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The prevalence, risk factors and serotypes of GBS in Libyan women at labour and the rate of vertical transmission of GBS from mother to infant.

By

Lubna Mohamed Said Elmahaishi

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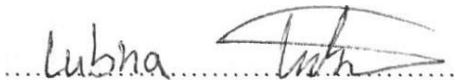
DECLARATION

I, Lubna Mohamed Said Elmahaishi, do hereby declare that this dissertation is the result of my investigation and research and that this has not been submitted in part or full for any degree to any other University.



L. Elmahaishi

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ABSTRACT

Background: Group B *Streptococcus* (GBS) also known as *Streptococcus agalactia*, is one of the most important causes of serious neonatal infections. Early detection of GBS colonisation in the mother is thus of primary importance to prevent neonatal infection.

Aim and objectives of this study were to detect the prevalence, risk factors and serotypes of GBS in Libyan women at labour and to determine the rate of vertical transmission of GBS from mother to infant with the use of real-time PCR.

Methods: We assessed 200 pregnant women at labour at Said Hospital in Misrata, Libya between July 2020 and May 20201. Two samples (vaginal samples and rectal samples) were collected from 100 mothers delivering preterm and 100 mothers delivering full term, as well as one sample from the infant at birth. The study conformed with the Declaration of Helsinki (2013) and ethics requirements of Said Hospital in Libya from which participants were recruited. Data regarding maternal demography and reproductive health history were collected through a questionnaire and GBS was detected using RT-PCR.

Results: GBS was detected in 36 (18%) of the 200 mothers, with serotype VI being the predominant serotype, followed by serotypes III, IV and V. Vertical transmission of serotypes III and V were observed in the neonates. Maternal GBS colonization was associated with tertiary education (p = 0.003), weight (p = 0.002), gravidity (p = 0.048). Neonatal GBS colonisation was associated with full term delivery (FTD) and low birth weight (LBW) (p = 0.001).

Conclusion: This study found that the prevalence of GBS in Libya was not significantly different from other Middle Eastern and African countries although the distribution of serotypes differed.

The application of RT-PCR affords a rapid and accurate detection of GBS serotypes and could inform the use of intra-partum antibiotic prophylaxis (IAP) to reduce neonatal infection.

Keywords: *Streptococcus* agalactiae, group B streptococcus, vertical transmission, preterm delivery, full-term delivery

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LIST OF ABBREVIATIONS

ANBW: Above normal birth weight.

AV: Aerobic vaginitis.

BMI: Body mass index.

ELBW: Extremely low birth weight.

CDC: Centres for Disease Control.

cm: Centimetre.

CPS: Capsular polysaccharide.

CT: Cycle threshold.

DNA: Deoxyribonucleic acid.

EOD: Early onset disease.

FTD: Full term delivery.

GBS: Group B *Streptococcus.*

IAP: Intra-partum antibiotic prophylaxis.

LBW: Low birth weight.

LOD: Late onset disease.

LP: Lancefield precipitation test.

Min: Minutes.

ML: Milliliter

NBW: Normal birth weight.

ng: Nanogram.

PCR: Polymerase chain reaction.

PTD: Preterm delivery.

PROM: Premature rupture of membranes.

RNA: Ribonucleic acid.

rpm: revolutions per minute.

TH: Todd Hewitt broth.

µl: microliter.

USA: United States of America.

UTI: Urinary tract infections.

VLBW: Very low birth weight.

WHO: World Health Organization.

WGS: Whole genome sequencing.

CHAPTER 1. INTRODUCTION

Group *B Streptococcus* (GBS), also known as *Streptococcus agalactiae*, is a Gram-positive bacterium found in 20% of the normal flora in the human gastrointestinal and genital tracts (Mcgee *et al*., 2013; Russell *et al*., 2017). GBS is a part of the genus *Streptococcus* of the family *Streptococcacea*. Microscopically, it is a Gram-positive diplococcus that divides in one plane and accordingly occurs in chains or pairs. When cultured on sheep blood agar, bacterial colonies appear as flat, grey-white mucoid colonies measuring 1-3 mm in diameter and surrounded by a narrow, clear zone (Remington *et al*., 2011).

GBS has ten serotypes based on type-specific capsular polysaccharides (CPS, Ia, Ib, II to IX) (Schrag *et al*., 2013, Edwards *et al*., 2016). The CPS is a major virulence factor involved in bacterial evasion of phagocytes (Melin and Efstratiou, 2013; Beigverdi *et al*., 2014).

GBS rarely causes disease in healthy individuals, but it is pathogenic in cases of immune-compromised, pregnant women, neonates and the elderly (Gerolymatos *et al*., 2018).
Between 10% and 35% of pregnant women have GBS colonisation (Edwards and Baker, 2016). This condition increases the risk of a vertical transmission of GBS from the mother to the infant at labour. Usually women who carry GBS do not present with any clinical symptoms, but it has been associated with adverse pregnancy outcomes in women with aerobic vaginitis (AV) (Morozumi *et al*., 2014). GBS can also cause urinary tract infections (UTI), chorioammionitis and puerperal endometritis, especially following a Caesarean delivery and wound infections (Morozumi *et al*., 2014).

