarriage 5(2.4%), as illustrated in **Table 3.2.** Further, these findings demonstrated that 3 in 100 pregnant women with history of miscarriage are colonized, while 2 in 100 pregnant women with no miscarriage history are colonized.

Table 3.2 demonstrated the association between maternal *E. coli* carriage and parity (P< 0.05) with *E. coli* frequently isolated from pregnant women gave birth for the first or second time. This is comparatively in agreement with Tameliene and coworkers who reported high *E. coli* colonization rate in women who gave birth for the second time (Tameliene *et al.*, 2012).

The last factor analysed in the study was stillbirth as displayed in **Table 3.2**. Even though there was significance between *E. coli* and stillbirth, this factor could also not be linked to *E. coli* colonization, as women with no history of stillbirth were largely colonized 29(13.9%) compared to pregnant women with previous history of stillbirth 2(1%).

A quite low number of responses were obtained regarding previous HIV and rapid plasma regain (RPR) test results of pregnant women screened in this study. Information regarding HIV and Syphilis status was therefore inadequate to perform a meaningful interpretation between co-infection with HIV, Syphilis and *E. coli* colonization.

In current study, presumptive identification of *E. coli* isolates was done using the Chromogenic agar, and all isolates were confirmed as *E. coli* by real time PCR, in which the target gene *uspA* and *uidA* were amplified. As presented in **Figure 3.1**. A total of 36 positive isolates have shown presence of an amplicon of size 884 base pairs on the electrophoresis gel, representing *uspA* gene confirming the organism as *E. coli*. The results of the two tests have shown 100 % correlation. Furthermore, the current study result is comparable to the study conducted by Anastasi and coworkers, who reported 83% of strains confirmed to be *E. coli* with *uspA* gene (Anastasi *et al*. 2010). Moreover, none of the isolates have shown the presence of *uidA*, this means that all *E. coli* isolates do not contain this gene; therefore it was not suitable for *E. coli* isolates confirmation.

In our study, 5 of the participants were found with two *E. coli* isolates. This was executed based on colony morphology and colour on plates; therefore total isolates to be tested for sensitivity added up to 36 as shown in **Table 3.3**. For our study *E. coli* isolates were highest resistance to Ampicillin 72.2% which was relatively in agreement with Tameliene and coworkers who also reported that 22.7% of *E. coli* isolates were resistant to ampicillin. Furthermore, in another study conducted by and coworkers, *E. coli* isolates were reported resistant to ampicillin with 57.5% (Tameliene *et al.*, 2012; Devi *et al.*, 2014).

E. coli isolates in this study presented with 61.1% resistance against Trimethoprim sulphamexazole. The Trimethoprim sulphamexazole resistance to *E. coli* isolates in this study was also higher as compared to the resistance of 26.3% reported by Devi and coworkers (Devi *et al*. 2012). Our study had fewer cases of resistance against Ciprofloxacin 2.7%, Nalidixic acid 8.3% and Gentamicin 13.9% respectively. The study conducted by Devi *et al* showed the resistance against Ciprofloxacin 5.0%, Nalidixic acid 20.5% and Gentamicin 5.0% separately (Devi *et al*. 2014). A study by Tameliene *et al*

showed no resistance to Gentamicin resistance 0% (Tameliene *et al*. 2012), which was different from our findings and that of other studies.

Our study detected intermediate resistance against PTZ 2.7%, Ampicillin 25%, Gentamicin 2.8% and Nalidixic acid 2.8%. The intermediate resistance reported in the current study is comparable to that reported by Devi *et al* except for PTZ which was not tested for in their study.

In the current study Ceftradzime, Cefuroxime, Cefoxitin, Amikacin, Imipenem, Meropenem, Ertapenem and Cefepime presented with 100% susceptibility against *E. coli* isolates. Our findings agree with the findings of a study in Luthuania (Tameliene *et al*. 2012), except for Cefoxitin, Ertapenem and Cefepime which were not used in their study. Ampicillin was highly resistant to *E. coli* isolates from pregnant women in our study, this could be due misuse of the drug in the respective region. Even though ampicillin was resistant, other drugs such as the carbapenems can still be administered according to CLSI, 2018.

As far as we know, the present study is the first of its kinds to be conducted in Namibia. Furthermore, there is no available data to elucidate the genes encoding *E. coli* resistance against antimicrobial substances in Namibia; therefore, comparison of our findings with previous data is limited. It is known that *E. coli* belonging to the Enterobacteriaceae family that ESBL enzymes cause resistance (Odjana *et al*. 2014; Ayodele & Adeniyi, 2016). Although our study did not determine the ESBL in our *E. coli* isolates, we evaluated the presence of *bla*-genes in all the isolates detected in the study.

