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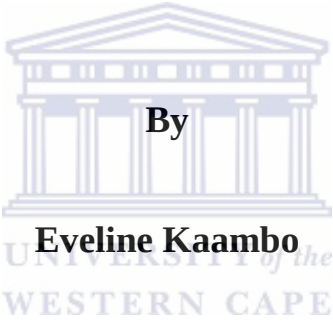
The frequency and characterization of streptococci in aerobic vaginitis (AV) and its association with pregnancy outcomes.



By

Eveline Kaambo

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Medical Biosciences, University of the Western Cape.

Supervisor: Prof. C. Africa

December 2014

The frequency and characterization of streptococci in aerobic vaginitis (AV) and its association with pregnancy outcomes.

Eveline Kaambo

KEYWORDS

Aerobic vaginitis
Group B Streptococci
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Risk factors
Adverse pregnancy outcomes
Serotypes
Polymerase Chain Reaction (PCR)
Antimicrobial sensitivity testing
Trek sensititre system
Pulse Field Gel Electrophoresis (PFGE)

ABSTRACT

Aerobic vaginitis has been implicated in preterm delivery of low birth weight infants. The presence of aerobic vaginitis (AV) -associated bacteria such as group B streptococci (GBS) and *E. faecalis* in pregnant women are regarded to be the leading cause of neonatal mortality and morbidity world-wide.

The aim of the study was to detect the prevalence of AV and its associated bacteria with preterm delivery in the Western Cape, South Africa. Furthermore, it sought particularly to examine and investigate the predictive value of GBS and *E. faecalis* for preterm delivery (PTD). It also aimed to establish other factors which may predict adverse pregnancy outcomes.

Three hundred and one pregnant women were recruited from four different antenatal in the Western Cape, South Africa. The study conformed with the Declaration of Helsinki (2013). Maternal data was collected from a questionnaire and maternal medical records. Vaginal and rectal swabs were collected and microscopically examined for AV, followed by culture characterization of GBS and *E. faecalis.* Antimicrobial susceptibility testing was also performed.

In this study, AV was detected in 79 (26.2%) of the 301 pregnant women, and GBS and *E. faecalis* isolated from 50 (16.6%) and 21 (7.0%) respectively.

GBS serotype V was the predominant serotype, followed by serotype III. Pulse field gel electrophoresis (PFGE) profile analysis for both GBS and *E. faecalis* yielded a total of 24 restrictions profiles for GBS and 16 for *E. faecalis*. Multivariable analysis revealed that parity, gravidity, vaginal discharge, urinary tract infection, and smoking were significantly associated with PTD. The results from the study provides improved guidelines maternal screening of pregnant women. The early detection of AV-related bacteria may significantly reduce maternal and neonatal morbidity.

DECLARATION

I declaration that *The frequency and characterization of streptococci in aerobic vaginitis (AV) and its association with pregnancy outcomes* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have use or quoted have been indicated and acknowledged by complete references.

Eveline Kaambo **December 2014**

Signed: ……

CONFERENCE PRESENTATION WHICH HAVE EMINATED FROM THIS RESEARCH

1. Kaambo E, Stemmet M, Nco S, and Africa CWJ. (2012). The association of asymptomatic vaginal infections with preterm delivery. (**Won the best poster award**) at Infection Control Africa Network (ICAN) conference, 2012. Conventional Center, V & A, Waterfront, Cape Town

2. Eveline Kaambo, Charlene WJ Africa (2011). A comparison of Polymerase Chain Reaction (PCR) with culture for the detection of *Streptococcus agalactiae* in vaginal swabs from women with a history of preterm delivery. Microbiology and Biotechnology: rising to the challenges of the times. 6th Asia – Pacific Biotechnology Congress. 40th PSM Anual Convention and Scientific Meeting, 2011. Philippine International Convention Center (PICC), Manila, Philippines

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DEDICATION

I dedicate this thesis to the memory of my loving brother Lord Uaundjae Kaambo

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LIST OF ABBREVIATIONS USED IN THIS STUDY

AV: Aerobic Vaginitis

BLAST: Basic Local Alignment Search Tool

Bp: Base pairs

BPD: bronchopulmonary dysplasia

BV: Bacterial vaginosis

CNA: Colistin Nalidixic Acid

CDC: Centers for Disease Control

CLSI: Clinical and Laboratory Standard Institute

CPS: Capsular Polysaccharide

DNA: Deoxyribonucleic Acid

E. coli: *Escherichia coli*

EDTA: Ethylenediamine Tetraacetic Acid

E. faecalis: *Enterococcus faecalis*

ELBW: Extremely Low Birth Weight (< 1 000g)

EOS: Early Onset Sepsis

EOD: Early Onset Disease

FT: Full term

FTD: Full Term Delivery

GBS: Group B Streptococci

GC-clamp: Guanosine-cytosine clamp

GenBank: Nucleotide sequence database

HIV: Human Immunodeficiency Virus

HPV: Human Papilloma Virus

I: Intermediate

Kb: Kilobase pairs

LOS: Late onset neonatal sepsis

LBW: Low Birth Weight

LGT: lower genital tract

LS SIL: Low grade squamous intraepithelial lesion

m: Meter

min: Minutes

M: Molar

mM: Millimolar

MRSA: methicillin resistant *Staphylococcus aureus*

NEC: necrotizing enterocolitis

NCBI: National Center for Biotechnology Information

ng: nanogram

nm: nanometer

NICU: neonatal intensive care units

OD: Optical Density

PAP: Papinocolaou

PCR: Polymerase Chain Reaction

PFGE: Pulse-field Gel Electrophoresis

PROM: Premature Rupture Of Membranes

PT: Preterm

PTB: Preterm birth

PTD: Preterm Delivery

PPROM: Preterm prelabour rupture of membranes

rDNA: ribosomal DNA

RNA: Ribonucleic acid

rRNA: ribosomal RNA

rpm: revolutions per minute

R: Resistant

RFLP: Restriction Fragment Length Polymorphism

s: seconds

S: Sensitive

S. aureus: *Staphylococcus aureus*

SDS: Sodium dodecyl sulphate

T_m: Melting temperature

T-RFLP: Terminal Restriction Fragment Length Polymorphism

Tris: 2-amino-2 (hydroxymethyl) 1,-3-propanediol

TSST-1: toxic shock syndrome toxin-1

µl: microliter

µm: micromolar

UGT: upper genital tract

UTI: urinary tract infections

v/v: volume per volume

VLBW: Very Low Birth Weight (< 1 500g)

w/v: weight per volume

WHO: World Health Organization

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The vaginal microbiota is important for maintaining vaginal health and preventing infections of the reproductive tract (Stojanovic *et al*., 2012). The composition of the human vaginal microbiota can be affected by numerous host factors, such as poor hygiene, age, diabetes, change in hormone levels, infections, menopause, sexual activity, pregnancy and the use of contraceptives or spermicides, plus individual habits such as douching (Aila *et al.*, 2009). A number of bacterial species are known to colonize both the gastrointestinal and the reproductive tract, and the rectum has been suggested to play an essential role as a source or reservoir for organisms that colonize the vagina (Aila *et al.,* 2009). The vaginal microbiota of a healthy asymptomatic woman consists of an extensive diversity of anaerobic and aerobic bacterial genera and species dominated by the microaerophilic, genus *Lactobacillus* (Donati *et al.*, 2010), known to produce lactic acid (reducing the vaginal pH to < 4.5), bacteriocines and hydrogen peroxide (H_2O_2) which inhibit the growth of potentially pathogenic non – acid tolerant microorganisms (Mijac *et al.,* 2006; Li *et al*., 2011; Stojanovic *et al.,* 2012). Amongst these, hydrogen peroxide is postulated to have a crucial role in protecting against the overgrowth of pathogens in the vagina, since it can be inhibitory to bacteria, fungi, viruses and mammalian cells (Mijac *et al.,* 2006), either alone or in combination with halide and peroxidise that are present in vaginal secretions (Mijac *et al.,* 2006; Donati *et al.,* 2010). In addition, other mechanisms of lactobacilli include surface – binding proteins that inhibit adhesion to uroepithelial cells (Stojanovic *et al*., 2012).

There are a variety of different strains of lactobacilli present in the vagina, the most frequent being *Lactobacillus jensenii, L. gasseri, L. iners* and *L.crispatus*, and the species and relative numbers of species differ according to the population studied (Donati *et al.*, 2010).

Lactobacillus – deficient conditions are believed to be associated with the development of several infections such as AV(Aerobic Vaginitis), which in turn promotes the transmission of sexually transmitted disease, such as *Chlamydia*, Syphilis, HIV (Human Immunodeficiency Virus), HPV (Human Papilloma Virus), *Gonorrhoea* and *Trichomonasis* (Donati *et. al*., 2010; Marconi *et al.*, 2013). An alteration in the balance of lactobacilli in the vaginal microbiota has also been associated with bacterial vaginosis (BV), a condition which may be differentiated from AV by the lack of inflammation and the predominance of anaerobic vaginal species (Donders *et al*., 2002).

1.2. Definition of Aerobic Vaginitis (AV)

'Aerobic vaginitis' is a new vaginal pathology that is neither classifiable as specific vaginitis nor as bacterial vaginosis (Donders *et al*., 2002; Tempera *et al*., 2004). AV is also considered or described as a disruption of lactobacilli, accompanied by signs of inflammation and the presence of a rather scarce, mainly aerobic microbiota, composed of enteric commersals or pathogens (Donders *et. al*. 2011; Tansarli *et. al,* 2013). The clinical features which are considered to be associated with AV and BV in regards to the host response is specific and clearly different for each individual condition (Donders *et al*., 2011). When the vaginal ecosystem is disrupted during AV, the pH increases to >6, with a decrease in lactic acid and an increase in inflammatory cytokines such as IL – IB (Hemalatha *et al*., 2012).

AV is caused by the overgrowth of aerobic pathogens including *Escherichia coli*; Group B Streptococcus, *Staphylococcus aureus*, and *Enterococcus faecalis* that trigger a localized vaginal inflammatory immune response as evidenced by clinical signs and symptoms, which includes the presence of vaginal discharge, an elevation of the vaginal pH, inflammation with leukocyte infiltration and a marked depletion of healthy *Lactobacillus* species (Donati *et. al*., 2010; Donders *et. al*. 2011; MacPhee *et al*., 2013; Tansarli *et. al,* 2013). AV has been implicated in complications of pregnancy such as ascending chorioamnionitis, premature rupture of the membranes and preterm delivery (Rabiee *et al.,* 2006; Najmi *et al.,* 2013; Donders *et. al*. 2011).

1.3. Diagnosis of Aerobic Vaginitis (AV)

AV diagnosis is based on wet mount microscopy (Donders 2007). Aerobic vaginitis is diagnosed when vaginal smears are deficient in lactobacilli, positive for cocci or coarse bacilli, positive for parabasal epithelial cells, and positive for vaginal leucocytes (plus their granular aspect) (Tansarli *et. al.,* 2013). The diagnosis of AV is also based on molecular diagnostic methods and microscopic criteria (Table 1) graded as, not present (0 points), moderate (1 points) or severe (2 points) (Tansarli *et. al*., 2013; Donders *et. al*., 2011). The number of points establishes the composite AV score, with the maximum score being 10 (Donders *et. al*., 2011). Furthermore, comparable to the Nugent score used for grading BV, the AV score may be normal or slight, moderate or severe AV (Donders *et. al*., 2011).

Table 1: Classification of AV

Classification	**Grade**	**Description**
Normal	1	Increased lactobacilli, decreased cocci
Intermediate	II	Corresponds to a diminished Lactobacillary microbiota, mixed with other bacteria (Donati *et al.,* 2010). Due to their specific link to pathology, this group is further subdivided into different groups such as slightly disturbed, fairly normal (LBGIIa) and moderately disturbed, rather abnormal (LBGIIb) lactobacillary microbiota (Donders *et al.,* 2011).
Abnormal	III	Consists of a variety of of other bacteria that has no lactobacilli present (Donders *et al.,* 2011).

Studies have shown that the normal and abnormal lactobacillary microbiota are divided into three or four types called "Lactobacillary grades" (LBG's) (Donati *et al.,* 2010). Lactobacillary grades (LBGs) are the basis for a composite score to which the following four variables have been added.

a) Proportional number of lactobacilli
b) The presence of toxic leukocytes

c) The presence of parabasal epithelial cells
d) The type of background microbiota

In this classification, the immune reaction of the host is also taken into account for the diagnosis (Donders 2007). Parabasal cells are considered a sign of severe epithelial inflammation as they are encounted only in moderated or severe forms of AV such as in desquamative inflammatory vaginitis (Donders 2007).

1.4. Treatment for AV

The paramount approach for treating AV in both pregnant and non-pregnant women is unknown.

Antibiotics may not be adequate for most patients with AV, due to the inflammatory component, including leukocytes and parabasal cells (Donders *et al*., 2011). For this reason, the appropriate diagnosis and distinction between these infectious conditions is very crucial as their treatment is different; for example, AV does not respond well to metronidazole which is commonly used for the treatment of *Trichomonas* vaginitis (TV) and BV (Donders 2007). Clindamycin is therefore considered to be a better choice than metronidazole for pregnant women with an abnormal vaginal microbiota.

Optimal treatment includes antibiotics that have little consequence on the normal microbiota, usually the *Lactobacillus* species, while eradicating the Gram-negative enteric organisms such as *E. coli*, and Gram-positive GBS, *S. aureus*, and *E. faecalis* successfully (Subramaniam *et al.*, 2012; Zarbo *et al*., 2013). Conventional treatment for AV consists of a course of broad-spectrum antibiotics that target anaerobic and aerobic bacteria. One such antibiotic or drug of choice is the aminoglycoside, kanamycin, the efficacy of which has been demonstrated (Zarbo *et al*., 2013; Donders *et al.*, 2011). An additional antibiotic used for aerobic vaginitis (AV) is the quinolone, moxifloxacin (Zarbo *et al*., 2013).

Several studies have shown that the effects of screening and treatment for abnormal vaginal flora in order to reduce preterm delivery (PTD) remain controversial (Honda *et al.,* 2014).

1.5. Aerobic Vaginitis (AV) and its association with pregnancy outcomes

Pregnancy is considered to be a period in which the vaginal microbiota, conditioned by high estrogen levels, have a good supply of glycogen and an elevated proportion of lactobacilli, which can surpass 90% of the microorganisms present in the vagina during fertile age (Zarbo *et al*., 2013).

It is suggested that the presence of abnormality in the vaginal microbiota in early pregnancy is considered to be one of the most significant risk factors for preterm delivery, premature rupture of membranes and low birth weight (Donders *et al.,* 2009; Donders *et al*., 2011).

Other risk factors for spontaneous preterm births include a previous preterm birth, black ethnicity, periodontal disease, and low maternal body-mass index (Goldenberg *et al.*, 2008). In addition, a short cervical length and a raised cervical-vaginal foetal fibronectin concentration are considered to be predictors of spontaneous preterm birth (Goldenberg *et al*., 2008).

The prevalence of AV is still largely unknown. Besides the prevalence studies on GBS colonization (Rocchetti *et al.,* 2011), which ranges between 7 and 25% in women between 35 and 37 weeks of gestation, only sporadic papers have been published on the frequency of AV during pregnancy (Donders *et al*., 2011). Because of the high concentrations of circulating estrogens, severe AV, typically with increased numbers of parabasal cells, is reported to be infrequent in pregnancy (Donders *et al*., 2011). However, there is a clear indication that, even when asymptomatic, these vaginal infections represent a risk factor for preterm delivery (Stojanovic *et al*., 2012; Honda *et*

al., 2014). However, the mechanism by which abnormal vaginal microbiota results in a defined risk for preterm birth remains elusive (Stojanovic *et al*., 2012).

Not only are the microscopic details different between AV and BV, the local immune response elicited in the host is unique in each individual condition (Donders *et al*., 2011). In pregnant women, the concentration of pro-inflammatory cytokines, IL-1b (Interleukin – 1b), IL-6 (Interleukin - 6) and IL-8 (Interleukin – 8), are evidently linked to LBG, and are inversely proportional to the numbers of lactobacilli (Donders *et al*., 2011). While IL-6 and IL-8 are associated with adverse pregnancy outcomes, patients with AV may similarly be at increased possibility of having preterm delivery (Donders *et al*., 2011; Donati *et al*., 2010). Interleukin 1 - b increases significantly more in AV (eight fold increase) compared to bacterial vaginosis (BV). IL-6 increases fivefold in those diagnosed with AV (Donati *et al.,* 2010; Hemalatha *et al.,* 2012). Previous studies have shown that IL-6 is a well recognized marker for bacterial amnionitis and imminent term and preterm delivery (Donati *et al.,* 2010). In addition, as a result of the increased immune response in AV, symptoms are related to the thinned vaginal mucosa and increased inflammatory reaction (Donders *et al*., 2011).

1.6. AV associated bacteria implicated in PTD

Among the organisms implicated in AV and associated with PTD are Group B Streptococci, Group D Streptococci, *Staphylococcus* aureus and *Escherichia coli* (Fatemi *et al*., 2009). Perinatal infection causes septicemia, meningitis or pneumonia, which are associated with a high mortality (Fatemi *et al.,* 2009; Taminato *et al.,* 2011).

1.6.1. *Escherichia coli* (*E. coli*)

One of the most commonly isolated microorganisms in AV is *E. coli*, frequently isolated as the sole organism (Donders *et al*., 2011). Even though the role of *E. coli* in vaginitis is particularly controversial, it is known to alter the micro – environment (Zarbo *et al*.,

2013) and remains one of the most common causes of neonatal sepsis, often being recovered from placental tissues in chorioamnionitis (Donders *et al*., 2011).

The physiologic changes in pregnancy are believed to predispose women to UTI. Reduced immunological status during pregnancy may also contribute. In the literature *E. coli* is described as the most common pathogen associated with both symptomatic and asymptomatic bacteriuria, accounting for 80 to 90% of infections in pregnant women (Schnarr *et al*., 2008). Other Gram negative bacteria and Group B streptococcus (Schnarr *et al*., 2008) are also reported.

Specific virulence determinants in uropathogenic strains of *E. coli* are considered to be associated with invasive infection and pyelonephritis in pregnancy (Schnarr *et al*., 2008). These are comprised of toxins and adhesions, pili or fimbriae which allow adherence to uroepithelial cells preventing bacteria from urinary lavage, and allowing for multiplication and tissue invasion (Schnarr *et al*., 2008).

E. coli has more often been reported in women who deliver PTD than full term delivery (FTD) (Krohn *et al*., 1997; McDodald *et al.,* 2000; Cray *et al*., 2005). Neonates born at preterm gestational ages are believed to be more likely to develop neonatal sepsis and meningitis caused by *E. coli* in comparison with neonates born at term (Krohn *et al.*, 1997). The high rates of *E coli* infection were also reported in a study by Stoll *et al*., (2011) where the rates of GBS were lower amongst very low birth weight (VLBW) infants (5.09 vs 2.08 per 1000 LBs) and infants with a BW of 1501 to 2500 g (0.54 vs 0.38 per 1000 LBs).

Early onset neonatal sepsis (EOS) remains an uncommon but significant cause of morbidity and mortality among the very low birth weight (VLBW) infants (Hornik *et al*., 2012; Stoll *et al*., 2005). Gram-negative organisms such as *E. coli* continue to be the predominant pathogens associated with EOS (Stoll *et al*., 2005)

Some studies have reported an emerging trend of *E. coli* predominating in PTD and in early neonatal sepsis following prophylaxis for GBS colonization during pregnancy (Baltimore *et al*., 2001; Hyde *et al*., 2002; Stoll *et al*., 2002; Towers *et al*., 2002; Lin *et al*., 2001; Cordero e*t al.*, 2004). EOS caused by *E. coli* was reported to increase slightly by 3.17 to 3.20 episodes per 1000 NICU admissions (Hornik *et al*., 2012). While most of EOS cases were reported to be caused by Gram-negative organisms, Hornik *et al*., (2012) associated LOS with Gram-positive organisms.

Late onset neonatal sepsis (LOS) in the neonate is believed to contribute significantly to the morbidity and mortality of hospitalized neonates worldwide (Chu *et al*., 2012). The risk factors for LOS include prematurity, low birth weight, and invasive interventions (Chu *et al*., 2012). A study by Bizzaro *et al*., (2008) revealed that there were significant increases in both EOS and LOS which could be attributed to *E. coli*. Changes in the incidence or antibiotic resistance patterns of *E. coli* EOS were observed. Increased rate of early onset *E. coli* sepsis was also revealed in a study by Stoll *et al*., (2011) amongst the preterm neonates in particular. A significant increase in ampicillin-resistant *E. coli* was reported with very low birth weight infants while in *E. coli* LOS no significant change was reported (Krohn *et al.,* 1997; Bizzarro *et al*., 2008).

1.6.2. *Staphylococcus aureus* (*S. aureus*)

Staphylococcus aureus is a prominent human pathogen contributing to a variety of infections, ranging from mild skin lesions to life - threatening toxic shock syndrome, breast abscesses, postoperative infections and menstrual toxic shock syndrome (Bourgeois-Nicolaos *et al*., 2010; Li *et al*., 2012). In most previous studies it has been demonstrated that *Staphylococcus aureus* infections are increasing among pregnant women, postpartum women and neonates, but the risk factors for *S. aureus* colonization in pregnancy and the association between maternal colonization and infant infections are not really well defined (Top *et al*., 2012). The rate of vaginal carriage of *S. aureus* has been reported to be 4% – 22% of the vaginal microbiata of pregnant women (Top *et al*., 2012; Bourgeois-Nicolaos *et al*., 2010). Imperative factors that are considered to increase

the risk for neonates due to S. *aureus* colonization, include breastfeeding, number of household members, low birth weight, early gestation age at birth and the duration of antibiotic or ventilator days (Seybold *et al*., 2008; Jimenez - Truque *et al.,* 2012; Lazenby *et al*., 2012).

According to the literature, increased oxygen levels and a neutral pH increases the chances of toxic shock syndrome toxin-1 (TSST-1) production (MacPhee *et al* 2013). The production of TSST-1, encoded by the tst gene, is regulated in large part by the agr system of *S. aureus* (Jarraud *et al*., 2002; MacPhee *et al* 2013). AV – associated bacteria such as *E. faecalis* are considered to increase tst expression leading to increased production of TSST-1 while lactobacilli reduce *S. aureus* virulence (MacPhee *et al* 2013). *S. aureus* accounts for > 90 % of late-onset sepsis in neonates. Late-onset sepsis is noted to be four times higher in very low birth weight (VLBW) infants (Grass *et al.,* 2013).

The factors that contribute to *S. aureus* rectovaginal colonization in pregnant women are not well understood, but it is believed that being from black race is one of the risk factors for MRSA rectovaginal colonization (Andrews *et al.,* 2008; Top *et al.,* 2012). MRSA is noted as a significant pathogen in neonatal intensive care units (NICUs) (Seybold *et al*., 2008). Community – acquired MRSA infections have been reported to occur in both neonates and pregnant women or postpartum women, as a result of vaginal colonization of S. *aureus* during pregnancy (Li *et al*., 2012). Traditional antibiotic therapy, even though highly effective, has led to the emergence of antibiotic-resistant strains, such as methicillin resistant S. *aureus* (MRSA) (Li *et al*., 2012). Studies report the prevalence of methicillin - resistant S. *aureus* (MRSA) in the range of 0.5% - 10% in infants and mothers even though in some areas, higher rates of colonization have been reported (Chen *et al.,* 2006; Top *et al*., 2012; Jimenez – Truque *et al.,* 2012). The literature reveals that 1.8% of neonates are MRSA colonized and 16 out of 50 (32%) of these colonized neonates went on to develop MRSA infection (Lazenby *et al*., 2012).

1.6.3. **Streptococci**

1.6.3.1. Lancefield Group D Streptococci (*Enterococcus faecalis*)

Lancefield group D Streptococci includes the enterococci-organisms now classified in a separate genus from other streptococci and nonenterococcal group D streptococci. Group D Enterococci and non-enterococci are part of the genital tract, but are a common cause of UT (Urinary tract) infections, subacute bacterial endocarditis, abdominal abscesses, and wound infections (Hufnagel *et al.*, 2007; Al-Abbas 2012). The predominance of asymptomatic genital tract infection in pregnant women has been widely reported in the literature (Akerele *et al*., 2002), with enterocci causing life threatening infections in preterm infants and other immuno-compromised patients, (Hufnagel *et al.*, 2007).

The enterococcal species which are considered to be significant pathogens for humans are *E. faecalis* and *E. faecium*. These organisms are likely to affect patients who are elderly or whose normal microbiota has been altered by antibiotic treatment (Hufnagel *et al.*, 2007). Furthermore, E. *faecalis* is associated with 6% mortality rate in early onset septicaemia which increases to 15% in late – onset infections, whilst in general it is implicated in 7% to 50% of fatal cases (Al-Abbas 2012).

Enterococci, have been previously reported to be harmless inhabitants of the gastrointestinal tract flora initially, and has emerged as one of the main causes of health care – associated infections (Coombs *et al.*, 2014). Enterococci are believed to be difficult to treat because of their intrinsic resistance to antibiotics that notably include beta – lactams and aminoglycosides which are frequently used to treat infections with Gram-positive cocci (Al-Abbas 2012; Gaca *et al.*, 2012).

An association was found between enterococcal colonization and prematurity (Hufnagel *et al.*, 2007). *E. faecalis* colonization rates were found to be higher in the age groups of < 32 weeks of gestation (Hufnagel *et al*., 2007).

Seliga-Siwecka *et al.*, (2013) has shown that the presence of *E. faecalis* in the amniotic fluid considerably increases the risk of histological inflammation of the placenta (OR 10.7, 95% CI 1.27–89.9) and also increases the risk for necrotizing enterocolitis (NEC) and bronchopulmonary dysplasia (BPD).

Preterm birth neonates are considered to be more likely to be suffering from necrotizing enterocolitis (NEC) than neonates born at term (Wang *et al*., 2014). NEC is described as the most serious gastrointestinal disorder in newborn infants with an incidence of 10 – 15% in those with very low birth weight (VLBW <1500 g, Normann *et al.,* 2013). Neonates who have developed NEC are believed to carry *E. faecalis* (Wang *et al*., 2014; Normann *et al.,* 2013).

The survival rate of the preterm neonates has improved considerably over the last 20 years or so even with neonates who are born with VLBW (Thompson *et al*., 1992). However, preterm neonates and those with very low birth weight may require a prolonged ventilation and stay in neonatal intensive care units (NICU) (Thompson *et al*., 1992). The defense mechanisms of these infants are considered to have been impaired against infections putting them at a high risk of nosocomial infection caused by microorganisms such as *E. faecalis*, GBS and *S. epidermidis* (Thompson *et al*., 1992). *E. faecalis* is believed to be the leading cause of the nosocomial infections (Gaca *et al.,* 2012; Thompson *et al*., 1992).

Serum concentrations of IL-8 were elevated in severe cases of NEC from its onset through the first 24 hours (Wang *et al*., 2014). IL-8 are considered to play a significant role in infant infections (Wang *et al*., 2014).

E. faecalis is described as one of the first colonizers that could suppress the inflammatory responses and also shape the immune system of the neonates (Wang *et al*., 2014). In the neonates, the intestines are likely to undergo acute inflammation when exposed to Gram-negative bacteria (Wang *et al*., 2014). The presence of *E. faecalis* is believed to help the

intestines maintain the immune balance in response to such challenges (Wang *et al*., 2014). However, the therapeutic effects of *E. faecalis* must be examined thoroughly, especially as *E. faecalis* was reported to be an opportunistic pathogen in hospital infections (Wang *et al*., 2014).

At birth, an infant's gastrointestinal tract is sterile. However it rapidly becomes colonized with organisms from the mother and the local environment, with enterococci and members of the family *Enterobacteriaceae* being the predominant organisms found in the stools during the first days of life in full-term infants (Hallstrom *et al*., 2004; Magne *et al.*, 2005). In the literature it is also reported that in the preterm neonates, especially those with a very low birth weight (weighting less than 1,000 g at birth), bacterial colonization and other factors may influence the biodiversity of the intestinal flora and may also be considered to increase the risk of gastrointestinal disease such as necrotizing enterocolitis (NEC) (Magne *et al.,* 2005). Other factors, include the immaturity of the main crucial functions, the mode and environment at which the delivery took place, the feeding regimen and the type of drug therapy (i.e. antibiotics, corticoids) (Magne *et al.,* 2005).

Low grade squamous intraepithelial lesion (LS SIL) aerobic vaginitis in women is considered to be incredibly common although not believed to be an indicator of human papilloma virus (HPV) infection (Jahic *et al*., 2013). LS SIL and AV were revealed in 9 cases of *E. coli*, while 4 had *E. faecalis*. *E. coli* was isolated in 3 cases of women who had a normal Papinocolaou (PAP smear) (Jahic *et al.*, 2013).

1.6.3.2. Group B Streptococci (GBS)

GBS (also known as *Streptococcus agalactiae* and referred to as GBS for the remainder of this thesis) exists as normal microbiota in the female genital tract and anal areas of healthy adults (Uh *et al.,* 1997; Dermer *et al.,* 2004; Stronati *et al.,* 2004; Domelier *et al.*, 2006; van der Mee-Marquet *et al.,* 2006). However, even though the majority of women are unaware of their status, an estimated one out of three women is a carrier of GBS. The gastrointestinal tract serves as the natural reservoir for GBS and is expected to be the

source of vaginal colonization (O'Reilly *et al*., 1998; Dermer *et al.,* 2004; Najmi *et al.*, 2013).

Women who are colonized with GBS might develop infections associated with pregnancy including urinary tract infection, bacteremia, chorioamnionitis, postpartum endometritis (Campbell *et al*., 2000; Najmi *et al.*, 2013; Milyani *et al.,* 2011; Konikkara *et al.,* 2013). Intrauterine infection has been associated with the ability of GBS to ascend from the lower genital tract (LGT) and colonize the upper genital tract (UGT) (Rocchetti *et al.*, 2011, Zhou *et al*., 2010; Romero *et al*., 2003).

Pregnant women may express signs and symptoms of GBS colonization during gestation as well as during labour (Rallu *et al* 2006), increasing the risk of preterm labour (PTL), preterm prelabour rupture of membranes (PPROM) and perinatal transmission of the organism (Varma *et al.,* 2006; Hiller *et al.,* 2005; Maghaddam 2010).

GBS has significant occurrence in septicaemia in elderly individuals and immunocompromised patients (Kong *et al*., 2002; Hajare *et al*., 2012) and emerged in the 1960s as a significant human pathogen that caused neonatal sepsis and meningitis (Bohnsack *et al.,* 2008; Domelier *et al.,* 2006; Ramaswamy *et al.,* 2006).

In the newborn, GBS infection is divided into two diverse types, viz congenital and acquired (Rabiee *et al.,* 2006; Baltimore 2007), and remains the foremost cause of neonatal mortality and morbidity in the world despite a recent decline in occurrence (Bohnsack *et al.,* 2008; Li *et al.,* 2006; Bergeron *et al.,* 2004*;* Tsolia *et al.,* 2003; Konikkara *et al.,* 2013).

In neonates and infants, GBS disease is defined as either early-onset disease (EOD, age, 0-6 days) or late-onset disease (LOD, age, 7 to 90 days) (Martins *et al.,* 2007; Mhaskar *et al.,* 2005; Konikkara *et al.,* 2013). EOD is associated with the presence of GBS in the vagina of the mother, and transmission is thought to take place vertically through

aspiration of infected amniotic fluid or passage through the birth canal (Martins *et al.*, 2007).

Colonization of GBS in pregnant women is the single principal risk factor for early-onset newborn diseases (EOD) caused by vertical transmission and colonization of the infant during delivery (Bergseng *et al.*, 2007; Rallu *et al* 2006). Intrapartum colonization is strongly associated with early onset GBS sepsis resulting in approximately 4% of the fatalities and serious morbidities that include sepsis, pneumonia, meningitis, illness and death in infants, but also long - term disabilities such as loss of hearing, impaired vision, developmental problems, cerebral palsy, osteomyelitis and septic arthritis (Rabiee *et al.*, 2006; Bergeron *et al.*, 2004).

Well-established risk factors for early-onset invasive GBS in newborns are rupture of membranes for more than 18 h before delivery, fever in the mother during labour, preterm delivery and a history of GBS disease in previous infants (Bergh *et al.*, 2004; Bergseng *et al*., 2007; Rabiee *et al.*, 2006).

The source of bacterial strains causing late-onset disease (LOD) is less well understood and may involve community or nosocomial acquisition (Martins *et al.*, 2007). However, there is evidence that in several infants with LOD, the GBS causing the infection share identical serotypes as the GBS isolated from their mothers, suggesting a maternal source (Martins *et al.*, 2007; Konikkara *et al.*, 2013).

1.7. Justification of the study

This study will provide valuable data which can be used in determining neonatal diseases by establishing the prevalence of AV amongst women of childbearing age, and identifying disparities that may exist between the different ethnic groups within the Western Cape, South Africa. The results obtained from this study could also possibly lead to the development of intervention programmes to target specific population groups,

to decrease vertical transmission of AV-associated bacteria from the mother to the neonate.

In brief, this study will evaluate risk factors including the prevalence of AV, maternal demography and particularly, a history of previous preterm delivery as predictors of pregnancy outcomes in a population from the Western Cape. This study will also aid in the increased public awareness of AV and may inform the screening based prevention protocol within our communities, this reducing the treatment costs incurred for infants with EOD and LOD.

1. 8. Null hypothesis

Endogenous bacteria are not risk factors for adverse pregnancy outcomes.

1.8.1. Objectives

1.8.1. Objectives of this study:

- To detect the prevalence of AV.
- To identify and characterize the predominant facultative anaerobes implicated in AV.
- To examine and investigate the predictive value of AV-associated GBS and *E. faecalis* in PTD
- To establish whether maternal demography and reproductive history may predict current pregnancy outcomes.

CHAPTER 2: MATERIALS AND METHODS

2. 1. Study population

Patients were recruited from four Maternity Obstetric Units (MOU) in the Western Cape, South Africa. These were located in Mowbray, Mitchells Plain, Guguletu and Khayelitsha. The participants were from lower socioeconomic groups representative of four different ethnic groups. The Mowbray (MOU) serves as referral to communities of black and coloured ethnic groups, whereas Guguletu and Khayelitsha serve members predominantly from the black ethnic group and Mitchells Plain clinics serve predominantly the coloured ethnic group.

2.2. Ethical consideration

Participants were randomly recruited. The content and objectives of the study were explained and informed consent was obtained from all participants. Ethics approval for this study was obtained from the human ethics committee of the University of the Western Cape (UWC). The study complied with the declaration of Helsinki (2013).

2.3. Patient Data collection

After obtaining written informed consent, data were collected by means of a standard structured questionnaire (see appendix 2 A) designed to obtain socio - demographic data and other relevant information. This included information such as maternal age, gestational age, previous obstetric history, history of current pregnancy, parity, marital status, mode of delivery, and neonatal birth weight of previous deliveries (see appendix 2 A). All 301 pregnant women received and completed the questionnaires. The women were recruited from the four MOU metioned aboved (Mowbray, Mitchells Plain, Guguletu and Khayelitsha). The women were not interviewed during labour nor after delivery.

2.4. Sample collection

Samples were collected from 301 women at 28 to 37 weeks of gestation (Fig.1) (see appendix 2 D). A modification of the sample collection procedure described by Uh *et al* (1997) was used.

Women who had received antibiotic therapy two weeks prior to recruitment were not included in the study.

A vaginal and anorectal swab were collected from each patient (Bergeron *et al.,* 2000) using sterile cotton-tipped swabs and transported to the laboratory in liquid Amies with charcoal medium (Sterilin, 18114CST, Italy).

2.5. Criteria used for the microscopic diagnosis of AV

A guideline was established in order to characterize the presence and severity of AV (Donders *et al.,* 2009; Donders *et al.,* 2011). This was based on Nugent scoring methods used for BV determination, employing a Gram – stained microscopic assessment that numerated specific bacterial morphotypes (Donders *et al.,* 2011). The "AV" score, was calculated using high – power field light microscopy to evaluate the presence or absence of lactobacilli, leukocytes, (including toxic leukocytes) other vaginal flora and parabasal epithelial cells (Donders *et al.,* 2009; Donders *et al.,* 2011**).** The presence of healthy Gram–positive lactobacilli were compared to the presence of aerobic or facultative anaerobic Gram–positive cocci (such as Streptococci, Staphylococci, or *Enterococcus*) and Gram-negative bacilli ,such as *Klebsiella* species and *E. coli* (Donders *et al.,* 2009).

Table 2 shows the scoring criteria for AV. Lactobacillary grades (LBG) I, consisted of numerous pleomorphic lactobacilli and no other bacteria; LBG IIa, showed a mixed microbiota, but predominantly lactobacilli; LBG IIb, was also a mixed microbiota, but the percentage of lactobacilli was severely decreased as a result of an increased number of other bacteria; whilst, LBG III, lactobacilli were considered to be severely depressed or absent because of the overgrowth of other bacteria.

AV score: <3, no signs of aerobic vaginitis (AV); 3 or 4, slight AV; 5 or 6, moderate AV; ≥6, severe AV (Donders *et al*., 2011).

Table 2: Microscopic diagnosis criteria for AV (Donders *et. al.*, 2011)

AV score	Lactobacillary grades (LBG)	Number of leukocytes	Proportion of toxic leukocytes	Background flora	Proportion of parabasal epitheliocytes (PBC)
0	I and IIa	≤ 10 / hpf	None or sporadic	Unremarkable or cytolysis	None or <1%
1	IIb	> 10 / hpf and ≤ 10 / epithelial cell	≤ 50 % of leukocytes	Small coliform bacilli	≤ 10%
2	III	> 10 / epithelial cell	> 50% of leukocytes	Cocci or chains	> 10%

Lactobacillary grates (LBG); parabasal epitheliocytes (PBC); high – power field (hpf) (400X magnification).