GBS infection is a common cause of neonatal morbidity and mortality worldwide. Globally in 2017, around 0.49 cases per 1000 live births and a related case mortality rate of 8.4% was reported. Vertical acquisition of GBS during labour from recto-vaginally colonised mothers is the major risk factor for early onset disease (EOD) in infants (Verani *et al*., 2010). The most serious of these GBS infections is meningitis 24% and pneumonia 13% (Lamagni *et al*., 2013; Kohli-Lynch *et al*., 2017; Seale *et al*., 2017). Long - term effects of early onset GBS neonatal disease (EOGND) such

as neurodevelopmental defects as psychomotor retardation, spasticity, hemiparesis and seizures in around 8.7% to 15.8% of cases. (Eastwood *et al.*, 2015).

This is an epidemiological study, which aims to establish the prevalence of GBS colonisation in a cohort of pregnant women in Libya and examine for vertical transmission from mother to infant at birth. In addition, the associated risk factors for GBS colonisation will be determined as well as the predominant serotype.

CHAPTER 2. REVIEW OF THE LITERATURE

This chapter provides a review about epidemiology of maternal GBS colonisation and risk factors as well as explaining the neonatal infections (prevalence, prevention, serotyping). The chapter reviews literature from a theoretical and empirical perspective to unlock research and knowledge towards understanding the prevalence of GBS colonisation in pregnant women and vertical transmission from mother to infant at birth.

2.1. Epidemiology of maternal GBS colonisation

Between 10% and 35% of pregnant women are reported to have GBS colonisation, depending on differences in sample and detection methods and population differences relating to age, ethnicity, socioeconomic status and geography (Edwards and Baker, 2016). This condition increases the risk of vertical transmission of GBS from the mother to the infant at labour. Recent studies report maternal GBS prevalence to be higher in pregnant women in North Africa and the Middle East than in other parts of Africa and in regions further East (Table 2.1) with a lower prevalence in India (2%) and a very low prevalence reported in Sudan (0.5%).

All GBS serotypes have the ability to cause neonatal infections. Statistics show that in the United States of America (USA), around 95% of neonatal infections are caused by serotypes Ia, Ib, II, III and V (Woldu *et al*., 2014). Serotype III is the most common serotype in EOD (Madzivhandila *et al.,* 2011; Emaneini *et al*., 2014; Citation *et al.,* 2016), but a study done in Canada in 2014 highlighted that 57% of serotype III was isolated from both EOD and late onset disease (LOD) (Public Health Agency of Canada, 2014).

2.2. Maternal factors which may influence GBS colonisation

2.2.1. Maternal demography

2.2.1.1. Age

Differences in colonisation rates have been recorded for different age groups (Table 2.2) with some studies reporting higher GBS colonisation rates in younger women aged < 30 years (Sadaka *et al*., 2018; Girma *et al*., 2020; Goel *et al*., 2020), while others reported increased GBS colonisation in older women > 30 years (Khan *et al*., 2015; Yaseen *et al*., 2021). Reasons for these differences remains unclear; however younger women are more sexually active and since it is known that GBS colonisation can be sexually transmitted, it may explain the increase in GBS colonization with younger age (Lekala et al., 2015).

Table 2.1. Epidemiology of GBS prevalence among pregnant women in different parts of the world.

Country	GBS prevalence	Reference
Brazil	26%	**(Wollheim *et al.*, 2017)**
China	8.2%	**(Ji *et al.*, 2017)**
Egypt	26.25% 11.25%	**(Sadaka *et al.*, 2018)** **(Wassef *et al.*, 2017)**
Ethiopia	10.4%	**(Mengist *et al.*, 2016)**
Jordan	19.5%	**(Clouse *et al.*, 2019)**
India	2%	**(Khatoon *et al.*, 2016)**
Iran	11.8%	**(Darabi *et al.*, 2017)**
Israel	31.0%	**(Hakim *et al.*, 2018)**
Kuwait	20.7%	**(Ghaddar *et al.*, 2014)**
Lebanon	18.4%	**(Ghaddar *et al.*, 2014)**
Morocco	24.0%	**(Moraleda *et al.*, 2018)**
Saudi Arabia	19%	**(Musleh and Al Qahtani, 2018)**
South Africa	16.6%	**(Africa and Kaambo, 2018)**
Sudan	0.5%	**(Abdullahi *et al.*, 2017)**

Table 2.2. Age differences in maternal GBS colonisation

Country	Number of cases	GBS colonisation >30 years of age	GBS colonisation < 30 years of age	Total number with GBS colonisation	Reference
Egypt	200	3	50	53	Sadaka *et al*., 2018
Ethiopia	135	4	18	22	Girma *et al*., 2020
India	450	4	11	15	Goel *et al*., 2020
Pakistan	150	13	3	24	Yaseen *et al*., 2021
Saudi Arabia	1328	46%	29%	178	Khan *et al*., 2015

2.2.1.2. Weight

To assess the theory that maternal obesity is an independent risk factor for recto-vaginal GBS colonisation, a retrospective study in Washington, USA, revealed a prevalence of GBS colonisation in 25.8% of 10 564 patients in labour (Kleweis *et al*., 2015). This study reported a significant relationship ($p < 0.001$) between body mass index (BMI) and GBS (OR, 1.39; 95% CI, 1.25-1.55).

Najmi *et al*., (2013) studied the prevalence of GBS colonisation in 405 pregnant women at the Aga Khan University Hospital in Karachi, Pakistan and found a GBS prevalence of 17%. The colonisation was found to be inversely associated with BMI of the patient (OR, 0.91; 95% CI, 0.08-1.0).

Reasons for these apparent discrepancies may include geographical disparities in risk factor profiles and demographics as well as the different range in socioeconomic status between countries. In addition, prevention strategies for maternal GBS colonization may differ between countries (Kram *et al*., 2016).