We found a total number of 36 PCR positive isolates which were amplified and tested for genes as β-lactamase demonstrated in **Table 3.4**. We detected 6 isolates with *blaTEM* and 9 isolates with *blaSHV*. Moreover, we observed that most *E. coli* strains were negative in PCR for the following genes: *blaMOX*, *blaCTXM* and *blaFOX*. The most commonly detected *bla* genes were *blaTEM* and *blaSHV* among *E. coli* isolates. The current study findings agree with Ayodele & Adeniyi findings reported *blaTEM* and *blaSHV* in their study (Ayodele & Adeniyi, 2016). Our study data demonstrated the association between high ampicillin and Trimethoprim sulphamexazole resistance and beta-lactamase genes (*blaSHV* and *blaTEM*) among *E. coli* isolates.

Our study findings are not in agreement with the study conducted in Poland by Odjana *et al* (Odjana *et al*. 2014) that reported *E. coli* strains carrying the *blaCTXM* gene, which was associated with resistance towards ciprofloxacin, levofloxacin, tobramycin, and trimethoprim/sulfamethoxazole.

Conclusion

Maternal colonization with *E. coli* was relatively lower in this study than other studies (14.9%). Maternal *E. coli* carriage was high in pregnant women between the age range of 20-39, in pregnant women with low level of education, unemployed, unmarried and who reside in rural areas. The study has also revealed that *E. coli* was largely isolated from pregnant women with no history of miscarriage and stillbirth, while *E. coli* was prevalent in women with 1-2 parity.

Our study findings have revealed that there was significant statistical association between *E. coli* colonization and age, educational level, employment, marital status and habitat as observed with *P* values of <0.05. Similarly, there was also statistically significant association between *E. coli* colonization and parity. However, there was no significant difference between *E. coli* colonization and history of still births as well as miscarriages.

The resistance of *E. coli* to ampicillin was high (72.2%) and disturbing. However, the study detected susceptibility to other antibiotics including the carbapenems, which can be used as the drugs of choice to treat *E. coli*. Further studies in pregnant women are recommended to further elucidate the role of these organisms in infections in both pregnant women and their babies.

5. LIMITATIONS AND RECOMMENDATIONS

A demonstrative number of pregnant women in Ohangwena and Oshikoto regions of Namibia could not be obtained, as only one hospital was studied in Oshikoto region and two hospitals in Ohangwena region due to ease of accessibility.

The prevalence for this study cannot be generalized to the entire Namibian rural population of pregnant women, as the samples were obtained from some selected areas in Northern Namibia. To avoid misinterpretation of the results, the prevalence of *E. coli* is only applicable to the selected areas which were included in the study.

Furthermore, specimen delivery to the laboratory for analysis was at times delayed due to long distance transportation. To ensure reliability of results, specimens were transported at 2 – 8°C (cold chain management) in Amies transport medium. The transport medium preserves microbiological specimens and maintains viability of micro-organisms without allowing growth of contaminants thus compromising the quality of the specimens.

A total of 408 samples were expected to be collected, but due to financial constraints, only 208 samples were collected for the study

Different regions in Namibia need to be screened to obtain the *E. coli* prevalence amongst pregnant women in the country.

Additional genes encoding resistance associated with *E. coli* resistance should be analysed using molecular techniques.

6. SIGNIFICANCE OF THE STUDY

The study results will provide some knowledge about the prevalence of *E. coli* amongst pregnant women in rural settings in Namibia.

The study will also provide valid information concerning prevalence, and antimicrobial resistance patterns of *E. coli* to the Ministry of Health and Social Services. This data can be used by the ministry to implement proper intervention programs.

Our study findings will also provide useful information to national diagnostic laboratories regarding the advantages of performing gene based resistance of *E. coli* to antibiotics.

This study will serve as baseline information to *E. coli* colonization in pregnant women.

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APPENDICES
APPENDIX A: *ESCHERICHIA COLI* STUDY QUESTIONNAIRE / DATA COLLECTION TOOL

PLEASE NOTE: NO PARTICIPANT NAME SHOULD BE WRITEN ON THIS QUESTIONNAIRE

PURPOSE: This questionnaire is designed to derive information related to *Escherichia coli* colonization in pregnant women attending antenatal screening in the selected rural areas in Namibia.