2.6. Sample processing

Swabs were inoculated onto enriched blood agar (Colistin nalidixic agar, Becton Dickinson, Catalog number 63954, Bio-Rad Laboratories and MacConkey agar (Catalog number CM0109B, Oxoid, Basingstoke, Hampshire, England) and incubated at 37ºC for 24 h. Plates showing no growth were incubated for another 24 h before being declared as negative for growth. After Gram - staining and agar inoculation, swabs were incubated in enriched broth (Todd-Hewitt Broth; Catalog number T1438, Sigma Aldrich, Deisenhofen, Germany) and GNS broth for 24 h at 37ºC to ensure recovery of suspected pathogens. GNS broth (Gentamicin Nalidixic Supplement**),** is a standard selective broth medium that consists of Todd–Hewitt broth supplemented with gentamicin (8 µg per milliliter) and nalidixic acid (15 µg per milliliter) (Bergeron *et al*., 2004).

Bacterial suspensions were smeared onto a glass slide, fixed and stained according to Kopeloff's modification of the Gram stain (Libman *et al*., 2006). Bacterial morphology was microscopically observed by light microscopy and recorded. Gram-positive cocci and Gram–negative bacilli were further identified as described in the following sections.

2.7. Identification of Gram–negative bacilli

MacConkey agar allowed for the growth and differentiation between lactose fermenters and non-lactose fermenters. Lactose – fermenting colonies appeared pink on the MacConkey agar and were picked for further identification using the API 20E test API 20 E test kit (BioMerieux ®SA, Marcyl; Etoile - France; Ref 20 160).

A fresh culture for the API 20 E test was prepared by culturing fresh colonies from MacConkey grown overnight at 37^{o}C for 24 h in a sterile glass tube containing 5 ml saline. Post incubation, the suspension was pipetted into API 20E strip (BioMerieux ® SA, Marcyl; Etoile - France; Ref 20 160) according to the manufacturer's instructions.

2.7. 1. Inoculation and incubation of the API strip

In order to make it easier to transfer the bacterial suspension to the API 20 E strip, thereby avoiding creating bubbles, the strip was held at a slight angle from the bench, placed on the chamber top to hold it up - right and the bacterial suspension pipetted into each well using a sterile pipette. Care was taken to ensure that the end of pipette touched the side of the well, allowing for the fluid to be drawn into the well while slowly squeezing the bulb. The vials containing CIT, VP, and GEL wells were each filled to the top with the suspension, while for LDC, ODC, ADH, H_2S, and URE (Table 3) wells, each was filled with half the suspension and the other half with sterile mineral oil.

Prior to starting the test, the small indented wells in the bottom of the incubation chamber were filled with 5 ml of sterile water. The API 20 E strip was placed into this bottom chamber tray and the cover labeled with the sample number and the date. The strip was incubated at 37^{o}C for 24 h.

Table 3: Abbreviations used for API 20 E Strip Test

Abbreviations	**Full name**
ADH	Arginine Dihydrolase
CIT	Citrate
GEL	Gelatin
IND	Indole
GLU	Glucose
H_2S	Hydrogen sulfide
LDC	Lysine Decarboxillase
ODC	Omithine Decarboxillase
TDA	Tryptophane Deaminase
URE	Urea
VP	Pyruvate

2.7. 2. Reading and interpretation of the API 20 E Strip:

After overnight incubation, the strip was examined for at least 3 or more positive tests before proceeding with the adding of the specific reagents to the test wells. The results were interpreted according to the manufacturer's instructions. A pink colour following the addition of one drop of James agent (BioMerieux ®SA, Marcyl; Etoile - France; Ref 70 542) indicated a positive indole (IND) after 1 min. One drop of TDA reagent was added to the TDA test and after 1 min a red colour indicated a positive result. Two drops of each of VP 1 and VP 2 reagents were added to the VP test, and allowed to stand for 10 min at room temperature. A red colour indicated positive and yellow indicated negative results. Lastly, a yellow reaction was considered to be positive results for GLU glucose fermentation and blue or green was negative. Two drops of each nitrite test reagent was added to the GLU tube, and the reaction observed. A positive red result observed within 3 min, indicated nitrate reduction.

2.8. Identification of Gram–positive cocci

2.8. 1. Catalase test

The test was performed in order to differentiate between staphylococci and streptococci. The principle of the catalase test is that the enzyme, catalase acts as a catalyst by converting 3% hydrogen peroxide (H_2O_2) into oxygen and water, resulting in immediate bubbling. A single colony taken from 5% sheep blood agar plate grown overnight at 37°C was transferred to a slide and mixed to a smooth suspension in saline. A hundred microliters (100 µl) of 3% hydrogen peroxide (H_2O_2) was added to the suspension. Effervescence indicated a positive catalase test (i.e. *Staphylococcus*) and the absence of effervescence indicated a negative catalase result i.e. *Streptococcus* and *Enterococcus* (Fig. 1).

2.8. 2. Identification of Catalase+ve cocci

The DNAse plate (Catalog number CM0321, Oxoid, Basingstoke, Hampshire, England) was used to differentiate between *Staphylococcus aureus* and *Staphylococcus epidermidis*. The DNAse test is used for detecting deoxyribonuclease activity of bacteria and particularly for identification of pathogenic *Staphylococcus aureus* which produces deoxyribonuclease (DNA - ase) enzymes (Reynolds 2011). The DNAse plate was inoculated by spotting or streaking a heavy inoculum of test organism. The plates were incubated at 37°C for 24 h. After 24 h incubation, the plates were flooded with 1 N HCl. A zone of clearing around the spot or streak organisms indicated DNAse activity (*Staphylococcus aureus*).

2.8. 3. Identification of Catalase–ve cocci

Lancefield Grouping

A latex slide agglutination grouping kit (Catalog number DR0585A, Oxoid, Basingstoke, Hampshire, England) was used to classify catalase–negative Gram-positive cocci into Lancefield groups. Grouping is dependent on the carbohydrate contained in the cell wall of the G+C. Depending on the Lancefield serology, species were further identified with confirmatory tests.

2.8.3. 1. GBS typing

Isolates producing a positive Lancefield grouping of B were further serotyped using Hemolytic *Streptococcus* Group B typing sera (Catalog number SSI-LATX-54985/7; Statens Serum Institut, Denmark) for the identification of the capsular polysaccharide antigens Ia, Ib, II, III, IV, V, VI, VII VIII and IX, according to the manufacturer's instructions.

Briefly, a colony of GBS was incubated overnight in 5 ml of THB (Todd-Hewitt broth). Approximately 10 µl of the bacterial suspension was added to the latex suspension on the circle card and thoroughly mixed using a sterile stick. The slide was rocked back and forth for a few seconds and examined for agglutination within 5 – 10 seconds. Agglutination tests were carried out on ten antisera (Ia, Ib, II-IX). Any agglutination after 30 seconds was not considered to be a positive reaction. If no designation could be made, the isolate was then determined to be non-typeable.

2.8.3. 2. Christie Atkins Munch-Petersen (CAMP) Test for GBS

The CAMP test was performed to aid with the identification of GBS. GBS are able to produce CAMP factor (a diffusible extracellular protein) that synergistically acts with beta-lysin of *Staphylococcus aureus* (*S. aureus*) and enhances the lysis of red blood cells.

A known GBS strain served as a positive control. The CAMP test was performed on Columbia agar plate containing 5% sheep blood. A toxin - producing *S. aureus* was inoculated diagonally on to a sheep blood agar plate. A single colony of the beta hemolytic streptococci from overnight culture was streaked perpendicularly approximately 2 mm spaces away from *S. aureus* without touching. The inoculated plates were incubated anaerobically at 37ºC overnight.

The isolates were considered to be GBS if they produced an enhancement of haemolysis in the form of a distinct arrowhead shape at 1 to 2 mm from the *S. aureus*, with the widest portion of the arrowhead on the GBS side.

2.8.3. 3. Hippurate Test for GBS

The hippurate test was used to detect the enzyme hippurate hydrolase which hydrolyzes sodium hippurate to form benzoic acid and glycine. The addition of ferric chloride to benzoic acid forms an insoluble brown ferric benzoate precipitate. The hippurate broth was inoculated with the fresh culture and the broth was then incubated for 24 h at 37ºC. Post incubation, the tube of broth was centrifuged for 20 min to sediment the bacteria. Clear supernatant (800 µl) was transferred to a 1.5 ml Eppendorf tube or small clear tube before adding 200 µl of ferric chloride reagent and thoroughly mixing. A positive test was indicated by a heavy precipitation that didn't clear within 10 min. A clear golden-brown liquid indicated a negative test.

2.8.3. 4. Bacitracin Sensitivity Test for GBS

All beta hemolytic colonies on CNA were tested for Bacitracin (0.04 units) sensitivity (Catalog number X3484D, Oxoid, Basingstoke, Hampshire, England).

The bacitracin test was performed in order to differentiate between GBS and *S. pyogenes* (GAS). Fresh culture suspensions (100 µl for each individual isolate) were evenly spread on Mueller-Hinton agar plates containing 5% sheep blood. The plates were anaerobically

incubated at 37ºC overnight. A negative (resistant) test was indicated by no zone of inhibition around the bacitracin disc while susceptibility was indicated by the appearance of a zone of inhibition.

2.8.3. 5. *Enterococcus*

Confirmatory tests for *Enterococcus faecalis* included:

I. Colonial morphology on blood agar and MacConkey agar. On blood agar, *Enterococcus* appears as a pale, non – haemolytic colony. While on MacConkey agar, *E faecalis* appears as tiny magenta – coloured colonies.

II. Bile Esculin Agar slants for *Enterococcus*

The bile esculin test was performed to aid with the differention of GBS from the *Enterococcus*, by demonstrating the ability of *Enterococcus* to hydrolyze esculin in the presence of bile. Using a sterile loop, a colony from a pure overnight culture was transferred to the bile esculin slant (Lot 7H2418, F92430, Bio-Rad, Marnes-la-Coquette). The tubes were incubated anaerobically for 18 h at 37ºC. The tube was examined for definite blackening of the agar indicating a positive test. No colour change in the agar indicated a negative test.

2.9. Antimicrobial susceptibility

2.9. 1. Kirby-Bauer Method

The antimicrobial susceptibility test was based on the procedure described by Lopardo *et al* (2003). Isolates were tested for antibiotic sensitivity by performing the Kirby-Bauer disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines. Pure colonies from each individual isolate from 5% sheep blood agar plates containing CNA were suspended in 5 ml of saline. The inoculum was adjusted to the turbidity of a 0.5 McFarland standard. Fresh culture suspensions (100 µl) were evenly spread on the Mueller-Hinton agar plates containing 5% sheep blood. The following eight antibiotics were tested viz., Penicillin G (PG) 1 Unit; Clindamycin (CD) 2 µg;

Gentamicin (GM) 10 µg; Fusidic Acid (FC) 10 µg; Erythromycin (E) 5 µg; Trimethroprin (TM) 1.25 µg; Sulphamethoxazole (SMX) 25 µg; Tetracycline (T) 10 µg. Discs were placed on Mueller-Hinton agar streaked plates containing 5% sheep blood. Plates were anaerobically incubated at 37ºC overnight. Strains were considered resistant or susceptible to each individual antibiotic when the inhibition zones from the individual plates were interpreted. The zones of inhibition were measured from the edge of the disk to the edge of the inhibition zone using a ruler and reported as sensitive. Cultures showing no zones of inhibition were interpreted as resistant.

2.9. 2. Sensititer trek system

A fresh culture for the TREK system (Thermo, Scientific, Waltham, MA, USA) was prepared by culturing fresh colonies grown overnight at 37^{o}C for 24 h. The inoculum was prepared by the direct colony suspension: colonies grown overnight on CNA agar with 5% sheep blood were suspended in sterile glass tubes containing PBS. For the Gram-negative isolates, the inoculum was prepared using colonies grown on MacConkey agar plates. The suspension was vortexed for 20 s adjusted to a 0.5 McFarland turbidity standard and read on the nephelometer (12 V DC. 1.25A: SN 437R03n098). Each PBS tube containing the suspension was vortexed for further 10 s and left to stand for 15 min at room temperature. One hundred microliters (100 µl) of the suspension from the top third of the tube was transferred and dispersed in the Mueller-Hinton Broth with TES/Lysed Horse Blood. Tubes were inverted and vortexed for a further 20 s. The inoculated tubes were again inverted and the suspension dispensed into a sterile petri dish lid. Using a multichannel pipette, 100 µl aliquotes of the suspension were delivered into the Trek microtiter plate (Catalog number STP6F, Trek Diagnostic System Inc, 982 Keynote Circle, Suite 6, Cleveland, OH 44131), the plate sealed with the film supplied and the plate incubated at 37^{o}C for 24 h. The Trek microtiter plates were placed into the Vizion Trek Diagnostic System (Magellan Biosciences; Model NO. 436 – A; Serial No. 436/VR02N82) for reading.

The breakpoints were defined by the Clinical and Laboratory Standards Institute (CLSI); when breakpoints were unknown, breakpoints for comparable drugs or comparable microorganisms were taken. Quality control was performed using American Type Culture Collection (ATCC) strains as controls.

2. 10. Molecular biology

2.10. 1. Isolation of DNA

DNA isolation was based on a procedure described by Sambrook *et al.,* (2001). DNA extraction was carried out for all samples collected**.** Total genomic DNA was extracted from vaginal and rectal swabs as follows: A standard loop of Group B streptococci was inoculated into 5 ml of Todd Hewitt broth and grown for 24 h at 37ºC. The culture (1.5 ml) was transferred to a 2.0 ml Eppendorf tube and centrifuged at 4000 x g for 2 min using a microcentrifuge (Geratebau Eppendorf Centrifuge GmbH, 04436 Engelsdorf, No. 5415 C 65232, Germany**)**. In some cases, centrifugation was carried out until a visible pellet was observed. The supernatant was discarded and the pellet resuspended in 567 µl buffer by repeated pipetting. Afterwards 20% SDS (30 µl) and 20 mg/ml protease K (6 µl) were added. The samples were mixed thoroughly by inverting and incubated for 1 h at 37ºC. Thereafter, 5 M NaCl (100 µl) was added and thoroughly mixed, followed by incubation for 2 min at 65ºC and the addition of 80 µl CTAB/NaCl (10% CTAB, hexadecylytrimethyl ammonium bromide; 0.7M NaCl, sodium chloride). The samples were mixed thoroughly and then incubated for 10 min at 65ºC. The suspension was extracted with an equal volume (700 µl) of chloroform/isoamyl alcohol (24:1) solution, mixed thoroughly and then centrifuged at 10,000 x g for 5 min. This step was done in order to remove the CTAB-protein/polysaccharide complexes. The upper aqueous phase was transferred to a new 2.0 ml Eppendorf tube and an equal volume (700 µl) of phenol/chloroform/isoamyl alcohol (25:24:1) added. After thoroughly mixing, the tube was centrifuged at 15,000 x g for 5 min and the supernatant or upper (aqueous) phase containing nucleic acid was transferred to a new 2.0 ml Eppendorf tube. Isopropanol (0.6 volumes) was added to precipitate the DNA. After thoroughly mixing, the solution was

allowed to stand for 15 min to allow for precipitation before centrifugation at 12,000 x g for 15 min resulted in a visible pellet. The pellet was washed with 500 µl of 70% ethanol and then centrifuged for 5 min after being thoroughly mixed by inverting. The supernatant was carefully removed. The pellet was air-dried and finally resuspended in 100 µl of TE buffer. The DNA concentration (calculated as OD260nm x 50*ng*/µl) and purity (ratio OD260nm/ OD280nm) was determined by using the spectrophotometer (Thermo Scientific Nanodrop 2000/2000C) and the purity determined by electrophoresis on a 1% agarose gel at 100 volts for 1 h. The genomic DNA was used for the PCR assay.

2.10. 2. Agarose gel electrophoresis

DNA extracts were separated in 0.8% (w/v) agarose gels and PCR products in 1% (w/v) agarose gels using 0.5 x TBE buffer (Sambrook *et al.*, 2001). Samples were prepared by mixing with loading buffer 1x (0.2 ml/ml glycerol and 5 mg/ml bromophenol). Electrophoresis was performed in 0.5 x TBE buffer at 100 V. Ethidium bromide (0.5 µg/ml) was added to the agarose gel during the preparation for the staining procedure. The DNA fragments were sized according to their migration in the gel compared to the DNA molecular weight markers (λ P*st*1 DNA restriction fragments, Catalog no.Fe-SM0361, and FE-SM0613 O'RangeRuler 50 bp DNA Ladder, Fermentas, Inc). The gels were analyzed with the Doc-ItLS® Image Acquisition Software, Version 5.5.5a (Doc-It Life Sciences Software, UVP™, Inc., San Gabriel, USA). The gels were visualized using ultraviolet (UV) illumination (UVP™, Inc., San Gabriel, CA 91778, USA).

2.10. 3. DNA Amplification by Polymerase Chain Reaction (PCR)

Pairs of primers (Table 4) were designed for the PCR amplification of the universal bacterial and GBS-specific 16S rRNA genes. A standard 50 µl PCR reaction solution contained 1 x PCR amplification buffer (10 x buffer being 200 mM Tris pH 7.6, 100 mM KCL, 100 mM $(NH_4)_2$ SO_4, 20 mM $MgSO_4$, 1% (w/v) Triton X- 100), 0.2 mM each of dATP, dCTP, dGTP and dTTP, 0.5 µM of each primer, 1U of *Taq* DNA polymerase, and 10 ng template DNA.

A total of 30 cycles were run under the following conditions after the initial heating step of 94ºC for 4 min: denaturing at 94ºC for 30 s, primer annealing at 50ºC for 30 s and extension 72ºC for 1 min 30 s and final extension at 72ºC for 10 min. The universal bacterial 16S rRNA gene primer pair of E9F (McInerney *et al*., 1995) and U1510R (Reysenbach *et al.*, 1995) was used to amplify the nearly complete 16S rRNA gene from the majority of bacterial organisms (Baker *et al*., 2003). Amplified products were separated on 0.8% agarose gels using electrophoresis in 0.5 x TBE buffer. The products were stained with ethidium bromide and visualized under UV using a transilluminator.

The pair of GBS specific primers, Sag 40 and Sag 445, was used for PCR amplification under the following conditions: A pre – PCR step was use at 94ºC for 2 min. Initial denaturation at 94ºC for 45 s followed by 35 cycles of three temperature cycles involving denaturation at 95ºC for 30 s, annealing at 60ºC for 1 min, extension at 72ºC for 2 min. This was followed by a final extension at 72ºC for 10 min. The presence of each PCR product was then verified using electrophoresis in 1% agarose gel and stained with ethidium bromide.

Table 4: Oligonucleotide specific primers used in this study for the amplification of the universal bacterial and GBS specific 16S rRNA genes.

Primers	Nucleotide sequences (5'-3')	Position (16S rRNA gene)	Annealing temp.	Specificity	References
E9F	GAGTTTGATCCTGGCTCAG	9-27	50ºC	Universal	McInerney *et al*., 1995
U1510R	GGTTACCTTGTTACGACTT	1510-1492	50ºC	Universal	Reysenbach *et al*., 1995
Sag 40	CGCTGAGGTTTGTGTTTACA	40-61	60ºC	Bacteria	Riffon *et al*., 2001
Sag 445	CACTCCTACCAACGTTCTTC	445-465	60ºC	Bacteria	Riffon *et al*.,2001

DNA molecular analysis such as pulsed-field gel electrophoresis (PFGE), PCR-based typing methods, and sequence analysis were used to determine molecular relatedness of isolates for epidemiologic investigation.

2.10. 4. Pulse Field Gel electrophoresis (PFGE)

A PFGE method was based on a procedure described by Manning *et al*, (2005). Contour clamp homogenous electric field (CHEF) gel electrophoresis is a molecular technique used to separate large, restricted DNA fragments (>30kb) (Benson *et al*., 2002). The bacterial DNA is embedded in agarose, and cell lysis and DNA restriction occurs *in situ*. This prevents DNA shearing. The restricted fragments are separated on an agarose gel matrix by alternating the electric fields for optimal separation of large DNA fragments. The CHEF gel electrophoresis is traditionally used for molecular typing in medical epidemiology. In this particular study, the technique was used to determine the relatedness amongst the GBS strains.

2.10.4.1. Preparation of unsheared DNA

Two to three colonies of GBS from pure cultures were picked and incubated in 5 ml of Todd Hewitt broth for 24 h at 37°C. The microorganisms were suspended in TE buffer (10 mM NaCl, 1 mM EDTA, pH 8.0) to a turbidity equivalent to 2.5 McFarland or 0.27 Absorbance. Four hundred microliters (400 µl) of this suspension was pipetted into a separate microcentrifuge tube, then 20 µl Lysozyme/Lysostaphin (20 mg/ml) was added to the suspension before being incubated in water bath at 56-60ºC for 15 min. Twenty microliters (20 µl) Proteinase K (20 mg/ml) and 400 µl of embedding 1% agarose was added to each individual sample (cell suspension) and carefully mixed. The suspension (100 µl) was immediately transferred into one well of the plug mold avoiding creating any air bubbles. The agarose was allowed to solidify for 15-20 min at room temperature. The plugs were transferred into sterile 15 ml tubes containing 5 ml of Lysis buffer (50 mM Tris: 50 mM EDTA, pH 8.0 + 1% Sarcosyl) and 25 µl of Proteinase K (20 mg/ml). The plugs were thoroughly mixed by inversion. The plugs were incubated overnight (16-20 hrs) at 50ºC without agitation. The Lysis buffer was removed using a pipette and the plugs washed with approximately 5 ml of sterile water for 15 min at 50ºC in water bath (repeat twice). Thereafter, the plugs were washed again four times in 5 ml TE buffer (10 mM Tris: 1 mM EDTA, pH 8.0) for 15 min at 50ºC in water bath without agitation. The

plugs were stored for 3-6 months at 4ºC in 1 x TE buffer for later use (the plugs should be stable).

2.10.4. 2. DNA Restriction (Enzyme Digestion of Samples)

Restriction endonuclease digestion was performed by transferring slices of the plugs into a sterile 2.0 ml microcentrifuge tube. Portions of plugs (2 mm) were cut, covered with 180 µl of sterile water and 20 µl 10 x Buffer Tango in a 2.0 ml Eppendorf tube, and incubated for 15 min at 30°C. The reaction buffer was replaced with 175 µl of sterile water, plus 20 µl 10 x Buffer Tango and 5 µl of 50 U Sma *I* were added before incubation at 30ºC for 3 h or overnight. The buffer was discarded and 500 µl of 1 x TE buffer added to the plugs. The plugs were stored at 4ºC for 2-4 weeks for later use.

2.10.4. 3. Pulse field Separation of DNA Restriction Fragments

Agarose gel (1%) was freshly prepared in 100 ml 0.5 x TBE (Tris Borate EDTA). Digested plugs were loaded into wells with standard strains at either end of the gel and after every 5 lanes. PFGE performed with a CHEF DR II (Catalog number 170-3670, Bio-Rad) in 0.5 x TBE under the following conditions: 14ºC; initial start time 4 s; final start time 45 s; gradient 6 V; run time 24 h. Lambda DNA ladder was used as a standard marker (Catalog number 170-3635, Bio-Rad, Basingstoke, Hampshire, England) for all the gels and *S. aureus* and two of the GBS type strains (V and III) were used as controls. DNA fragments generated from GBS strains were run in every 2nd and 3 rd lane to allow normalization of gels and comparisons with database. The gels were stained for 15 min in ethidium bromide solution (5 drop off ethidium bromide to 300 ml deionized water), and destained in a dark room for 40 min in 500 ml distilled water, with a water change every 20 min. Gels were visualized under UV illumination, photographed and the image was saved.

2.10.4. 4. PFGE analysis

The PFGE profiles were analyzed by visual assessment of bands. The sample profiles of DNA fragments generated using PFGE were matched using the The AlphaEase FC image processing and analysis software (AlphaInnotech Comporation ™San Leondro, CA), Reference controls were used to merge profiles generated from different gels. The resulting nominal data was coded into presence (coded 1) or absence (coded 0) data creating a binary file for multivariate statistical analysis. The binary file was used to create a similarity matrix that was used for cluster analysis and Multidimensional Scaling (MDS) analysis using the Primer v6 software, also known as the Primer – E software (Clarke *et al*., 2001). The Unpaired Group Mean Weight Average (UPGMA, average) pairing was used to create hierarchical clustering based on Euclidean distance methods. While the MDS analysis was based on the original binary data according to Bray – Curtis similarity matrix.

Cluster analysis methods are used to group objects according to their similarities and dissimilarities, based on measures of association.

2.11. Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) for window version 19.0. The Chi square test was used to determine the associations between two categorical variables, but when the number in a cell was less than five**,** instead of chi-square, Fisher exact test was used. For both tests variables were not associated (not statically significant) if the p-value was greater than 0.05. The Chi square test/ Fisher exact test was used to evaluate associations between delivery status and marital status, social demographics, and obstetrics history.

To measure the relationship between numerical variable (age, gravity, and parity) and a binary variable (delivery status) the Mann-Whitney U test was used because the numerical variable is not normally distributed (nonparametric test). To determine whether or not the numerical variable was normally distributed we used Shapiro-Wilk test. For the

Shapiro-Wilk test if the p value was greater than 0.05, it was considered to be normally distributed. For the Mann-Whitney U test, values less than 0.05, indicated a statistically significant difference between groups.

A specimen was considered positive for GBS if it was positive by culture or by PCR assays. Sensitivity and specificity, positive predictive value, and negative predictive value were then calculated for each of the assays. Statistical work was performed by using the following formulae:

Sensitivity = TP/ (TP+ FN), Specificity = TN/ (FP + TN), positive predictive value (PPV) = TP/ (TP + FP), negative predictive value (NPV) = TN/ (FN + TN). The following formula: Sensitivity/1–specificity was used in order to determine the positive odd ratio; while for the negative odd ratios the following formula was use (FN/TP + FN divided by TN/FP+TN.

The percentage accuracy for each of the methods was determined using the following formula: TP + TN/sample number X 100, where TP = True positive, TN = true negative, FN = false negative, FP = false positive

CHAPTER 3: DEMOGRAPHICS OF STUDY POPULATION AS ESTABLISHED FROM THE QUESTINNAIRE

Not all of the 301 recruited pregnant women responded to all of our questions in the questionnaire handed to the patients at recruitment. Therefore, the results in this section are exclusively based on the number of responses we obtained to each question rather than the total number of participants in the study. The women were not interviewed during labour nor after delivery. Pregnancy outcomes were extracted from the patients folders post delivery. The questionnaire covered possible risk factors associated with adverse pregnancy outcomes.

3.1. Population locations

Socioeconomic factors between the mothers from different locations were analyzed. The participants were from Guguletu (16.9%), Khayelitsha (KMOU, 24.6%), Mowbray (MMH, 29.9%) and Mitchells Plain (MP, 28.6%).

3.2. Maternal Age

The age distribution of the 301 pregnant women ranged from 17 - 42 years (Table 5). The mean age of the entire study population was 28.8 (± 5.2) years (Table 5).

The results of the age distribution analysis show that among the 301 pregnant women the majority, 36.52% were from the age group 26 - 30 years, 27.65% were from the age group 17 - 25 years, 24.57% were age group 31 - 35 years, 8.87% were from age group 36 - 40 years, and 2.39% were aged group 41 years and over (Table 5).

Table 5: Demographics of the study population

Variables/ Categories	Frequency (%)	Mean (SD):
Location of the population (MOU)		
MMH	90 (29.9%)	
KMOU	74 (24.6%)	
MP	86 (28.6%)	
GUG	51 (16.9%)	
Total	**301 (100.0%)**	
Age groups		28.8 (± 5.2)
17-25	81 (27.65%)	
26-30	107 (36.52%)	
31-35	72 (24.57%)	
36-40	26 (8.87%)	
41 and over	7 (2.39%)	
Total	**293 (100.0%)**	
Marital status		
Married	134 (44.52%)	
Single	160 (53.15%)	
Missing	7 (2.33%)	
Total	**301 (100.0%)**	
Education		
Primary school (G1-G7)	14 (4.65%)	
High school(G8-12)	274 (91.03%)	
University	10 (3.32%)	
Missing	3 (1.0%)	
Total	**301 (100.0%)**	
Race		
Black	149 (78.4%)	
Coloured	41 (21.6%)	
White	-	
Total	**190 (100.0%)**	
Height (cm)		158.07 (± 7.81)
≤ 150	28 (9.30%)	
151 – 155	51 (16.94%)	
156 – 160	70 (23.26%)	
161 – 165	54 (17.94%)	
>165	28 (9.30%)	
Missing	70 (23.26%)	
Total	**301 (100.0%)**	
Weight (kg)		74.18 (± 15.41)
≤ 50	10 (3.32%)	
51 – 55	12 (4.0%)	
56 – 60	29 (9.63%)	
61 – 65	37 (12.29%)	
>65	176 (58.47%)	
Missing	37 (12.29%)	
Total	**301 (100.0%)**	
Employment		
Yes	118 (39.60%)	
No	180 (60.40%)	
Total	**298 (100.0%)**	

Mowbray (MMH), Mitchells Plain (MP), Guguletu (GUG) and Khayelitsha (KMOU)

3.3. Marital Status

The subjects did not differ significantly with marital status, with 44.52% being married and 53.15% being single women. Seven women (2.33%) did not respond to this question (Table 5).

3.4. Education levels

Ninety - nine percent of our study population had some form of education ranging from primary to university level (Table 5). The majority of subjects indicated that they had high school education (91.03%) with less than 5% having completed primary school and even less (3.32%) having had tertiary education (Table 5). One percent failed to reveal their educational background in this study population.

3.5. Ethnicity

The sample group comprised of mostly black (78.4%) and coloured (21.6%) mothers (Table 5).

3.6. Maternal Height

Out of the 301 women, only 231 women revealed their height in the study. Seventy women did not respond to this question. The overall mean (SD) height of the women was 158.07 (± 7.81) cm (Table 5).

3.7. Maternal Weight

Two hundred and sixty - four women recorded their weights while the remaining 37 women did not respond to the question. The mean (SD) weight was 74.18 (± 15.41) kg. The majority of mothers had a weight of >65 kg (Table 5).

3.8. Employment levels

The majority of the women (180) in our studied population were unemployed (60.40%), and118 (39.60%) women were employed (Table 5).

3.9. Behavioural patterns of the pregnant women

Table 6: Behavioural patterns

Variables/ Categories	Frequency (%)
Number of sexual partners	
1	263 (98.9%)
2 and more	3 (1.1%)
Total	**266 (100.0%)**
Personal Hygiene	
Bath	72 (24.16%)
Hand wash	207 (69.46%)
Shower	18 (6.04%)
Douching	1 (0.34%)
Total	**298 (100.0%)**
Smoking	
Yes	41 (13.62%)
No	260 (86.38%)
Total	**301 (100.0%)**
Alcohol use	
Yes	29 (9.63%)
No	272 (90.37%)
Total	**301 (100.0%)**

3.9.1. Number of sexual partners

Of the 301 women investigated, the majority (98.9%) reported having one sexual partner while less than 2% had multiple sexual partners (Table 6).

3.9.2. Personal hygiene habits

Hand washing of the body was the most frequently reported means of hygiene practice (69.46%), followed by bathing (24.16%) and shower (6.04%). Only 1 mother reported douching (Table 6).

3.9.3. Smoking

Two hundred and sixty women (86.38%) did not smoke and 41 (13.62%) smoked while pregnant (Table 6).

3.9.4. Alcohol consumption

Two hundred and seventy - two (90.37%) women did not consume alcohol and a small percentage (9.63%) admitted to consuming alcohol while pregnant (Table 6).

3.10. Emotional well – being of the pregnant women

The overall perinatal history of emotional well - being among the 301 pregnant women revealed that 111 (36.9%) women claimed to have experienced difficult situations not related to domestic violence in the previous year. They did not elaborate on what these situations were. Ninety - three percent of the women claimed that they did not experience any type of domestic violence while pregnant, while 21 (7.0%) reported experiencing violence at home (Table 7).

Ninety – three point seven percent of the women were pleased about their pregnancy, and 94.7% claimed that their partners were equally supportive of their pregnancy. Only 5.32% claimed that the partners were not supportive (Table 7).

Table 7: Maternal emotional well–being

Variables/ Categories	Frequency (%)
A) Experienced difficult situation in the previous year	
Yes	111 (36.9%)
No	190 (63.12%)
Total	**301 (100.0%)**
B) Pleased about this pregnancy	
Yes	282 (93.7%)
No	19 ((6.3%)
Total	**301 (100.0%)**
C) Partner supportive	
Yes	285 (94.7%)
No	16 ((5.32%)
Total	**301 (100.0%)**
D) History of depression, anxiety or panic attacks	
Yes	142 (47.18%)
No	159 (52.82%)
Total	**301 (100.0%)**
E) Experienced any type of domestic violence	
Yes	21 (6.98%)
No	279 (92.69%)
Missing	1 (0.33%)
Total	**301 (100.0%)**

One hundred and forty – two (47.18%) women had a history of depression, anxiety or panic attacks, while 52.8% did not suffer from any of these conditions in our studied population (Table 7).

3.11. Obstetric and gynaecological history

3.11.1. Parity

The mean (SD) parity for women who participated in this study was 1.43 (± 1.04). Most of the mothers reported a parity of 0 – 3 (Table 8).

3.11.2. Gravidity

The overall mean (SD) gravidity was 2.72 (± 1.09) (Table 4). Here again, the most frequently reported gravidity was 0 – 3 (Table 8).

Table 8: Obstetric and Gynecological history

Variables/Categories	Frequency (%)	Mean (±SD):
Parity		1.43 (± 1.04)
0 – 3	285 (94.68%)	
4 – 5	9 (3.0%)	
≥ 6	2 (0.66%)	
Missing	5 (1.66%)	
Total	**301 (100.0%)**	
Gravidity		2.72 (± 1.09)
0 – 3	241 (80.07%)	
4 – 5	45 (14.95%)	
≥ 6	10 (3.32%)	
Missing	5 (1.66%)	
Total	**301 (100.0%)**	
Previous pregnancy outcomes		
Preterm	151 (50.2%)	
Full term	150 (49.8%)	
Total	**301 (100.0%)**	
Infant weight of previous delivery		4.87 g (± 1.46)
≤ 499 g	3 (1.0%)	
500 - 999 g	8 (2.66%)	
1000 – 1499 g	19 (6.31%)	
1500 – 1999 g	21 (6.98%)	
2000 – 2499 g	28 (9.30%)	
2500 g +	76 (25.25%)	
Missing	146 (48.50%)	
Total	**301 (100.0%)**	

3.11.3. Previous pregnancy outcomes

According to their obstetrics history, the 301 pregnant women were divided into two groups; 151 constituted those with a history of Preterm (PTD) delivery (<37 weeks of gestation), and the control group consisted of 150 mothers who had a history of Full term (FTD) delivery (>37 weeks of gestation). Preterm delivery was reported by 50.2% of the study group, while the remaining 49.8% reported previous deliveries to be at term (Table 8).

3.12. Medical History

3.12.1. Maternal Medical history

Table 9: Medical history

Variables/ Categories	Frequency (%)
Medical History	
Hypertension	30 (9.97)
Diabetes	1 (0.33%)
Urinary tract infection	4 (1.33%)
Other: low blood pressure, asthmatic; anaemic, TB	9 (2.99%)
No symptoms	253 (84.05%)
Hypertension & Diabetes	2 (0.66%)
Hypertension & urinary tract infection	1 (0.33%)
Hypertension & low blood pressure	1 (0.33%)

Thirty women had a history of hypertension (Table 9). Nine women (2.99%) reported asthma, anaemia or low blood pressure. Two women had a history of both hypertension and diabetes, and one woman had hypertension and low blood pressure (Table 9). The majority (253) reported no diabetes or blood pressure disorders. Four women reported having had a urinary tract infection in the past.

3.12.2. Maternal history of reproductive infections

Of 301 women who participated in this study, 201 (66.8%) were HIV-negative, 74 (24.6%) were HIV-positive and 26 (8.6%) did not disclose their HIV-sero-status. When asked about other STDs, the majority of the women (83.46%) in this study reported no history of STD (Table 10). Thirty - five of the women did not respond to the question.

Table 10: Maternal Infections

Variables/ Categories	Frequency (%)
HIV status	
Positive	74 (24.6%)
Negative	201 (66.8%)
Unknown	26 (8.6%)
Total	**301 (100.0%)**
Previous STD	
Yes	44 (14.62%)
No	222 (73.75%)
Unknown	35 (11.63%)
Total	**301 (100.0%)**
Current Symptoms of infections	
Vaginal discharge	196 (65.12%)
Urinary tract infection	21 (6.98%)
Both	3 (1.0%)
No symptoms	81 (26.91%)
Total	**301 (100.0%)**

There were no symptoms of current infection reported in 81 (26.58%) of the women while 196 (65.12%) reported a vaginal discharge, and 21 (6.98%) reported current urinary tract infections (Table 10).

Table 11: Logistic regression analysis used to assess the influence of Obstetric factors associated with previous PTD

Variables	S. E.	OR	95% CI for OR	p - value
Gravidity	0.431	0.2116	0.093 - 0.503	0.000**
Parity	0.442	5.914	2.489 - 14.054	0.000**
Infant weight	0.157	1.616	1.89 - 2.197	0.002**
Current Symptoms (Ref = no symptoms)				
Vaginal discharge	0.560	8.955	2.988 - 26.842	0.000**
Urinary tract infection	1.308	42.078	3.242 - 546.131	0.004**
Symptoms (Both)	40192.970	.000	.000 -	1.000
Smoking (Ref = no smoking)				
Yes	0.643	0.215	0.061 - 0.759	0.016**
Experienced any type of domestic violence	1.041	2.216	3.150 – 189.190	0.002**
Experienced difficult situation in the previous year	0.380	24.264	1.076 – 4.764	0.031**
Cox and Snell R Square	37.8%			
Chi-square (Hosmer and Lemeshow Test sig)	12.823 (0.118)			

A p-value of less than ($p < 0.05$) was considered statistically significant. SE standard error, OR odd ratio, CI confidence interval

3.13. Multivariate analysis of confounding risk factors associated with previous PTD

Since it was previously established that some mothers had a history of preterm delivery, we elected to compare them with the mothers who had previously delivered at term in order to establish whether any of the above factors may indeed have influenced their obstetrics and gynaecological history.

A multivariable analysis were undertaken to identify and examine all the risk factors of PTD. Results of multiple logistic regression analysis are shown in Table 11. The multivariate analysis method used is the binary logistic regression because the dependent variables "delivery status" is qualitative with two categories (Preterm and Full term). The Homers – Lemeshow test for logistic regression analysis demonstrated a good model fit with $p = 0.118 > 0.05$. The independent variables included in the model explained 37.8% (Cox and Snell R square) of the variance of delivery history. Table 11 shows that gravity, parity, infant weight, smoking, vaginal discharge, urinary tract infection, domestic violence and stress (experiencing difficult situation in the previous year) were significantly associated with a history of previous PTD (Table 11).