2.2.2. Socio-economic factors

Several studies in Africa have differed in GBS prevalence and on the socio-economic factors (educational levels, urban or rural living, and access to medical care) which may influence GBS colonisation. Idih *et al*., (2019) reported a 6.1% prevalence of vaginal GBS in 180 mothers in Nigeria and found an association with tertiary education, while Mitima *et al*., (2014), in a study on 509 mothers in the third trimester of pregnancy in Congo, reported a 20% GBS prevalence which was significantly associated with primary education (OR, 2.5; 95% CI, 1.57-3.97). However, Namugongo *et al*., (2016) in a cross sectional study on 309 pregnant women in 35-37 weeks of gestation in South Western Uganda, showed no significant association between educational level and GBS colonisation.

In Zimbabwe, rural habitation was noted to be significantly related with GBS colonisation ($p < 0.001$) with a high prevalence of 60.3% in a study of 1037 pregnant woman in 20–26 weeks gestation and at delivery (Mavenyengwa *et al*., 2010). However, a study on 139 pregnant women in Hawassa, Ethiopia, reported an overall GBS prevalence rate of 20.9% with no statistically significant association observed between GBS colonisation and any of the socio-demographic factors including age, residential location and occupation (Mohammed *et al*., 2012).

2.2.3. Medical history

Aerobic vaginitis (AV) is an endogenous infection that occurs as a result of a disturbance of the normal vaginal microbiota. AV is the result of the overgrowth of aerobic opportunistic pathogens including *Escherichia coli*, GBS, *Staphylococcus aureus*, and *Enterococcus faecalis* that activate a contained vaginal inflammatory immune response as demonstrated by clinical symptoms including a vaginal discharge, an increased vaginal pH and noticeable decrease of healthy *Lactobacillus* species (Tansarli *et al*, 2013). AV has been associated with difficulties in pregnancy such as ascending chorioamnionitis, premature rupture of the membranes and preterm delivery (Najmi *et al*., 2013), thus its early diagnosis and treatment during pregnancy may decrease the risk of adverse pregnancy outcomes.

Kim *et al*., (2011) studied maternal factors associated with GBS colonisation in 2644 pregnant women between 35 to 37 weeks of gestation during 2006 – 2008 in Korea. GBS colonisation was 8.3% and a significant association was found between GBS and a maternal history of vaginitis (OR, 1.50; 95% CI, 0.98-2.29). Munir *et al*., (2016) reported on risk factors for GBS colonisation in 200 women in the third trimester of pregnancy from October 2014 to March 2015 in Lahore. GBS colonisation was 14% and significantly associated with vaginal discharge (p = 0.027) and previous history of miscarriage (p = 0.010).

A historical cohort study collected data from electronic health records of all births between 2003 and 2015 in USA (Edwards *et al*., 2019). This study compared the risk of adverse pregnancy outcomes with GBS colonisation and invasive GBS disease in 60029 mothers. Overall, GBS colonisation was 21.6% and 0.1% had invasive GBS disease. Chronic hypertension (aRR, 1.03; 95% CI, 0.96-1.09) and gestational diabetes (aRR, 1.21; 95% CI, 1.11-1.32) were associated with GBS colonisation. In addition Kwetukia, (2020) did a cross sectional study on 131 women during labour in March 2020 and July 2020 at Iringa Regional Referral Hospital. This study determined the prevalence, predictors and antimicrobial susceptibility pattern of GBS colonization among pregnant women and their newborns. GBS colonisation was 23% and a significant association was found between GBS and hypertension (AOR=11.433, 95% CI=2.721-48.038, p=0.001).

2.2.4. Gynecological history

Goel *et al*., (2020) analysed GBS colonisation and related risk factors in 450 pregnant women during 35-38 weeks of gestation in India and observed a significant association among GBS colonisation and nulliparous women (p = 0.026). On the other hand, Musleh and Al Qahtani, (2018) studied 457 pregnant woman between October 2011 and September 2016 in Saudi Arabia and reported GBS colonisation in 19% of pregnant women, with no association noted between GBS colonisation and parity.

2.2.5. Adverse pregnancy outcomes

Patil *et al*., (2013) analysed data from 905 pregnant woman in India to demonstrate that screening is essential in pregnant women (35-37 weeks gestation) and reported that GBS colonisation was significantly related with preterm birth (OR, 8.3; 95% CI, 1.11-5.5), intrapartum temperature of more than 38°C (OR, 3.1; 95% CI, 0.43-6.66), premature rupture of membranes (OR, 7.5; 95% CI, 1.11-3.4) and prolonged period (up to 10 hours) of rupture of membranes (OR, 21; 95% CI, 15.2-34.2).

A cross-sectional study on 135 woman (35-37 weeks gestation) conducted between May to August 2015 in Southwest Ethiopia (Girma *et al*., 2020), revealed recto-vaginal GBS colonisation in 16.3% of the women studied and significantly associated GBS colonisation with a history of preterm delivery (AOR, 6.3; 95% CI, 1.42-28.3) and urinary tract infection (AOR, 6.4; 95% CI, 1.95-21.1).

In Turkey, Alp *et al*., (2016) administered a questionnaire to measure risk factors for GBS carriage in 500 women and reported GBS colonisation in 13.6% of women, of whom 9.8% were pregnant. A significant association was reported between a history of PROM and GBS colonisation (p = 0.022).