INCLUSION CRITERIA: A Participant should **ONLY** be included in this study if her current pregnancy is 35 weeks and onwards of gestation.

EXCLUSION CRITERIA: A participant **SHOULD BE EXCLUDED** if she has taken antibiotics within the last 14 days prior to date of contact. A participant whose pregnancy is below 35 weeks of gestation **SHOULD BE EXCLUDED** from this study.

PART I: DEMOGRAPHIC INFORMATION

Unique identification number	:	
Date attended	:	
Hospital number	:	
Name of hospital attended	:	
Date of Birth	:	

PART II SOCIO-ECONOMIC CHARACTERISTICS

Cross (x) the appropriate box with the correct answer:

	Married	**Single**	**Divorced**	**Windowed**	**Cohabiting**
Marital status					

	Below matric	**Matric**	**Tertiary**
Highest level of education reached			

	Employed	**Self-employed**	**Unemployed**
Participant's employment status			

	Professional	**Skilled**	**Semi-skilled**
Participant's occupation			

	Employed	**Self-employed**	**Unemployed**
Husband's employment status			

	Below matric	**Matric**	**Tertiary**
Husband's highest level of education reached			

	Rent-whole/alone	**Rent-sharing**	**Own**
Accommodation			

	Urban	Semi-urban	Rural
Where do you dwell most yearly?			
State the name of the place			

PART III: OBSTETRIC HISTORY

1. Did you take antibiotics taken in the last 14   **YES** ☐   **NO** ☐   days?

2. Indicate number of successful pregnancies   ☐☐   (parity)

3. Indicate number of previous miscarriages (if any)

4. Indicate number of previous stillbirths (if any)   ☐☐

5. Indicate number of previous normal vaginal deliveries (if any)   ☐☐

6. Do you have any history of pain in the lower abdomen during   **YES** ☐   **NO** ☐

   pregnancy?

If yes, was the pain treated or not?

Treated	Untreated

PART IV: Current pregnancy

Please complete in writing or indicate   *with a*

cross (x) where required: ☐☐☐☐☐☐☐☐

Expected date of delivery (EDD)   :

Gestational age at current visit (weeks) : _____

Any trauma in current pregnancy?   :   **YES** ☐   **NO** ☐

If yes, specify   : _____

Height (cm)   : _____

Weight (to the nearest kg)   : _____

Mid-arm circumference (cm)   : _____

RPR test done   :   **YES** ☐   **NO** ☐

TPHA test done   :   **YES** ☐   **NO** ☐

HIV test done   :   **YES** ☐   **NO** ☐

(Adapted from Moyo *et al.*, 2000)

The patient identification number should be preceded with the first 3 letters of the hospital name example: **Eenhana:** *EEN001* and **Onandjokwe:** *ONA001* and should be recorded in the patient's

health passport for traceability purposes, in circumstances where a positive *E. coli* isolation was found.

APPENDIX B: CONSENT FORM

Research topic: Distribution of *Escherichia coli* in pregnant women and antimicrobial susceptibility patterns of the isolates in selected rural areas in Namibia

Investigators: Nangolo. L. (BSc), Mukesi M. (MSc) & Moyo, S. R. (PhD)

Institutions: Namibia Institute of Pathology, Namibia University of Science and Technology

Email: leanangolo@gmail.com, mmukesi@nust.na, srmoyo@nust.na

Procedure

This is a study focusing on determination of *Escherichia coli* rate and its gene-based resistance which is an organism known to cause complications on pregnancy and neonates.

1. This study will be conducted over a period of two years. Participants will include pregnant women who are between 35 – 37 weeks of gestation.
2. Participants will be screened for *E. coli* colonization in the vagina.
3. One swab, (vaginal) will be collected from each participant by registered nurses.
4. A questionnaire will be used to collect demographic and clinical data. Voluntary informed and written consent must be obtained from you, for this information to be eligible for use in this study. A unique identification number will be allocated to you to protect your identity and all information will be handled with strict confidentiality. All data will be stored on a protected device.

Purpose and benefits

The study will contribute valuable information that can be used to guide policy on *Escherichia coli* therapy and prevention in pregnant women in Namibia.

Risks, Stress or Discomfort

If at any stage of the investigation you feel uncomfortable, you are free to withdraw. Finally, we would like to review your medical record for information about your health history and treatment.

Full name and surname   ....

Signed (signature of parent if minor)   ....

Date   ....