CHAPTER 4: ISOLATION AND CHARACTERIZATION OF AV – ASSOCIATED AEROBES

4.1. Microscopic diagnosis of AV

AV was detected in 79 (26.2%) of the 301 pregnant women, and 222 (73.8%) presented with normal vaginal microbiota. Of those with AV, 67 were diagnosed with light AV, 11 had moderate AV and severe AV was detected in 1 woman (Table 12).

Table 12: Microscopic diagnostic criteria for AV (Donders *et. al.*, 2011)

Variable/categories	Frequency (%)
AV score	
Normal flora (< 3**)**	222 (73.8%)
Light AV (3-4)	67 (84.8%)
Moderate AV (5-6)	11 (13.9%)
Severe AV (≥6)	1 (1.3%)
Total of AV positive	79 (26.2%)

Aerobic vaginitis (AV); high – power field (hpf) (400X magnification). “Severe AV” or “desquamative inflammatory vaginitis”: score > 6; “moderate AV”: score 5–6; “light AV”: score; “no signs of AV”: score < 3.

4.2. Identification of AV – associated bacteria isolated from samples

4.2.1. Identification of Gram-positive cocci

Gram- positive cocci were examined for ß-hemolysis on Colistin nalidixic acid (CNA) blood agar containing 5% sheep blood (Fig. 2), catalase test, bacitracin sensitivity, hippurate and aesculin hydrolysis.

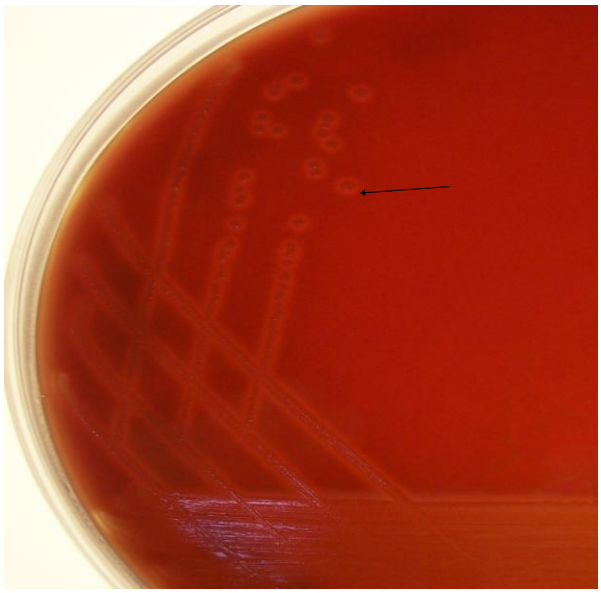

Fig. 2: Identification of GBS**.** Isolates were grown on CNA blood agar containing 5% sheep blood. The colonies are surrounded by narrow zones of ß-hemolysis.

Table 13: Confirmatory tests for Gram-positive cocci

	Positive (+ ve)	Negative (- ve)
Catalase	6	82
Bacitracin	-	82
Hippurate	25	57
Aesculin	26	56
DNAse	4	2

4. 2. 1.1**.** Catalase Test

The catalase test was done to confirm that Gram-positive cocci isolates were streptococci (catalase-negative) or *Staphylococcus* (catalase-positive). Results were interpreted as shown in Fig. 3. Four isolates were catalase – positive and 82 isolates were catalase negative 82 (Table 13).

Catalase negative Gram-positive cocci were further characterized using bacitracin, aesculin hydrolysis and hippurate hydrolysis tests, while catalase positive Gram-positive cocci were further characterized using the DNAse test.

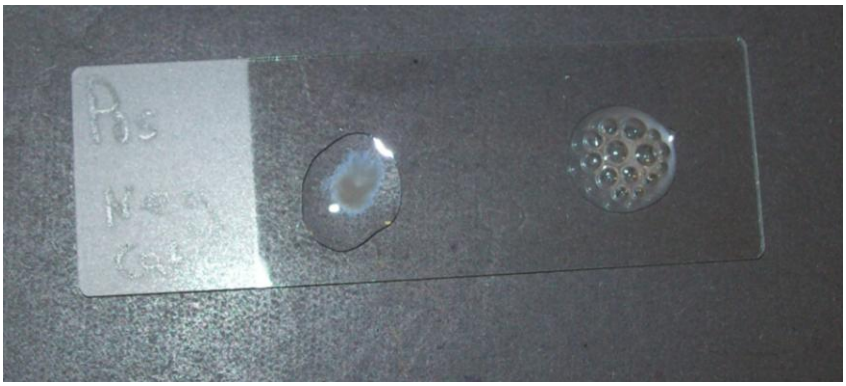

Fig. 3: The Streptococci (left, absence of effervescence) are catalase negative, and *Staphylococci* are catalase positive (right), indicated by the production of effervescence.

4. 2. 1.2**.** Bacitracin Test

ß-hemolytic, catalase-negative Gram positive cocci were tested for bacitracin sensitivity to exclude *Streptococcus pyogenes*. Colonies were considered to be sensitive by observation of a zone of inhibition around the bacitracin disk and negative (resistant) if no zone of inhibition was observed around the disc. Positive (*S. pyogenes*) and negative controls (GBS) were included in the all tests performed. *Staphylococcus*, GBS, and *Enterococcus* show no zone of inhibition around the bacitracin disc. None of the isolates showed sensitivity to bacitracin (Table 13).

4. 2. 1.3**.** HippurateTest

The hippurate test in this study was used to determine the presence of the enzyme hippurate hydrolase, which hydrolyzes sodium hippurate to form benzoic acid and glycine. The addition of ferric chloride to benzoic acid forms an insoluble brown ferric benzoate precipitate (Fig. 4).

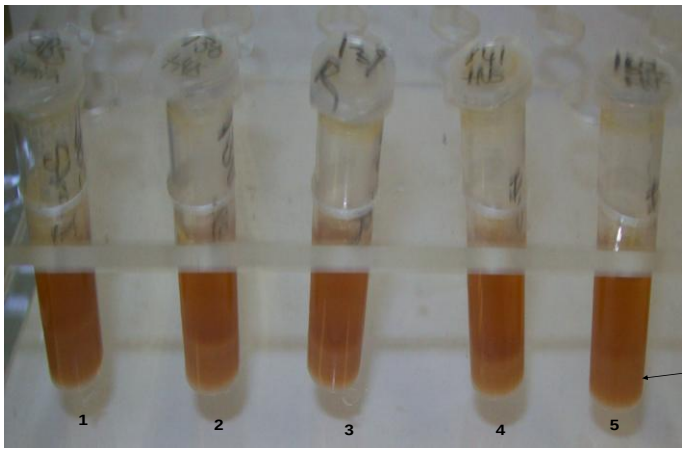

Fig. 4: The hippurate hydrolysis test. A positive test is shown by the heavy brown precipitate at the bottom of the tube. Tube 1, positive control; and tube 2 – 5, represent test samples. The arrow indicates a heavy precipitate that lasts longer than 10 min, which is an indication of a positive test.

4. 2. 1.4**.** Aesculin Test

The bile aesculin test was performed to determine if the bacteria were able to hydrolyze aesculin in the presence of bile (Fig. 5). All *E. faecalis* isolates tested had an esculin positive reaction in this study.

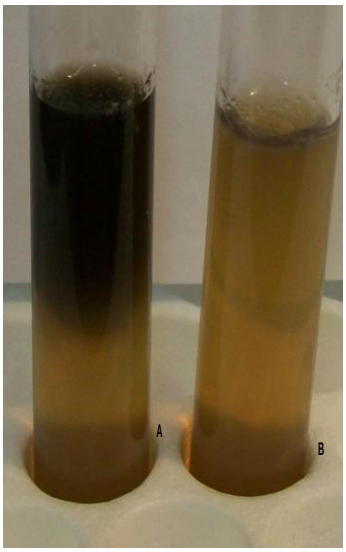

Fig. 5: The aesculin test reaction showing blackening of the medium (indicating a positive reaction, Tube A) and no change in color of the medium (negative reaction, Tube B).

4. 2. 1.5. Latex Agglutination Test

Lancefield grouping of catalase-negative Gram-positive cocci was achieved using latex agglutination (Fig. 6). The intensity of the agglutination reaction (Fig. 6) was measured using a qualitative scale from weak (+) to strong (++++). Results showed that 57 of the isolates were GBS and 24 were *Enterococcus* (Table 14).

Table 14: Tests used for the presumptive identification of GBS and *E. faecalis*

Test	**GBS**	***E. faecalis***
Haemolysis on Blood Agar	β	Y
Catalase	-	-
Growth on McConkey (6.5% NaCl)	-	+
Aesculin hydrolysis	-	+
Hippurate hydrolysis	+	-
Lancefield groups	B	D/G
Frequency of isolates	57	24/1

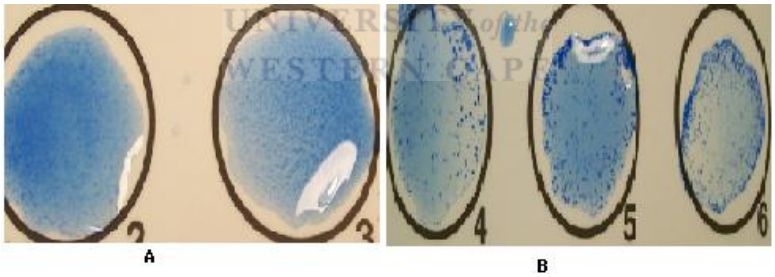

Fig. 6: Slide agglutination test used to identify the group antigens of GBS and *Enterococci*. A positive agglutination reaction is indicated by clumping and grades as weak (+) to strong (++++) while a negative reaction is smooth with no apparent clumps. 2 – 3 are graded as weak (+), while 4 – 6 are strong (++++)

4. 2. 1.6. Growth on MacConkey Agar

MacConkey agar contains 6.5% NaCl and bile salts. Growth on MacConkey agar confirmed the identification of *E. faecalis* which grew as tiny magenta colonies. GBS did not grow on MacConkey agar.

4.2.1.7. Identification of *Staphylococcus aureus*

Following overnight incubation of the DNAse plate, the plate was flooded with 1 N HCL. The presence of *Staphylococcus aureus* was confirmed by clear zones appearing on the plate indicating the production of DNAse while no clearing of *staphylococcal* growth was reported as *Staphylococcus epidermidis*. Four isolates were DNAse positive, while 2 isolates were DNAse negative (Table 13).

4.2.2. Identification of Gram-negative bacilli (enterobacteriaceae)

The colonies from the MacConkey after overnight incubation at 37^{o}C were picked for further identification using the API 20 E test.

The identification of *enterobacteriaceae* Gram-negative bacteria was achieved with the 7-digit numerical profile obtained according to prescribed values for each positive test (Fig. 7). This test was performed using the database (1.2.1) with the api*web* TM identification software. The 7-digit numerical profile was entered manually to obtain the profile of the microorganism.

Fig. 7: API 20 E test was performed in order to identify the enterobacteriaceae

Of the 301 pregnant women who participated in the study, GBS and *Enterococcus* (Group D) were isolated from 50 and 21, with AV diagnosed in 10 and 9 respectively. *E. coli*, *S. aureus*, *S. epidermidis*, Group G, *Citrobacter* and *Candida* were isolated from 4, 4, 2, 1, 1, 2 respectively (Table 15).

Table 15: Number of women presenting with AV – associated microorganisms

Organisms	**Frequency (%) of women**
E. coli	4 (1.3%)
Citrobacter feundii	1 (0.33%)
S. aureus	4 (1.3%)
S. epidermidis	2 (0.66%)
GBS	50 (16.6%)
Enterococcus faecalis (Group D)	21 (7.0%)
Enterococcus (Group G)	1 (0.33%)
Candida species	2 (0.66%)

With GBS and *E. faecalis* being the 2 species isolated from most women, the remainder of this study will be dedicated to the characterization of these two species, with particular emphasis on GBS which, as discussed in Chapter 1, has been frequently associated with maternal and neonatal morbidity.

4.2.3. Distribution of AV – associated species

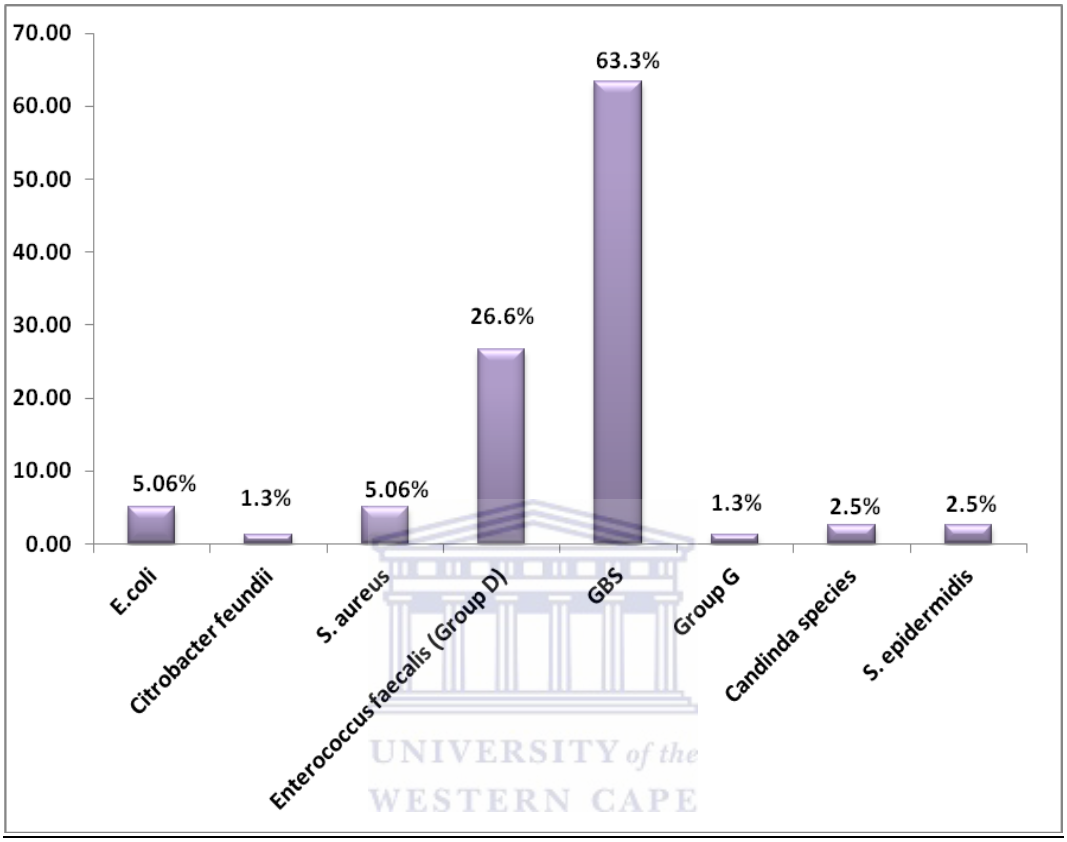

Fig. 8: Frequency of women with AV - associated microorganisms isolated in this study

Figure 8 represents the number of women with AV – associated bacteria expressed as a % of the total number of women with AV (n = 79). As demonstrated in Table 15, GBS and *E. faecalis* were the species isolated from most women while other species were isolated from ≤ 5.06% of the women.

4. 3. Distribution of GBS serotypes

The distribution of GBS serotypes is believed to differ from one country to next and also from one province to another with changes in prevalence over time. With GBS being the predominant isolate in this population, we investigated the distribution of serotypes using GBS capsular polysaccharide antigens (CPA) typing (Fig. 9). Six different serotypes of

GBS were identified with serotype V predominating, followed by serotype III. Of the GBS positive isolates GBS serotype III and serotype V comprised 87.7% of the isolates. Only 1 (1.75%) isolate belonged to each of serotypes Ia, II, IV and IX in our study. One woman harboured more than 1 serotype of GBS (IV and V) in our population. No strains belonging to serotypes Ib, VI, VII, and VIII were found in any of the isolates in this study. Serotyping was not possible in 3 cases (5.3%), and defined as nontypeable (NT) (Fig. 9). In eight cases where GBS was cultured from both rectal as well as vaginal samples, serotyping resulted in identical findings for all of the paired samples, except for three.

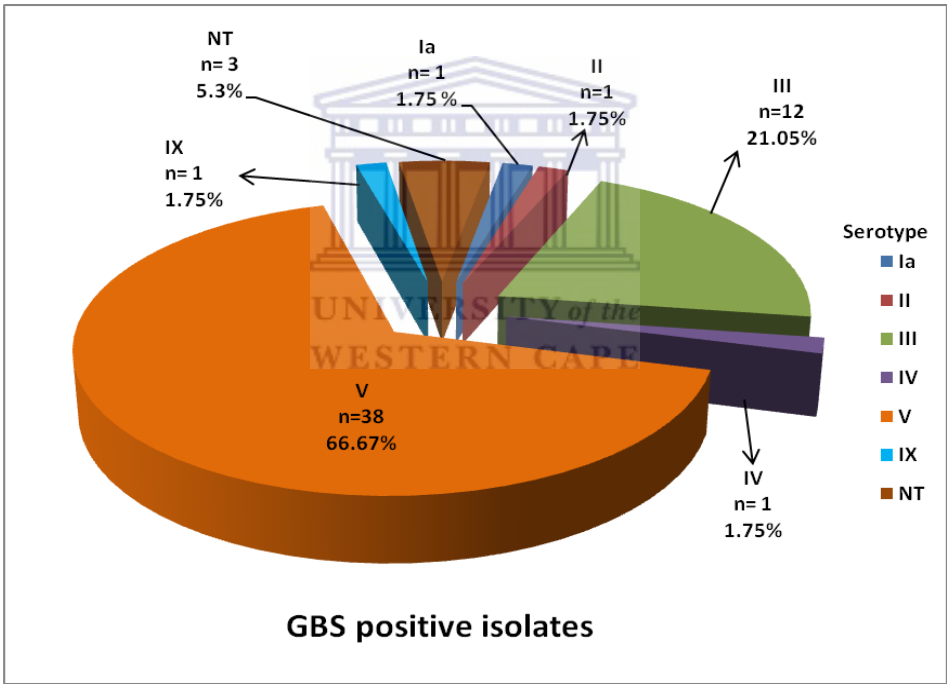

Fig. 9: Distribution of capsular antigen types among GBS culture positive isolates from women who had previous history of PTD of N=151 and of history of FTD of N=150 pregnant women from Western Cape. NT, not typeable

4.4. Culture – independent detection of GBS

In addition to culture, the use of molecular diagnostic techniques such as PCR and PFGE for the genomic analysis of GBS clinical isolates were employed.

4.4.1. Extraction of metagenomic DNA from Ano-vaginal samples

Fig. 10 shows purified metagenomic DNA from both the vaginal and rectal specimens. The DNA extraction using CATAB/NaCl method yielded DNA of high molecular weight of the metagenomic DNA (Fig. 10). Eight microliters (8 µl) of DNA was loaded from all the samples, and 2 µl of loading dye was loaded and viewed on 8% agarose gel. The DNA obtained in this present study had absorbance of 260nm, and the A260/280nm was varied from different ranges.

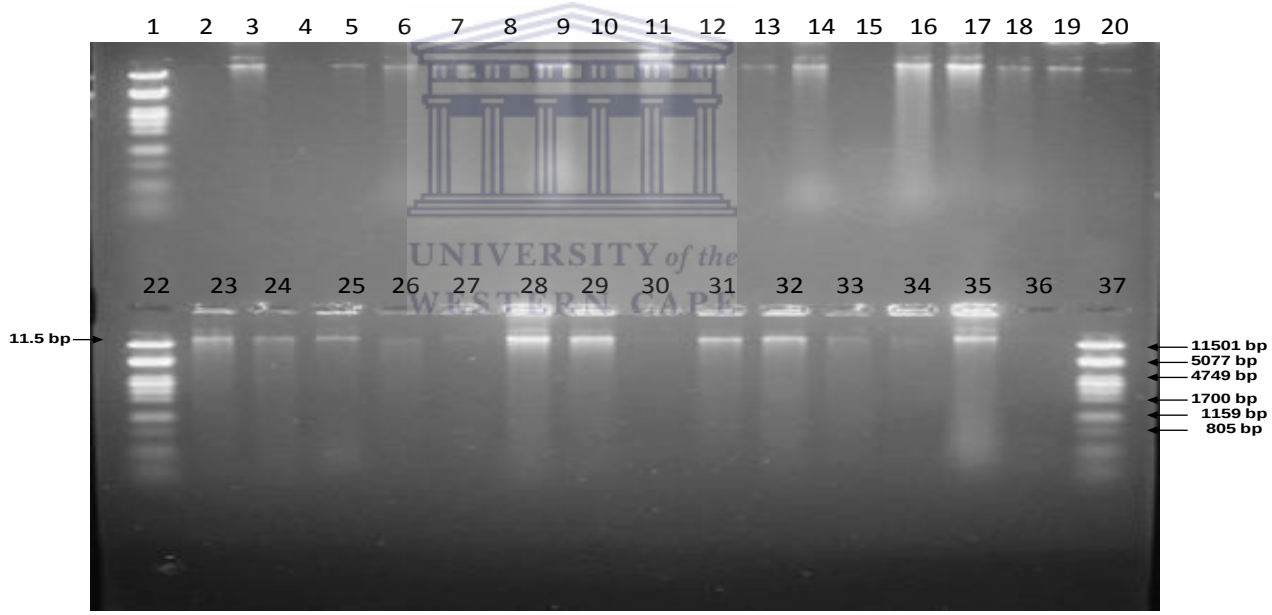

Fig. 10: Genomic DNA run on Agarose gel (1%) comparing the distribution of GBS diversity in selected clinical samples from MOU in the Western Cape. Top gel: Lanes 1: Marker (λ Pst1), 2: sample # 111HVS, 3: sample # 08 HVS , 4: ample #50 HVS, 5: sample # 62 HVS, 6: sample # 84 R, 7: sample # 95 R, 8: sample # 99 R, 9: sample # 130 HVS,10: sample # 116 HVS, 11: sample # 139 HVS; 12: sample # 139 R, 13: sample # 141 HVS, 14: sample # 167 HVS, 15: sample # 193 HVS, 16: sample # 193R, 17: sample 3# 194 HVS 18: sample # 211 HVS; 19: sample # 211 HVS, 20: sample # 216 HVS. Bottom gel: Lanes 22 & 37: Marker (λ Pst1), Lanes 22-35: Clinical samples from vaginal and rectal specimens.

4.4.2. PCR amplification of 16S rRNA genes

PCR analysis was positive for fifty women and negative for 251 women. No samples were positive by PCR and negative by culture. The overall GBS colonization rate by PCR was 16.6% amongst the 301 pregnant women.

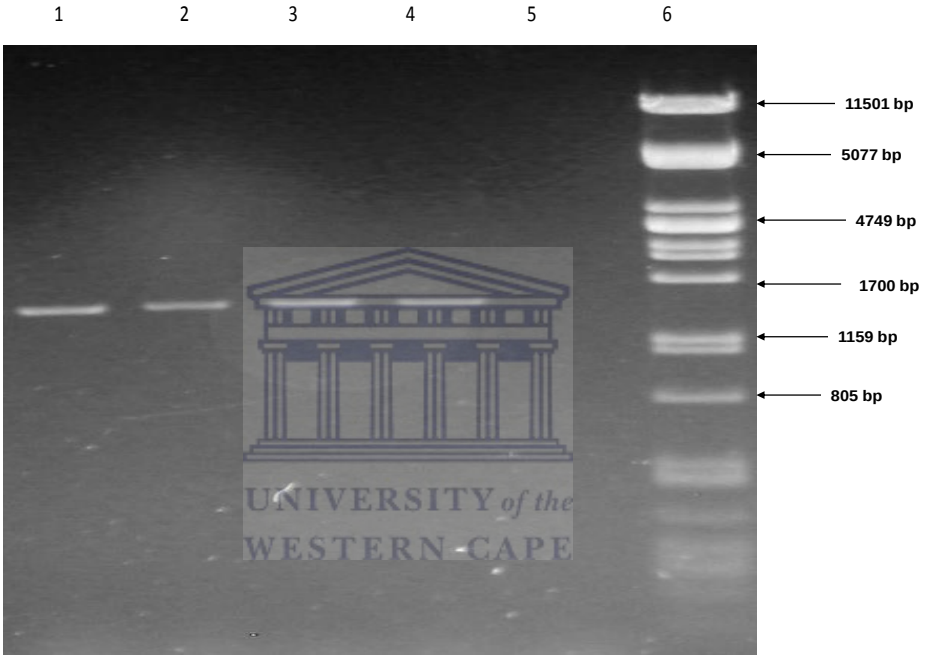

Fig. 11: Agarose electrophoresis gel (1%) showing the gel purified PCR products from the clinical samples using universal bacteria primers (E9F and U1510R). Lanes 1: Sample #139 HVS; 2: sample # 139 R, 3: sample # 447 HVS; 4: Positive control (GBS 11 strain); 5 Negative control; 6: Marker (λ Pst1).

In order to check if the extracted DNA was suitable for PCR, the universal bacteria primers (U1510R) and (E9F) were used (Fig. 11). This shows that the DNA was indeed suitable for PCR amplification. It also did aid on the amount of template to be used, 5 ng of metagenomic was used in all PCR experiments.

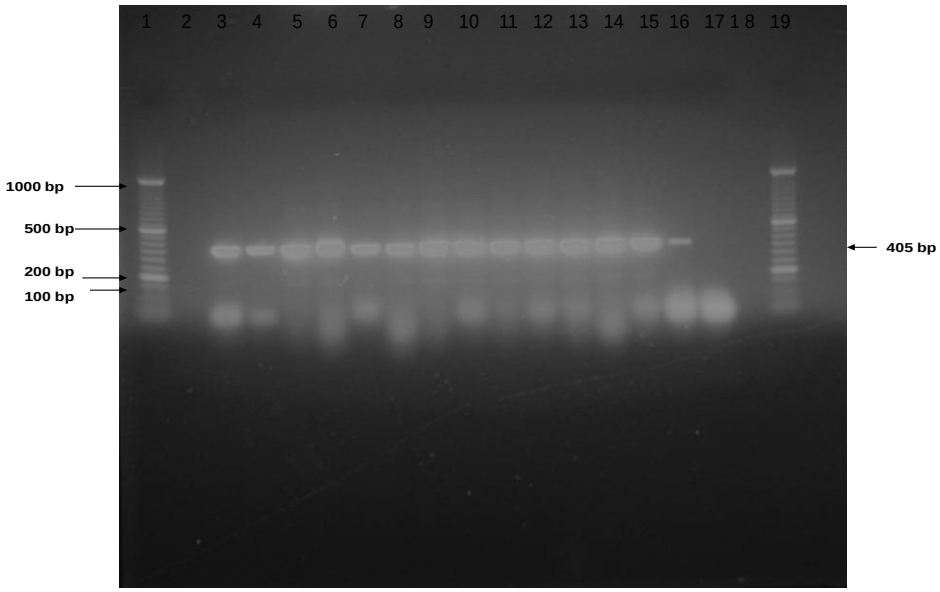

Fig. 12: PCR amplification with the GBS-specific primers (405 bp). The purified DNA was run on agarose gel (1%). Lanes 1: Marker (50bp) 2: Negative control, 3: Positive control (GBS strain V) 3: sample # 95 R, 5: sample # 112 HVS, 6: sample # 116 HVS, 7: sample # 139 HVS, 8: sample # 139R, 9: sample # 141 HVS, 10: sample # 167 HVS, 11: sample 193 HVS, 12: sample # 193 R, 13: sample # 194 HVS, 14: sample # 219 HVS, 15: sample # 219 R; 16: sample # 221 R.

The results in Fig. 12 show the PCR amplification obtained from genomic 16S rDNA from the clinical samples. The PCR product was amplified with GBS-specific primers (sag 40 and sag 445) and viewed on 1% agarose gel. The PCR product yielded a strong band which is observed (Fig. 12). After PCR analysis the size of GBS amplification fragment of expected size of 405 bp was obtained (Fig. 12). DNA template from GBS strains was used as a positive control and no target DNA was added to the negative control. The results obtained from the PCR assay resulted in fifty - seven isolates identified as GBS - positive from the vaginal and rectal swabs.

4.4.3. Comparison of rectal and vaginal colonization

The GBS were more frequently isolated from the vagina than the rectum in this study (Table 16). Of the total 57 GBS positive cultures, 26 derived from the vagina resulting in a frequency rate for this site of 45.6%. Twenty - three samples with negative vaginal cultures were positive by their anorectal samples (Table 16). This could have resulted in an error of 40.4% if only vaginal cultures had been considered (Table 16).

Table 16: Rates of detection of colonization with GBS culture positive according to the sources of specimens among the 301 (N=301) pregnant women

SITES	Women (N)	GBS culture positive	Positive anatomical culture site %		
			HVS specimen	Rectal specimen	Combined high vaginal and rectal swabs
Total	301	**57**	26	23	8

HVS (High Vaginal Specimen); Rectal (Anal Specimen)

4.4.4. GBS culture and PCR assay comparison

The PCR assay was compared to the culture methods (gold standard) for 301 pregnant women who were recruited in our study population. In order to ensure that we achieved the highest detection levels of GBS colonization using the PCR assay, both high vaginal samples (HVS) and rectal samples were collected from the pregnant women and tested. The results show that amongst the 301 pregnant women who participated in this study, 16.6% (50/301) of the women were identified as GBS carriers.

All specimens found to be positive by culture methods (N = 57) were also positive by PCR assay (Table 17).

Table 17: Rates of GBS colonization detected among the 301 pregnant women by comparing the PCR technique versus culture for GBS detection.

Methods of Detection	Delivery history	Frequency GBS positive specimen No. (%)	Sampling sites		
			High Vaginal Specimen	Rectal Specimen	Combine anatomical site specimen (high vaginal and rectal)
Culture					
Total		**57 (100.0%)**	**26 (100.0%)**	**23 (100.0%)**	**8 (100.0%)**
Conventional PCR assay					
Total		**57 (100.0%)**	**26 (100.0%)**	**23 (100.0%)**	**8 (100.0%)**

HVS (High Vaginal Specimen); Rectal (Anal Specimen) and PTD= Preterm delivery; FTD =Full delivery

Specificity, sensitivity, and predictive values

When the PCR assay was compared with culture, a sensitivity of 100% and specificity 100% was obtained (Table 18).

Table 18: Detection of GBS colonization by culture and PCR. The diagram was used for sensitivity, specificity, positive and negative predictive value.

	CULTURE		
	Culture negative	**Culture positive**	**Frequency (%)**
PCR			
negative	251	0	251 (83.4%)
positive	0	50	50 (16.6%)
Total	251	50	301 (100.0%)

Both the culture and PCR methods were negative for 251 women (vaginal swabs; rectal swabs; and from both combined anatomical sites). None of the isolates which were GBS-positive for culture were negative by PCR. The PCR was repeated to confirm the results. Sensitivity, specificity, negative and positive predictive values of the PCR assay were estimated in this study by comparing the PCR assay results with culture method. The results revealed sensitivity, specificity, negative and positive predictive values of the PCR assay was 100% (Table 18).

Similar results were obtained when culture was used as the reference standard. The sensitivity, specificity, negative and positive predictive values of culture method when compared with PCR assay were all 100% (Table 18).

4.5. Antimicrobial susceptibility of isolates

4.5.1. Kirby-Bauer disk diffusion method

All culture positive isolates obtained from the pregnant women were tested for antibiotic susceptibility by performing the Kirby-Bauer disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. For Gram-positive cocci the following eight antimicrobial drugs were tested: penicillin G (PG) 1unit, clindamycin (CD) 2µg, gentamicin (GM) 10 µg, fusidic acid (FC) 10 µg, erythromycin (E) 5 µg, tetracycline (T) 10 µg, trimethroprin (TM) 1, 25 µg and sulphamethoxazole (SMX) 25 µg. Strains were considered as resistant or sensitive to specific antibiotics according to the inhibition zones measured (Fig. 13). Zones of inhibition were measured from the edge of the disk to the edge of the inhibition of bacterial growth using a ruler.

Critical breakpoints for the interpretation of results are summarized in appendix (see appendix 2D)

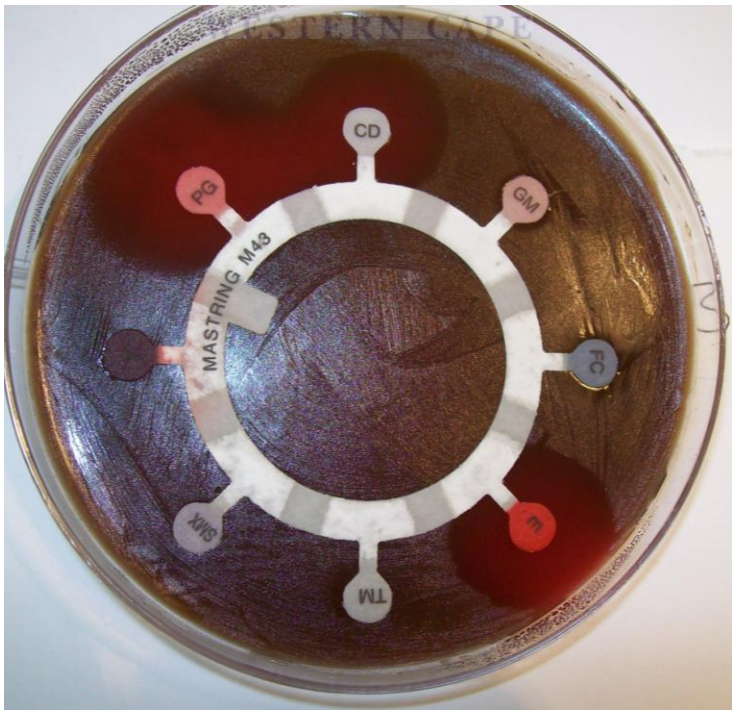

Fig. 13: Disc diffusion analysis of GBS antimicrobial susceptibility test

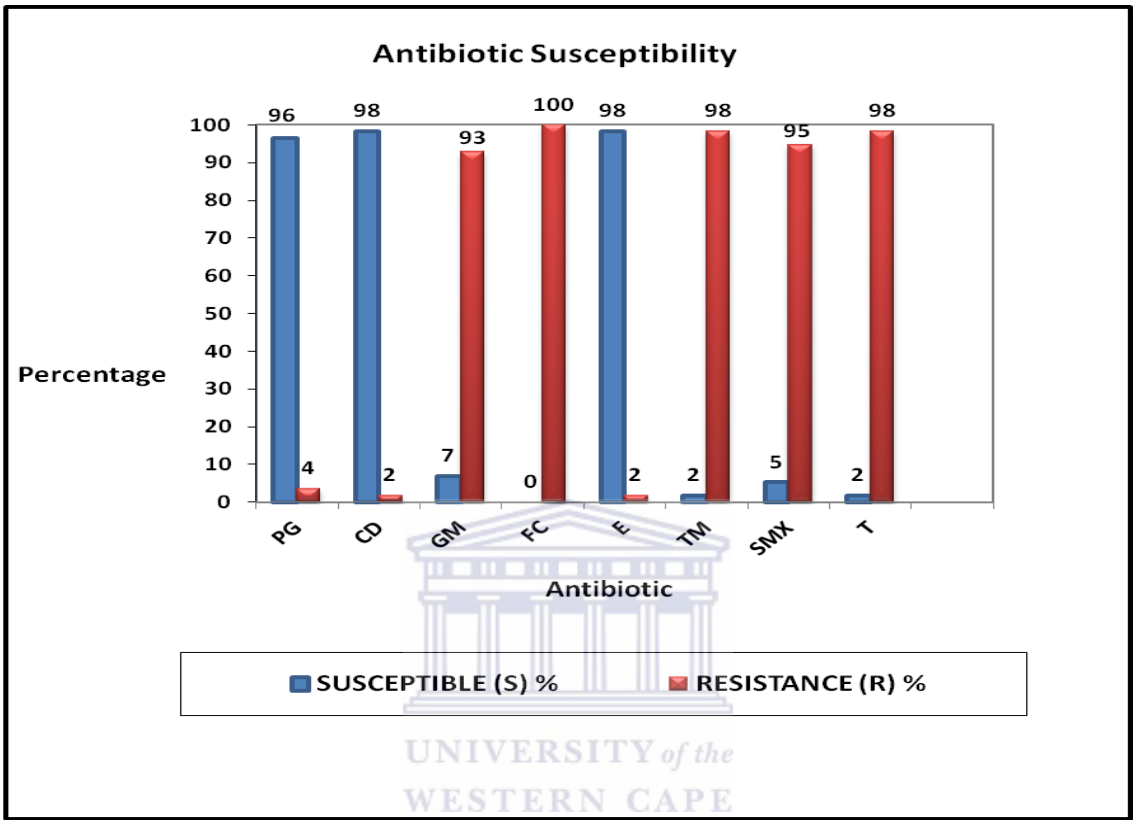

Fig. 14: Proportions of the responses of the 57 GBS culture positive isolates tested against eight different antibiotics: penicillin G (PG), clindamycin (CD), gentamicin (GM), fusidic acid (FC), erythromycin (E), trimethroprin (TM), sulphamethoxazole (SMX), and tetracycline (T).

Fifty – six out of fifty - seven (98%) of GBS positive isolates were susceptible to clindamycin and erythromycin, followed by 96% (55/57) susceptible to penicillin G in this study. Only one isolate 2% (1/57) was found susceptible to each of tetracycline (T) and trimethroprin (TM) (Fig. 14).

Total resistance of GBS isolates (100%) was demonstrated to fusidic acid and 98% (56/57) were resistant to tetracycline and trimethroprin (Fig. 14). Ninety - five percent of the isolates (54/57 isolates) were resistant to sulphamethoxazole, and 93% were resistant to gentamicin (Fig. 14).

4.5.2. MIC analysis using the TREK system

The trek system was used to determine the MIC profiles of GBS and *E. faecalis* isolates since most of our study population were colonized with GBS followed by *E faecalis*. For the breakpoint EUCAST/ CLSI was used see (Appendix 2E).