2.3. Identification of risk factors for neonatal infection

Risk factors for vertical transmission include women with a temperature ≥ 38°C during labour, preterm labour, women with preterm PROM ≥ 18 h, women with a previous infant with GBS infection and women with GBS bacteriuria during the present pregnancy (National Institute for Health and Care Excellence, 2012; Al-Kadri *et al*., 2013; Kim *et al*., 2014).

GBS colonisation in the infant may ascend *in utero* by penetrating into the amniotic cavity regardless of whether the membrane has been ruptured or not (Whidbey *et al*., 2013). A study exposed a fast onset of neonatal EODat delivery or a few hours after labour, suggesting that the GBS infection procedure may have started *in utero* rather than during delivery (Madhi *et al*., 2013).

2.4. Neonatal GBS infections

2.4.1. Prevalence of GBS neonatal infections

Between 40% to 60% of children are likely to be colonised by GBS at labour and only 1-2% develop early GBS infection. The average number of neonatal GBS infections globally in the 1990s was 12.7 million (The United Nations Inter Agency Group for Child Mortality Estimation, 2014). After introduction of the prophylaxis programme where antibiotics were administered during childbirth, GBS neonatal infections dropped in 2013 (Madhi *et al*., 2013; Wang *et al*., 2014; Dongorz *et al*., 2017). Despite these efforts, GBS infections still continue as the main reason for neonatal death. There are variances in the rates of GBS infections amid countries. In 2013, it was 8 per 1000 live births in USA and 6 per 1000 live births in Europe. In Africa, the GBS infection ratio was 31 per 1000 live births (World Health Organization Global Health Observatory, 2014), while in Canada during 2000 to 2014, the GBS infection ratio was 31 per 1000 live births (Surveillance and Epidemiology Division, 2016). In Brazil, the GBS infection ratio was reported to be between 0.39 and 1.0 per 1000 live births (Evangelista and Freitas, 2015).

GBS in the neonate is categorised as early-onset disease (EOD, age 0 to 6 days) or late-onset disease (LOD, age 7 to 90 days) (Romero *et al*., 2014; Arain *et al*., 2015; Le Doare *et al*., 2016; Kwatra *et al*., 2016).

2.4.2. Early Onset Disease

Early-onset GBS neonatal disease (EOGND) may occur as a result of preterm labour and delivery (PTD), during passage of the foetus through the vaginal canal or through amitotic fluid infection after rupture of the membrane 18 or more hours before delivery (Mendz *et al*., 2013; Helmig *et al*., 2017).

GBS causes early onset GBS neonatal septicaemia (EOGNS) in approximately 80% of GBS infected neonates (Madhi *et al.,* 2013). EOGND includes pneumonia and meningitis in 13% and 24% of infected neonates respectively (Surveillance and Epidemiology Division, 2016). In addition, neonates with EOGND who survive, can suffer from neurodevelopmental defects such as psychomotor retardation, spasticity, hemiparesis and seizures in around 8.7% to 15.8% of cases. (Eastwood *et al.*, 2015).

2.4.3. Late-Onset Disease

LOD occurs in infants between 7 days to 3 months of life (Konnikkara *et al*., 2013). The pathogenesis and risk factors of LOD are uncertain, with some studies suggesting nosocomial infection or environmental contact (Burianová *et al*., 2013; Brandolini *et al*., 2014), while others identify breast milk as a source of infection (Zimmermann *et al*., 2017).

The most commonly reported LOD is meningitis (Edwards *et al*., 2011). Signs include irritability, seizures and lethargy (Porta and Rizzolo, 2015). Moreover, long-term estimation of infants who survive GBS meningitis appearances that 50% of children have deficit and 30% have severe neurological sequelae, including hearing loss or vision, mental retardation and hydrocephalus (Melin, 2011).

In contrast to EOD, LOD is unaffected by the use of intrapartum antibiotic prophylaxis (IAP) during labour (Schrag and Verani, 2013).

2.5. Prevention of neonatal infection

2.5.1. Universal routine antenatal GBS screening.

The Centres for Disease Control and Prevention (CDC) recommend GBS screening for pregnant women during 35 to 37 weeks gestation, by laboratory culture, to identify pregnant women who carry the bacteria. Treatment in this strategy includes administration of antibiotic prophylaxis during labour of all GBS positive women (Centres for Disease Control and Prevention, 2014). A disadvantage of the 35 to 37-week screening programme, is the intermittent GBS colonisation during pregnancy. Therefore, there is a poor correlation between antenatal screening results and intrapartum maternal GBS colonisation (Poncelet *et al*., 2013).

2.5.2. Prophylaxis

The use of intravenous intrapartum antibiotic prophylaxis (IAP) during labour is used to decrease early-onset GBS (EOGBS) in the neonate by 80% (Edmond *et al*., 2012). The CDC recommends a minimum duration of 4 hours between IAP administration and delivery (Centres for Disease Control and Prevention, 2014), although 1-2 hours before delivery was found to be sufficient to prevent or reduce GBS infection (Barber *et al*., 2008).

Studies in Australia recommend risk factor–based IAP strategies, rather than universal screening based on culture, to decrease EOGBS (Poncelet *et al*., 2013) while a study in the UK confirmed that instead of EOGBS incidence decreasing, it increased from 0.48 to 0.57 per 1000 live births (Edmond *et al*., 2012).