APPENDIX C: ETHICAL CLEARANCE BY MINISTRY OF HEALTH AND SOCIAL SERVICES

REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198	Ministerial Building	Tel: 061 – 2032150
Windhoek	Harvey Street	Fax: 061 – 222558
Namibia	Windhoek	Email: shimenghipangelwa71@gmail.com

OFFICE OF THE PERMANENT SECRETARY

Ref: 17/3/3 **LN**
Enquiries: Mr. J. Nghipangelwa

Date: 19 October 2017

Ms. Lea Nangolo
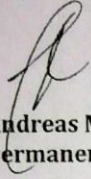**University of Science and Technology**
Windhoek

Dear Ms. Nangolo

Re: The distribution of Escherichia coli in pregnant women and antimicrobial susceptibility pattern of the isolates in selected rural areas in Namibia.

1. Reference is made to your application to conduct the above-mentioned study.

2. The proposal has been evaluated and found to have merit.

3. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**

3.1 The data to be collected must only be used for academic purposes;

3.2 No other data should be collected other than the data stated in the proposal;

3.3 Stipulated ethical considerations in the protocol related to the protection of Human Subjects' should be observed and adhered to, any violation thereof will lead to termination of the study at any stage;

3.4 A quarterly report to be submitted to the Ministry's Research Unit;

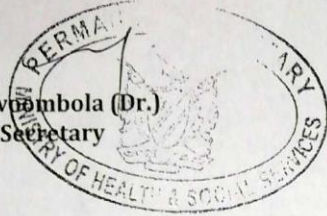3.5 Preliminary findings to be submitted upon completion of the study;

3.6 Final report to be submitted upon completion of the study;

3.7 Separate permission should be sought from the Ministry of Health and Social Services for the publication of the findings.

Yours sincerely,

Andreas Mwoombola (Dr.)
Permanent Secretary

"Your Health Our Concern"

APPENDIX D: ETHICAL CLEARANCE BY THE FACULTY OF HEALTH AND APPLIED SCIENCES

NAMIBIA UNIVERSITY OF SCIENCE AND TECHNOLOGY

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W: www.nust.na

FACULTY OF HEALTH AND APPLIED SCIENCES

DECISION/FEEDBACK ON RESEARCH PROPOSAL ETHICAL CLEARANCE

Dear ~~Prof/Dr/Mr~~/Ms/~~Other(s)~~:   Lea Nangolo

Student No (if applicable): 201049015

Research Topic:	distribution of *Escherichia coli* in pregnant women and antimicrobial susceptibility pattern of the isolates in selected rural areas in Namibia
Supervisor (if applicable):	Prof SR Moyo
Co-supervisor(s): if applicable	N/A
Qualification registered for (if applicable):	Master of Health Sciences

Re: Ethical screening application No: REC -00011/2017

The Research Ethics Screening Committee has reviewed your application for the above-mentioned research project. Based on the recommendation of the expert reviewer, the research as set out in the application is hereby:

(Indicate with an X)

Approved: i.e. may proceed with the project			
Approved provisionally: subject to compliance with recommendation(s) listed below	X		
Not approved: Not to proceed with the project until compliance with recommendation(s) listed below and resubmit ethics application for consideration			
IS MINISTRY OF HEALTH & SOCIAL SERVICES (MoHSS) APPROVAL REQUIRED?	YES:	X	NO:

It is important to note that as a researcher, you are expected to maintain ethical integrity of your research, strictly adhere to the ethical policy of NUST, and remain within the scope of your research proposal and supporting evidence as submitted to the REC. Should any aspect of your research change from the information as presented, which could have an impact or effect on any research participants/subjects/environment, you are to report this immediately to your supervisor or REC as applicable in writing. Failure to do so may result in withdrawal of approval. Kindly consult your supervisor or HoD if you need further clarification.

We wish you success in your research endeavour and are of the belief that it will have positive impact on your career as well as the development of NUST and the society in general.

Ethical issues that require compliance/ must be addressed		
No.	**Ethical issues**	**Comment/recommendation**
1.	Research activities involve collection of samples/specimen from pregnant women	To obtain permission from the MoHSS of Namibia and submit copy to FHAS-REC secretariat*.
2.		

NB: May attach additional page as required; * Failure to do so will invalidate research outcome

Full Name (reviewer): Ms Berta E van der Colf... Signature: ...*B.End.Colf*...... Date: 14-08-2017......

Full Name: PROF OMOTAYO AWOFOLU............... Signature: .... Date: 15-08-2017......
Chair: Ethics Screening Committee