4.5.2. 1. GBS

Susceptibility to antibiotics

The results of the antimicrobial susceptibility analysis are summarized in Table 19. All the GBS strains were susceptible to tigecycline (100%), followed by 55 (96.5%) to linezolid, and moxifloxacin. Forty - eight (84.2%) isolates were found to be susceptible to trimethroprim/sulphamethoxazole (Table 19). Thirty – six of fifty – seven (63.2%) of GBS positive isolates were susceptible to ceftriaxone, clindamycin, and meropenem followed by 32 (56.1%) to erythromycin. The results showed that 45 (78.9%) of GBS isolates were susceptible to levofloxacin, to chloramphenicol 44 (77.2%), to vancomycin 40 (70.2%), to daptomycin 38 (66.7%), to ceftriaxone, cefotaxime and meropenem 36 (63.2%), to azithromycin 33 (57.9%), and to penicillin 31 (54.4%) in our studied population (Table 19). Three isolates (5.3%) were found to be susceptible to tetracycline (Table 19).

Intermediate sensitivity to antibiotics

Intermediate sensitivity to levofloxacin was found in 11 (19.3%) of the GBS isolates, with intermediate sensitivity to trimethroprim/sulphamethoxazole in 5 (8.8%) and 1 (1.8%) isolate showed intermediate sensitivity to linezolid and moxifloxacin (Table 19).

Resistance to antibiotics

A high proportion of GBS isolates were resistant to tetracycline 94.7% (54/57) in this study, followed by 26 (45.6%) to penicillin (Table 19). Twenty – four (42.1%) of the GBS isolates were resistant to azithromycin. Twenty – two isolates (38.6%) were

resistant to ertapenem, followed by 23 (40.4%) to clindamycin, 21 (36.8%) to cefotaxime and meropenem respectively. Nineteen (33.3%) isolates were resistant to daptomycin, while 17 (29/8%) were resistant to vancomycin (Table 19). Twenty – two point eight percent of the isolates (13/57) were resistant to chloramphenicol.

Whilst resistance to trimethroprim/sulphamethoxazole was detected in 4 (7.0%) of the GBS isolates (Table 19), 1 isolate (1.8%) was found to be resistant to each of the following levofloxacin, linezolid and moxifloxacin (Table 19).

GBS isolates were divided into penicillin sensitive and penicillin resistant groups for further assignment into specific antimicrobial profile groups. If the profiles different by less than 3 antibiotics they were assigned to the same alphabetical group with a subgroup number i.e. a_1, a_2 etc (Table 20).

Table 19: Antimicrobial susceptibility patterns for GBS and *E faecalis*

ANTIMICROBIC		Frequency (%) GBS N = 57	Frequency (%) *E. faecalis* N = 24
AMOXICILLIN/CLAVULANIC ACID	Susceptible	-	-
	Intermediate	-	-
	Resistant	-	-
AZITHROMYCIN	Susceptible	33 (57.9%)	-
	Intermediate	-	1 (4.2%)
	Resistant	24 (42.1%)	23 (95.8%)
CEFEPIME	Susceptible	-	-
	Intermediate	-	-
	Resistant	-	-
CEFOTAXIME	Susceptible	36 (63.2%)	-
	Intermediate	-	-
	Resistant	21(36.8%)	24(100%)
CEFTRIAXONE	Susceptible	36 (63.2%)	-
	Intermediate	-	-
	Resistant	21(36.8%)	24(100%)
CEFUROXIME	Susceptible	-	-
	Intermediate	-	-
	Resistant	-	-
CHLORAMPHENICOL	Susceptible	44 (77.2%)	20 (83.3%)
	Intermediate	-	-
	Resistant	13 (22.8%)	4(16.7%)
CLINDAMYCIN	Susceptible	34 (59.6%)	2 (8.3%)
	Intermediate	-	-
	Resistant	23 (40.4%)	22 (91.7%)
DAPTOMYCIN	Susceptible	38 (66.7%)	2 (8.3%)
	Intermediate	-	-
	Resistant	19 (33.3%)	22 (91.7%)
ERTAPENEM	Susceptible	35 (61.4%)	-
	Intermediate	-	-
	Resistant	22 (38.6%)	24(100%)
ERYTHROMYCIN	Susceptible	32 (56.1%)	1 (4.2%)
	Intermediate	-	1 (4.2%)
	Resistant	24 (42.1%)	22 (91.7%)
LEVOFLOXACIN	Susceptible	45 (78.9%)	19 (79.2%)
	Intermediate	11 (19.3%)	3 (12.5%)
	Resistant	1 (1.8%)	2 (8.3%)
LINEZOLID	Susceptible	55 (96.5%)	21 (87.5%)
	Intermediate	1 (1.8%)	2 (8.3%)
	Resistant	1 (1.8%)	1 (4.2%)
MEROPENEM	Susceptible	36 (63.2%)	-
	Intermediate	-	-
	Resistant	21(36.8%)	24 (100%)
MOXIFLOXACIN	Susceptible	55 (96.5%)	23 (95.8%)
	Intermediate	1 (1.8%)	-
	Resistant	1 (1.8%)	1(4.2%)
TETRACYCLINE	Susceptible	3 (5.3%)	2 (8.3%)
	Intermediate	-	-
	Resistant	54 (94.7%)	22 (91.7%)
TIGECYCLINE	Susceptible	57 (100%)	24 (100%)
	Intermediate	-	-
	Resistant	-	-
PENICILLIN	Susceptible	31 (54.4%)	-
	Intermediate	-	-
	Resistant	2 6 (45.6%)	24 (100%)
TRIMETHOPRIM/SULPHAMETHOXAZOLE	Susceptible	48 (84.2%)	22 (91.7%)
	Intermediate	5 (8.8%)	-
	Resistant	4 (7.0%)	2 (8.3%)
VANCOMYCIN	Susceptible	40 (70.2%)	20 (83.3%)
	Intermediate	-	-
	Resistant	17 (29.8%)	4 (16.7%)

The breakpoints were defined by the EUCAST/ CLSI

Table 20: Breakdown of antibiotic profile grouping based on penicillin sensitivity and penicillin resistance

Number of resistant isolates in the all pregnancy outcomes																		
Isolate	**Antibiotic Profiles**	PG	CD	E	T	CH	VA	DAP	CEF	CEFT	AZI	MER	ER	MOX	TRM/SMX	LIN	LEV	TIGER
P 59 H	a	S	S	S	R	S	S	S	S	S	S	S	S	S	R	I	S	S
P 92 H	a	S	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S
P 92 R	a	S	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S
P 93 R	b	S	S	S	R	S	S	S	R	R	S	S	S	S	S	S	S	
P 50 H	b	S	S	S	R	S	S	S	R	R	S	S	S	S	S	S	S	S
P 08 H	c_1	S	R	S	R	S	S	S	S	S	S	S	S	S	I	S	S	
P 04 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
P 84 R	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	I	S	S	S
112 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
114 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
114 R	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
221 R	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
139 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
139 R	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
116 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
130 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
99 R	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
194 H	c_1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
118 R	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
193 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
P 123 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	I	S
P 143 R	c_1	S	S	S	R	S	S	S	S	S	S	S	S	I	S	S	I	S
95 R	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
130 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
167 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
P 62 H	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	I	S	S	S
193 R	c_1	S	S	S	R	S	S	S	S	S	S	S	S	S	I	S	S	S
P 147 H	c_2	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S	S

Table 20continues

Number of resistant isolates in the all pregnancy outcomes

Isolate	**Antibiotic Profiles**	PG	CD	E	T	CH	VA	DAP	CEF	CEFT	AZI	MER	ER	MOX	TRM/SMX	LIN	LEV	TIGER
P 130 H	d	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	S	S
P 43 R	d	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	S	S
P 133 H	d	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	S	S
P 133 R	d	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	S	S
P 131 R	d	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	S	S
P 132 H	e	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	I	S
P 127 H	e	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
P 131 R	e	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
P 107 R	e	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
P 12 R	f	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
P 151 R	f	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
P 144 R	f	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
P 115 R	j_1	R	R	R	R	R	S	R	R	R	R	R	R	S	S	S	I	S
104 R	j_2	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
P 129 R	i	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	S	S
P 125 R	i	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	I	S
120 R	g	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R	I	S
111 R	h_1	R	R	R	R	S	S	S	S	S	R	S	R	S	I	S	I	S
P 53 R	h_2	R	R	R	R	S	S	R	R	R	R	R	R	S	R	S	S	S
P 112 H	k_1	R	S	S	R	S	S	S	S	S	S	S	R	S	S	R	R	S
P 93 H	k_2	R	S	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S
P 145 R	l	R	R	R	R	R	R	S	R	R	R	R	R	S	S	S	S	
P 94 H	m	R	R	R	R	S	S	S	S	S	R	S	S	S	S	S	I	S

NT = non-typeable, CEF= cefotaxime, CEFT= ceftriaxone, ERT= ertapenem, TRM/SMX= TRM/SMX=trimethroprin/sulphamethoxazole, CH chloramphenicol, CD = clindamycin, PG= penicillin G, MERO= meropenem, E= erythromycin, DAP= daptomycin, T= tetracycline, LEVO= levofloxacin, AZI= azithromycin, MOXI= moxifloxacin, LIN= linezolid, TIGER= tigecycline and VA= vancomycin

4.5.2. 2. Antibiotic susceptibility profiles of *E. faecalis* isolates

Because the TREK system was found to give more accurate results the disc – diffusion, we elected to use only the TREK system for the *E. faecalis* antibiotic susceptibility testing.

The antimicrobial susceptibility of the 24 *E. faecalis* isolates showed that all 24 (100%) isolates of *E. faecalis* were susceptible to tigecycline, followed by 95.8% (23/24) susceptible to moxifloxacin, 91.7% (22/24) to trimethroprim/sulphamethoxazole, 87.5% (21/24) to linezolid and 83.3% (20/24) to chloramphenicol and vancomycin in this study. Seventy – nine point two percent (19/24) of the isolates were susceptible to levofloxacin. Two isolates 8.3% (2/24) presented susceptibility to clindamycin, and daptomycin, and 4.2% (1/24) to erythromycin (Table 19).

Intermediate sensitivity to levofloxacin was found in 12.5% (3/24 isolates), to linezolid in 2 isolates (8.3%), and to erythromycin and azithromycin in 4.2% (1/24 isolates) (Table 19).

Breakpoints for amoxicillin / clavulanic acid; cefepime and cefuroxime could not be established and were listed as none interpretable (NI).

All 24 *E. faecalis* isolates (100%) were resistant to penicillin, ertapenem, cefotaxime, ceftriaxone, and meropenem, followed by 95.8% (23/24) resistant to azithromycin. Resistance to clindamycin, erythromycin, daptomycin and tetracycline was demonstrated by 91.7% (22/24) (Table 19). Four isolates were resistant to vancomycin and chloramphenicol. Two isolates were resistant to trimethroprim/sulphamethoxazole.

4.6. Comparison of specific serotypes of GBS with antibiotic profiles

4.6. 1. Antibiotic susceptibility using the Kirby-Bauer disk diffusion method

The susceptibility profiles of the GBS serotypes are summarized in Table (21).

GBS serotypes Ia, II, IX and the three non-typeable strains showed susceptibility to clindamycin, erythromycin and penicillin G only. Serotype III was susceptible to Clindamycin, erythromycin, penicillin G and sulphamethoxazole. Serotype IV was susceptible to clindamycin, erythromycin, penicillin G, sulphamethoxazole and trimethroprin. Serotype V showed susceptibility to clyndamycin, erythromycin, penicillin G and gentamicin with increasing resistance to gentamicin, tetracycline, trimethroprin and sulphamethoxazole.

Table 21: Antibiotic susceptibility profiles of GBS serotypes using disc diffusion

Antibiotic (µg/disc)												
No (%) of susceptible isolates by serotype	**Clindamycin (CD) 2 µg**			**Erythromycin (E) 5 µg**			**Fusidic acid (FC) 10 µg**			**Gentamicin (GM) 10 µg**		
	Susceptible (%)	**Resistant (%)**	**Total**	**Susceptible (%)**	**Resistant (%)**	**Total**	**Susceptible (%)**	**Resistant (%)**	**Total**	**Susceptible (%)**	**Resistant (%)**	**Total**
Ia [N = 1]	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)
II [N = 1]	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)
III [N = 12]	12 (21.05%)	0 (0%)	12 (21.05%)	12 (21.05%)	0 (0%)		0 (0%)	12 (21.05%)	12 (21.05%)	0 (0%)	12 (21.05%)	12 (21.05%)
IV [N = 1]	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)
V [N = 38]	37 (64.91%)	1 (100%)	38 (66.67%)	37 (64.91%)	1 (1.75%)	38 (66.67%)	0 (0%)	38 (66.67%)	38 (66.67%)	4 (7.0%)	34 (59.6%)	38 (66.67%)
IX [N = 1]	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)
NT [N = 3]	3 (5.26%)	0 (0%)	3 (5.26%)	3 (5.26%)	0 (0%)	3 (5.26%)	0 (0%)	3 (5.26%)	3 (5.26%)	0 (0%)	3 (5.26%)	3 (5.26%)
Total (Frequency %)	**56** (98.2%)	**1** (1.8%)	**57** (100.0%)	**56** (98.2%)	**1** (1.8%)	**57** (100.0%)	**0** (0%)	**57** (100.0%)	**57** (100.0%)	**4** (7.0%)	53 (93.0%)	**57 (**100%)

Antibiotic (µg/disc)												
No (%) of susceptible isolates by serotype	**Penicillin G (PG) 1 unit**			**Tetracycline (T) 10 µg**			**Trimethroprin (TM) 1.25**			**Sulphamethoxazole (SMX) 25 µg**		
	Susceptible (%)	Resistant (%)	**Total**	Susceptible (%)	Resistant (%)	**Total**	Susceptible (%)	Resistant (%)	Total	Susceptible (%)	Resistant (%)	**Total**
Ia [N = 1]	1 (1.75%)	0 (0%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.85%)	1 (1.75%)
II [N = 1]	1 (1.75%)	0 (0%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.85%)	1 (1.75%)
III [N = 12]	12 (21.05%)	0 (0%)	12 (21.05%)	0 (0%)	12 (21.05%)	12 (21.05%)	0 (0%)	12 (21.05%)	12 (21.05%)	22 (38.6%)	10 (17.54%)	32 (56.14%)
IV [N = 1]	1 (1.75%)	0 (0%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)
V [N = 38]	36 (63.16%)	**2** (3.5%)	38 (66.67%)	1(1.75%)	37 (64.91%)	38 (66.67%)	0 (0%)	38 (66.67%)	38 (66.67%)	0 (0%)	38 (66.67%)	38 (66.67%)
IX [N = 1]	1 (1.75%)	0 (0%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.75%)	1 (1.75%)	0 (0%)	1 (1.85%)	1 (1.75%)
NT [N = 3]	3 (5.26%)	0 (0%)	3 (5.26%)	0 (0%)	3 (5.26%)	3 (5.26%)	0 (0%)	3 (5.26%)	3 (5.26%)	0 (0%)	3 (5.26%)	3 (5.26%)
Total Frequency (%)	**55** (96.5%)	**2** (3.5%)	**57** (100.0%)	**1 (**1.8%)	**56** (98.2%)	**57** (100.0%)	**1 (**1.8%)	**56** (98.2%)	**57** (100.0%)	**3** (5.26%)	**54** (94.7%)	**57** (100.0%)

NT, nontypeable

*The numbers in brackets mean the total number of GBS isolates by serotype

4.6.2. Comparison of specific serotypes of GBS with antibiotic profiles from TREK system

Antibiotic susceptibility was performed in all 57 GBS positive isolates and 24 *E. faecalis* isolates. The TREK susceptibility profiles of the GBS serotypes are summarized in Table 22.

GBS serotypes Ia, II, IX and the three non-typeable strains showed susceptibility to clindamycin, erythromycin daptomycin, erythromycin and penicillin G, trimethroprim/ sulphamethoxazole, cefotaxime, ertapenem, meropenem, moxifloxacin, tigecycline, linezolid, chloramphenicol, vancomycin, daptomycin and ceftriaxone excluding serotypes IX isolate which was intermediate. These serotypes were all resistant to tetracycline. Of the 3 isolates which were non-typeable (NT), 1 was susceptible to trimethroprim/sulphamethoxazole. One isolate (1/3) was also resistant to trimethroprim/sulphamethoxazole and intermediate susceptibility to levofloxacin and trimethroprim/sulphamethoxazole was also detected in 1 (1/3) isolate.

Serotype III was susceptible to clindamycin, erythromycin, penicillin G, linezolid, chloramphenicol, ceftriaxone, cefotaxime, ertapenem, meropenem, vancomycin, daptomycin and azithromycin. Resistance was observed in 1 isolate (1/12) to levofloxacin (Table 22). Ten isolates belonging to serotype III were resistant to tetracycline (Table 22). One out of twelve isolates (1/12) was resistant to moxifloxacin. Intermediate susceptibility to trimethroprim/sulphamethoxazole was observed in one (1/12) isolate.

Serotype IV was susceptible to clindamycin, erythromycin daptomycin, erythromycin and penicillin G, trimethroprim/ sulphamethoxazole, cefotaxime, ertapenem, meropenem, moxifloxacin, tigecycline, linezolid, chloramphenicol, vancomycin, daptomycin and ceftriaxone.

Isolates of serotype V were all susceptible to moxifloxacin, tigecycline, and 36 (36/38) isolates were susceptible to linezolid. Thirty – two (32/38) isolates were susceptible to

trimethroprim/ sulphamethoxazole. The majority of isolates (28/38) isolates were susceptible to levofloxacin, followed by 25 isolates susceptible to chloramphenicol. Twenty - one isolates (21/38) were susceptible to vancomycin. Susceptibility to the following antibiotics was also observed namely, 19 isolates (19/38) to daptomycin, 18 isolates (18/38) to cefotaxime, 16 isolates (16/38) to ertapenem, 15 isolates (15/38) to clindamycin, 14 isolates (14/38) to azithromycin, 13 isolates (13/38) to erythromycin, and 12 isolates (12/38) to penicillin G in this study.

Thirty - seven out of thirty - eight (37/38) isolates belonging to serotype V were resistant to tetracycline. Resistance to penicillin G was detected in 26 isolates (26/38). Twenty – four isolates were resistant (24/38) to erythromycin and to azithromycin respectively. Resistance to clindamycin was observed in 23 (23/38) isolates. While 22 isolates were resistant to ertapenem, 20 were resistant to cefotaxime, and resistance to daptomycin was found in 19 isolates (19/38).

Of the 38 isolates belonging to serotype V, 17 were resistant to vancomycin, and 13 isolates were resistant to chloramphenicol. Resistance to trimethroprim/ sulphamethoxazole was found in fewer isolates (3/38), and 1 isolate was resistant to levofloxacin.

Intermediate susceptibility to levofloxacin was found in 9 isolates from serotype V. Three of the thirty – eight isolates showed intermediate susceptibility to trimethroprim/ sulphamethoxazole, and 1 showed intermediate susceptibility to linezolid and erythromycin.

Table 22: Antibiotic susceptibility profiles of GBS serotypes using TREK

	Antibiotic															
No (%) of susceptible isolates by serotype	**Clindamycin**				**Tetracycline**				**Trimethroprin/ Sulphamethoxazole**				**Vancomycin**			
	S	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**
Ia [N = 1]	1	0	0	1	0	1	0	1	1	0	0	1	1	0	0	1
II [N = 1]	1	0	0	1	0	1	0	1	1	0		1	1	0	0	1
III [N = 12]	12	0	0	12	2	10	0	12	11	0	1	12	12	0	0	12
IV [N = 1]	1	0	0	1	0	1	0	1	1	0	0	1	1	0	0	1
V [N = 38]	15	23	0	38	1	37	0	38	32	3	3	38	21	17	0	38
IX [N = 1]	1	0	0	1	1	1	0	1	1	0	0	1	1	0	0	1
NT [N = 3]	3	0	0	3	0	3	0	3	1	1	1	3	3	0	0	3
Frequency	34	23	0	57	3	54	0	57	48	4	5	57	40	17	0	57
Percentage	59.6	40.4	0	100.0	5.3	94.7	0	100.0	84.2	7.0	8.8	100.0	70.2	29.8	0	100.0

	Antibiotic															
No (%) of susceptible isolates by serotype	**Chloramphenicol**				**Erythromycin**				**Ertapenem**				**Penicillin G**			
	S	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**
Ia [N = 1]	1	0	0	1	1	0	0	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**1**
II [N = 1]	1	0	0	1	1	0	0	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**1**
III [N = 12]	12	0	0	12	12	0	0	**12**	**12**	**0**	**0**	**12**	**12**	**0**	**0**	**12**
IV [N = 1]	1	0	0	1	1	0	0	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**1**
V [N = 38]	25	13	0	38	13	24	1	**38**	**16**	**22**	**0**	**38**	**12**	**26**	**0**	**38**
IX [N = 1]	1	0	0	1	1	0	0	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**1**
NT [N = 3]	3	0	0	3	3	0	0	**3**	**3**	**0**	**0**	**3**	**3**	**0**	**0**	**3**
Frequency	44	13	0	57	32	24	1	**57**	**35**	**22**	**0**	**57**	**31**	**26**	**0**	**57**
Percentage	77.2	22.8	0	100.0	56.1	42.1	1.8	**100.0**	**61.4**	**38.6**	**0**	**100.0**	**54.4**	**45.6**	**0**	**100.0**

NT= non-typeable, R = Resistant, S= Susceptible, I = Intermediate

*The numbers in brackets mean the total number of GBS isolates by serotype

Table 22 (continued): Antibiotic susceptibility profiles of GBS serotypes using TREK

No (%) of susceptible isolates by serotype	Antibiotic															
	Cefotaxime				Azithromycin				Daptomycin				Levofloxacin			
	S	R	I	Total	S	R	I	Total	S	R	I	Total	S	R	I	Total
Ia [N = 1]	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1
II [N = 1]	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	1
III [N = 12]	12	0	0	12	12	0	0	12	12		0	12	11	0	1	12
IV [N = 1]	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1
V [N = 38]	18	20	0	38	14	24	0	38	19	19	0	38	28	1	9	38
IX [N = 1]	0	1	0	1	1	0	0	1	1	0	0	1	1	0		1
NT [N = 3]	3	0	0	3	3	0	0	3	3	0	0	3	2	0	1	3
Frequency	**36**	**21**	**0**	**57**	**33**	**24**	**0**	**57**	**38**	**19**	**0**	**57**	**45**	**1**	**11**	**57**
Percentage	**63.2**	**36.8**	**0**	**100**	**57.9**	**42.1**	**0**	**100**	**66.7**	**33.3**	**0**	**100**	**78.9**	**1.8**	**19.3**	**100**

No (%) of susceptible isolates by serotype	Antibiotic															
	Linezolid				Meropenem				Moxifloxacin				Tigecycline			
	S	R	I	Total	S	R	I	Total	S	R	I	Total	S	R	I	Total
Ia [N = 1]	1	0	0	1	1	0	0	1	1	0	0	1	**1**	**0**	**0**	**1**
II [N = 1]	1	0	0	1	1	0	0	1	1	0	0	1	**1**	**0**	**0**	**1**
III [N = 12]	12	0	0	12	12	0	0	12	11	0	1	12	**12**	**0**	**0**	**12**
IV [N = 1]	1	0	0	1	1	0	0	1	1	0	0	1	**1**	**0**	**0**	**1**
V [N = 38]	36	1	1	38	18	20	0	38	38	0	0	38	**38**	**0**	**0**	**38**
IX [N = 1]	1	0	0	1	1	0	0	1	1	0	0	1	**1**	**0**	**0**	**1**
NT [N = 3]	3	0	0	3	3	0	0	3	2	1	0	3	**3**	**0**	**0**	**3**
Frequency	**55**	**1**	**1**	**57**	**36**	**21**	**0**	**57**	**55**	**1**	**1**	**57**	**57**	**0**	**0**	**57**
Percentage	**96.5**	**1.8**	**1.8**	**100**	**63.2**	**36.8**	**0**	**100**	**96.5**	**1.8**	**1.8**	**100**	**100**	**0**	**0**	**100**

NT = non-typeable, R = Resistant, S= Susceptible, I = Intermediate

*The numbers in brackets mean the total number of GBS isolates by serotype

Table 23: Antibiotic susceptibility profiles of *E. faecalis* using TREK

	Antibiotic															
	Clindamycin				Tetracycline				Trimethroprin/ Sulphamethoxazole				Vancomycin			
Susceptibility	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**
Number of isolates	2	22	0	24	2	22	0	24	22	2	0	24	20	4	0	24
Percentage	8.3	91.7	0	100	8.3	91.7	0	100	91.7	8.3	0	100	83.3	16.7	0	100
	Chloramphenicol				Erythromycin				Ertapenem				Penicillin G			
Susceptibility	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**
Number of isolates	20	4	0	24	1	1	22	24	0	24	0	24	0	24	0	24
Percentage	83.3	16.7		100	4.2	4.2	91.7	100	0	100		100	0	100	0	100

	Antibiotic															
	Cefotaxime				Azithromycin				Daptomycin				Levofloxacin			
Susceptibility	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**
Number of isolates	0	24	0	24	0	23	1	24	2	22	0	24	19	2	3	24
Percentage	**0**	**100**	**0**	100	0	95.8	4.2	100	**8.3**	**91.7**	**0**	100	**79.2**	**8.3**	**12.5**	100
	Linezolid				Meropenem				Moxifloxacin				Tigecycline			
Susceptibility	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**	**S**	**R**	**I**	**Total**
Number of isolates	21	1	2	24	0	24	0	24	23	1	0	24	24	0	0	24
Percentage	**87.5**	**4.2**	**8.3**	**100**	**0**	**100**		**100**	**95.8**	**4.2**	**0**	**100**	**100**	**0**	**0**	**100**

R = Resistant, S= Susceptible, I = Intermediate

Table 24: Breakdown of antibiotic profile grouping based on vancomycin sensitivity and vancomycin resistance

Number of resistant isolates in the all pregnancy outcomes																		
Isolate	**Antibiotic Profiles**	PG	CD	E	T	CH	VA	DAP	CEF	CEFT	AZI	MER	ER	MOX	TRM/SMX	LIN	LEV	TIGER
P 96 H	n_1	S	I	R	S	S	S	R	R	R	I	S	S	S	S	S	S	S
P 75 R	n_2	S	R	R	S	S	S	R	R	R	S	S	R	S	S	S	S	S
P 61 H	o_1	R	S	R	R	S	S	R	R	R	R	R	R	S	S	S	R	S
P 72 H	o_2	R	R	S	S	R	S	R	R	R	I	R	R	S	S	S	S	S
P119 R	o_3	R	R	R	R	S	S	R	R	R	S	R	R	R	S	S	S	S
P 98 H	p	R	R	R	R	S	S	R	R	R	R	R	R	S	S	S	S	S
P 85 H	p	R	R	R	R	S	S	R	R	R	R	R	R	S	S	S	I	S
217 H	p	R	R	R	R	S	S	R	R	R	R	R	R	S	S	S	S	S
217 R	p	R	R	R	R	S	S	R	R	R	R	R	R	S	S	S	S	S
P 91 H	p	R	R	R	R	S	S	R	R	R	R	R	R	S	S	S	S	S
231 H	p	R	R	R	R	S	S	R	R	R	R	R	R	S	S	S	S	S
P 91 R	q	R	R	R	R	S	S	S	R	R	R	R	R	S	S	I	S	S
P 88 H	q	R	R	R	R	S	S	S	R	R	R	R	R	S	S	S	I	S
P 95 R	r	R	R	R	R	S	R	R	R	R	R	R	R	S	S	R	R	S
P 09 H	r	R	R	R	R	S	R	R	R	R	R	R	R	S	S	R	R	S
175 R	s_1	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
232 H	s_2	R	R	I	R	R	R	R	R	R	R	R	R	S	S	S	S	R
P 82 R	t_1	R	S	R	R	S	R	R	R	R	R	R	R	R	S	I	R	S
234 H	t_2	R	I	R	R	S	R	R	R	R	R	R	R	R	R	S	S	S
P 82 H	t_3	R	R	R	S	S	R	S	R	R	R	R	R	S	S	S	I	S
P 03 H	u	R	I	S	R	S	R	R	R	R	S	R	R	S	R	S	R	S
P 80 H	v	R	I	R	R	S	R	R	R	R	S	R	R	S	S	S	S	S

NT = non-typeable, CEF= cefotaxime, CEFT= ceftriaxone, ERT= ertapenem, TRM/SMX= TRM/SMX=trimethroprin/sulphamethoxazole, CH chloramphenicol, CD = clindamycin, PG= penicillin G, MERO= meropenem, E= erythromycin, DAP= daptomycin, T= tetracycline, LEVO= levofloxacin, AZI= azithromycin, MOXI= moxifloxacin, LIN= linezolid, TIGER= tigecycline and VA= vancomycin

*The numbers in brackets mean the total number of GBS isolates by serotype

All *E faecalis* isolates were susceptible to tigecycline and most were susceptible to linezolid, moxifloxacin, chloramphenicol, trimethroprim/ sulphamethoxazole and vancomycin (Table 23).

Total resistance was observed to penicillin G, ertapenem, cefotaxime, and meropenem with increased resistance noted for clindamycin, tetracycline, azithromycin, and daptomycin (Table 23).

E. faecalis isolates were divided into vancomycin sensitive and vancomycin resistant groups for further assignment into specific antimicrobial profile groups as explained previously for Table 20. The predominant antimicrobial profiles for *E. faecalis* was observed in AP profile p, followed by o (Table 24).

4.8. PFGE analysis

The PFGE analysis of the 57 GBS positive isolates and 24 *E faecalis* was carried out using CHEF – DR II systems from Bio–Rad according to the protocol of Gautom *et al*., (1997) with a few modifications. Figures 15 - 24 show fingerprints or pulsotypes using *Sma I* restriction enzyme. PFGE patterns were analyzed by both computer – assisted program Primer V6 (Clarke *et al*., 2001) and by manual or visual comparison of each banding pattern using criteria developed by Tenover *et al*., (1995).

One of the objectives of this study was to establish the diversity of the GBS and *E faecalis* strains in the population. Genomic DNA was extracted and separated using PFGE. In order to determine if the strains were similar or different, restriction digestion was used to generate fragments that were separated on agarose gel. Their patterns were analyzed using cluster analysis (and MDS ordination).

In all cases, the GBS and *E. faecalis* samples were of diverse bacterial strains as shown by the presence of different restriction patterns arising from the DNA that had been separated using PFGE (see diagrams below). The significance of the clusters was to assess if there was any pattern that related the strains according to their distribution in an individual and geographic location or other biological significance.

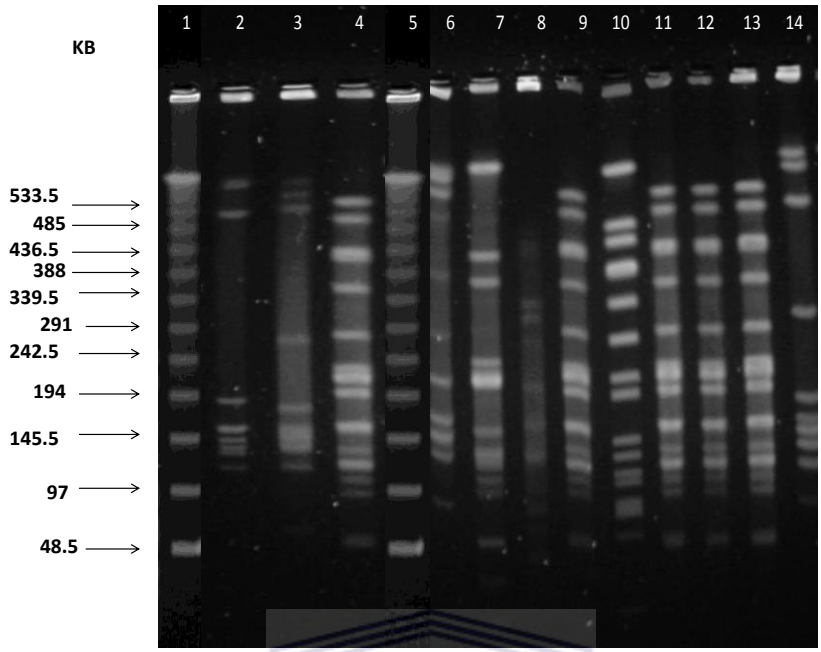

Fig. 15: PFGE patterns for Group B streptococcus PTD isolates obtained from pregnant women. Lambda ladder was used as a standard size marker. Lanes 1& 5: Marker, 2: GBS strain V, 3: P04 H, 4: P12 R, 6: P14 R, 7: P84 R, 8: P94 H, 9: P125 R, 10: Control (*S. aureus*), 11: P131 R, 12: P133 H, 13: P133 R, 14: P143 R. Each lane shows the patterns obtained from an isolate from different individuals. Isolates which were obtained from single individuals yielded identical respective patterns.

Figure 15 shows fingerprints or pulsotypes using *SmaI* restriction enzyme. Different band patterns are demostrated in lanes 6 and 7 (Fig. 15), while lanes 11 to 13 had identical band patterns (Fig. 15). Lane 6 was a NT and lane 7 belonged to serotype V. They both were susceptible to penicillin G, clindamycin, erythromycin, cefotaxime, ceftriaxone, moxifloxacin, levofloxacin, meropenem, ertapenem, tigecycline, azithromycin, daptomycin, linezolid, trimethroprin/sulphamethoxazole, and vancomycin, and resistant to tetracycline. Both were isolated from women who had a previous history of PTD.

The serotypes in Lanes 12 and 13 were isolated from a woman who had a previous history of PTD and all isolates were susceptible to, moxifloxacin, trimethroprin/sulphamethoxazole, tigecycline and linezolid, and resistant to penicillin G, clindamycin, erythromycin tetracycline, cefotaxime, ceftriaxone, chloramphenicol, meropenem, ertapenem, azithromycin, daptomycin,

trimethroprin/sulphamethoxazole with vancomycin, and intermediate susceptibility to levofloxacin observed in one isolate.

Lanes 8 and 14 (Fig. 15) represent serotypes, V and serotype II respectively. The isolates belonging to serotype II were susceptible to penicillin G, clindamycin, erythromycin, cefotaxime, ceftriaxone, chloramphenicol, meropenem, ertapenem, azithromycin, daptomycin, tigecycline, linezolid, trimethroprin/sulphamethoxazole, moxifloxacin, and vancomycin. Resistance to, tetracycline was observed in both isolates.

The isolate belonging to serotype V (lane 8) was also resistant to penicillin G, clindamycin, azithromycin and erythromycin, with intermediate susceptibility to levofloxacin.

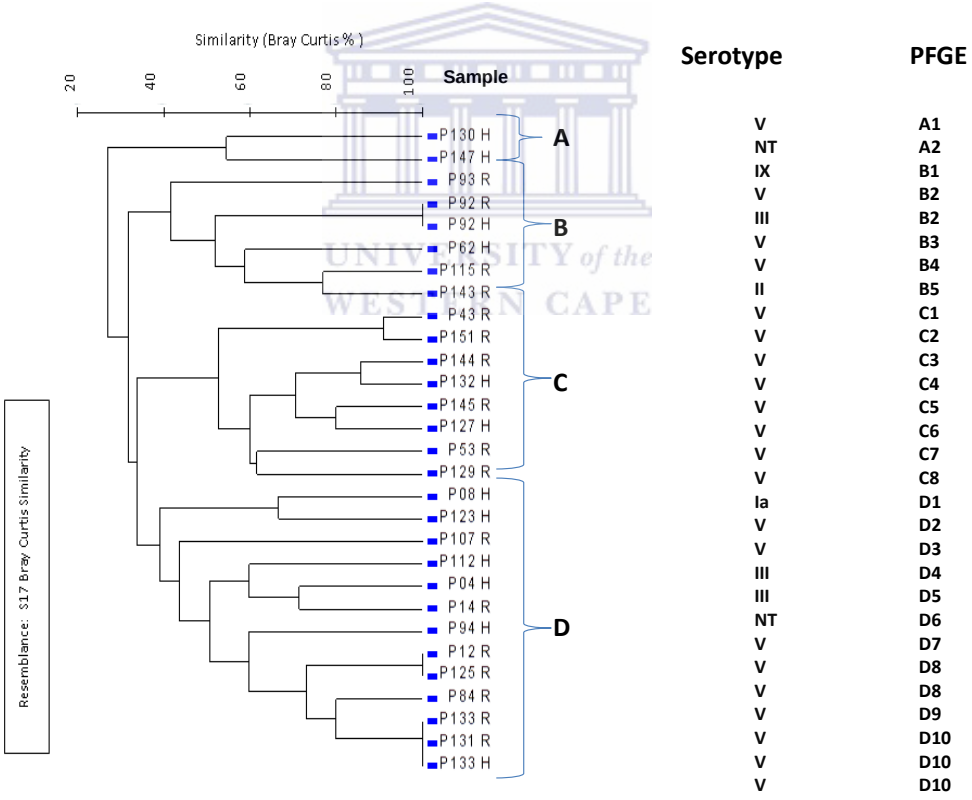

Fig. 16: Cluster analysis of the GBS-PTD diversity.

Figure 16 shows the dendogram based on PFGE of the GBS isolates, based on the Hierarchical clustering, average/UPGMA (Unpaired Group Mean Weight Average) and Euclidian distance methods using PRIMER V6. As per the generated dendogram, the results show that the isolates were categorized into 3 major cluster groups based on 60% similarity of the genetic profiles of the restriction patterns. Most of the GBS strains were genetically diverse except for P92 H, P92 R and R131 R, P133 H and P133 R, which had 100% similarity and clustered together (Fig. 16). Four clusters (A, B, C and D) and 2 isolates with distinct GBS diversity profiles were identified (Fig. 15 and 16).

The first cluster (labeled A) had 2 subgroups, A1 and A2. Subgroup A1 belonged to serotype V, which included isolate (P130 H), and the second subgroup (A2) consisted of 1 non - typeable (NT) isolate (P147 H) (Fig. 16). All the isolates in this cluster were susceptible to the following antibiotics tigecycline, chloramphenicol, linezolid, and levofloxacin as well as trimethroprin/sulphamethoxazole. The isolate (P130 H) was resistant penicillin G, erythromycin, clindamycin, cefotaxime, ceftriaxone, meropenem, ertapenem, daptomycin and vancomycin.