Penicillin is the antibiotic of choice for IAP. Ampicillin is an alternative for women who are allergic to penicillin and without a history of anaphylaxis. Clindamycin and erythromycin can be used in some countries. However, because of high resistance, erythromycin is no longer recommended in the USA for pregnant women who are allergic to penicillin (Verani *et al*., 2014; Sinha *et al.*, 2016).

The disadvantage of the IAP strategy is that a large proportion of women receive antibiotics when they do not need to, thus increasing the risks of antibiotic-resistant GBS related with widespread

antibiotic use in both women and infants (Banno *et al*, 2014; National Screening Committee, 2017).

2.6. Methods to detect GBS colonisation

2.6.1. Cultural studies

One of the most widely used methods for detecting GBS colonisation is to collect rectal/vaginal swabs and inoculate these onto artificial culture media that will select for the growth of GBS. Although the time for culture is approximately 2 days, the results are reliable and allow for the assessment of antimicrobial profiles when needed. Differences in demography as well as sampling and cultural methods may yield results that are not necessarily comparable with other studies.

Furfaro *et al*., (2019), collected vaginal and rectal swabs at ≤ 22weeks (n= 814) and ≥33weeks (n= 567) gestation from 814 pregnant women during 2015–2017 at King Edward Memorial Hospital, Subiaco, Western Australia. Rectal and vaginal swabs were cultured on to Strep B Carrot Broth and subsequently sub-cultured on StrepB CHROM agar. Overall GBS colonisation rate was 24%, 24.9 % at ≤ 22 weeks and 24.7 % at ≥33 week's gestation. Rectal colonisation was higher compared to vaginal colonisation at ≤22 weeks (3.7% and 2.5 % respectively) as well as at 33 weeks (3.2% and 1.6%, respectively).

A similar cross-sectional study was conducted at Jimma University Hospital in Ethiopia (Mengist *et al*., 2016) on 126 pregnant women at 35–37 weeks of gestation. Rectal and vaginal swabs were cultured onto Todd-Hewitt broth medium supplemented with Gentamicin and Nalidixic acid and subsequently sub-cultured on 5% sheep blood agar. They reported a 19.0% rate of GBS colonisation (24 mothers), with rectal GBS colonisation reported in 18 out of 126 (14.3%) cases and vaginal colonisation reported in 13/126 (10.4%) cases.

Sadaka *et al*. (2018) studied 200 pregnant women at 35–37 weeks of gestation at Al-Shatby University Hospital in Egypt. Using CHROMagarTM StrepB and sheep blood agar plates, GBS

was isolated from 53 (26.5%) GBS carriers. Vaginal colonisation was 100%, while only four women (7.5%) had rectal colonisation.

More sensitive and faster methods for detecting GBS colonisation are needed, especially during labour, to reduce postpartum complications and severe infections in the neonate, thereby avoiding the unnecessary use of antibiotic prophylaxis in women who are not colonised (Kugelman *et al*., 2021.

Molecular biology is fast replacing culture as the method of choice for epidemiological and surveillance studies and although more expensive, it affords early detection and diagnosis. The disadvantages of the culture method include limited sensitivity and a characterisation time of 36 – 72 hours (Daniels *et al*., 2011).

2.6.2. Real-time PCR

Previous studies have confirmed the importance of PCR testing in diagnosing GBS at birth to reduce mortality and morbidity for the foetus (Clarke *et al*., 2016; Koppes *et al*., 2017). The PCR tests have the ability to recognise GBS colonisation in pregnant women at delivery within 1-2 hours with high specificity (84.6% to 100%) and 62.5% to 100% sensitivity (El Helali *et al*., 2012; Di Renzo *et al*., 2015).

Real-time PCR amplifies a specific DNA target and then detects and monitors the production of amplicons in real-time using a fluorescent-labelled hybridisation probe. The advantage of using Real-time PCR is that the level of GBS colonisation can be quantitated as the degree of fluorescence can be compared to calibrated known standards.

A literature review on studies in France, Brazil and Demark found that Real-time PCR had better test accuracy than antenatal culture when intrapartum culture was used as the reference standard. In France, a study of 157 pregnant woman (at the Grenoble University Hospital Centre), reported a sensitivity of real-time PCR of 94.4% (95% CI, 72.7– 99.9%) and 50% (95% CI, 26–74%) with

antepartum culture (Defez *et al*., 2016). In Brazil, they studied 204 pregnant woman and reported a sensitivity of real-time PCR of 100% and a specificity of 95.6% (Wollheim *et al*., 2017). The biggest of the three studies, on 902 women in Denmark, reported a sensitivity of real-time PCR of 83%, specificity of 97%, PPV of 78% and NPV of 98% (Khalil *et al*., 2017).

2.6.3. GBS Serotyping

GBS serotyping is based on antigenic changes of the polysaccharide capsule (Slotved *et al.,* 2017). At this time, ten different capsular types are recognized, including Ia, Ib, II-IX (Spellerberg and Brandt, 2015). The capsule represents the main virulence factor, which helps GBS avoid host protection mechanisms by defensive bacteria from opsonisation, thereby interfering with phagocytic clearance (Chen *et al.,* 2013). Serotyping is commonly used for epidemiological determinations and pathogenicity studies and establishes a valuable tool to predict the influence of supposed polysaccharide-based GBS vaccines (Lin *et al*., 2018).

There are many methods for serotyping GBS including latex agglutination, Lancefield precipitation (LP), enzyme-based immunoassays and flow cytometry (Rosini *et al.,* 2015). The disadvantages for using these methods include the need for high titer serotype-specific antisera, which are costly, as well as limited type ability, thus resulting in a high number of non-typeable isolates. However, real-time PCR can overcome this limitation by recognising genetic variants in the *cps* locus to allot isolates to a particular serotype (Breeding *et al*., 2016). In addition, whole-genome sequencing (WGS) data can provide a possible method to determine GBS serotyping (Sheppard *et al*., 2016).

Epidemiological reviews around the world have shown that the distribution of GBS serotypes differs geographically (Mukesi *et al*., 2019) as shown in Table 2.3. Serotype III was found to predominate in Canada and Jorden, while serotype V was more commonly reported from Morocco, Egypt, Kuwait and Gambia and Ia predominated in Saudi Arabia and Brazil (Table 2.3).

Differences in serotype distribution have been reported between maternal isolates and invasive isolates from neonates, with serotype III and Ia found to be more invasive than serotype Ib, II and V in Africa and Europe (Mukesi *et al*., 2019).

Table 2.3. Distribution of the most prevalent serotypes among GBS isolates from pregnant women in different countries.

GBS serotyping	Region	Reference
V (38.5%), III (21%), Ia 8%), and II (11%)	Kuwait	Boswihi *et al*., 2012
V (33%), II (17%), III (15%), Ia 14%, VI (12%), Ib (8%) and IV (1%)	Egypt	Shabayek *et al*., 2014
V (55%), II (16%), III (10%), Ia (8%) and Ib (8%).	Gambian	Le Doare *et al*., 2017
III (25%), Ia (23%), V (19%), II (13%), Ib (9%), IV (6%) and VI (1%)	Canada	Teatero *et al*., 2017
Ia (37.3%), II (19.9%), Ib (11.1%), V (9.1%), III (6.8%) and IV (3.5%).	Brazil	Botelho *et al*., 2018
III (48%), Ia (24%), II (20%), V (8%)	Jorden	Clouse *et al*., 2019
Ia (30%) III and V (25%) each	Saudi Arabia	Mohamed *et al*., 2020
V (36%), II (25%), III (18%), Ia (9%), IV (7%) and IX (5%)	Morocco	Moraleda *et al*., 2018

2.7. Justification of the study

The current study is the first study on GBS screening of Libyan women during delivery and provides data on the prevalence of GBS colonisation in pregnant woman and neonatal infection in Libya. This study is important because a knowledge of the distribution of GBS serotypes in a region establishes a valuable tool to predict the influence of proposed polysaccharide-based GBS vaccines. Moreover, the current study provides information on risk factors related to maternal and neonatal GBS prevalence as well as confirming the importance of PCR for the rapid diagnosis of GBS at labour with high specificity and sensitivity. Research findings that emerge from undertaking the study will help to increase public awareness of GBS colonisation thereby reducing the prevalence of GBS and preventing vertical transmission from the mother to the neonate.

2.8. Aim of study

The aim of this study is to test the following hypothesis:

H_1: Vertical transmission occurs from mothers colonised with GBS to their infants at delivery.

This will be achieved through the following objectives:

i. To describe the risk factors associated with GBS colonisation.
ii. To determine the prevalence of GBS colonisation in Libyan women at labour.
iii. To determine the rate of vertical transmission of GBS from mother to infant.
iv. To determine the predominant GBS serotypes in maternal and neonatal samples.

CHAPTER 3. MATERIALS AND METHODS

This part of the research was focused on sample, data collection and sample processing as well as ethical clearance.

3.1. Ethical Approval

Ethical clearance for the study was obtained from the Human Ethics Committee at the University of the Western Cape (UWC BM20/3/3) and approval to conduct the study was obtained from the Ethics Committee of Said Hospital, Libya.

Informed consent was obtained from the 200 mothers who agreed to participate in the study. The study complied with the Declaration of Helsinki (2013).

3.2. Data Collection

Data regarding maternal demography and reproductive history were collected through the use of a questionnaire. The questionnaire collected data pertaining to maternal age, weight, socio-economic status, medical and gynaecological history.

3.3. Study area

The proposed study was conducted at Misrata, Libya. Misrata is located in the north western region of Libya and it is the third-largest city in Libya, after Tripoli and Benghaz. The population in 2022 was estimated at around 285759. The hospital serves patients around the Libyan cities, not only in Misrata. An average of 250-300 women deliver at Said Hospital every month.

3.4. Sample Collection

The study sample included a total of 200 delivery cases and their neonates. One hundred (100) samples were collected from mothers who delivered preterm and 100 samples were collected from

mothers who delivered full term and served as the control group from July 2020- April 2021. Exclusion criteria were caesarean deliveries and women who had received antibiotic treatment during two weeks prior to sample collection.

Sample collection comprised three maternal swabs (vaginal swabs, rectal swabs and recto-vaginal swabs) collected at delivery using rayon swabs in transport media (ESwab, Copan Diagnostics, Brescia, Italy 480C). A vaginal sample was collected from the mucosa of the lower third of the vagina. A rectal sample was collected from the rectal mucosa around 2.5 cm beyond the anal sphincter, while the vaginal-rectal sample was collected by first inserting a swab into the vagina and then into the rectum. All samples were tested within 24 h of collection.

Neonatal swabs were collected at birth. Swabs were collected from the external ear canal of the infant since this is the most sensitive indicator of neonatal colonisation, and determines transmission rates (Kunze *et al.*, 2011).