The nontypeable isolate (P147 H) was susceptible to penicillin G, clindamycin, erythromycin, cefotaxime, ceftriaxone, meropenem, ertapenem, azithromycin, daptomycin, and vancomycin, and resistant to tetracycline and moxifloxacin.

The second cluster (labeled B) had 1 subgroup, which included serotype II isolate P143 R and serotype V isolate P115 R plus 2 isolates (P92 H, and P62 H) (Fig. 17). All the isolates in this cluster had different serotypes but were all susceptible to the following antibiotics penicillin G, clindamycin, erythromycin, cefotaxime, ceftriaxone, chloramphenicol, meropenem, ertapenem, azithromycin, daptomycin, tigecycline, linezolid, trimethroprin/sulphamethoxazole, moxifloxacin, and vancomycin was observed in all isolates. All were resistant to tetracycline, however one isolate (P62 H) was also resistant to trimethroprin/sulphamethoxazole and showed intermediate susceptibility to linezolid and isolate P115 showed intermediate susceptibility to levofloxacin. In addition, 1 isolate (P115 R), was also resistant to penicillin G, clindamycin, azithromycin, cefotaxime, ceftriaxone, chloramphenicol, meropenem, ertapenem, daptomycin and clindamycin.

The third cluster (labeled C) included serotype V, and had 3 subgroups which included isolates (P43 R and P151 R) in the first subgroup, isolates (P144 R, P132 H, P145 R, and P127 H), plus 2 isolates (P53 R and P129 R) in the third subgroup (Fig. 16 and Fig. 17). All the isolates in this third cluster were susceptible to the following antibiotics moxifloxacin, tigecycline, linezolid, trimethroprin/sulphamethoxazole and levofloxacin. One isolate was susceptible to chloramphenicol.

The fourth cluster (labeled D) included 3 subgroups, one of which included serotype V, isolates (P84 R, P12 R, P125 R, P133 H), the second subgroup consisted of 3 isolates (P94 H, P14 H, P04 H), and the third subgroup included serotype Ia (P08 H) and serotype V isolate (P123 H) (Fig. 16). In the fourth cluster, all the isolates in the second subgroup were susceptible to penicillin G, clindamycin, erythromycin, moxifloxacin, tigecycline, linezolid, trimethroprim/sulphamethoxazole, levofloxacin, cefotaxime, ceftriaxone, daptomycin, meropenem, ertapenem, and vancomycin excluding isolate P94 H which was resistant to penicillin G, clindamycin, azithromycin and erythromycin and intermediate to levofloxacin. They were all resistant to tetracycline. Furthermore, serotype V which included isolate P112 H, was the only one in this cluster which was resistant to ertapenem.

The third subgroup in the fourth cluster, comprised of serotype Ia (P08 H) and serotype V (P123 H). All the isolates in this subgroup were susceptible to penicillin G, clindamycin, erythromycin, moxifloxacin, tigecycline, linezolid, levofloxacin, trimethroprim/sulphamethoxazole, cefotaxime, ceftriaxone, daptomycin, meropenem, ertapenem, vancomycin, chloramphenicol, and tigecycline, and they were resistant to tetracycline.

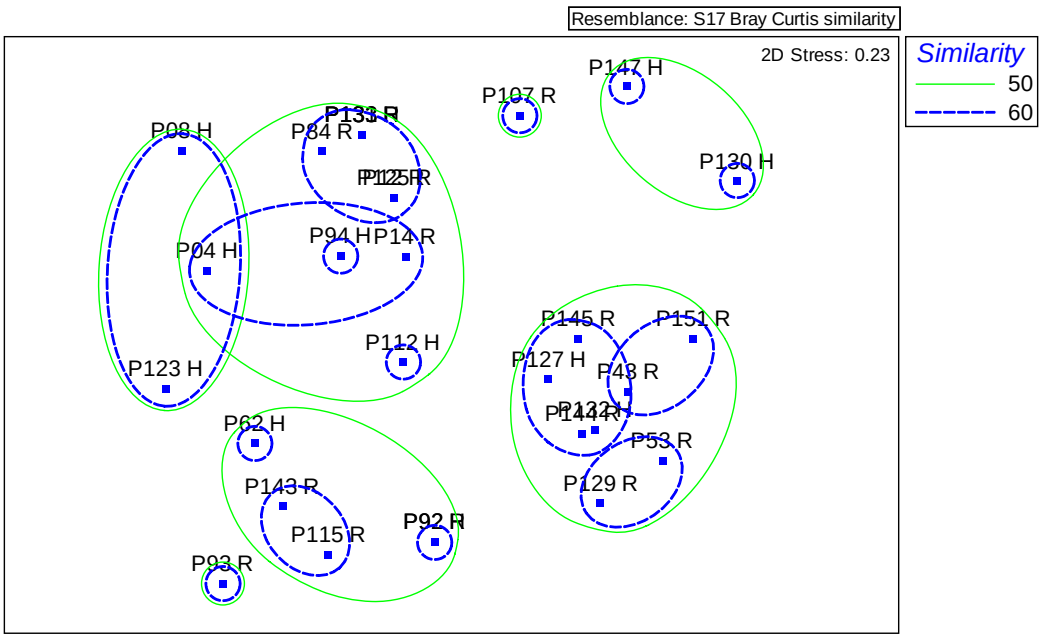

Fig 17: MDS analysis of the GBS PTD isolates diversity profiles based on a 50% and 60% (middle small circles) similarity. The distance between the points shows the relative differences between the isolates diversity profiles. An identical symbol shows the isolates that cluster together at 60% identical threshold.

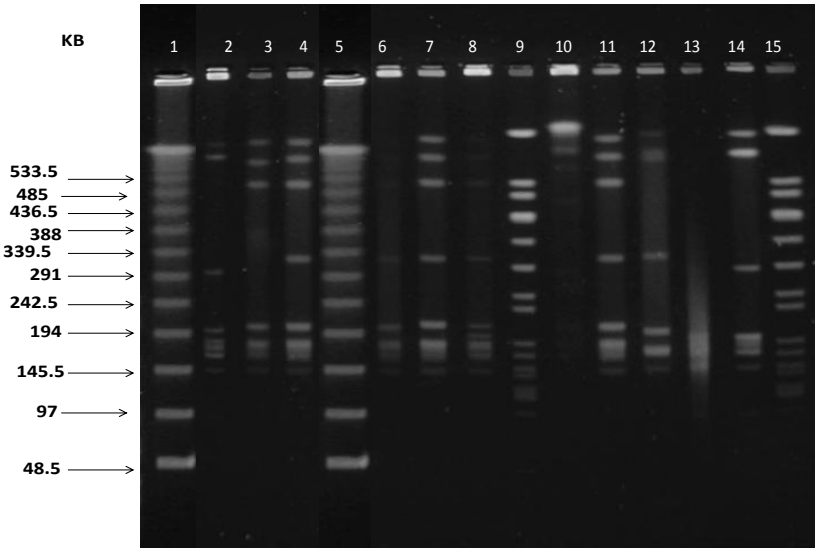

Fig. 18: PFGE profiles for Group B streptococcus FTD obtained from clinical samples. Lambda ladder was used as a standard size marker. Lanes 1& 5: Marker, 2: GBS strain III, 3: 130 H, 4: 139 H, 6: 141 H, 7: 139 R, 8: 167 H, 9&15: Control (*S. aureus*), 10: 193 H, 11: 193 R, 12: 194 H, 13: 216 H, and 14: 219 H. Each lane shows the patterns obtained from an isolate from different individuals. Isolates which were obtained from single individuals yielded identical respective patterns.

Figure 18 shows the fingerprints of the GBS strains of which some demonstrated distinct band patterns. The isolates in lanes (4 and 7) presented identical PFGE fingerprint patterns (Fig. 18). For this reason they are considered to be possibly related. Furthermore the PFGE fingerprint patterns of isolates number P141 H and sample number 167 H (lanes 6 and 8) shows more than 4 band differences, consistent with two genetic events. The 2 isolates belong to GBS serotype V, and they were isolated from women who had a previous history of FTD. Both isolates were susceptible to penicillin G, clindamycin, cefotaxime, ceftriaxone, chloramphenicol, linezolid, levofloxacin, ertapenem, vancomycin, tigecycline, trimethroprim/sulphamethoxazole and erythromycin excluding the isolate P141 H which showed intermediate susceptibility. Resistance to tetracycline was observed in both isolates.

PFGE fingerprint patterns of isolate sample numbers 139 R and sample number 219 H (lanes 7 and 14) shows more than 4 band differences, consistent with two genetic events of serotypes V and III respectively. Susceptibility to penicillin G, clindamycin, levofloxacin, ceftriaxone, cefotaxime, chloramphenicol, meropenem, ertapenem, tigecycline, trimethroprim / sulpha-

methoxazole, vancomycin and erythromycin was detected in both isolates with resistance to tetracycline.

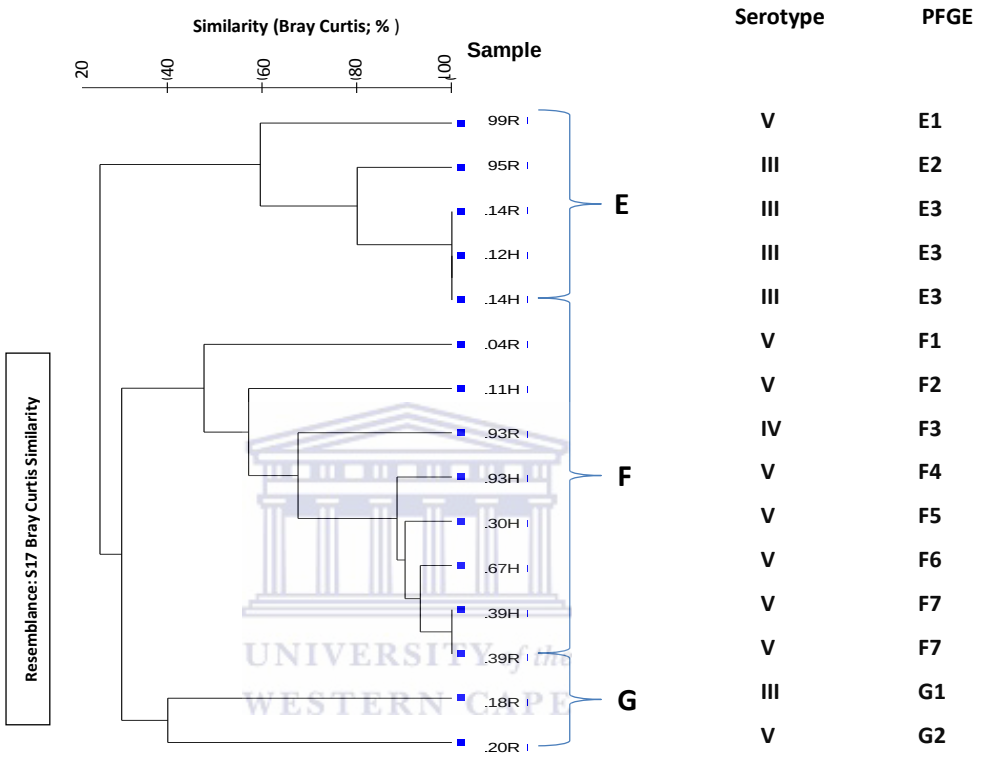

Fig. 19: Cluster analysis of the GBS-FTD diversity.

The dendogram shown in Fig. 19 was based on PFGE of the GBS isolates, the dendogram drawn based on the Hierarchical clustering, average/UPGMA (Unpaired Group Mean Weight Average) and Euclidian distance methods using PRIMER V6. Figure 19 show the dendogram presented using the cluster analysis. The results show that the isolates fall into 3 clusters based on 60% similarity of the genetic profiles of the restriction patterns (Fig. 19). As per the generated dendogram, most of the GBS strains here were genetically diverse except for those found in isolates number 114 H, 112 H and 114 R and also isolates number 139 H and 139 R, which had 100% similarity and clustered together (Fig. 19). The results indicate that all individuals

harboured different strains of GBS in the rectal (R) and vaginal (H) samples. Three major clusters of the GBS strains were identified but they did not have a notable characteristic to explain the similarities between the isolates.

The first cluster (labeled E) included one subgroup, which had serotypes III, consisting of 3 isolates (114 H, 114 R 95 R and 112 H), and 1 isolate belonging to serotype V (99 R).

The second cluster (labeled F) included one subgroup which consisted mostly of serotypes V and included 7 isolates (F1 to F7) (139 H, 139 R, 167 H, 130 H, and 193 R) excluding one isolate (193 H) belonging to serotype IV, plus 2 distinct isolates, the belonging to serotype V (104 R and 111 H) (Fig.19 and Fig.20). All the isolates in this cluster belonging to serotype V were susceptible to penicillin G, clindamycin and erythromycin. However, the distinct isolates were only susceptible to penicillin G and erythromycin.

The third cluster (labeled G) included 1 subgroup, (G1) which included serotype III, isolate 118 R, and the second one (G2) had serotype V, isolate 120 R (Fig. 19). All the isolates belonging to this cluster were susceptible to penicillin G, clindamycin and erythromycin.

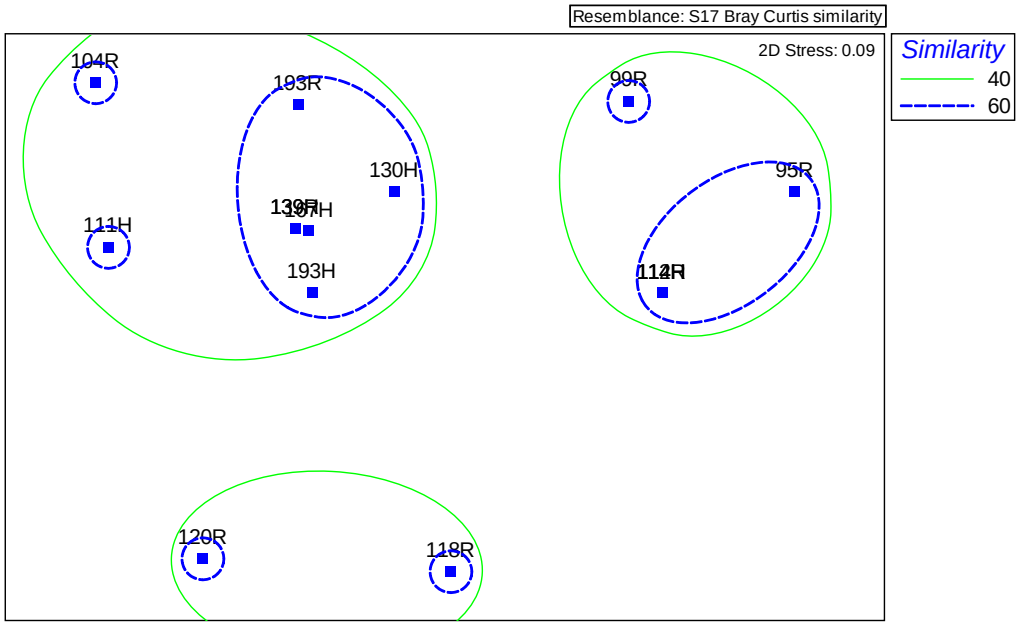

Fig 20: MDS analysis of the GBS FTD isolates diversity profiles based on a 40% and 60% (middle small circles) similarity. The distance between the points shows the relative differences between the isolates diversity profiles. An identical symbol shows the isolates that cluster together at 60% identical threshold.

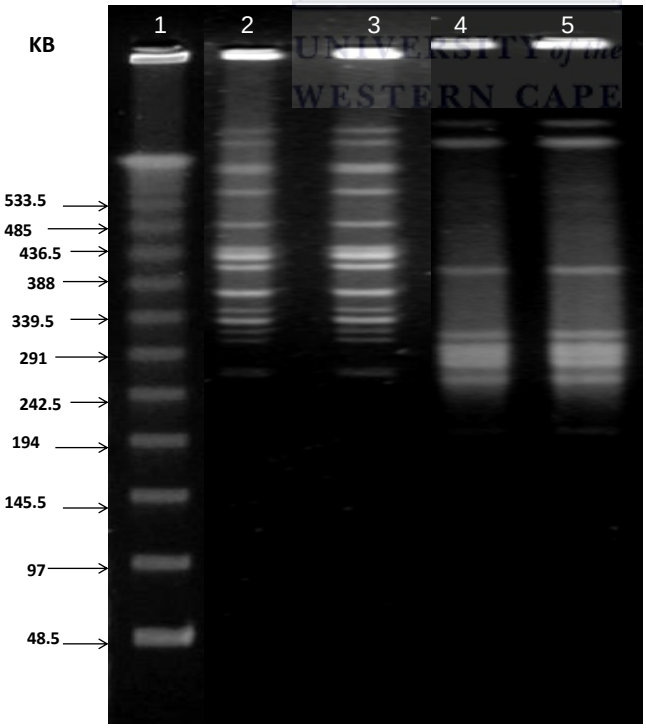

Fig. 21: PFGE patterns of three isolates with different DNA profiles. Lanes 1: Marker, Lanes 2 and 3, PFGE of two different isolates from samples with same patterns belonging to serotype V isolates Lanes 4 – 5, PFGE from strains with GBS serotypes III isolates

The results demonstrate that GBS colonized women may harbour different serotypes. Distinguishable PFGE profiles were observed for serotypes III and V (Fig. 21). The two isolates from serotype III were susceptible to penicillin G, clindamycin, erythromycin, tigecycline azithromycin, cefotaxime, ceftriaxone, ertapenem, meropenem, daptomycin, linezolid and vancomycin. Resistant was observed to tetracycline. Intermediated was detected in moxifloxacin and levofloxacin. These two isolates were isolated from women who had a previous history of FTD.

Lanes 2 and 3 represent patterns of GBS serotype V (Fig. 21). They were susceptible to penicillin G, clindamycin, erythromycin, ertapenem, meropenem, moxifloxacin, chloramphenicol, daptomycin, trimethroprin/sulphamethoxazole, levofloxacin, linezolid, tigecycline azithromycin, cefotaxime, ceftriaxone, and vancomycin and they were resistant to tetracycline (Fig. 21). The isolates in Figure 21 were isolated from women who had a previous history of PTD.

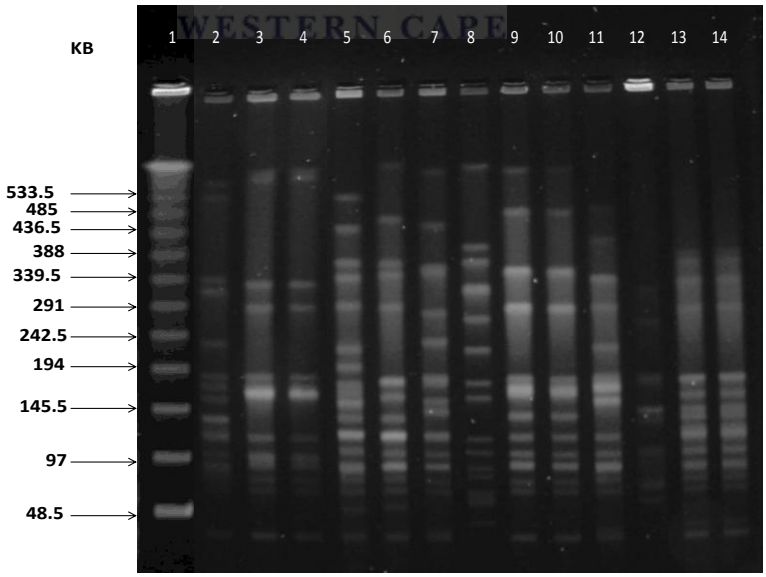

Fig. 22: PFGE patterns for *E. faecalis* (Group D) obtained from clinical samples. Lanes 1& 5: Marker, 2: 175 R, 3: 217 H, 4: 217 R, 6: 231 H, 7: 232 H, 8: P88 H, 9: Control (*S. aureus*), 10: P91 H, 11: P91 R, 12: P95 R, 13: P96 H, 14&15: P98 H. Each lane shows the patterns obtained from an isolate from different individuals. Isolates which were obtained from single individuals yielded identical respective patterns.

Figure 22 shows the fingerprints of the epidemiologically unrelated strains (lanes 6 to 14) demonstrated distinct patterns. Both isolates were susceptible to trimethroprin/sulphamethoxazole, moxifloxacin, chloramphenicol, levofloxacin, linezolid, tigecycline and vancomycin. Resistance to penicillin G, clindamycin, erythromycin, tetracycline, ertapenem, meropenem, daptomycin, azithromycin, cefotaxime, and ceftriaxone was observed.

The isolates of the two strains (lanes 3 and 4) as well as isolates in lanes (10 and 11) presented identical PFGE fingerprint patterns (Fig. 22). These isolates were found to be susceptible to trimethroprin/sulphamethoxazole, moxifloxacin, chloramphenicol, levofloxacin, linezolid, tigecycline and vancomycin. They were also resistant to penicillin G, clindamycin, erythromycin, tetracycline, ertapenem, meropenem, daptomycin, azithromycin, cefotaxime, and ceftriaxone.

Sample numbers 231 H and 232 H (lanes 6 and 7) show more than 4 band differences, consistent with two genetic events. Susceptibility was detected in both isolates to trimethroprin/sulphamethoxazole, moxifloxacin, levofloxacin, linezolid, tigecycline. The 2 isolates were resistant to penicillin G, clindamycin, tetracycline, ertapenem, meropenem, daptomycin, azithromycin, cefotaxime, and ceftriaxone. On the other hand the isolate in lane 7 was resistant to chloramphenicol, vancomycin, and intermediate to erythromycin.

E. faecalis and GBS

The analysis of *E. faecalis* isolates revealed 19 pulsotypes. Their antimicrobial susceptibility patterns have already been described above.

The results from the PFGE profiles revealed the diversity amongst the GBS strains by serotypes, serotypes Ia, II, and IV had 1 band patterns in this study. Serotype V strains had 32 patterns, serotype III had 9 patterns. The non-typeable strains had 2 patterns in the current pregnancy outcomes.

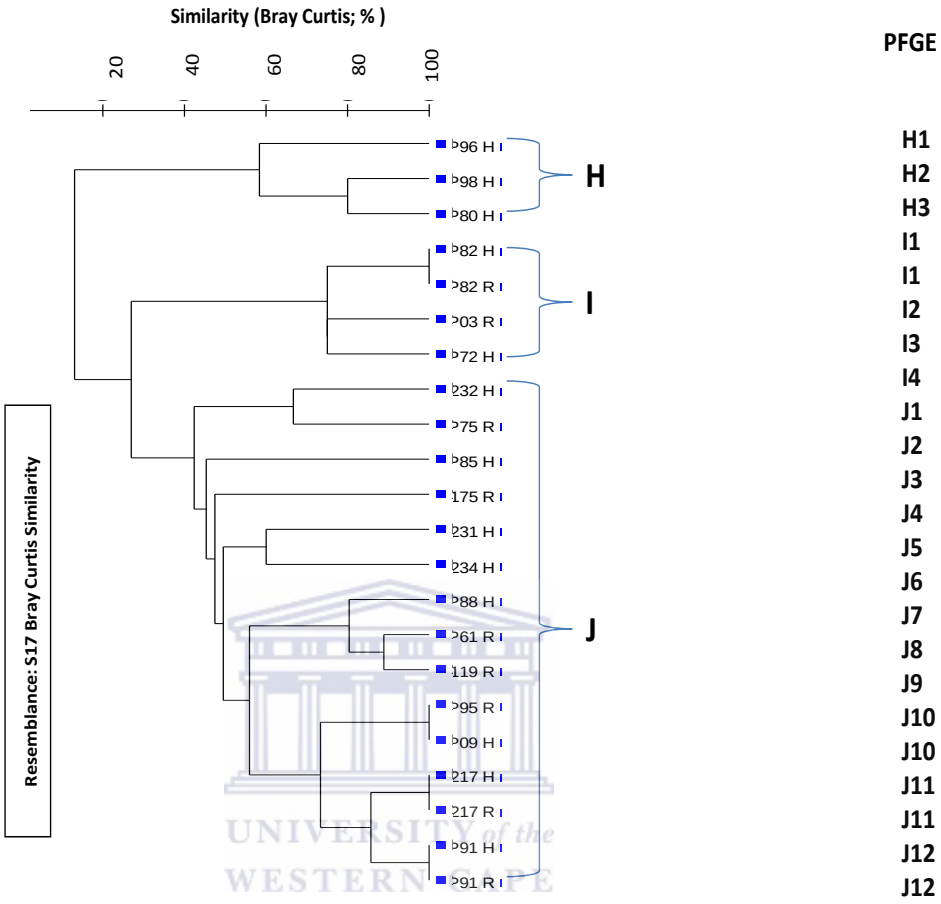

Fig 23: Cluster analysis of the *E. faecalis* diversity.

Figure 23 shows that most of the *E. faecalis* strains were genetically diverse except for those found in isolates number P91 H, P91 R and 217 R, 217 H and also isolates number P82 H and P82 R, which had 100% similarity and clustered together (Fig. 23).

As indicated by the generated dendogram, isolates were categorized into 3 major cluster groups based on 60% similarity of the genetic profiles of the restriction patterns (Fig. 23). The results shows that all individuals were infected with different strains of *E. faecalis* in the rectal (R) and also in the vaginal (H) samples except for the isolates number P91, P 82 and 217 which had the same strain infecting the three isolates (Fig. 23). The first cluster had two subgroups, with

isolates (P98 H and P80 H) and isolate (P96 H) (Fig. 23). All isolates in this cluster were susceptible to trimethroprin/sulphamethoxazole, moxifloxacin, chloramphenicol, levofloxacin, linezolid, tigecycline and vancomycin, and resistant to penicillin G, clindamycin, tetracycline, ertapenem, meropenem, daptomycin, azithromycin, cefotaxime, and ceftriaxone. They were isolated from women who had a previous history of PTD.

The second cluster included isolates (P72 H, P03 R and P82 H, P82 R). These isolates were susceptible to tetracycline, trimethroprin/sulphamethoxazole, moxifloxacin, chloramphenicol, linezolid, tigecycline and vancomycin, levofloxacin and erythromycin. All isolates were resistant to penicillin G, clindamycin, ceftriaxone, cefotaxime, ertapenem, meropenem. Furthermore, 2 isolates in this cluster showed intermediate susceptibility to levofloxacin and the second, to azithromycin, and erythromycin. They all were isolated from women with a previous history of PTD.

The third cluster had two subgroups, one which included isolates (P91 H, and 127 H) and the second subgroup, consisted of 5 isolates (232 H, P88 H, P61 R, P09 H, P119 R) (Fig. 23), plus 5 distinct isolates (P75 R, P85 H, 175, 231 H, 234 H) (Fig. 24).

In this cluster, all the isolates were susceptible to tigecycline and resistant to penicillin G, meropenem. The isolates in the first subgroup (P91 H, and 127 H) were susceptible to trimethroprin/sulphamethoxazole, moxifloxacin, levofloxacin, linezolid, tigecycline. They were all resistant to penicillin G, clindamycin, ceftriaxone, cefotaxime, ertapenem, meropenem, azithromycin, daptomycin, erythromycin, and ertapenem.

The second subgroup, consisted of 5 isolates (232 H, P88 H, P09 H, P61 R, P119 R), 4 of which were isolated from women who had a previous history of PTD. All the isolates in the second subgroup were susceptible to trimethroprin/sulphamethoxazole, linezolid, tigecycline, chloramphenicol, and vancomycin and were all resistant to penicillin G, tetracycline, ertapenem, meropenem, daptomycin, cefotaxime, and ceftriaxone. However, one isolate (P61 R) was resistant to levofloxacin, azithromycin and daptomycin, while susceptibility to moxifloxacin and clindamycin was observed. Meanwhile, isolate (P88 H) was resistant to azithromycin,

clindamycin, and intermediate to levofloxacin, and was found to be susceptible to moxifloxacin. Isolate (P119 R) was susceptible to azithromycin and levofloxacin, while resistance to moxifloxacin, clindamycin and daptomycin was observed.

The results of the 5 distinct isolates demonstrated that these isolates were very diverse, for example isolate 231 H showed susceptibility to trimethroprin/sulphamethoxazole, moxifloxacin, levofloxacin, linezolid, and tigecycline. Two (2) isolates were resistant penicillin G, clindamycin, tetracycline, ertapenem, meropenem, daptomycin, azithromycin, cefotaxime, and ceftriaxone.

Isolate 234 H was susceptible to chloramphenicol, levofloxacin, linezolid, and tigecycline and resistant to penicillin G, clindamycin, tetracycline, ertapenem, meropenem, daptomycin, azithromycin, cefotaxime, trimethroprin/sulphamethoxazole, ceftriaxone and vancomycin.

Isolate P85 H was susceptible to chloramphenicol, moxifloxacin, linezolid, tigecycline, trimethroprin/sulphamethoxazole, and vancomycin and resistant to penicillin G, clindamycin, erythromycin, tetracycline, ertapenem, meropenem, daptomycin, azithromycin, cefotaxime, ceftriaxone. Intermediate susceptibility to levofloxacin was also detected. This isolate was from a woman who had a previous history of PTD.

Sample number 175 R was susceptible to moxifloxacin, linezolid, tigecycline, trimethroprin/sulphamethoxazole and vancomycin and resistant to penicillin G, clindamycin, erythromycin, chloramphenicol, tetracycline, ertapenem, meropenem, daptomycin, azithromycin, cefotaxime, and ceftriaxone.

The isolate 75 R was susceptible to penicillin G, clindamycin, erythromycin, chloramphenicol, tetracycline, meropenem, azithromycin, cefotaxime, and ceftriaxone, tigecycline, trimethroprin/sulphamethoxazole, linezolid and vancomycin and was only resistant to ertapenem, daptomycin, erythromycin, cefotaxime and ceftriaxone.

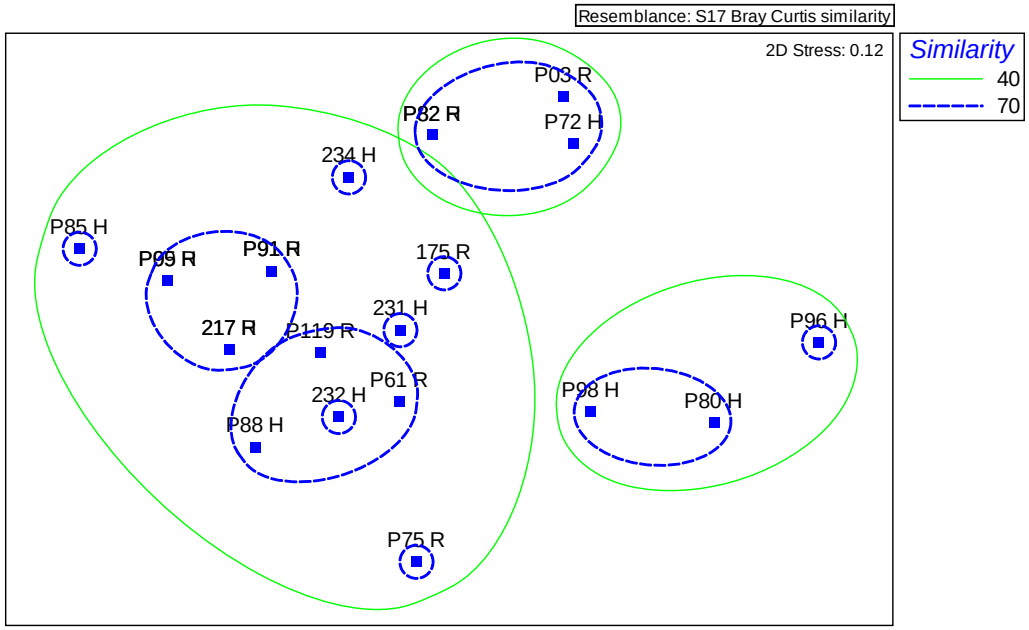

Fig 24: MDS analysis showing the clustering of the *E. faecalis* isolates diversity profiles based on a 40% and 70% (middle small circles) similarity. The distance between the points shows the relative differences between the isolates diversity profiles. An identical symbol shows the isolates that cluster together at 70% identical threshold.

CHAPTER 5: COMPARISON OF GBS AND *ENTEROCOCCUS FAECALIS* ISOLATION WITH PATIENT DEMOGRAPHICS AND PROPOSED RISK FACTORS

5.1. Association with maternal demography

Because GBS and *E. faecalis* were the predominant Gram-positive cocci isolated, the following chapter will focus mainly on these 2 species. Furthermore, maternal height, weight, hygiene practices and ethnicity have frequently been associated with adverse pregnancy outcomes due to vaginal colonization of GBS and *Enterococcus.*

5.1.1. Association with marital status

No significant association (p = 1.000) was observed between GBS positive women and marital status although GBS was observed slightly more in single women than married women (Table 25). Six percent of the GBS positive women did not reveal their marital status. This applied to *E. faecalis* as well (Table 25), where *E. faecalis* was more frequently isolated from single women (66.7%) than married women (33.3%).

5.1.2. Association with ethnicity

GBS and *E. faecalis* appeared to be more prevalent in black than coloured or white women (Table 25). Thirty - three of the 50 women who were colonized with GBS did not reveal their ethnicity or race group nor did 3 women colonized by *E. faecalis*.

5.1.3. Association with age

The mean (SD) age of GBS positive women was 28.6 (±5.3) years, with an age range from 17-42 years. The study revealed a higher GBS colonization rate among the women in the age group 26-30 years (34.0%), followed by age group 17-25 years (30.0%). GBS colonization appeared to decrease with increased age (Table 25). Lower rates were reported from age groups 31-35 years (20.0%), 36-40 years (10.0%), and ≥ 41 years (4.0%). One woman did not reveal her age and

despite the inverse proportion noted between increased age and decreased colonization, this was not statistically significant (p = 0.613).

Unlike GBS, *E. faecalis* colonization was more frequently observed in women between 31-35 years (47.6%), with a smaller percentage (23.8%) in the age groups of 17-25 years and 26-30 years (Table 25).

5.1.4. Correlation with maternal height

The mean (SD) height of the GBS positive women was 158.3 cm (± 7.81), with only 30 women having recorded their height and the remaining 20 (40.0%) not responding to this question.

No significant correlation was found between maternal height and *E. faecalis* colonization (Table 25), and 2 women who were *E. faecalis* positive, did not reveal their height.

5.1.5. Correlation with maternal weight

No significant correlation could be found between maternal weight and GBS nor with *E. faecalis* colonization, although their prevalence increased in mothers weighing ≥ 61 kg (Table 25).

5.1.6. Association with personal hygiene habits

Personal hygiene habits were not significantly associated with GBS colonization, nor with *E. faecalis* colonization. Because most mothers reported hand-washing as their means of hygiene practice, the prevalence of both species was much higher in women who hand-washed (Table 25). Similar proportions were noted for both species in women who bathed, while the lowest prevalence occurred in women who showered, since this was not the most frequently used method of keeping clean.

Table 25: Association between maternal demography and GBS/*E. faecalis* colonization

Categories/ Variables	**GBS positive (n = 50) No. (%)**	***E. faecalis* positive (n = 21) No. (%)**
Marital status		
Married	21 (42.0%)	7 (33.3%)
Single	26 (52.0%)	14 (66.7%)
Missing	3 (6.0%)	0 (0%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	1.000	0.501
Ethnicity		
Black	16 (32.0%)	15 (71.4%)
Coloured	1 (2.0%)	3 (14.3%)
White	0 (0%)	0 (0%)
Missing	33 (66.0%)	3 (14.3%)
Total	50 (100.0%)	21 (100.0%)
p – value	1.000	0.256
Age groups Mean (SD): 28.6 (**±**5.3)		
17-25	15 (30.0%)	5 (23.8%)
26-30	17 (34.0%)	5 (23.8%)
31-35	10 (20.0%)	10 (47.6%)
36-40	5 (10.0%)	1 (4.8%)
≥41	2 (4.0%)	0 (0%)
Missing	1 (2.0%)	0 (0%)
Total	50 (100.0%)	21 (100.0%)
p – value	0.613	0.291
Height (cm) Mean (SD): 158.03 (**±**7.81)		
≤ 150	5 (10.0%)	3 (14.29%)
151 – 155	8 (16.0%)	4 (19.05%)
156 – 160	5 (10.0%)	7 (33.33%)
161 – 165	8 (16.0%)	4 (19.05%)
>165	4 (8.0%)	1 (4.76%)
Missing	20 (40.0%)	2 (9.52%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	0.644	0.721
Weight (kg) Mean (SD):72.8 (**±**15.41)		
≤ 50	1 (2.38%)	0 (0%)
51 – 55	3 (7.14%)	2 (10.0%)
56 – 60	4 (9.52%)	2 (10.0%)
61 – 65	9 (21.43%)	3 (15.0%)
>65	25 (59.52%)	13 (65.0%)
Total	**42 (100.0%)**	**21 (100.0%)**
p – value	0.630	1.000
Personal Hygiene		
Bath	13 (26.0%)	5 (23.8%)
Hand wash	34 (68.0%)	15 (71.4%)
Shower/douche	3 (6.0%)	1 (4.8%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	0.950	0.616

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (****p** < 0.01 and ***p** < 0.05).). P-value obtained by Chi-square test with Yates corrections and Fisher exact test

5.2. Association with socio – economic standards

All participants in this study were from lower socio–economic groups. Locations of residence, level of education and employment status are closely inter–connected, since they provide evidence of the socio – economic standards of the mothers. This has a direct bearing on income and access to medical care. A significant correlation was found between GBS colonization and location but not for *E. faecalis* (Table 26).

Table 26: Influence of socio – economic standards

Categories/ Variables	**GBS positive (n = 50) No. (%)**	***E. faecalis* positive (n = 21) No. (%)**
Location of the population (MOU)		
MMH	18 (36.0%)	4 (19.0%)
KMOU	16 (32.0%)	7 (33.3%)
MP	12 (24.0%)	6 (28.6%)
GUG	4 (8.0%)	4 (19.0%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	**0.000****	0.836
Education		
Primary school (G1-G7)	2 (4.0%)	3 (14.3%)
High school (G8-12)	48 (96.0%)	18 (85.7%)
University	0 (0%)	0 (0%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	0.499	0.808
Employment		
Yes	19 (38.0%)	6 (30.0%)
No	31 (62.0%)	14 (70.0%)
Total	**50 (100.0%)**	**20 (100.0%)**
p – value	0.925	0.480

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test with Yates corrections and Fisher exact test

Although the prevalence of both GBS and *E faecalis* was considerably increased in mothers with high school education (96.0% and 85.7% respectively) and in those who were unemployed (62.0% and 70.0% respectively), this association was not significant (Table 26).