3.5. Sample Processing

3.5.1. Isolation of DNA

For sample lysis, 200 µl of swab sample was transferred into a 2.0 ml collection tube, 200 µl of lysis buffer added, followed by the addition of 20 µl carrier RNA and 20 µl proteinase K to the collection tube. The tube contents were mixed using a Vortex mixer (FOUR E's Scientific, model MI0101002D, China) and the tubes were incubated for 10 min at 65 °C followed by 10 min at 95 °C. A volume of 260 µl of binding buffer was added to the collection tube, mixed, and incubated at room temperature for 5 min. The lysate was transferred to the Mini Spin Column and centrifuged for 1 min at 11.100 × g. First and second washings were achieved by adding 600 µl of wash buffer I, centrifuging for 1 min at 11.100 × g, discarding the RTA collection tube with filtrate and then placing the mini spin column into a new RTA collection tube. After adding 700 µl wash buffer II, the tube was centrifuged for 1 min at 11,100 × g, the filtrate discarded and the mini spin column put back into the used RTA collection tube. This washing step was repeated once. Ethanol was removed by centrifuging for 5 min at 11.100 × g and the RTA collection tube with filtrate was discarded. DNA was eluted by placing the mini spin column into a new 1.5 ml collection tube. A

volume of 100-200 µl of Elution buffer was added and the tube incubated at room temperature for 1 min, followed by centrifuging for 1 min at 11,100 × g. The mini spin column was discarded and the DNA sample stored at -20 °C to -80 °C.

3.5.2. Real-Time PCR for GBS detection

All samples were analysed with the Agilent AriaMx Real-Time System (Agilent Technologies, model G8830A, USA). Normally the strip has eight holes, two holes for controls and six holes for samples. Controls consisted of 5 µl negative control and 15 µl rehydration control in one hole in the strip and 5 µl positive control and 15 µl rehydration control in another hole followed by 5 µl sample with 15 µl rehydration control in the remaining six holes. The system took 1:15:37 min to yield a result.

The optimal reaction conditions were 95 °C for 2 min followed by 44 cycles at 95 °C for 10 min, then 60 °C for 50 min.

3.6. Real-time PCR serotyping

Probes and primers were intended to amplify special areas of the polysaccharide capsular genes of each of the serotypes of GBS (Table 3.1) from the National Centre for Biotechnology Information databases. Probes and oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA). Probes were labelled with a fluorescent probe (5′ 6-carboxyfluorescein (6-FAM)) and two quenchers (internal ZENTM, and 3′ Iowa Black® FQ) and purified by high-pressure liquid chromatography. PCR reactions were done in a last volume of 20 µl and contained of 10 µl Taqman Universal Mastermix, 7.4 µl sterile water, 0.2 µl forward primer, 0.2 µl reverse primer, 0.2 µl probe, and 2 µl GBS DNA. Triplicate reactions were done on the Agilent AriaMx Real-Time System (Agilent Technologies, model G8830A, USA) and analysed by ARIA software. Positive reactions were clear as a cycle threshold (CT) < 30 for 50 ng DNA template/reaction. Negative control reactions (no DNA template) were included with each run.

Table 3.1: Primers used for GBS serotyping by **RT-**PCR.

	Sequence (5′-3′)	Target gene	Size
Ia-F	GTTTAAAAATCCTGATTTTGATAGAATTTTAGCAGCTTTTAAC	cpsH	207
Ia-R	CTGATATTTTGAATATTATTATGCAAACAATAATAATATGTTCCCCCTA		
Ia-P	6-FAM-TCGTTGATT/ZEN/ATCGGTATAGTATCATTGGCT-IAbFQ		
Ib-F	GTATTAAAT TCGTTATTTAGAAGTCCAGAATTTCATAGAGTCATTGC	cpsH	195
Ib-R	GGCATAATAATATAGAAATCCTAAACAAGACAAAATAATTGCATTAAAC		
Ib-P	6-FAM-TGC ATT CAA/ZEN/T TCACTGGCAGTAGGG-IAbFQ		
II-F	CACATATATATTAAAGTTCACCCTAGAGATAACATTGACTACTCTAATC	cpsK	151
II-R	CTAATGCCGTGGAAAAATATGTAATCCCAACATCAAATT		
II-P	6-FAM-AATGCAACA/ZEN/GTAATACAAAGGAACATCCCT- IAbFQ		
III-F	GGAATTGTTCTTTATTTTTCTGCCT	cpsI	170
III-R	ACTATACCAAAAGTTGAGAATAATAATACAATACTCCAATGA		
III-P	6-FAM-ATGTTACAC /ZEN/GCTCTTTGAGGAAATAGATCC- IAbFQ		

IV-F	GAAGAAAATATATATTTGCCATACAGTATATCATCTC CTTATTACAATTATC	cpsK	159
IV-R	CATAGAATACCTTCTTTATTGGTACGTTTACATAAATC ATCAATATTAAC		
IV-P	6-FAM-AGGGAACAG /ZEN/AGGAGATCAATAATTATATTGGC- IAbFQ		
V-F	CAAAATTCAATGAGAGAATGTTGTATTTTTTTGAGGC AATTC	cpsO	153
V-R	CAATCATCTTCCCACATATATCTATTCCACCAAATACT TC		
V-P	6-FAM-ATTTTCCAC /ZEN/ATAATACATCTTTAATCTCTGCTG T- IAbFQ		
VI-F	GACAGTCTATTACGAAAGTATAAGAGCGATT	cpsH	219
VI-R	AGCTTGTAGATTATCCTGTTTTGTTTGATAGCTTCTCT ATATAG		
VI-P	6-FAM-CCCTCCAGT/ZEN/GTGGGAATATTTTTAGGTTCAC-IAbFQ		
VII-F	GAGGGCTTACCTCACGACAGGAGAAGTAAAAAATAT AAAG	cpsK	160
VII-R	GCTGCGTTAATAACAATACTGACTTTGGAGC		
VII-P	6-FAM-AGTCTTACC/ZEN/CAAGAACAAAAGTCTCTGATT-IAbFQ		
VIII-F	GACTAATGGTTAAGTATGCTAACTTGCTAATT TGTGATAGTAA	cpsR	152