5.3. Medical history of the GBS – positive women

5.3.1. Association with previous STD

Seven (14.0%) of the women with GBS reported a history of STD. Six (12.0%) of the women did not respond to the question. There was no significant association between reported history of previous STD (p = 1.000) and GBS colonization (Table 27).

Only 4 of the 21 women with *E. faecalis* reported a history of STD (19.0%) and 3 (14.3%) did not respond to the question.

5.3.2. Association with HIV

Fifteen (30.0%) women with GBS reported that they were HIV-positive and 4 (8.0%) refused to disclose their HIV-sero-status in this study. As with other STD history, no statistically significant association was revealed (p = 0.440) between HIV–sero-status and GBS (Table 27).

Eleven of 21 (52.4%) of the women colonized with *E. faecalis* were HIV–positive, while 9.5% of women with *E. faecalis* refused to disclose their HIV–sero-status. *E. faecalis* colonization was not significantly associated with HIV infection.

5.3.3. Association with current symptoms of genito – urinary tract infection

GBS colonization was significantly associated (p = 0.000) with the presence of a vaginal discharge or current urinary tract infection (Table 27).

Of those who haboured *E. faecalis*, 13 (61.9%) reported a vaginal discharge and 1 (4.8%) had a urinary tract infection. The association of *E. faecalis* with current symptoms of genito – urinary tract infection was not significant (p = 0.265).

Table 27: Maternal Medical History and GBS/*E. faecalis* colonization

Categories/ Variables	**GBS positive (n = 50) No. (%)**	***E. faecalis* positive (n = 21) No. (%)**
Previous STD		
Yes	7 (14.0%)	4 (19.0%)
No	37 (74.0%)	14 (66.7%)
Unknown	6 (12.0%)	3 (14.3%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	1.000	0.332
HIV status		
Positive	15 (30.0%)	11 (52.4%)
Negative	31 (62.0%)	8 (38.1%)
Unknown	4 (8.0%)	2 (9.5%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	0.440	0.061
Current Symptoms of Genito – urinary tract infection		
Vaginal discharge	19 (38.0%)	13 (61.9%)
Urinary tract infection	2 (4.0%)	1 (4.8%)
Both	1 (2.0%)	1 (4.8%)
No symptoms	28 (56.0%)	6 (28.6%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	**0.000****	0.265
Smoking		
Yes	9 (18.0%)	2 (9.5%)
No	41 (82.0%)	19 (90.5%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	0.446	0.750
Alcohol use		
Yes	4 (8.0%)	1 (4.8%)
No	46 (92.0%)	20 (95.2%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	0.798	0.705
Lifestyle illnesses		
Hypertension	4 (8.0)	3 (14.3%)
Diabetes	1 (2.0%)	0 (0%)
other: low blood pressure, asthma, anaemia, TB , previous UTI	4 (8.0%)	1 (4.8%)
No symptoms	41 (82.0%)	17 (81.0%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	0.326	0.477

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$). P-value obtained by Chi-square test with Yates corrections and Fisher exact test

5.3.4. Association with substance use

No statistically significant association was observed between alcohol consumption and smoking with GBS nor *E. faecalis* colonization (Table 27). Nine (18.0%) of the 50 pregnant women colonized with GBS reported smoking and 4 (8.0%) consumed alcohol (Table 27). Only 1 woman with *E. faecalis* reported consuming alcohol while pregnant and 2 admitted to smoking.

5.3.5. Association with diseases of lifestyle

Forty - one (82.0%) GBS positive women in this study had no medical history of hypertension, diabetes, urinary tract infection, asthma, TB nor low blood pressure. Four (8%) GBS-positive and 3 *E. faecalis*-positive women reported a medical history of hypertension. One (2.0%) GBS-positive woman was diabetic (Table 27). Of those who reported a history of low blood pressure, asthma, anaemia, TB, urinary tract infection, 4 were GBS-positive and 1 was *E. faecalis*-positive (Table 27). No statistically significant association was observed with past medical history of the women and GBS or *E. faecalis* colonization in this study (Table 27).

5.4. Reproductive histories of the women

5.4.1. Association with parity

The mean (SD) parity of the 50 GBS positive women in this study was 1.28 (±1.11), with a range from 0 - 5. A marginal significant difference was observed between GBS colonization and parity ($p = 0.046$).

The mean (SD) parity of the 21 women who were colonized with *E. faecalis* in this study was 1.42 (± 0.06), with a parity range from 0 – 3. No significant association was found between parity and *E faecalis* (Table 28).

5.4.2. Association with gravidity

Gravidity ranged between 0 – 3 for GBS-positive women as well as for *E. faecalis*-positive women (Table 28). Although the colonization rate for both species decreased as the gravidity increased, this was not statistically significant.

Table 28: Reproductive histories associated with GBS and *E. faecalis* prevalence

Categories/ Variables	**GBS positive (n = 50)** **No. (%)**	***E. faecalis* positive (n = 21)** **No. (%)**
Parity Mean (SD): 1.28 (±1.11)		
0 – 3	49 (98.0%)	21 (100.0%)
4 – 5	1 (2.0%)	0 (0%)
≥ 6	0 (0.0%)	0 (0%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	**0.046****	1.000
Gravidity Mean (SD): 2.69 (±1.2)		
0 – 3	42 (84.0%)	17 (80.95%)
4 – 5	6 (12.0%)	3 (14.28%)
≥ 6	2 (4.0%)	1 (4.76%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	0.150	0.685
Previous Infant weight **Mean (SD):** 4.62 (±1.46)		
≤ 499 g	1 (3.4%)	0 (0%)
500 - 999 g	2 (6.9%)	2 (10.5%)
1000 – 1499 g	4 (13.8%)	1 (5.3%)
1500 – 1999 g	5 (17.2%)	1 (5.3%)
2000 – 2499 g	5 (17.2%)	4 (21.1%)
2500 g +	12 (41.4%)	11 (57.9%)
Total	**29 (100.0%)**	**19 (100.0%)**
p – value	0.176	0.917
Previous pregnancy outcomes		
PTD	29 (58.0%)	14 (66.7%)
FTD	21 (42.0%)	7 (33.3%)
Total	**50 (100.0%)**	**21 (100.0%)**
p – value	0.290	0.117

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test with Yates corrections Fisher exact test and Wilcoxon – Man U test

5.4.3. Association with previous infant weight

Of the 29 women with GBS who reported on the weight of previous infant delivery, 17 (58.6%) delivered infants weighing < 2500 g, i.e. low birth weight infants. Only 8/19 (42.1%) women

with *E. faecalis* reported previous delivery of low birth weight infants (Table 28). However, neither of these associations were found to be statistically significant.

5.4.4. Association with previous preterm delivery

A history of preterm delivery is considered by many to be a risk factor for future adverse pregnancy outcomes. Of the mothers who were GBS positive, 58.0% reported a history of PTD (Table 28). In the case of *E. faecalis* 66.7% reported a history of PTD. This association was not found to be significant (Table 28).

5.5. Association of GBS and *E. faecalis*-positive AV with obstetric history

The results show that in this studied population AV was detected in 79 women. Of these, 60 (75.9%) presented without any GBS or *E. faecalis*, and 39.2% (31/79) had a history of previous PTD. AV with GBS was detected in 10 of 79 (12.7%) of the women (Table 29) and AV with *E. faecalis* was detected in 9 (11.4%) of the women. No statistically significant correlation between AV and GBS colonization nor with AV and *E. faecalis* colonization was observed with previous pregnancy outcomes (Table 29).

Table 29: Compared AV with GBS in previous pregnancy outcomes (N = 79)

Variables/ Categories	Frequency (%)	Previous pregnancy outcomes		p – value
		PTD (n = 42) No. (%)	FTD (n = 37) No. (%)	
GBS-associated AV	10 (12.7%)	5 (11.9%)	5 (13.5%)	1.000
***E. faecalis*-associated AV**	9 (11.4%)	6 (14.3%)	3 (8.1%)	0.490
GBS and *E. faecalis*-negative AV	60 (75.9%)	31 (73.8%)	29 (78.4%)	0.833
Total AV	**79 (100.0%)**	**42 (100.0%)**	**37 (100.0%)**	

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test (with Yates corrections for 2 x 2 tables) and Fisher exact test

GBS and *E faecalis* were simultaneously detected in 1 (1.3%) pregnant woman in this study population.

CHAPTER 6: CURRENT PREGNANCY OUTCOMES OF THE STUDY POPULATION

Although not present at the time of delivery, we obtained the pregnancy outcomes of mothers who participated in the study from their folders. Not all of the folders could be retrieved, so this chapter will report only on the 199 of the 301 mothers (66.1%) whose folders we were able to access. One hundred and one of the 199 mothers (51.0%) delivered PT while the remaining (49.0%) delivered FT.

Table 30: Current pregnancy outcomes

Variables/ Categories	**Frequency (%)**	**Current pregnancy outcomes**		**p – value**
		PTD (n = 101) No. (%)	**FTD (n = 98) No. (%)**	
Current Infant weight (kg) Mean (SD): 5.3 (±7.41)		**Mean (SD):** 4.96 (**±** 6.3)	**Mean (SD):** 5.65 (**±** 8.30)	0.348
≤ 499 g	0 (0%)	0 (0%)	0 (0%)	
500 - 999 g	5 (2.55%)	5 (5.0%)	0 (0%)	
1000 – 1499 g	3 (1.53%)	2 (2.0%)	1 (1.1%)	
1500 – 1999 g	8 (4.08%)	6 (5.9%)	2 (2.1%)	
2000 – 2499 g	16 (8.16%)	15 (14.9%)	1 (1.1%)	
2500 g +	164 (83.67%)	73 (72.3%)	91 (95.8%)	
Total	**196 (100.0%)**	**101 (100.0%)**	**95 (100.0%)**	
Current mode of delivery				0.753
Abortion	1 (0.5%)	0 (0%)	1 (1.0%)	
Caesarian section	46 (23.1%)	32 (31.7%)	14 (14.3%)	
Normal vaginal delivery	146 (73.4%)	**67 (66.3%)**	**79 (80.6%)**	
Born before arrival at hospital	4 (2.0%)	1 (1.0%)	**3 (3.1%)**	
Still born	2 (1.0%)	1 (1.0%)	**1 (1.0%)**	
Total	**199 (100.0%)**	**101(100.0%)**	**98 (100.0%)**	

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (****p** < 0.01 and ***p** < 0.05).). P-value obtained by Chi-square test (with Yates corrections for 2 x 2 tables) and Fisher exact test

6.1. Gestation age of infants

One hundred and one (101) infants were born preterm (i.e. <37 weeks gestation) and 98 were born full – term (i.e. >37 weeks gestation) (Table 30).

6.2. Infant birth weight

The frequency of infants born with normal birth weight (>2500 g) and low birth weight (≤ 2500 g) are listed in (Table 30). Thirty - two of the 196 recorded births were low birth weight (LBW) infants. Of these, 28 (88.0%) were delivered preterm (PT) and 4 were delivered full – term (FT). LBW infants constituted 28.0% of those born PT.

6.3. Mode of delivery

Thirty – two (31.7%) of the PTD infants were delivered by Caesarian section and 67 (66.3%) were by normal vaginal delivery (Table 30). One PTD infant and 1 FTD infant were still born. Only 14 FT infants were delivered by Caesarian section.

6.4. Association of maternal demography with current pregnancy outcomes

The questionnaire responses of the mothers were used to associate perceived risks for PTD with the current pregnancy outcomes. Maternal age, weight, height, ethnicity, personal hygiene habits and obstetric history were analyzed using Chi – square and Fisher's exact tests.

6.4.1. Association with Age

Only 99 of the 101 mothers who delivered preterm recorded their ages. There was no significant difference between those who delivered PT and those who delivered FT (Table 31).

If we separated these into the groups according to current delivery status, those among PTD group, demonstrated an age range of 19 – 42 years. Of these women in PTD group, higher frequency was revealed among the age group 26 – 30 years (38.4%), followed by 27.3% from age group 17 – 25 years, 25.3% from 31 – 35 years, and 8.1% from age group 36 – 40 years (Table 31). One woman (1.0%) in the age group of ≥ 41 years delivered PT. Two women (2.0%) did not reveal their age in PTD group.

In women from the FTD group, their ages ranged from 17 – 42 years (Table 31). The results showed that the majority of the women were from the age group 26 – 30 (35.8%), followed by 25.3% aged 31 – 35 years and 22.1% from age group 17 – 25 years. Eleven (11.6%) women were from the age group 36 – 40 years, and 5 women aged ≥ 41 (5.3%).

6.4.2. Association height

Only 150 of the 199 mothers recorded their height. Of the 64 who delivered PT, most (70.3%) measured ≥ 165 cm in height. FTD increased in mothers with >160 cm in height but this difference was not significant (Table 31).

6.4.3. Association with weight

Although 7/10 mothers (70.0%) weighing between 51 – 55 kg delivered PT, maternal weight could not be significantly associated with pregnancy outcomes in this group (Table 31). Sixty percent of mothers delivering PT weighed > 65 kg.

6.4.4. Personal hygiene

Personal hygiene practices significantly affected pregnancy outcomes (p = 0.000). Thirteen of the mothers reported cleaning themselves by showering, of whom 9 (69.2%) delivered PT. Fifty – one point one percent (69/135) of mothers who reported hand washing, delivered PT constituting 68.3% of PT deliveries (Table 31).

Table 31: Pregnancy outcomes compared with maternal personal demography

Variables/ Categories	Frequency (%)	Current pregnancy outcomes		p – value
		PTD (n = 101) No. (%)	FTD (n = 98) No. (%)	
Age groups Mean (SD): 29.3 (± 5.4)		**Mean (SD):** 28.3 (± 5.11)	**Mean (SD):** 29.8 (± 5.66)	0.390
17-25	48 (24.7%)	27 (27.3%)	21 (22.1%)	
26-30	72 (37.1%)	38 (38.4%)	34 (35.8%)	
31-35	49 (25.3%)	25 (25.3%)	24 (25.3%)	
36-40	19 (9.8%)	8 (8.1%)	11 (11.6%)	
≥41	6 (3.1%)	1 (1.0%)	5 (5.3%)	
Total	**194 (100.0%)**	**99 (100.0%)**	**95 (100.0%)**	
Missing	5 (2.5%)	2 (2.0%)	3 (3.1%)	
Height (cm) Mean (SD): 157.7 (± 8.2)		**Mean (SD):** 156.4 (± 9.58)	**Mean (SD):** 158.7 (± 6.95)	0.063
≤ 150	18 (12.0%)	9 (14.1%)	9 (10.5%)	
151 – 155	36 (24.0%)	17 (26.6%)	19 (22.1%)	
156 – 160	43 (28.7%)	19 (29.7%)	24 (27.9%)	
161 – 165	37 (24.7%)	14 (21.9%)	23 (26.7%)	
>165	16 (10.7%)	5 (7.8%)	11 (12.8%)	
Total	**150 (100.0%)**	**64 (100.0%)**	**86 (100.0%)**	
Missing	49 (24.6%)	37 (36.6%)	12 (12.2%)	
Weight (kg) Mean (SD):73.4 (± 14.89)		**Mean (SD):** 71.07 (± 13.73)	**Mean (SD):** 75.53 (± 15.63)	0.937
≤ 50	4 (2.3%)	2 (2.5%)	2 (2.2%)	
51 – 55	10 (5.8%)	7 (8.6%)	3 (3.3%)	
56 – 60	22 (12.8%)	8 (9.9%)	14 (15.4%)	
61 – 65	25 (14.5%)	15 (18.5%)	10 (11.0%)	
>65	111 (64.5%)	49 (60.5%)	62 (68.1%)	
Total	**172 (100.0%)**	**81 (100.0%)**	**91 (100.0%)**	
Missing	27 (13.6%)	20 (19.8%)	7 (7.1%)	
Personal Hygiene				
Bath	48 (24.5%)	23 (22.8%)	25 (26.3%)	**0.000****
Hand wash	135 (68.9%)	69 (68.3%)	66 (69.5%)	
Shower	13 (6.6%)	9 (8.9%)	4 (4.2%)	
Total	**196 (100.0%)**	**101 (100.0%)**	**95 (100.0%)**	
Missing	3 (1.5%)	0 (0%)	3 (3.1%)	
Ethnicity				**0.000****
Black	147 (78.2%)	70 (73.7%)	77(82.8%)	
Coloured	41 (21.8%)	25 (26.3%)	16 (17.2%)	
Total	**188 (100.0%)**	**95 (100.0%)**	**93 (100.0%)**	
Missing	11 (5.5%)	6 (5.9%)	5 (5.1%)	

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test (with Yates corrections for 2 x 2 tables) and Fisher exact test

6.4.5. Ethnicity

A significant difference between PT and FT deliveries was observed for the different ethnic groups (Table 31). Of the 95 PT deliveries, 73.7% were blacks, compared with 26.3% who were

coloured. This represented 78.2% of black mothers and 21.8% of coloured mothers whose records we were able to access (Table 31).

6.4.6. Association with socio – economic status

MMH and GUG were the 2 areas with more PTD than FTD (Table 32). It was not surprising since most complicated pregnancies are referred to MMH and patients in GUG were found to be extremely disadvantaged socio-economically, with poor education resulting in frequent unemployment.

Marital status did not significantly affect pregnancy outcomes. Nor did education and employment status. Five of 7 mothers who had university education delivered FT infants suggesting a greater awareness of antenatal care among the higher educated.

Table 32: Correlation between maternal socio – economic status and current pregnancy outcomes

Variables/ Categories	**Frequency (%)**	**Current pregnancy outcomes**		**p – value**
		PTD (n = 101) No. (%)	**FTD (n = 98) No. (%)**	
Location of the population (MOU)				0.152
MMH	15 (7.5%)	10 (9.9%)	5 (5.1%)	
KMOU	56 (28.1%)	23 (24.8%)	33 (33.7%)	
MP	94 (47.2%)	47 (46.5%)	47 (48.0%)	
GUG	34(17.1%)	21 (20.8%)	13 (13.3%)	
Total	**199 (100.0%)**	**101 (100.0%)**	**98 (100.0%)**	
Marital status				0.495
Married	91 (46.2%)	46 (45.5%)	45 (46.9%)	
Single	106 (53.8%)	55 (54.5%)	51 (53.1%)	
Total	**197 (100.0%)**	**101 (100.0%)**	**96 (100.0%)**	
Missing	2 (1.0%)	0 (0%)	2 (2.0%)	
Education				0.537
Primary school (G1-G7)	9 (4.6%)	3 (3.0%)	6 (6.19%)	
High school (G8-12)	181 (91.9%)	95 (95.0%)	86 (88.66%)	
University	7 (3.6%)	2 (2.0%)	5 (5.15%)	
Total	**197 (100.0%)**	**100 (100.0%)**	**97 (100.0%)**	
Missing	2 (1.0%)	1 (1.0%)	1 (1.02%)	
Employment				
Yes	78 (39.8%)	40 (40.0%)	38 (39.6%)	0.160
No	118 (60.2%)	60 (60.0%)	58 (60.4%)	
Total	**196 (100.0%)**	**100 (100.0%)**	**96 (100.0%)**	
Missing	3 (1.5%)	1 (1.0%)	2 (2.0%)	

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test (with Yates corrections for 2 x 2 tables) and Fisher exact test

6.5. Maternal medical history and pregnancy outcomes

Table 33: Maternal medical history relative to current pregnancy outcomes

Variables/ Categories	Frequency (%)	Current pregnancy outcomes		p – value
		PTD (n = 101) No. (%)	FTD (n = 98) No. (%)	
Previous STD				0.278
Yes	30 (15.08%)	18 (17.8%)	12 (12.2)	
No	143 (71.86%)	69 (68.3%)	74 (75.51%)	
Unknown	26 (13.1%)	14 (13.9%)	12 (12.24%)	
Total	**199 (100.0%)**	**101 (100.0%)**	**98 (100.0%)**	
HIV status				**0.037****
Positive	50 (25.1%)	26 (25.7%)	24 (24.5%)	
Negative	133 (66.8%)	68 (67.3%)	65 (66.3%)	
Unknown	16 (8.0%)	7 (7.0%)	9 (9.2%)	
Total	**199 (100.0%)**	**101 (100.0%)**	**98 (100.0%)**	
Current Symptoms of Genito – urinary tract infection				**0.001****
Vaginal discharge	128 (64.3%)	51 (50.5%)	77 (78.6%)	
Urinary tract infection	13(6.5%)	2 (2.0%)	11 (11.2%)	
Both	3 (1.5%)	2 (2.0%)	1 (1.0%)	
No symptoms	55 (27.6%)	46 (45.5%)	9 (9.2%)	
Total	**199 (100.0%)**	**101 (100.0%)**	**98 (100.0%)**	
Smoking				**0.011****
Yes	21 (10.6%)	15 (14.9%)	6 (6.1%)	
No	178 (89.4%)	86 (85.1%)	92 (93.9%)	
Total	**199 (100.0%)**	**101 (100.0%)**	**98 (100.0%)**	
Alcohol use				0.445
Yes	17 (8.5%)	7 (6.9%)	10 (10.2%)	
No	182 (91.5%)	94 (93.1%)	88 (89.8%)	
Total	**199 (100.0%)**	**101 (100.0%)**	**98 (100.0%)**	
Lifestyle illnesses				0.935
Hypertension	15 (7.5%)	11 (10.9%)	4 (4.1%)	
Diabetes	1 (0.5%)	1 (1.0%)	0 (0%)	
Other: low blood pressure, asthma, anaemia, TB , previous UTI	9 (4.5%)	7 (6.9%)	2 (2.0%)	
No symptoms	170 (85.4%)	79 (78.2%)	91 (92.9%)	
Hypertension and diabetes	2 (1.0%)	1 (1.0%)	1 (1.0%)	
Hypertension and urinary tract infection	2 (1.0%)	2 (2.0%)	0 (0%)	
Total	**199 (100.0%)**	**101 (100.0%)**	**98 (100.0%)**	

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test (with Yates corrections for 2 x 2 tables) and Fisher exact test

6.5.1. History of STD

Sixty percent of the mothers (18/30) who reported a history of STD delivered PT. This formed 17.8% of the total PTD (Table 33). There was no significant association between a history of STD and pregnancy outcomes. Fourteen of the women who delivered PTD did not respond to the question on STD history.

6.5.2. HIV status

Fifty of the 199 recorded births (25.1%) were mothers who were HIV-positive (Table 33). Of these, 52.0% delivered PT. Although the frequency of HIV-positive mothers appeared very similar when comparing PTD and FTD, a significant difference was evident ($p = 0.037$) when comparing them with HIV-negative mothers. Seven of the women who delivered PT did not reveal their HIV status.

6.5.3. Genito - urinary tract infections

One hundred and twenty – eight women (64.3%) presented with a vaginal discharge, of whom 51 (40.0%) delivered PT. Vaginal discharge and UTI constituted 50.0% and 2.0% respectively of all PTD which differed significantly from the 78.6% and 11.2% with similar symptoms who delivered FT (Table 33). Of the 3 mothers who reported both vaginal discharge and UTI, 2 delivered PT and 1 delivered FT. Fifty – five mothers reported no symptoms which may have indicated a genito - urinary tract infection. Of these, 46 (83.6%) delivered PT and 16.4% delivered FT. This may indicate the high prevalence of asymptomatic pregnancy complications which could be attributed to endogenous microflora imbalance.

6.5.4. Smoking and alcohol consumption

Twenty – one of 199 mothers (10.6%) reported smoking while pregnant. Fifteen of the 21 mothers (71.4%) delivered PT even though PTD constituted only 14.9% of the total PTD (Table 33). There was a significant association with smoking between PTD and FTD ($p = 0.011$). Among the 98 who delivered FT, 92 (93.9%) were non- smokers.

No significant association was observed for alcohol consumption and pregnancy outcomes (Table 33). Only 6.9% acknowledged that they consumed alcohol while pregnant.

6.5.5. Lifestyle illnesses

Eleven of the 15 mothers (73.3%) who reported hypertension delivered PT. When hypertension was combined with diabetes, no difference was observed, but when hypertension and urinary tract infection were reported in the same individuals (in this case 2) they were both found to deliver PT (Table 33). Other illnesses such as low blood pressure, asthma, anaemia, TB or previous UTI were reported by 9 of the 199 mothers (4.5%). Despite the above mentioned association between hypertension and PTD, no statistically significant association was found ($p = 0.935$).

6.6. Previous obstetric histories

Previous obstetric histories were compared with current pregnancy outcomes in order to identify the relevant risk factors for PTD.

No significant difference was observed between pregnancy outcomes of the mothers reporting parity of 0 – 3. This group constituted 94.5% of the total of mothers whose pregnancy outcomes we managed to access (Table 34). The same applied to gravidity where similar frequency of PTD and FTD was reported in the 0 – 3 group. No significant association was found between gravidity and current pregnancy outcomes (Table 34).

Table 34: Correlation between previous obstetric history and current pregnancy outcomes

Variables/ Categories	Frequency (%)	Current pregnancy outcomes		p – value
		PTD (n = 101) No. (%)	FTD (n = 98) No. (%)	
Parity Mean (SD): 1.47 (±1.12)		**Mean (SD):** 1.30 (**±** 1.2)	**Mean (SD):** 1.65 (**±** 1.00)	0.788
0 – 3	188 (94.5%)	96 (95.0%)	92 (93.1%)	
4 – 5	9 (4.5%)	3 (3.0%)	6 (6.1%)	
≥ 6	2 (1.0%)	2 (2.0%)	0 (0%)	
Total	**199 (100.0%)**	**101 (100.0%)**	**98 (100.0%)**	
Gravidity Mean (SD): 2.8 (±1.15)		**Mean (SD):** 2.77 (**±** 1.26)	**Mean (SD):** 2.73 (**±** 1.03)	0.079
0 – 3	163 (81.9%)	80 (79.2%)	83 (84.7%)	
4 – 5	28 (14.1)	16 (15.8%)	12 (12.2%)	
≥ 6	8 (4.0%)	5 (5.0%)	3 (3.1%)	
Total	**199 (100.0%)**	**101 (100.0%)**	**98 (100.0%)**	
Previous gestation time of delivery				**0.001****
Preterm delivery (PTD)	101 (50.75%)	22 (21.78%)	79 (80.6%)	
Full term delivery (FTD)	98 (49.25%)	5 (4.95%)	93 (94.9%)	
Total	199 (100.0%)			

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test (with Yates corrections for 2 x 2 tables) and Fisher exact test

Ninety – three mothers with a history of FTD, delivered FT in the current pregnancy. A history of PTD did not appear to influence current pregnancy outcomes, since 80.6% delivered FT. Even though the majority of the women who had a history of PTD delivered full term, they gave birth to low birth weight infants in the current pregnancy outcome (Table 30). A statistically significant association was observed between obstetric history and current pregnancy outcome ($p = 0.001$).

6.7. The effect of AV, GBS and *E. faecalis* on current pregnancy outcomes

Seventy – nine women were found to have AV as per the microscopic criteria outlined in chapter 4. Of these, 50 were GBS – positive and 21 were *E. faecalis* positive. Of the 301 mothers who originally participated in the study, we managed to obtain the pregnancy outcomes for only 199 women and these will be described in the sections which follow.

Table 32 describes the distribution of total AV, GBS and *E. faecalis* in the 199 cases, while Table 34 describes the breakdown of the AV cases in Table 32 into GBS – associated AV, *E. faecalis* – associated AV and AV not associated with either of these species (n = 52).

6.7.1. Effect of AV on current pregnancy outcomes

Fifty – two of the 199 (26.13%) women had AV. Of the mothers with AV, 31 (59.6%) delivered PT infants and 21 (40.4%) delivered FT.

No significant difference was observed in pregnancy outcomes of the 52 mothers with AV (Table 35) even after these were divided into GBS – associated, *E. faecalis* – associated and non – GBS and non – *E. faecalis* – associated AV (Table 37).

6.7.2. Effect of GBS on current pregnancy outcomes

GBS was detected in 27 of the 199 women (13.6%) whose pregnancy outcomes were recorded (Table 35). Of these 18 (66.67%) delivered PT and 9 (33.33%) delivered FT. GBS accounted for 17.8% of PTD and 9.18% of FTD.

Four of the 52 cases of AV were GBS – associated (7.7%), all of whom delivered PT (Table 37). Because of the small sample number, this association was not considered to be significant.

Table 35: Effect of AV, GBS and *E. faecalis* on current pregnancy outcomes

		Current pregnancy outcomes (n = 199)		
Variables/ Categories	**Frequency**	**PTD (n = 101) No. (%)**	**FTD (n = 98) No. (%)**	**p – value**
AV	52	31	21	
Row %		59.6%	40.4%	0.185
Column %		30.7%	21.4%	0.185
GBS	27	18	9	
Row %		66.67%	33.3%	0.116
Column %		17.8%	9.18%	0.116
E. faecalis	17	11	6	
Row %		64.7%	35.3%	0.342
Column %		10.9%	6.1%	0.342
Total	**96**	**60**	**36**	
Non - GBS, Non - *E. faecalis* - AV	103	41	62	
Row %		39.8%	60.2%	**0.002****
Column %		40.6%	63.3%	**0.002****
Total	**199**	**101**	**98**	

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test (with Yates corrections for 2 x 2 tables) and Fisher exact test

Table 36 describes the GBS serotypes associated with the current pregnancy outcomes. While not significantly so, serotype V (80%) appears to be most frequently associated with PTD followed by serotype III (10%). One mother who delivered PT had serotypes III and IX (Table 36) while a NT species was also associated with PT.

Table 36: Correlation of specific GBS serotypes with current pregnancy outcomes

Variables/ Categories	**Frequency (%)**	**Current pregnancy outcomes (n = 32)**		**p – value**
		PTD (n = 20) No. (%)	**FTD (n = 12) No. (%)**	
Specific serotypes of GBS				
III	7 (21.9%)	2 (10.0%)	5 (41.67%)	0.073
V	23 (71.9%)	16 (80.0%)	7 (58.33%)	0.361
III & IX	1 (3.13%)	1 (5.0%)	0 (0%)	1.000
NT	1 (3.13%)	1 (5.0%)	0 (0%)	1.000
Total	**32 (100.0%)**	**20 (100.0%)**	**12 (100.0%)**	

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test (with Yates corrections for 2 x 2 tables) and Fisher exact test

6.7.3. Effect of *E. faecalis* on current pregnancy outcomes

E. faecalis was found in 8.54% (17 of 199) women (Table 35). Of the 17 mothers who were colonized by *E. faecalis*, 11 (64.7%) delivered PT and 6 (35.3%) delivered FT (Table 35).

Six mothers with *E. faecalis* had AV (Table 37), but this did not have a significant effect on pregnancy outcomes.

Table 37: Breakdown of AV – associated bacteria with current pregnancy outcomes

Variables/ Categories	**Frequency (%)**	**Current pregnancy outcomes (n = 52)**		**p – value**
		PTD (n = 31) No. (%)	**FTD (n = 21) No. (%)**	
Non – GBS, Non – *E. faecalis* associated AV	42 (80.8%)	24 (77.4%)	18 (85.7%)	0.721
GBS – associated AV	4 (7.7%)	4 (12.9%)	0 (0%)	0.138
***E. faecalis* – associated AV**	6 (11.5%)	3 (9.7%)	3 (14.3%)	0.675
Total	52 (100.0%)	31 (100.0%)	21 (100.0%)	

A p-value of less than ($p < 0.05$) was considered statistically significant at 95% significance level; and $p < 0.01$ at 99% significance level.*Highly Significant for the category (**$p < 0.01$ and *$p < 0.05$).). P-value obtained by Chi-square test (with Yates corrections for 2 x 2 tables) and Fisher exact test

6.8. Antimicrobial profiles of GBS associated with pregnancy outcomes

Antibiotic sensitivity patterns were compared with pregnancy outcomes in order to establish whether the pregnancy outcomes could be related to the antimicrobials to which the GBS and E. faecalis showed resistance. The numbers reflected below, therefore only refer to the positive cultures from the 199 mothers whose pregnancy outcomes we were able to record from the folders to which we had access.

The sensititre trek system results of the antimicrobial susceptibility analysis for both GBS and *E. faecalis* are shown in Tables 38 and 39 respectively.

GBS isolates showed susceptibility to levofloxacin, linezolid, moxifloxacin, tigecycline, and trimethroprim/sulphamethoxazole (Table 38). Increasing resistance was noted for azithromycin, cefotaxime, ceftriaxone, chloramphenicol, clindamycin, daptomycin, ertapenem, erythromycin, meropenem, tetracycline, penicillin, and vancomycin.

Significant associations were observed between resistant GBS strains and PTD for azithromycin, cefotaxime, ceftriaxone, ertapenem, erythromycin, meropenem, tetracycline, penicillin, and vancomycin.

The present study had demonstrated the prevalence of resistance among the isolates to antibiotics which are commonly used for treatment of GBS infections. When comparing PTD with FTD, there was a significant association between pregnancy outcomes and GBS antibiotic resistance to the following drugs: azithromycin ($p = 0.011$), cefotaxime, ($p = 0.000$), ceftriaxone ($p = 0.000$), meropenem ($p = 0.000$), ertapenem ($p = 0.000$), erythromycin ($p = 0.011$), clindamycin, ($p = 0.000$), chloramphenicol ($p = 0.012$), daptomycin ($p = 0.001$), penicillin ($p = 0.005$), tetracycline ($p = 0.44$) and vancomycin ($p = 0.002$) (Table 38).

Table 38: Antimicrobial susceptibility analysis for GBS isolates in the current pregnancy outcomes

		Current pregnancy outcomes			
ANTIMICROBIC		Frequency (%) PTD GBS N = 20	Frequency (%) FTD GB N = 12	Frequency (%) (N=32)	P – value
AMOXICILLIN/CLAVULANIC ACID	Susceptible	-	-	-	
	Intermediate	-	-	-	
	Resistant	-	-	-	
AZITHROMYCIN					0.011
	Susceptible	6 (30.0%)	10 (83.33%)	16 (50.0%)	
	Intermediate	-	-	-	
	Resistant	14 (70.0%)	2 (16.67%)	16 (50.0%)	
CEFEPIME					
	Susceptible	-	-	-	
	Intermediate	-	-	-	
	Resistant	-	-	-	
CEFOTAXIME					0.000
	Susceptible	6 (30.0%0	12 (100.0%)	18 (56.3%)	
	Intermediate	-	-	-	
	Resistant	14 (70.0%)	-	14 (43.8%)	
CEFTRIAXONE					0.000
	Susceptible	6 (30.0%)	12(100.0%)	18(56.3%)	
	Intermediate	-	-	-	
	Resistant	14 (70.0%)	-	14 (43.8%)	
CEFUROXIME					
	Susceptible	-	-	-	
	Intermediate	-	-	-	
	Resistant	-	-	-	
CHLORAMPHENICOL					0.012
	Susceptible	11(55.0%)	12 (100.0%)	23 (71.9%)	
	Intermediate	-	-	-	
	Resistant	9 (45.0%)	-	9 (28.1%)	
CLINDAMYCIN					0.000
	Susceptible	6 (30.0%)	12 (100.0%)	18 (56.3%)	
	Intermediate	-	-	-	
	Resistant	14 (70.0%)	-	14 (43.8%)	
DAPTOMYCIN					0.001
	Susceptible	8 (40.0%)	12 (100.0%)	20 (62.5%)	
	Intermediate	-	-	-	
	Resistant	12 (60.0%)	-	12 (37.5%)	
ERTAPENEM					0.000
	Susceptible	7 (35.0%)	12 (100.0%)	19 (59.4%)	
	Intermediate	-	-	-	
	Resistant	13 (65.0%)	-	13 (40.6%)	
ERYTHROMYCIN					0.011
	Susceptible	6 (30.0%)	10 (83.33%)	16 (50.0%)	
	Intermediate	-	-	-	
	Resistant	14 (70.0%)	2 (16.67%)	16 (50.0%)	
LEVOFLOXACIN					0.433
	Susceptible	16(80.0%)	8 (66.67%)	24 (75.0%)	
	Intermediate	4(20.0%)	4 (33.33%)	8 (25.0%)	
	Resistant	0 (0%)	-	-	
LINEZOLID					
	Susceptible	20 (100.0%)	12 (100.0%)	32 (100.0%)	
	Intermediate	0 (0%)	-	-	
	Resistant	0 (0%)	-	-	
MEROPENEM					0.000
	Susceptible	7(35.0%)	12 (100.0%)	19 (59.4%)	
	Intermediate	-	-	-	
	Resistant	13 (65.0%)	-	13 (40.6%)	
MOXIFLOXACIN					
	Susceptible	19 (95.0%)	11 (91.67%)	30 (93.8%)	
	Intermediate	-	1 (8.33%)	1 (3.1%)	
	Resistant	1 (5.0%)	-	1 (3.1%)	
TETRACYCLINE					0.044
	Susceptible	0 (0%)	3 (25.0%)	3 (9.4%)	
	Intermediate	-	-	-	
	Resistant	20 (100.0%)	9 (75.0%)	29 (90.6%)	
TIGECYCLINE					
	Susceptible	20 (100.0%)	12 (100.0%)	32 (100.0%)	
	Intermediate	-	-	-	
	Resistant	-	-	-	
PENICILLIN					0.005
	Susceptible	5 (25.0%)	10 (83.33%)	15 (46.9%)	
	Intermediate	-	-	-	
	Resistant	15 (75.0%)	2 (16.67%)	17 (53.1%)	
TRIMETHROPRIM/SULPHAMETHOXAZOLE					1.000
	Susceptible	19 (95.0%)	12 (100.0%)	31 (96.9%)	
	Intermediate	-	-	-	
	Resistant	1 (5.0%)	-	1 (3.1%)	
VANCOMYCIN					0.002
	Susceptible	9 (45.0%)	12 (100.0%)	21 (65.6%)	
	Intermediate	-	-	-	
	Resistant	11(55.0%)	-	11 (34.4%)	

The breakpoints were defined by the EUCAST/ CLSI

Susceptibility to antibiotics in *E. faecalis* isolates

The antimicrobial susceptibility of the 20 *E. faecalis* isolates shows that 95.0% (19/20) isolates of *E. faecalis* were susceptible to moxifloxacin, followed by susceptible 90.0% (18/20) to tigecycline, 85.0% (17/20) to trimethroprim/sulphamethoxazole, 85.0% (17/20) to linezolid and 75.0% (15/20) to chloramphenicol and vancomycin in this study. Sixty percent (12/20) of the isolates were susceptible to levofloxacin. Two isolates 10.0% (2/20) expressed susceptibility to clindamycin, 15.0% to daptomycin, and 10.0% (2/20) to erythromycin (Table 39).

Intermediate sensitivity to antibiotics in *E. faecalis* in the current pregnancy outcomes

For *E. faecalis*, intermediate sensitivity to levofloxacin was found in 10.0% (2/20 isolates), to linezolid in 2 isolates (10.0%), to erythromycin and azithromycin in 5.02% (1/20 isolates) (Table 39).

All 20 *E. faecalis* isolates (100%) were resistant to ertapenem, cefotaxime, and ceftriaxone, followed by 95.0% (19/20) resistant to penicillin, meropenem, azithromycin. Seventeen out of twenty isolates 85.0% (17/20) were resistant to, erythromycin, daptomycin and tetracycline (Table 37). Resistance to azithromycin was observed in 15 (75.0%), to clindamycin in 13 (65.0%), to levofloxacin (30.0%), and to tigecycline and linezolid 5.0% respectively. Five isolates were resistant to vancomycin. Three isolates were resistant to trimethroprim/sulphamethoxazole and chloramphenicol (Table 39). A significant association was observed between chloramphenicol resistance and PTD (Table 39). PTD was also associated with *E. faecalis* resistance to ertapenem, cefotaxime, and ceftriaxone, ertapenem, meropenem, tetracycline and penicillin.

Table 39 Antimicrobial susceptibility patterns for *E faecalis isolates* (N = 20) in the current pregnancy outcomes

		Current pregnancy outcomes			
ANTIMICROBIC		Frequency (%) PTD *E. faecalis* isolates N = 13	Frequency (%) FTD *E. faecalis* isolates N = 7	Frequency (%) (N=20)	P –value
AMOXICILLIN/CLAVULANIC ACID	Susceptible	-	-	-	
	Intermediate	-	-	-	
	Resistant	-	-	-	
AZITHROMYCIN	Susceptible	4 (30.8%)	-	4 (20.0%)	
	Intermediate	1	0 (0%)	1 (5.0%)	
	Resistant	8 (7.7%)	7(100.0%)	15 (75.0%)	
CEFEPIME	Susceptible	-	-	-	
	Intermediate	-	-	-	
	Resistant	13 (100.0%)	7(100.0%)	20 (100.0%)	
CEFOTAXIME	Susceptible	-	-	-	
	Intermediate	-	-	-	
	Resistant	13 (100.0%)	7 (100.0%)	20 (100.0%)	
CEFTRIAXONE	Susceptible	-	-	-	
	Intermediate	-	-	-	
	Resistant	13 (100.0%)	7(100.0%)	20 (100.0%)	
CEFUROXIME	Susceptible	-	-	-	
	Intermediate	-	-	-	
	Resistant	-	-	-	
CHLORAMPHENICOL	Susceptible	13 (100.0%)	4 (57.1%)	17 (85.0%)	**0.031**
	Intermediate	-	-	-	
	Resistant	-	3 (42.9%)	3 (15.0%)	
CLINDAMYCIN	Susceptible	2 (15.4%)	0 (0%)	2 (10.0%)	
	Intermediate	4 (30.8%)	1(14.3)	5 (25.0%)	
	Resistant	7 (53.8%)	6 (85.7%)	13 (65.0%)	
DAPTOMYCIN	Susceptible	3 (23.1%)	0 (0%)	3 (15.0%)	0.521
	Intermediate	-	-	-	
	Resistant	10 (76.9%)	7 (100.0%)	17 (85.0%)	
ERTAPENEM	Susceptible	-	-	-	
	Intermediate	-	-	-	
	Resistant	13 (100.0%)	7 (100.0%)	20 (100.0%)	
ERYTHROMYCIN	Susceptible	2 (15.4%)	0 (0%)	2 (10.0%)	
	Intermediate	-	1 (14.3%)	1 (5.0%)	
	Resistant	11 (84.6%)	6 (85.7%)	17 (85.0%)	
LEVOFLOXACIN	Susceptible	6 (46.2%)	6 (85.7%)	12 (60.0%)	
	Intermediate	2 (15.4%)	0 (0%)	2 (10.0%)	
	Resistant	5 (38.5%)	1 (14.3%)	6 (30.0%)	
LINEZOLID	Susceptible	10 (76.9%)	7 (100.0%)	17 (85.0%)	1.000
	Intermediate	2 (15.4%)	0 (0%)	2 (10.0%)	
	Resistant	1 (7.7%)	0 (0%)	1 (5.0%)	
MEROPENEM	Susceptible	1(7.7%)	-	1 (5.0%)	
	Intermediate	-	-	-	
	Resistant	12 (92.3%)	7 (100.0%)	19 (95.0%)	
MOXIFLOXACIN	Susceptible	11 (84.6%)	6 (85.7%)	17 (85.0%)	1.000
	Intermediate	-	-	-	
	Resistant	2 (15.4%)	1 (14.3%)	3 (15.0%)	
TETRACYCLINE	Susceptible	3 (23.1%)	0 (0%)	3 (15.0%)	0.521
	Intermediate	-	-	-	
	Resistant	10 (76.9%)	7 (100.0%)	17 (85.0%)	
TIGECYCLINE	Susceptible	12 (92.3%)	6 (85.7%)	18 (90.0%)	
	Intermediate	1 (7.7%)	-	1 (5.0%)	
	Resistant	0 (0%)	1(14.3%)	1(5.0%)	
PENICILLIN	Susceptible	1 (7.7%)	-	1 (5.0%)	1.000
	Intermediate	-	-	-	
	Resistant	12 (92.3%)	7 (100.0%)	19 (95.0%)	
TRIMETHOPRIM/SULPHAMETHOXAZOLE	Susceptible	12 (92.3%)	5 (71.4%)	17 (85.0%)	0.270
	Intermediate	-	-	-	
	Resistant	1 (7.7%)	2 (28.6%)	3 (15.0%)	
VANCOMYCIN	Susceptible	10 (76.9%)	5 (71.4%)	15 (75.0%)	1.000
	Intermediate	-	-	-	
	Resistant	3 (23.1%)	2 (28.6%)	5 (25.0%)	

The breakpoints were defined by the EUCAST/ CLSI

6.9. Comparison of serotypes, antibiotic profiles and PFGE profiles with pregnancy outcomes

Summarized results of the antimicrobial pulsotypes using *SmaI* restriction enzymes for all GBS are shown in (Tables 40). Profiles were indistinguishable if they showed no difference in bands and had the same band size. One to 2 band differences were considered to be closely related in this study. Profiles within four band differences were considered to be possibly related. Profiles that has ≥ 4 or more band differences were considered to be genetically unrelated.

Of the 199 folders we managed to access, only 28 of the 57 GBS isolates were represented with 24 distinct PFGE profiles (Table 40). Serotypes Ia, II, and IV will not be discussed here because we could not access the folders of the mothers from whom they were isolated in order to record their pregnancy outcomes. Serotype V had 18 PFGE patterns of which only 4 were associated with PTD. Serotype III had 4 patterns which were all FTD, along with the one isolate of serotype IX which was also associated with FTD. One NT strain was associated with PTD and was distinctly different from the other PFGE patterns (Table 40) and antimicrobial profile (AP) groups. Six strains of serotype V, 4 of serotype III, I of serotype IV and 1 NT strain showed the same antibiotic profile (Table 40).

Twelve different antimicrobial profiles were observed, of which antimicrobial profile c predominated (Table 40), followed by profiles e and f (3 isolates each, 10.7%). Antimicrobial profiles a and i were represented (2 isolates each, 7.1%) with only one isolate (3.6%) from antimicrobial profiles b, d, j_1, h_2, l and m (Table 40).

Seven of the PFGE profiles designated C belonged to serotype V, the most common serotype, 2 of which belonged to antimicrobial profiles e and f each, and the remainder belonged to antimicrobial profiles i, h_2, and l respectively (Table 40).

Table 40: Distribution of GBS serotypes in PTD and FTD

Number of isolates in the current pregnancy outcomes						
Isolate	Serotype	Antibiotic Profiles (AP)	PFGE patterns	PTD	FTD	TOTAL
P 92 H	III	a	B2		1	1
P 92 R	V	a	B2		-	
P 93 R	IX	b	B1		1	1
P 04 H	III	c_1	D5		1	1
P 84 R	V	c_1	D9	1	-	1
114 H	III	c_1	E3		1	1
114 R	III	c_1	E3		1	1
139 H	V	c_1	F7		1	1
139 R	V	c_1	F7		-	
118 R	III	c_1	G1		1	1
193 H	V	c_1	F4		1	1
193 R	IV	c_1	F3		-	
P 123 H	V	c_1	D2		1	1
167 H	V	c_1	F6		1	1
P 147 H	NT	c_2	A2	1	-	1
P 130 H	V	d	A1		1	1
P 132 H	V	e	C4		1	1
P 127 H	V	e	C6		1	1
P 107 R	V	e	D3	1	-	1
P 12 R	V	f	D8		1	1
P 151 R	V	f	C2		1	1
P 144 R	V	f	C3		1	1
P 115 R	V	j_1	B4	1	-	1
P 129 R	V	i	C8	1	-	1
P 125 R	V	i	D8		1	1
P 53 R	V	h_2	C7		1	1
P 145 R	V	l	C5		1	1
P 94 H	V	m	D7		1	1

Only 19 of the outcomes recorded were *E. faecalis*-positive. For *E. faecalis* 16 distinct profiles was observed. *E. faecalis* similar profiles revealed similar resistance patterns. Of the 4 PTD, all had different PFGE and antimicrobial patterns (Table 41).

Nine diverse antimicrobial profiles were observed in 19 *E. faecalis* isolates of the mothers whose records we accessed. Of the19 *E. faecalis* isolates, 6 were from antimicrobial profile p (Table 41), 3 from antimicrobial profiles t and 0, and 2 each from antimicrobial profiles a and s respectively (Table 41).

PFGE profiles patterns J11 and J12 showed similar antibiotic profiles with all the other PFGE showing heterogeneity (Table 41).

Table 41: Distribution of *E. faecalis* isolates in the PTD and FTD

Number of isolates in the current pregnancy outcomes					
Isolate	**Antibiotic Profiles (AP)**	**PFGE patterns**	**PTD**	**FTD**	**TOTAL**
P 96 H	n_1	H1	-	1	1
P 98 H	p	H2	-	1	1
P 80 H	v	H3	-	1	1
P 82 H	t_1	I1	-	1	1
P 82 R	t_3	I2	-	-	
P 03 H	u	I3	1	-	1
P 72 H	o_2	I4	-	1	1
232 H	s_2	J1	-	1	1
175 R	s_1	J4	1	-	1
231 H	p	J5	-	1	1
234 H	t_2	J6	-	1	1
P88 H	q	J7	1	-	1
P61 H	o_1	J8	1	-	1
P119 R	o_3	J9	-	1	1
P 95 R	r	J10	-	1	1
217 H	p	J11	-	1	1
217 R	p	J11	-	-	
P 91 H	p	J12	-	1	1
P 91 R	p	J12	-	-	

A statistical significant association was observed between chloramphenicol (p = 0.031) and *E. faecalis* in current pregnancy outcome (Table 41).

6.10. Multivariate analysis of confounding risk factors associated with current PTD

There are various predisposing factors which have been previously associated with an increased risk of GBS colonization, which in turn causes the increased risk for GBS infections in neonates. The prevalence of GBS and *E. faecalis*, in AV was used to determine the confounding risk factors for PTD by comparing maternal personal demography of the women (such as location of the population (MOU), marital status, education, employment, ethnicity, maternal age, height, weight, and personal hygiene), reproductive histories (for example parity, gravidity, previous infant weight, and previous delivery history) and medical history of the women (for instance previous STD, HIV status, current symptoms of genito - urinary tract infection including AV, smoking, alcohol consumption and lifestyle illness).

The multivariate analysis method that was used is the binary logistic regression because the dependent variables “delivery status” is a categorical qualitative with two categories (Preterm

and Full term). The independent variables included in the model explained 37.4% (Cox and Snell R square) of the total variance of the model. The results in Table 42 show that gravidity (p = 0.003), parity (p = 0.000), current symptoms such as vaginal discharge (p = 0.000) and urinary tract infection (p = 0.000), other vaginal species (p = 0.000), and smoking (p = 0.021), are significantly associated PTD. When gravidity increases 1 unit (pregnancy) the likelihood of PTD decreases 2.624 (OR = [95% CI 1.380 – 4.988]). Similarly when parity increases 1 unit (child) women are 0.292 (OR = [95% CI 0.147 – 0.583]) times less at risk of PTD. All the other risk factors were found not to be insignificant associated with current PTD.

No statistical significant association was observed between alcohol consumption (p = 0.052), GBS (p = 0.061), GBS serotypes (p = 0.063) and current PTD (Table 42).

Table 42: Logistic regression analysis used to assess the influence of obstetric factors associated with current PTD

Variables	B	S.E.	OR	95% C.I. for OR	P-value
Race					
Black	-0.231	0.600	0.794	0.245 - 2.572	0.700
Coloured	0.718	0.851	2.050	0.387 - 10.872	0.399
HIV status	-0.198	0.463	0.820	0.331 - 2.033	0.669
Current Symptoms of the genitor – urinary infection					
Urinary tract infection	-4.049	0.965	0.017	0.003 - 0.116	**0.000****
Vaginal discharge	-2.547	0.541	0.078	0.027 - 0.226	**0.000****
Both (discharge and urinary tract infection)	-1.789	1.894	0.167	0.004 - 6.846	0.345
Alcohol consumption	-1.773	0.911	0.170	0.029 - 1.012	0.052
Smoking	2.002	0.868	7.403	1.352 - 40.535	**0. 021****
Parity	-1.230	0.352	0.292	0.147 - 0.583	**0. 000****
Gravidity	0.965	0.328	2.624	1.380 - 4.988	**0. 003****
Personal Hygiene	0.250	0.395	1.284	0.592 - 2.782	0.527
Previous PTD	0.491	0.672	1.634	0.438 - 6.097	0.465
GBS	-0.807	0.965	0.164	0.025 - 1.089	0.061
AV	-0.042	0.416	0.959	0.424 - 2.166	0.919
E. faecalis	0.781	0.706	2.184	0.547 - 8.715	0.268
Other vaginal species	1.768	0.419	5.858	2.575 - 13.326	**0. 000****
Serotypes	2.066	1.112	7.892	0.892 - 69.837	0.063
Constant	-0.025	1.006	0.975		0.980
Cox and Snell R Square		37.4%			
Chi-square (Hosmer and Lemeshow Test sig.)	93.281				**0. 000**

A p-value of less than (p < 0.05) was considered statistically significant. SE standard error, OR odd ratio, CI confidence interval, AV = Aerobic vaginitis, GBS = Group B Streptococci, *E. faecalis* = *Enterococcus faecalis*, PTD = Preterm Delivery

CHAPTER 7: DISCUSSION AND SUMMARY

The World Health Organization (WHO) defines preterm birth (PTB) as birth < 37 weeks gestation and low birth weight (LBW) as birth weight < 2500 g, the latter being determined by both duration of gestation as well as foetal growth rate (Kramer 1987).

The literature is filled with studies associating maternal infection with adverse pregnancy outcomes. Less frequently studied are maternal endogenous infections and the role they play in pregnancy outcomes.

This study investigated the prevalence of aerobic vaginitis (AV) in a cohort of pregnant women in the Western Cape, South Africa and focused particularly on the role of GBS and *Enterococcus faecalis* on the pregnancy outcomes of the study group. PTD is the leading cause of peri – natal mortality and long – term morbidity (Antsaklis *et al*., 2013), with up to 50% of PTD occuring in pregnancies with no known risk factors.

FACTORS ASSOCIATED WITH THE RISK FOR PTD

A number of studies have investigated the demographic factors and past medical history of pregnant women who have given birth to early - onset - disease infected neonates (EOD), age, 0-6 days) (Mellin 2011; Javanmanesh *et al.,* 2012). Risk factors include the maternal age (< 20 years), ethnicity, the number of previous pregnancies (multiparous), multiple partners, a neonate previously diagnosed with EOD, and also socio - economic status (Namavar Jahromi *et al*., 2008; Kim *et al.*, 2011; Chawanpaiboon 2011; Zhang *et al*., 2012). It is believed that women from low socio - economic background, women with diabetes, or women colonized with sexually transmitted infection (STI) or lower genital tract *Candida* species, are more likely to be at high risk of delivering preterm (Chawanpaiboon 2011; Mellin 2011; Zhang *et al*., 2012).

7.1. Patient demography

In the present study, demographic factors were examined by way of a questionnaire handed to mothers when they were recruited for the study and their responses correlated with the microbial analyses and pregnancy outcomes which follow.

7.1.1. Age

Mothers of very young or advanced age are at risk for adverse pregnancy outcomes (Maghaddam *et al.,* 2010; Rocchetti *et al.*, 2011). While some say that an association with age only becomes significant when the mother is older than 40 (Hoffman *et al*., 2007; Nybo Andersen *et al*., 2000), others maintain that ≥ 36 years poses an equal risk (Cnattingius *et al*., 1992; Delbaere *et al*., 2007).

In the present study, the majority of mothers (37%) were aged between 26 – 30 years, followed by 28% in the 17 – 25 year age group, of whom very few were aged < 20 years, ruling out the risk of adolescent pregnancies which have been associated with increased maternal and infant morbidity and mortality (Shrim *et al.*, 2011). A study comparing < 15 year – old mothers with > 20 year – olds found that mothers ≤ 15 years had a higher risk for both maternal and early neonatal death compared with their older counterparts (Conde – Agudelo *et al*., 2006; Offiah *et al.,* 2012), with a likelihood of more PTLBW deliveries (Jolly *et al*., 2000; Gilbert *et al*., 2004; Chen *et al*., 2007).

On the other hand, only 25 of the 199 recorded pregnancy outcomes (12.5%) were of mothers > 36 years of age, of whom 9 (36%) delivered PT and 16 (64%) delivered FT. Childbirth at advanced maternal age (i.e. > 35 years) has increased remarkably over the past 3 decades with the literature reporting it as 1/12 in 2006, compared with 1/100 in the 1970s (Mathews *et al*., 2002; Martin *et al*., 2009).

Reasons for this increase can be attributed to women becoming more career oriented and independent, thus delaying marriage and motherhood. The association of advanced age with PTD has also been attributed to associated hormonal factors (Offiah *et al*., 2012). With the majority of mothers in the present study coming from disadvantaged backgrounds, delayed pregnancy was

not a factor for consideration, particularly since they were unlikely to be pursuing a career which might necessitate such a delay.

Advanced maternal age has been reported with adverse pregnancy outcomes such as miscarriage, small for gestational age infants but not with still births and preterm delivery (Goldergberg *et al*., 2008; Offiah *et al*., 2012; Khalil *et al*., 2013), while in contrast, Kenny *et al* (2013) found a significant correlation between advanced maternal age and still birth, and extremely large for gestational age infants. Studies which looked at the relationship between advancing maternal age and preterm delivery, reported preterm births in 8% of mothers more than 35 years of age compared to less than 4% of births amongst women younger than 35 years (Offiah *et al.*, 2012). However there is not enough evidence to establish if advancing maternal age is an independent risk factor for preterm birth (Carolan *et al.*, 2010; T'sang – T'ang *et al*., 2010). Furthermore, even though advances in medical care have favoured healthy mothers of advanced age, the need for an awareness of possible risk should not be ignored (Seda *et al*., 2013).

Maternal age has also been significantly associated with caesarian section (Kenny *et al*., 2013; Khalil *et al*., 2013). Caesarian section in this study appeared to be largely due to concerns for the welfare of the infant rather than because of advanced maternal age. Age was therefore not found to be an independent risk factor for adverse pregnancy outcomes in this study.

7.1.2. Maternal height and weight

The literature suggests that mothers of shorter stature are more likely to deliver preterm than taller mothers with a better developed physical statute (Lao *et al*., 2000; Chan *et al*., 2009; Mykelstad *et al*., 2013; Vogel *et al.*, 2014). Increased height, on the other hand was associated with decreased PTB in a study by Dickey *et al*., (2012). It appears that shorter mothers have a smaller pelvis which directly influences pregnancy length. This is particularly true in Asian mothers and in malnourished underweight mothers of black ethnicity (Lao *et al* 2000; Patel *et al*., 2004).

Underweight and obesity are also recognized risk factors for adverse pregnancy outcomes such as preterm birth and LBW (Zhang *et al*., 2012; van den Broek *et al*., 2014), with underweight mothers showing a 3 – fold increase in risk for PTD (van den Broek *et al*., 2014). Obese mothers weighing > 65 kg are likely to carry heavier infants which may not be carried to term because of their weight (Dickey *et al*., 2012), thereby necessitating medically induced PTD (Parker *et al*., 2014). Likewise, underweight mothers have an increased risk of PTD of LBW infants (Han *et al*., 2011; Fujiwara *et al*., 2014).

Height did not appear to significantly affect pregnancy outcomes nor did colonization of AV – associated bacteria in this study although more mothers between 156 and 165 cm in height delivered FT infants. On the other hand, mothers weighing less than 55 kg appeared to deliver more PT than FT. However this association was not statistically significant, nor was a significant association found between weight and colonization of AV - associated GBS and *E. faecalis*.

7.1.3. Socio – economic status of the mothers

Ethnicity, location, education, employment, marital status and access to medical care are all important socio – economic indicators which have independently been implicated as risk factors for adverse pregnancy outcomes (Chawanpiaboon *et al*., 2011; Offiah *et al*., 2012).

Black ethnicity has been associated with PTD (Aveyard *et al*., 2002; Goldenberg *et al*., 2008; Subramaniam *et al*., 2011). However, the approach to ethnicity is often problematic in that there is often no standardized classification of ethnicity with some researchers basing their classification on skin colour (Sedaghatian *et al*., 2000) while others base it on area of origin or self – reporting. The way in which women assign themselves to a particular ethnic group may also bias the outcomes of a study. Mothers classifying themselves as coloured in this study, showed a greater risk for PTD then black mothers. This association was found to be statistically significant. The difference in proportions between black and coloured mothers (78.2% vs 21.8%) may have influenced this association significantly. Their ethnicity would also govern their areas in which they lived, which in turn would affect their access to prenatal care. With reduced access

to medical care reported in this study, social disadvantage rather than biological factors may explain the risk for PTD.

Access to prenatal care has been regarded as a way to reduce prematurity (Lams *et al*., 2008). This seems to originate from the high rate of preterm birth reported in women who receive no prenatal care than from the content of care (Lams *et al*., 2008). A neonate born from a mother of African origin was found to have a three times greater chance of developing late - onset disease (LOD, age 7 to 90 days) compared to infants of mothers from other ethnic groups (Patel *et al*., 2004).

Minority groups are often socio – economically deprived which may compound the issue since it includes multiple factors which collectively or independently may contribute to the risk of PTD of LBW infants. Other factors such as the small structure associated with mothers of Asian origin highlight differences in anatomical differences associated ethnicity (Patel *et al*., 2004).

All of the subjects in this study came from lower socio – economic areas. This would impact directly on their general health and well–being, particularly those from the black community. Adequate sanitation and availability of water sources are an important factor to consider. For many of the women, the location of their water source required long walking distances which could prevent weight gain during pregnancy while also putting a strain on them when carrying these water supplies.

Their socio – economic status would also be reflected in their education, employment and knowledge of risk factors for adverse pregnancy outcomes. Coming from low socio – economic backgrounds, many in this study did not receive any tertiary education and reported only having attended high school (91.9%). This directly influenced their employment status with > 60% reporting that they were unemployed. Added to this was the fact that the majority were unmarried (53.8%). Educational level could also be linked to personal hygiene habits.

With water facilities not being conveniently situated, their consumption of water for personal hygiene practices would also be affected. Cleanliness is an important basic concept for maternal

and neonatal health and adequate hygiene practices cannot be over – emphasized. Mothers in this study were asked to list their general personal hygiene practices. The majority reported that they washed by hand (68.9%) while others reported bathing (24.5%) and a few (6.6%) reported that they shower. A significant difference in pregnancy outcomes was noted with these different practices, ($p = 0.000$) with showering reflecting the lowest association with PTB.

7.1.4. Emotional well – being of the mother

Previous studies found that maternal exposure to severe life events contributed significantly to the risk of preterm birth (Dole *et al*., 2003; Kramer *et al*., 2009; Khashan *et al,* 2009; Zhang *et al*., 2012). These exposures could be defined as death or serious illness of close relatives prior to conception or in the first or second trimester of pregnancy (Khashan *et al,* 2009), increasing the preterm birth rate by 16%, while if the severe life event involved an older child, the risk of preterm and very preterm birth could be increased by 23% and 59% respectively (Khashan *et al,* 2009). This could also be exacerbated by the use of anti – depressants (Huybrechts *et al*., 2014).

In the present study, around 37% of the women claimed that they had experienced difficult situations in the past year but they did not elaborate on what the situations were. Domestic violence was only reported by 7.0% with 5% indicating that their partners were not supportive of having a baby. One can only assume that the difficult situations described included their living conditions and general low socio – economic status.

Besides maternal psychological and social stress, physically strenuous work and lack of social support may be related to PTD (Zhang *et al*., 2012). With the increasing number of mothers who carry on to work late into their pregnancy, occupational stress is hypothesized to be an important contributor to adverse reproductive outcomes, both for mother and baby (Mutambudzi *et al*., 2011). Physical stresses such as shift work, long hours of standing and heavy lifting have consistently been associated with increased risk of preterm birth (Mutambudzi *et al*., 2011). Theoretically, having and being able to maintain a job, is in itself an indicator of a higher socioeconomic status, which has an inverse effect on preterm birth rates (Offiah *et al.*, 2012). Recent studies have found no increase in the rate of preterm birth and employment, but these are

mainly studies carried out in developed countries, where physical strain and hazardous working conditions are not the norm (Offiah *et al.,* 2012).

With the majority of women in the present study being unemployed (60%), work – related stress was not found to be a risk factor.

7.1.5. Life – style habits

Habits such as smoking, alcohol and illicit drug use, poor eating habits, sexual and physical activity, are of enormous significance as modifiable risk factors which, if eliminated may lead to an effective reduction in the preterm birth rate (Aliyu *et al.,* 2009; Offiah *et al.,* 2012).

Cocaine use has been significantly associated with PTB, LBW, SGA and is associated with smoking (Gouin *et al*., 2011). Cocaine use can also lead to hypertension in the mother and foetus which may result in haemorrhages during gestation (Addis *et al*., 2001).

Cigarette smoking is considered to play a very crucial role in foetal growth restriction (Behrman *et al*., 2007; Murphy 2007; Lams, *et al*., 2008) and has been strongly associated with adverse pregnancy outcomes (Conter *et al*., 1995; D´onofrio *et al*., 2003; Knopik *et al*., 2005; Bada *et al*., 2005: Salihu *et al*., 2005; Kyrklund – Blomberg *et al*., 2005; Murphy 2007; Behrman *et al*., 2007; Lams *et al*., 2008: Stroud *et al*., 2009; McCowan *et al*., 2009; Thiriez *et al*., 2009). Fantuzzi *et al*., (2007) reported a dose – dependent relationship, while Andres *et al*., (2013) reported no association between smoking and adverse pregnancy outcomes. Smoking has also been associated with impaired learning (Cornelius *et al*., 2001; Batstra *et al*., 2003; Mortensen *et al*., 2005; Lambe *et al*., 2006). The effects are more pronounced in women with low socio – economic background (Odendaal *et al*., 2001).

Our results revealed a significant association between smoking and PTD ($p = 0.011$), where 15/21 mothers who smoked, delivered PT. Of the 98 who delivered FT, 92 (94%) were non – smokers. Furthermore, depression is considered to be associated with the increase in smoking, therefore the relation between depression and preterm birth might be mediated by this behaviour.

Alcohol consumption during pregnancy may have detrimental effects on foetal development and subsequent neonatal survival. Women who have more than one drink a day are at an increased risk of preterm delivery. According to a previous study, the risk of preterm delivery and very preterm birth (< 32 completed weeks) among women who drink while pregnant, is believed to be around 2 – 4% compared to women who do not drink (Offiah *et al.*, 2012). However, earlier studies (Shiono *et al*., 1986) showed no association between PTD and alcohol consumption. The impact of alcohol consumption in the present study was significantly associated with PTD ($p = 0.044$). Less than 7% of mothers in this study acknowledged that they consumed alcohol during pregnancy.

7.1.6. Medical history

Pre – existing hypertension is a major risk factor for adverse pregnancy outcomes. In the present study, 15 of 199 mothers (7.5%) reported pre – existing hypertension of whom 11 (73%) delivered PT. Of the 4 mothers who reported a combination of hypertension and diabetes, or hypertension and UTI, 3 (75%) delivered PT. However, these associations were not statistically significant. Since obesity is directly related to hypertensive disorders and diabetes, their control is essential to reduce the risk of PTD (Lepercq *et al.*, 2004).

No association between PTB and HIV has been established in recent studies (van den Broek *et al*., 2014; Ndirangu *et al*., 2014). Even though HIV infection has been reported to have little effect on pregnancy outcomes in the developed countries, previous studies from sub – Sahara Africa have suggested that infants of HIV infected mothers might be at risk for adverse pregnancy outcomes such as lower birth weight, prematurity and neonatal death (Rollins *et al.*, 2007).

In this study, although the difference between HIV positive and HIV negative mothers showed a statistical difference ($p < 0.05$), HIV could not be significantly associated with pregnancy outcomes.

This study showed that 60% of mothers with a history of STD delivered PT, but this did not significantly correlate with pregnancy outcomes although gonorrhea has previously been reported to contribute to PTD (Waight *et al*., 2010).

UTI can cause uterine contractions resulting in PTD (Schieve *et al*., 1994). A significant association (p = 0.001) was observed in this study between current symptoms of genito – urinary tract infection of the women and PTD. Women who had a vaginal discharge and urinary tract infection were more likely to deliver PT baby.

7.1.7. Obstetric and gynaecological history

A history of PTD has been reported to greatly increase the risk for future PTD (Giraldo *et al*., 2012; van den Broek *et al*., 2014). However, surveys demonstrated that only 24.3% of health professionals were aware of the risk of PTD recurrence and only 4% were aware of prophylaxis to prevent PTD (Clark *et al*., 2014). In the present study, only 22 of 101 women who delivered PT had a history of previous PTD, while 93/98 who delivered FT had previous FT deliveries. A significant difference between PT and FT deliveries was observed in this study (p = 0.001).

PTD was significantly associated with parity (p = 0.000) and gravidity (p = 0.001) in this study. Other studies reported that nulliparous women are at greater risk for PTD than multiparous women (Hannoun *et al*., 2012). Particularly if aged < 18 years and > 35 years (Seoud *et al*., 2002; Kozuki *et al*., 2013). Older primiparous women were at greater risk for PTD than older multiparous (Lisonkova *et al*., 2010), and primiparous teenage mothers do not have a risk for PTD (Ekwo *et al*., 2000). Mothers with parity ≥ 6 in the present study delivered PT. No association was found between parity and GBS colonization in a study from Turkey (Aynur *et al*., 2005)

Short pregnancy intervals may also influence pregnancy outcomes because the mothers body may need time to recover from the stress of the previous pregnancy (Howard *et al*., 2013), and the depletion of essential nutrients (Scholl *et al*., 2000; Smits *et al*., 2001). The questionnaire for

the present study did not interrogate pregnancy interval so no information is available in this regard.

7.2. AV –associated bacteria and pregnancy outcomes

7.2.1 Association of AV with pregnancy outcomes

The typical vaginal microbiota of a woman during her childbearing age is characterized by a prevalence of H_2O_2 – producing *Lactobacillus* which determine and regulate the vaginal pH, and also contribute to the creation and maintenance of a balanced microbiota hostile to the attack of microbial pathogens (Shamim *et al* 2008; Donati *et al*., 2010). The vagina is a complicated environment, containing many microbiological species in variable quantities and relative proportions (Shamim *et al.,* 2008). An imbalance of the normal vaginal microbiota has been linked to many severe gynecological and obstetric complications such as pelvic inflammatory disease, increased risk of acquiring sexually transmitted diseases (STD) and preterm birth (Marconi *et al.,* 2013).

Aerobic vaginitis is a vaginal endogenous infection characterized by an imbalance of the normal vaginal microbiota and is distinguished from bacterial vaginosis by its ability to promote an inflammatory response and its high prevalence of aerobic/facultatively anaerobic microbial community as opposed to the predominantly anaerobic microflora of bacterial vaginosis (Donders *et al*., 2011).

The prevalence of mild to moderate AV in pregnancy has been reported as 8 – 10% (Rezeberga *et al*., 2008; Donders *et al*., 2009; Zodzika *et al*., 2011). The prevalence in our study was 26%., which denotes a very high prevalence among the pregnant women.

The literature reports that AV has an association with miscarriage, preterm labor and preterm delivery, due to the inflammation derived from the cervical – vaginal environment (vaginitis) and urinary tract infections (Cribby *et al.,* 2008). The cellular components of bacteria such as GBS (peptidoglycan and hemolysin) and *E. coli* (lipopolysaccharide (LPS), are known mediators that

trigger the inflammatory reaction, and are proposed to be the contributing agents that could initiate preterm labour and neonatal sepsis (Donders *et al.* 2011).

In the present study, AV was diagnosed microscopically using lactobacilli grading (Donders *et al.*, 1999; Donders 2000). Of the 199 pregnancy outcomes which we were able to access, 52 (26%) were AV-positive. Thirty – one of the 52 (60%) delivered PT, constituting 30% of the total (n = 101).

Twenty – seven of the 199 mothers (13.6%) were GBS-positive of whom 18 (66%) delivered PT. *E. faecalis* was isolated from 17/52 (32%) mothers with AV of whom 11 (64%) delivered PT. This represented 18% and 11% of the total PTD for GBS and *E. faecalis* respectively.

The finding of enteropathogenens and *S. aureus* has previously been reported to be predictive for preterm birth (McDonald *et al*., 1991). In the present study, GBS and *E. faecalis* were predominating isolates and thus further investigation of these two species ensured.

7.2.2. Association of GBS with pregnancy outcomes

Of the bacteria implicated in the aetiology of AV, the presence of GBS in the maternal tract has been found to be the major determinant of both infection in the neonate and colonization of the newborn (Baltimore 2007). Approximately 10% to 40% of pregnant women are colonized with GBS, either in the vagina or rectum, and about 40% to 70% pass on the microorganisms to their newborns unknowingly (Votava *et al.*, 2001; Konikkara *et al.*, 2013). For this reason, this study will focus mainly on GBS and *E. faecalis* and their impact on pregnancy outcomes.

The presence of Group B *Streptococcus* (GBS) in pregnant women is regarded to be one of the leading causes of neonatal mortality and morbidity in the world, and is reported to occur in 5 – 35% of pregnant women (Giraldo *et al*., 2012). The present study was done in order to determine the prevalence of AV and the colonization of AV-associated bacteria in pregnant women in the Western Cape, South Africa. In this studied population, (n = 301) the overall GBS prevalence among the pregnant women was shown to be high (16.6%). These findings are in accordance

with reports from other developing or industrialized countries, such as Germany (16.5%, Dzowela 2005); Argentina (3.2%, Toresani *et al.*, 2001); Turkey (8.0%, Bararos *et al.*, 2005); India (2.3%, Sharmila *et al*., 2011); and Mozambique (1.8%, De Steenwinkel *et al.*, 2008), but lower than those reported in Ethiopia (20.9%, Mohammed *et al*., 2012); Tanzania (23%, Joachin *et al*., 2009); Zimbabwe (31.6%, Moyo *et al.*, 2002); the Netherlands (17.2%, Valkenburg – van den Berg *et al.*, 2006); Iran (22.76%, Javanmanesh *et al.*, 2013); Brazil (24%, Soares *et al.*, 2013) and Switzerland (21%, Rausch *et al*., 2009). Variations between different counties could be due to multiple factors including sampling and detection techniques, as well as maternal socio – economic and other personal demography (Fatemi *et al*., 2010). However, identification of risk factors are important for the prevention of maternal and neonatal infectious (Mohammed *et al.*, 2012).

Knowledge of the prevalence of GBS in women would lead to a greater understanding of EOD and LOD potential. Regardless of the fact that incidence rates of GBS disease and GBS carrier rates are known in numerous industrialized countries, this is often not the case in developing countries, although some information is available (Moyo *et al*., 2002). With prevalence rate varying between 12% and 25% in studies reported from South Africa (Schrang *et al.*, 2004; Dagnew *et al.*, 2012), little is known about the prevalence of GBS – related adverse pregnancy outcomes in the Western Cape, South Africa, making the present study a much needed and important one.

GBS isolation and characterization

Routine methods for the identification of GBS involves culture on blood agar supplemended with sheep blood, producing β haemolysis, CAMP testing and biotyping using agglutin reaction (Verani *et al.*, 2010).

Association of maternal risk factors for adverse pregnancy outcomes and GBS colonization revealed that ethnicity, medical history and personal hygiene habit did not appear to influence GBS colonization in this study. Contrary to the findings of Joachim *et al*., (2009) who reported a

higher rate of GBS in women with no education, the highest rate of GBS colonization in this study was found in women with a high school qualification (96%).

GBS colonization was also increased in women who were unemployed (62%) compared to those who were employed and more frequently isolated from women in the 26 – 30 year age group. Increased GBS colonization was reported from women aged > 20 years (Joachim *et al*., 2009; Dechen *et al*., 2010) while Kim *et al.,* (2011); Mahboobeh *et al*., (2010); Maghaddam *et al.,* (2010); and Rocchetti, *et al.*, (2011) reported higher rates of colonization in younger women (< 20 years).

The highest prevalence of GBS was found to be among women who reported a vaginal discharge (38.0%) in this study, significantly supporting the theory that GBS should be considered a vaginal pathogen (El-Kersh *et al.,* 2002; Donders *et al*., 2005; Nahaei *et al*., 2007).

In the present study, no association was observed between smoking, alcohol consumption and GBS colonization. Smoking was considered to be one of risk factors associated with GBS colonization. But this was not in agreement with a study by (Nasri *et al*., 2013). Terry *et al*., (1999) which showed no significant difference in GBS in colonization between smokers and non-smokers.

Other studies have associated the intake of alcohol during pregnancy with adverse pregnancy outcomes, including foetal growth restriction (Aliyu *et al*., 2009).

In our studied population, parity was significantly associated with GBS colonization, unlike another study involving Turkish pregnant women, where parity was observed to be unrelated to GBS colonization (Kucukercan *et al.,* 2005).

Colonization rates were reported to be significantly greater among multi gravidity women than primi gravidity women (Orrett 2003; Shamila *et al*., 2011) but our study showed that GBS

colonization rate was lower 8/50 (16.0%) in women who had delivered four or more times and higher in primigravid women.

In the current study, stressful life situations of the women also appeared to influence GBS colonization. Life changing events such as divorce, illness, injury, and passing of a family member cause stress which may threaten the maternal well being (Zhang *et al*., 2012).

It has been suggested that the gastro-intestinal tract may be the primary site of colonization by group B streptococci (Stronati *et al*., 2004; Moghaddam 2010; Khan *et al.,* 2011) and that vaginal colonization may occur through contamination from the rectum (Moghaddam, 2010). Swabbing of multiple sites, especially the lower vagina and anorectal region, increases the isolation rate (Aynur *et al*., 2005; Simoes *et al*., 2007).

In the present study, several isolates were obtained simultaneously from both the vaginal and rectal specimens, and some were isolated only from either rectal (23/57) specimens or vaginal specimens. In similar study, swabbing both the vagina and rectum was found to increase the yield substantially compared with sampling either the vagina or the rectum alone (Sharmila *et al*., 2011).

The fact that GBS was sometimes isolated from only one site and not the other shows how imperative it is to sample both anatomical sites (vagina and rectum) when screening for GBS carriage in pregnant women. The exclusion of rectal samples in our study would have resulted in false negative results in 40% of cases. Higher GBS detection rates in the rectum than the vagina have been previously reported (Moghaddam 2010).

A limitations of culture includes the possibility of mixed culture resulting in the overgrowth of competitive species and the labour intensity.

Molecular diagnostic techniques such as PCR have the advantage of rapidly identifying bacteria, which are difficult to grow and identifying newly emerging strains of bacteria (Bergeron *et al*., 2004; Lavigne *et al*., 2012). The benefit of rapid reporting of bacteria in pregnant women can

significantly impact their pregnancy outcomes, and improve mortality rate (Lavigne *et al*., 2012) and allowing for a simpler and more efficient prevention programme, which is speedy, sensitive, and precise test (Ke *et al.,* 2000; Manning et al., 2005; Riedlinger *et al*., 2010; Lavigne *et al*., 2012).

PCR offers a good alternative to culture in the absence of susceptibility testing (Vervi *et al*.2000) with 97% sensitivity and 100% specificity reported (Bergeron *et al*., 2000).

In the present study, the sensitivity and specificity of the PCR assay were 100% respectively when compared with culture. The overall performance of the PCR assay shows acceptable negative and positive predictable values at 100% also.

The use of Lancefield grouping as a confirmatory test for streptococci is well favoured with both sensitivity and specificity close to 100% (Platt *et al*., 1995; Guerrero *et al*., 2004).

Lancefield grouping identified both GBS and *E. faecalis,* in this study once Lancefield groups were established, the isolates were subjected to Serotyping in order to identify the predominant serotypes in this population.

Capsular serotype has been one of the mainstays in the explanatory epidemiology of the GBS (Jones *et al*., 2003). The polysaccharide capsule, which is the source for serotyping, is the most important known GBS virulence factor (Borchardt *et al.,* 2004; Manning *et al.,* 2005). Ten distinct capsular serotypes have been described in the classification of GBS (Ia, Ib, and II to IX) according to the immunologic reactivity of the polysaccharide capsule (Sellin *et al.,* 2000; Jones *et al.,* 2003; Lin *et al.,* 2006; Ramaswamy, *et al.,* 2006; Boswihi *et al*., 2012). Distributions of these serotypes differ by geographic location and study population (Borchardt *et al*., 2004, Boswihi *et al*., 2012). Although classified into ten distinct serotypes, 4 to 7% do not react with hyperimmune sera to the characterized capsular polysaccharides (CPS) and are referred to as nontypeable (Diedrick *et al.,* 2010; Afshar *et al*., 2011). Nontypeability might be explained if the

isolate is a nonencapsulated variant and if the isolate produces an uncharacterized polysaccharide for which antibodies are not yet available (a new serotype). Nontypeability can also be described if the isolate has an insertion or a mutation in genes that are essential for capsule expression (Afshar *et al*., 2011). Human error must also be considered.

Six different serotypes of GBS were identified in this study group with serotype V predominating, followed by serotype III. Three isolates were non-typeable. Serotypes Ib, VI – VIII were not present in our study despite being reported from other countries. Serotyoes Ia, II and IX were reported in lower numbers in our study, although Ia has been reported predominate in Finland and Sweden (Kalliola *et al*., 1999; Berg *et al.,* 2000) and serotype II was reported to predominate in Asia (Turner *et al*., 2012). Serotype VI and VIII are the predominant serotypes reported in Japan (Lachenauer *et al*., 1999) and are rarely found elsewhere.

Serotype III GBS strains are considered to be accountable for the majority of infections, in neonates worldwide (Jones *et al*., 2003; Manning *et al*., 2005; Melin 2011; Kiely *et al.*, 2011). With invasive strains reported in Nairobi (Huber *et al*., 2011) and Soweto, South Africa (Madzivhandila *et al.,* 2011).

Eight women tested positive for GBS in both vaginal and rectal sites in this study and 5 out of the 8 women had identical isolates in both sites. The remaining 3 women were colonized with two different serotypes namely, serotypes III/IX, serotypes IV/V and serotype s III/V.

7.2.3. Association of *Enterococcus* with pregnancy outcomes

Enterococcus has been associated with PTD (Miedema *et al.*, 2000; Hufnagel *et al*., 2007) and can be life threatening to the neonate (Maki 1988) with associated increased maternal morbidity and mortality (Gribby *et al.*, 2008).

Enterococcus was found to be the predominant aerobe along with *E. coli* in AV (Jahic *et al.*, 2013). Made up of > 40 species the most common enterococcal pathogens are *E faecalis* (80-90% of enterococcal infections), followed by *E. faecium* (5-10%) (Murray 1990; Hidron *et al*.,

2008). The cytolysin of *E faecalis* allows it to colonize different body sites, thereby contributing to its role in intrauterine infection and adverse pregnancy outcomes (van Tyre *et al*., 2013).

Having emerged as a multi-drug resistant organism (Huycke *et al* 1998; van Tyne *et al* 2013; Multrick *et al* 2003; McBride et al., 2007), significant associations have been reported between multi – drug resistant enterococci and gestational age as well as birth weight (Hufnagel *et al.,* 2007). Newborns have been reported to be colonized with more enterococci from the first day of life. This could be problematic if the infant developed subsequent infection. Prevention strategies targeting adverse pregnancy outcomes are an important area of study if these adverse outcomes are to be reduced or eliminated.

In the present study, *E. faecalis* was more frequently isolated from single than married women and more prevalent in black than coloured or white women. *E. faecalis* colonization was also more frequently observed in order women.

No significant correlation was found between *E. faecalis*-associated AV and pregnancy outcomes.

7.3. <u>Prevention and Treatment of AV – associated adverse pregnancy outcomes</u>

Bacterial infection can be attributed to half of all placentas delivered before the end of the second trimester (Onderdonk *et al.,* 2008). Most infections of the amniotic cavity are polymicrobial in nature (Steel *et al* 2005) with the vagina being the potential source of infection. Vaginal colonizers such as GBS and enterococci are transmitted from mother to infant intrapartum resulting in Early-onset sepsis (EOS) in the neonate within 6 hours of birth. Most cases of EOS are caused by GBS, *Enterococcus* and *E. coli*.

Screening of pregnant women for BV/AV revealed that AV was more frequently detected than BV (Tomusiala *et al.,* 2013). The failure of metronidazole treatment to prevent PTD in patterns with BV (Klebanoff *et al*., 2001; Odendaal *et al*., 2002) resulted in the use of broad spectrum antibiotics (Lamont *et al.,* 2003; Ugwumadu *et al.,* 2003; Larsson *et al*., 2006) to reduce PTB by

eliminating the overgrowth of organisms such as GBS and *Enterococcus* which have been implicated in AV, thought to occur as a result of improper treatment for BV (Donders *et al*., 2002; Tansarli *et al*., 2013). The ability of GBS and *E. faecalis* to induce the production of toxic shock syndrome -1 (TSST - 1) suggested that women colonized by AV-associated bacteria would benefit from prophylactic treatment (McPhee *et al.*, 2013) to prevent pregnancy complications (Hay *et al.*, 1994; Carey *et al*., 2005; Rezeberga et al., 2008; Donders *et al*., 2009).

Strategies to prevent GBS infection can be obtained by the elimination of exposure to GBS or the enhancement of the resistance of the host to the organism. Chemopromophylaxis and vaccines are the possibilities available to prevent GBS.

Neonatal GBS infection is often prevented by identifying and treating pregnant women who carry GBS or who are at highest risk of transmitting the bacteria to newborns (Keenan 1998; Artz *et al*., 2003). One recommended approach for preventing the transmission of GBS from mothers to neonates is to screen pregnant women by culture of combined vaginal and rectal regions at 35 to 37 weeks of gestation and to treat empirically those with positive cultures or risk factors for disease transmission (Schrang *et al*., 2002; Brimil *et al*., 2006; Rabiee *et al.*, 2006).

Intrapartum chemoprophylaxis has greatly reduced the risk of neonatal infection in women who carry GBS (Uy *et al.*, 2002; Borchardt *et al.*, 2004; Verani *et al.*, 2010), but despite these measures, GBS remains a leading cause of infectious neonatal morbidity in the world (Atkins *et al*., 2006).

The Centers for Disease Control recommend that only mothers with history of previous PTD should be treated with antibiotics if found to have vaginosis or vaginitis, since previous PTD is a powerful indicator of risk for PTD (Romero 2004).

In the present study, the Kirby – Bauer method of disc diffusion antimicrobial susceptibility was used. Zones of inhibition were used to classify isolates into susceptible, intermediate or resistant to a series of antibiotics and their MIC (minimum inhibitory concentrations) determined by using the Titretrel system.

Appropriate methods for susceptibility testing are very important because of reported resistance in strains that appear susceptible in broth susceptible tests. In this study the Kirby – Bauer disc diffusion method and TREK minimum inhibitory concentrations (MIC) methods were compared for agreement and where discordance was indicated, MIC was considered as the most reliable outcomes. PFGE was used to genotype sensitive and resistant strains.

Antibiotic resistance patterns of 57 GBS strains were tested for their susceptibility to eight different types of antibiotics that have been recommended for eradication of carriage and treatment of invasive GBS diseases**.**

Penicillin G remains the antibiotic of choice for the intrapartum prophylaxis for GBS (CDC 2002) with erythromycin and clindamycin used as alternatives for those allergic to penicillin (CDC 2002; Simores *et al*., 2004; Sharmila *et al.,* 2011). GBS has been frequently been reported to be susceptible to ampicillin (AMP) cefazolin, erythromycin, imipenem, norfloxacin penicillin vancomycin (Soares *et al.,* 2013).

GBS positive cultures isolated from colonized pregnant women demonstrated that few were resistant to penicillin (5%), erythromycin (2%) and clindamycin (2%). Although strains resistant to penicillin have been described in previous studies, they remain rare (Almedura *et al.,* 2011; Nakamura *et al*. 2011).

Resistance to clindamycin and erythromycin is on the increase and along with amoxicillin, have not been successful in reducing PTD (McGregory *et al*., 1990; Andrews *et al*., 2003; Goldenberg *et al*., 2006).

The frequency of clindamycin and erythromycin resistance was very low in our study compared to those reported in other studies (Movales *et al*., 1999; Andrews *et al*., 2000; Castello-Filho *et al.,* 2010; de Almida *et al.,* 2011). With respect to resistance to clindamycin, resistance

frequencies of 4% to 19% have been reported (Simores *et al*., 2007) compared to the 2% from this study. This resistance has been attributed to specific serotypes.

All the GBS strains isolated from the study population in the present study were resistant to fusidic acid (FC), while others were resistant to trimethroprim/sulphamethoxazole (98%), tetracycline (98%), and gentamicin (93%). Resistance to tetracycline, clindamycin, and trimethroprim/sulphamethoxazole has also been reported in asymptomatic GBS carriers (Soares *et al.,* 2013).

Resistance of GBS to tetracycline in our study population were similar to the results observed in other studies (Aynur *et al.,* 2005; Duarte *et al*., 2005; Soares *et al.,* 2013).

Sulfamethoxazole, has been found to be associated with high risks of adverse pregnancy outcomes, such as birth defects, placental abruption, foetal growth restriction, and foetal death. It has also been reported that maternal exposure to this folic acid antagonist is harmful (Yang *et al*., 2011). Resistance to sulphamethoxazole has also been reported in Brazil (Soares *et al*., 2013) and Crotia (Boris *et al*., 2003). In our study population, susceptibility to sulphamethoxazole was only found in three women, two of whom were HIV positive.

GBS is a common cause of UTI recommended therapy being trimethroprim/sulphamethoxazole (Sweith *et al.,* 2005). Our results stress the importance of epidemiological updates, particularly for those with penicillin allergies.

A significant association was established between pregnancy outcomes and GBS antibiotic resistance patterns for cefotaxime ($p = 0.000$), ceftriaxone ($p = 0.000$), meropenem ($p = 0.000$), ertapenem ($p = 0.000$), erythromycin ($p = 0.011$), clindamycin, ($p = 0.000$), chloramphenicol ($p = 0.012$), daptomycin ($p = 0.001$), penicillin ($p = 0.005$), tetracycline ($p = 0.44$), vancomycin ($p = 0.002$) and azithromycin ($p = 0.011$).

It has been suggested that GBS resistance to antimicrobials is related to specific serotypes (Motlava *et al*., 2004). Different serotypes predominate in different population groups with associated antimicrobial resistances. Serotypes III, V, II, Ia and Ib are prevalent among pregnant women and neonates with EOD in USA and in Europe (; Kvam *et al.*, 1995; Harrison *et al.*, 1998). Serotypes VI and VII are rarely found (Harrison *et al.*, 1998).

Serotype III and V are reported to be the most resistant (Oliviera *et al*., 2005; Soares *et al.*, 2013) particularly to erythromycin and clindamycin (Fernandez *et al*., 1998; De Azavedo *et al.*, 2001). In the present study, GBS serotype V was the most resistant with 26/38 showing resistance to penicillin, 24/38 resistant to erythromycin and azithromycin, 23/38 resistant to clindamycin, 22/38 resistant to ertapenem, 20 resistant to cefotaxime, 19/38 resistant to daptomycin, 17/38 resistant to vancomycin, 13/38 resistant chloramphenicol and few (3/38) resistant to trimethroprim/sulphamethoxazole. Both serotypes V and III were resistant to tetracycline, while serotype III demonstrated susceptibility to most of the other antimicrobials testes, as did Ia, II, and IX. How these antimicrobial profiles relate to the genetic heterogeneity of the GBS strains among and within serotypes, will be discussed later in this chapter in the section on PFGE.

E. faecalis

Of the 199 mothers whose pregnancy outcomes were available, 20 were colonized by *E. faecalis*. Of these, all showed susceptibility to tigecycline, 19/20 were susceptible to moxifloxacin, 17 to thrimethoprim/sulphamethoxazole, 17/20 to linezolid, 15/20 to chloramphenicol and vancomycin, 12/20 to levofloxacin. Only 2/20 were sensitive to clindamycin and erythromycin, demonstrating the increased resistance of *E. faecalis* to these antimicrobials.

Total resistance was observed to ertapenem, cefotaxime, ceftriaxone, penicillin, meropenem, azithromycin. Of the 20 E. faecalis strains, 17 were resistant to tetracycline.

PTD was associated with resistance to cephalosporins, ertapenem, meropenem, tetracycline, and penicillin in this study. Trimethroprim/sulphamethoxazole (co-trimoxazole) appears to be the

drug of choice for *E. faecalis* prophylaxis since most strains have demonstrated sensitivity, along with norfloxacin and ciprofloxacin (Peter *et al*., 1997; Delzell *et al*., 2000).

Ampicillin and cefuroxime administration was reported as safe and effective for the prevention of infections following caesarian delivery (Ziogos *et al.,* 2010). However, according to FDA, safety for use of amoxicillin, Ampicillin, augmentin, Cefuroxime, cephalexin has not been established with a recommendation that ciprofloxacin, catifloxacin, levofloxacin, and trimethroprim/sulphamethoxazole only be used if the potential benefit justifies the potential risk to the foetus.

Like GBS, *Enterococcus* has frequently been isolated from asymptomatic mothers delivering PT and much controversy exists regarding the administration of antimicrobial agents to mothers with asymptomatic infections as a means of preventing preterm birth since there is little convincing evidence for this theory (Carey *et al*., 2000; Kenyon *et al*., 2001; Larsson *et al*., 2005).

It is therefore advisable when antimicrobial agents are prescribed for prophylaxis, that not only the antimicrobial spectrum but also the presumed ecological disturbance on the anaerobic and aerobic vaginal and rectal microbiota should be taken into account (Tempera *et al.,* 2010). Because of their very low impact on the vaginal microbiota, kanamycin or quinolones are considered to be a good choice for therapy (Boswihi *et al.,* 2012).

7.4. PFGE analysis

Serotyping has for decades been of great significance in epidemiological studies of GBS disease and has more lately been supplemented by methods for molecular typing such as molecular finger printing PFGE and multi – locus enzyme genotyping (Moyo *et al*., 2002; Jones *et al*., 2003).

Molecular typing methods such as PFGE have distinct advantages over the original Serotyping methods in that it is far more reliable while also differentiating between strains of the same

serotypes (Blumberg *et al.,* 1992, Fasola *et al.,* 1993; Roland *et al.,* 1999; Le Thomas-Bories, *et al.,* 2001; Moyo *et al*., 2002; Thong *et al*., 2004).

PFGE is extensively used, is a sensitive technique for subtyping strains, and has been used to define distinct clusters of specific genotypes that have significant value for epidemiological studies (Murray 2010) despite its limitations of being expensive and technically challenging.

PFGE has turned out to be an influential tool for typing of diverse bacteria, both in relation to outbreak of infections in hospitals and also in the community (Skjaervold, *et al.,* 2004; Benson, *et al.,* 2002; de Almeida Correa *et al*., 2011; Thong *et al*., 2004). PFGE has furthermore been used for characterization of group B streptococci (Qasem *et al.,* 2009), and compared with other typing methods to observe the discriminatory power when applied to strains that were epidemiologically unrelated (Moyo *et al*., 2002) or non-typeable (Benson, *et al.,* 2002; Skjaervold *et al*., 2004).

PFGE might be used to study reinfection by antibiotic resistant strains of group B streptococcus (Benson *et al*., 2002) and compares genomic DNA fragments after being digested with a restriction enzyme (Benson *et al*., 2001) yielding numerous linear molecules of DNA (Jordan et al., 2005). Comparing clonal strains would reveal that the sites at which the restriction enzymes act on the DNA and the length flanked by these sites would be the same (Le Thomas-Bories, *et al.,* 2001). Alternatively, unlike strains would yield diversity at the sites at which the restriction enzymes act on the DNA and the length flanked by these sites (Le Thomas-Bories, *et al.,* 2001). In the present study, DNA was digested by *SmaI* restriction enzyme and submitted to PFGE for definition of clonality. Isolates of a specific serotype and displaying identical or related PFGE profiles were assigned to the same cluster.

In the present study, PFGE profiles have shown that a good number of the women studied were colonized by a single GBS strain and *E. faecalis* strain, although closely related strains may coexist in the vaginal and rectal samples collected from the pregnant women. PFGE was very useful to distinguish amongst the isolates of GBS and *E. faecalis*. The PFGE analysis for both GBS and *E. faecalis* were clustered into three major clusters with the similarity of 60%. The

majority of the isolates in *E. faecalis* were susceptible to trimethroprin/sulphamethoxazole, moxifloxacin, chloramphenicol, levofloxacin, linezolid, tigecycline and vancomycin. The *E. faecalis* isolates were resistant to penicillin G, clindamycin, tetracycline, ertapenem, meropenem, daptomycin, azithromycin, cefotaxime, and ceftriaxone was also observed amongst the majority of the *E. faecalis* isolates.

The PFGE profiles of all GBS isolates were distinguishable. The results also demonstrate the genetic diversity among the isolates from different body sites isolated from same individuals with 2 women who had two different serotypes also having different band patterns.

Different PFGE types in the same individual may be due to mutation, co-colonization with strains from different sources (i.e. different sex partners or contamination). This is important when genotypic information is used to establish proof of epidemiological linkage in disease transmission (Thong *et al*., 2004). The same serotypes belonging to different women were genetically heterogeneous. Fewer GBS strains were genetically similar with 100% similarity and clustered together. Additionally, strains belonging to different women were genetically heterogeneous even though they had the same serotypes.

7.5. CONCLUSION

AV and its association with PTD has not been extensively studied, nor has its prevention and treatment received the attention it deserves. It has been suggested that it may play a more important role than BV in maternal and neonatal morbidity.

The aim of the study was to detect the prevalence of AV and its association with preterm delivery in the Western Cape, South Africa. As well to identify and characterize the predominant facultative anaerobes implicated in AV. Furthermore, it sought to examine and investigate the predictive value of GBS and *E. faecalis* in PTD and determine the serotype distribution of GBS isolated from the women. It also aimed to establish whether maternal demography and reproductive history may predict current pregnancy outcomes. This aim was reached by establishing the risk factors which may contribute to preterm delivery. A significant association

between smoking, ethnicity, previous gestation time at delivery, personal hygiene, alcohol used, parity, gravidity and current symptoms of genito – urinary tract infection (such as urinary tract infection and vaginal discharge) and pregnancy outcome was observed in our studied population.

Furthermore, this study has demonstrated the serotype distribution in GBS isolated from pregnant women with serotype V followed by serotype III as the dominant GBS serotypes amongst the colonized women in this studied population.

Genetic and epidemiological relatedness defined strains for GBS and *E. faecalis*, into 24 GBS pulsotypes and 16 profiles for *E. faecalis* pulsotypes as follows: GBS serotype V strains had 18 patterns, serotype III had 4 patterns and serotype IX had 1 pattern in the current pregnancy outcomes. The non-typeable strains had 1 pattern. The degree of genetic heterogeneity of GBS strains among and within serotypes is an indication of endemic nature of the isolates in this study population.

Our results indicate that, the high prevalence of GBS colonization among pregnant women warrants for regular screening of this bacterium in women attending antenatal care.

A speedy and reliable detection of GBS colonization and *E. faecalis* would benefit mothers, especially those with poor prenatal care during pregnancy, and also will permit more effective prevention of GBS infections. The development of a rapid test for detection would aid in the instant identification and management of women those with unknown GBS and *E. faecalis* status at the onset of labour or rupture of membranes. Prevention of neonatal GBS morbidity and mortality is directly related to the care the mother has received during the prenatal and intrapartum period.

Limitations

The limitation of the study was we could not manage to get access to all the folders of the women who participated. Also not all the women gave birth at the specific MOUs were they

initially receive the antenatal care and some also gave birth at home and their information about their current pregnancy outcomes were not recorded.

Asymptomatic colonization present a challenge in that colonization may be largely transitory and may be intermittent or persistent, reaching a stable profile around 5 weeks prior to delivery (Motlava *et al*., 2004). We were only able to collect samples during the first trimester of pregnancy. Ideally sample collection should have been repeated at 35-37 weeks gestation (El Beitrie *et al*., 2005).

As in Brazil, South Africa has a "coloured" population of racial miscegenation, complicating and precise association with race or ethnic group.

Furthermore, we did not investigate birth interval in our study. Short interpregnancy interval has also been associated with PTD (Howard *et al*., 2013) due to insufficient time for the mother to recover from the stress of the previous pregnancy (Miller *et al.*, 1991; Winkuist *et al*., 1992; Scholl *et al*., 2000; Smits *et al*., 2001)

Previous infections are also self reported and often provided no indication if diagnosis resulted in treatment which may have impacted on pregnancy outcomes.

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APPENDIX 2 (A)

Sample number: _____
Folder number: _____ KMOU ☐ MPP ☐ GUG ☐
Residence: _____
Age: _____ **Grav**: _____ **Para**: _____
Height:_____ **Weight**: _____

PAST OBSTETRIC HISTORY

Year	Gestation/Birth weight[1]	Complications[2]	Neonatal Outcome[3]

Please code as follows:

1. **GESTATION/BIRTHWEIGHT**

500-999g	1
1000-1499g	2
1500-1999g	3
2000-2499g	4
2500g+	5
unknown	6

2. **COMPLICATIONS**

Extreme multi-organ immaturity	1
Hyaline membrane disease	2
Intraventricular haemorrhage	3
Necrotizing enterocolitis	4
Pulmonary haemorrhage	5
Hypoxic ischaemic encephalopathy	6
Meconium aspiration	7
Septicaemia	8
Pneumonia	9
Unknown	10

3. **NEONATAL OUTCOME**

Neonatal death	1
Admitted to NICU	2
Discharged	3

PAST MEDICAL HISTORY

Hypertension	1
Diabetes	2
Previous urinary tract infections	3
Other	4

If other, please specify: _____

PAST SURGICAL HISTORY

Myomectomy	1
Ectopic	2
Other uterine surgery	3
Other non-gyneacological surgery	4

If 3, please specify: _____
If 4, please specify: _____

DEMOGRAPHIC & LIFESTYLE FACTORS

MARITAL STATUS
Single ☐ Married ☐ Separated ☐ Divorced ☐

Other (Please specify) _____

HIGHEST LEVEL OF EDUCATION Please tick (√) the correct box

G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	UNIVERSITY	NONE

EMPLOYMENT YES☐ NO☐
If yes, please specify: _____

SMOKING HABITS
DO YOU SMOKE? Y ☐ N☐
HOW MANY A DAY? <10 ☐ 10-20☐ >20☐
HOW LONG HAVE YOU SMOKED? _____
DO YOU SMOKE DURING PREGNANCY? Y ☐ N ☐
DO YOU USE TIK? Y ☐ N ☐
DO YOU USE ANY OTHER SUBSTANCES? Y ☐ N ☐
IF YES, PLEASE SPECIFY_____

ALCOHOL USE
DO YOU DRINK ALCOHOL Y ☐ N ☐
IF YES, WHAT DO YOU CONSUME? _____
HOW MANY DRINKS PER WEEK DO YOU DRINK?
☐ <5 ☐ 5-10 ☐ >10

DO YOU DRINK DURING PREGNANCY? Y ☐ N ☐

REPRODUCTIVE HEALTH

HIV STATUS ☐ POS ☐ NEG ☐UNKNOWN CD4_____
HAART_____
HAVE YOU VER BEEN TREATED FOR A SEXUALLY TRANSMITTED DISEASE?
Y ☐ N☐

HOW MANY SEXUAL PARTNERS DO YOU HAVE CURRENTLY? _____
IS THIS A NEW PARTNER? Y☐ N ☐
CURRENT SYMPTOMS: ☐ VAGINAL DISCHARGE ☐URINARY SYMPTOMS

PERSONAL HYGIENE

BATH ☐ SHOWER ☐ HANDWASH ☐

OTHER _____

DOUCHING Y☐ N☐

OTHER VAGINAL PRODUCTS? _____

UNDERWEAR? NONE ☐ COTTON ☐ NYLON ☐

OTHER? _____

PERINATAL HEALTH SCREENING

1. Have you had some very difficult things happen in the last year?	**Yes**	**No**
2. Are you pleased about your pregnancy?	**Yes**	**No**
3. Is your partner supportive?		
4. Have you had problems with depression, anxiety or panic attacks before?	**Yes**	**No**
5. Is your partner or someone at home sometimes violent towards you?	**Yes**	**No**
	Yes	**No**

For total, add all shaded areas that are marked Total……

APPENDIX 2 (B)

CONSENT FORM FOR PARTICIPATION IN THE RESEARCH PROJECT

Title of project: The frequency and characteristic of Group B Streptococci (GBS) in aerobic vaginitis (AV) and its association with pregnancy outcomes in a sample of pregnant women attending Maternity Obstetric Units (MOU) in the Western Cape

Name of Researchers: Eveline Kaambo and Prof. Charlene W. J. Africa

I,…… (Full name), hereby consent to participate in the above mentioned research study. I fully understand the nature of the study, and have had all my questions answered by……. (name and rank).

I understand that all information supplied by me will be strictly confidential.

I further consent to have vaginal and rectal swabs taken from me by …… (name and rank).

These samples will be identified by a folder number only, and not by my name.

Signed this……..Day of……2009, at Cape Town

…….
Patient signature

…….
Witness

APPENDIX 2 (C)

INFORMATION SHEET

Prospective participants are requested to read this information sheet carefully and to ask questions where necessary, before signing the attached consent form. This sheet must be detached and retained by the participant and the consent form filed for record.

The vaginal microbiota is important for maintaining vaginal health and preventing infections of the reproductive tract. Women who are colonized with GBS, *E. faecalis*, and other AV-associated bacteria may causing adverse pregnancy outcomes. The aim of this study is to establish whether the prevalence of AV and its associated between bacteria can be linked to preterm delivery.

The clinical procedure will entail the collection of samples from the vagina and rectum using cotton swabs. The samples collections are safe and will be carried out with the utmost care to ensure the comfort of the patient.

Patients will be required to sign the attached form granting consent for the collection of swab samples and for the subsequent use of the samples and clinical parameters recorded. The patient will also be required to grant permission for her medical history to be disclosed. Participants will not be recorded by name, but samples and information will be coded to protect the identity of the individual. However, the coding will be used by the clinic to trace the individual if relevant information (as a result of the study) should be passed to her or her doctor. Permission will also be sought for the use of additional biological material collected in the clinic, which is usually discarded but which the researchers may find useful for future research.

Participation in this study is voluntary and refusal to participate will not prejudice the treatment of the patient in any way. Consent to participate will be recorded by completing the attached form. Should individuals agree to participate and later change their minds, they may withdraw by calling the following person: Prof C. Africa, University of the Western Cape, Department of Medical Biosciences, Tel: 021 9592342

APPENDIX 2 (D)

In this particular study, Figure 1 illustrates a schematic for the isolation and identification of Gram positive cocci in vaginal and rectal swabs cultures that was used in this study (Fig. 1). Two selective methods for detection of GBS from vaginal and rectal swabs were tested for all swabs.

For the reasons outlined in Chapter 1 the study focused on GBS for detailed characterization.

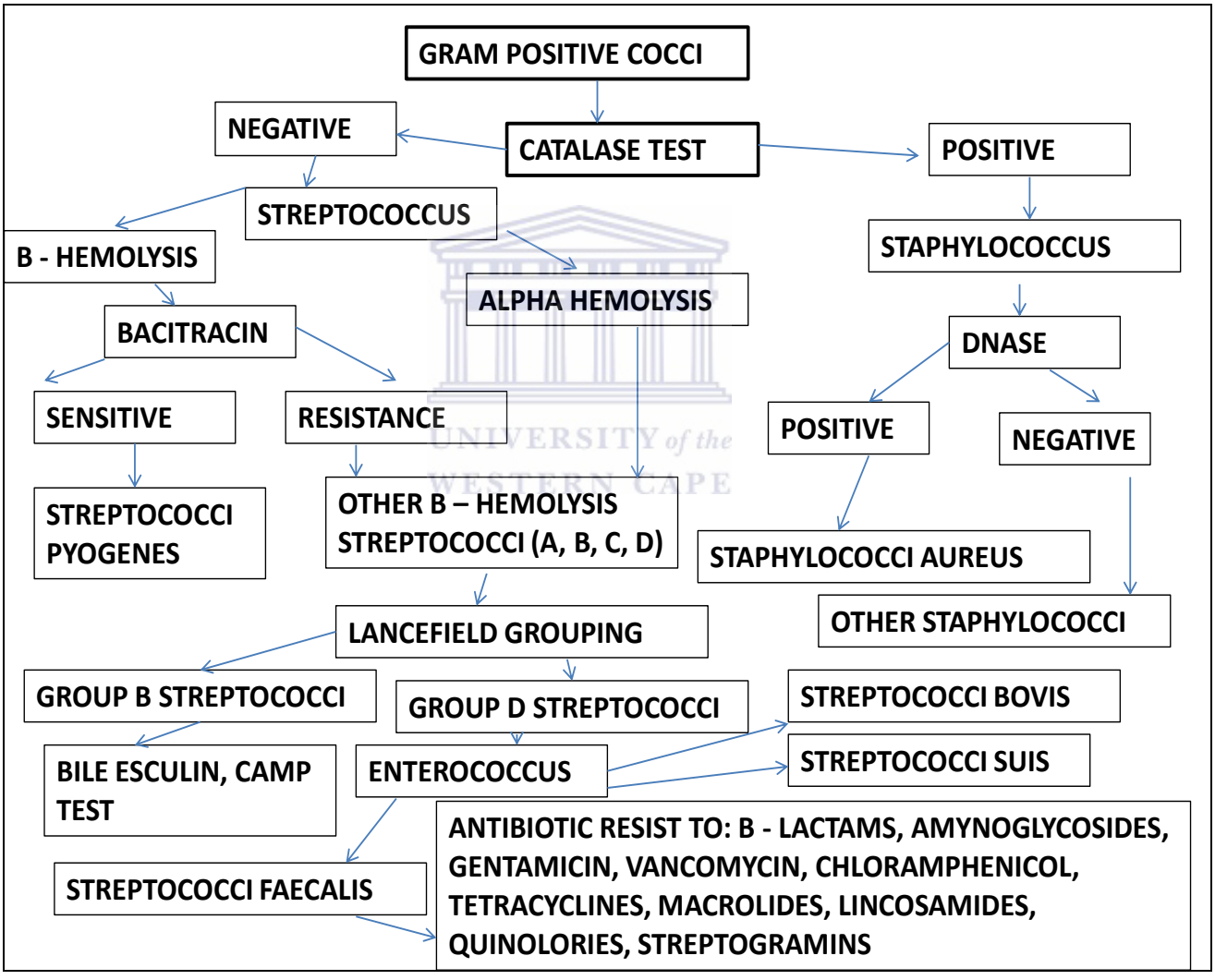

Fig. 1: Procedure used for the isolation and identification and detection of Gram positive cocci from the vaginal and rectal samples from the pregnant women.

APPENDIX 2 (E)

Table 7: EUCAST/ CLSI antibiotic susceptibilities of GBS isolates from both groups (N=57)

	STP6F MIC (µg/Ml)	Group B streptococcus		
ANTIMICROBIC	RANGE	Susceptible	Intermediate	Resistant
AMOXICILLIN/CLAVULANIC ACID	2- 16 µg/Ml	-	-	-
AZITHROMYCIN	0.25 – 2 µg/Ml	≤0.5 µg/mL	> 1 µg/mL	≥ 2 µg/mL
CEFEPIME	0. 5 – 8 µg/mL	-	-	-
CEFOTAXIME	0.12 - 4 µg/mL	≤0.5 µg/mL	-	-
CEFTRIAXONE	0.12- 2 µg/mL	≤0.5 µg/mL	-	-
CEFUROXIME	0.5 – 4 µg/mL	-	-	-
CHLORAMPHENICOL	1 – 32 µg/mL	≤ 4 µg/mL	> 8 µg/mL	≥ 16 µg/mL
CLINDAMYCIN	0.12 – 1 µg/mL	≤0.25 µg/mL	> 0.5 µg/mL	> 1 µg/mL
DAPTOMYCIN	0.06 - 2 µg/mL	≤1 µg/mL	-	>1 µg/mL
ERTAPENEM	0.5 - 4 µg/mL	≤ 1 µg/mL	-	> 1 µg/mL
ERYTHROMYCIN	0.25 – 2 µg/mL	≤0.25 µg/mL	0.25 - 0.5 µg/mL	≥ 1 µg/mL
LEVOFLOXACIN	≤0.5 – 4 µg/mL	≤ 2 µg/mL	>4 µg/mL	≥ 8 µg/mL
LINEZOLID	0.25 – 4 µg/mL	≤ 2 µg/mL	> 2 -4 µg/mL	> 4
MEROPENEM	0.25 – 2 µg/mL	≤ 0. 5 µg/mL	-	>0. 5 µg/mL
MOXIFLOXACIN	1 – 8 µg/mL	≤ 1 µg/mL	2 µg/mL	≤ 4 µg/mL
PENICILLIN	0.03 – 4 µg/mL	≤ 0. 25 µg/mL	-	> 0. 25 µg/mL
TETRACYCLINE	1 – 8 µg/mL	≤2 µg/mL	>4 µg/mL	≥ 8 µg/mL
TIGECYCLINE	0.015 – 0.12 µg/mL	≤0.25 µg/mL	> 0. 25 µg/mL	> 0. 5 µg/mL
TRIMETHOPRIM/SULPHAMETHOXAZOLE	0.5 – 4 µg/mL	≤ 1 µg/mL	> 1 - 2 µg/mL	> 2 µg/mL
VANCOMYCIN	0.5 – 4 µg/mL	≤ 2 µg/mL	-	> 2 µg/mL

The breakpoints were define by the EUCAST/ CLSI

APPENDIX 2 (F)

Buffers used in this study

10X TBE buffer
108 g Tris, 55 g boric acid and 9.3 g EDTA.
Dissolved and make up to 1 liter. Autoclave

0.5X TBE buffer
45mM Tris-borate (v/v)
1mM EDTA (w/v)
pH was adjusted to 8.3

TE buffer
10 mM Tris-HCl,
1 mM EDTA(w/v)
pH 8.0)

Lysis buffer
50 mM Tris: 50 mM EDTA, pH 8.0 + 1% Sarcosyl

Ethidium Bromide (EtBr)
1x (0.2 ml/ml glycerol and 5 mg/ml bromophenol). To dissolved shake it.

Loading buffer
0.25% (w/v) bromophenicol blue and 0.25% (w/v) xylene cyanol in 30% (v/v) glycerol in sterile distilled water.

10X TE
100mM Tris-Cl pH 7.5
10mM EDTA

Dissolve 12.11 g Tris and 3.72 g of EDTA in 700 ml distilled water. pH was adjusted to 7.5 with concentrated HCL. The volume was filled up to 1 L before autoclaving.

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