VIII-R	CTTGTCCTTAAAATTGTGTTTTGACTTTGTCAGATCAGTC		
VIII-P	6-FAM-ATGCTCCTA/ZEN/AAACAACCTACATCGCCTATG-IAbFQ		
IX-F	CATTGAGCAAAGAGAAAACAGTATATGTCAAAGGGC	cpsO	128
IX-R	ATGTTCAAGGATAAAATCTCTATTATGTTGCATTGCTTC		
IX-P	6-FAM-AGTACTACC/ZEN/AGACAGTCATACAAAGAGAAT-IAbFQ		

Sequences are obtainable 5′ to 3′ with probe modifications as designated (6-carboxyfuorescein [6-FAM] fluorescent probe, internal ZENTM and Iowa Black® FQ [IAbFQ] quenchers).

3.6. Statistical analysis of data

Statistical analysis employed the use of the Statistical Package for the Social Sciences (SPSS) version 28. Descriptive analyses were performed to describe demographic, medical and behavioural factors. Continuous variables were expressed as median (range) and category variables were expressed as proportions (%). Fisher exact test and Chi-square tests were used to test for a significant association of risk factors and to determine whether there was any significant difference between parameters and GBS proportions with a *p*-value of < 0.05 considered as significant. ANOVA was used to relate maternal risk factors with GBS colonisation and pregnancy outcomes.

CHAPTER 4. RESULTS

This section of the dissertation specifically focused on presenting results that were gathered in line with the research objective of the chapter and begins with the prevalence of maternal GBS colonisation, maternal risk factors, serotyping and neonatal data.

4.1. Prevalence of maternal GBS colonisation

Among the 200 pregnant women, 36 (18%) were identified as GBS carriers, and 164 (82%) mothers were GBS-negative. Vaginal-rectal swabs identified the most GBS carriers (18), followed by rectal swabs (11) with vaginal swabs detecting the least carriers (7). Mothers with GBS+ vaginal-rectal swabs, also yielded six positive rectal swabs and two positive vaginal swabs (Table 4.1). GBS was detected in 11 rectal swabs, of which six vaginal-rectal swabs were also positive, along with two vaginal swabs. GBS was detected in only seven vaginal swabs from mothers who also showed colonisation in two rectal swabs and two vaginal-rectal swabs (Table 4.1). There appeared to be no significant difference between sample sites and GBS prevalence (p = 0.520), although GBS was most frequently recovered from vaginal-rectal samples.

Table 4.1. Prevalence of GBS in maternal samples

Sample sit	Vaginal N (%)	Rectal N (%)	Vag-rectal N (%)
Vaginal	7 (3.5%)	2 (1%)	2 (1%)
Rectal	2 (1%)	11 (5.5%)	6 (3%)
Vaginal-rectal	2 (1%)	6 (3%)	18 (9%)

4.2. Maternal risk factors for GBS colonisation

4.2.1. GBS colonisation and maternal age

Of the women who participated in this research, the 26 to 35-year age group of mothers constituted 54.5% of the study group (Table 4.2.), followed by the 18 to 25-year-old group (37.5%) and 36 to

45-year-old group (8%). GBS prevalence stood at 36 (18%) of mothers, of whom the 26 to 35 age group had the highest colonisation rate (10.5%),followed by 7.5% in the 18 to 25-year-old group with no GBS detected in the 36 to 45 age group, (Table 4.2). Chi Square analysis showed no significant correlation between GBS carriage and maternal age (p = 0.147)

4.2.2. GBS colonisation and education.

The majority of respondents (84.5%) indicated that they had formal education (Table 4.2), with most of them having attained secondary education (71.5%). Only 15.5% did not have any recognised formal education. GBS colonisation was highest in those with secondary education (12.5%), but Chi Square and Fisher exact analysis showed a significant relationship with tertiary education (p = 0.003).

4.2.3. GBS Colonisation and employment.

The majority of respondents (164) indicated that they were unemployed, while only 36 had employment (Table 4.2). GBS colonisation was highest in unemployed mothers (29) with GBS colonisation reported in only seven employed mothers. Chi-squared showed no significant relationship between employment and GBS prevalence (p =0.735).

4.2.4. GBS colonisation and maternal weight

Two hundred mothers recorded their weights. The majority of respondents (35.5%) fell within the 66 to 70 kg group, followed by mothers who weighed >70 kg, 61 to 65 kg, 56 to 60 kg and 51 to 55 kg (Table 4.2). The mothers with the highest colonisation rate weighed between 66 and 70 kg, followed by those who weighed 61 to 65 kg. Few of the remaining mothers carried GBS (Table 4.2). Using the Chi Square and Fisher exact tests, GBS colonisation was significantly associated with maternal weight > 65 kg (Table 4.2).

Table 4.2. Maternal demography with GBS colonisation.

Variables/ Categories	Frequency (%)	GBS+ N (%)	*p* value
Age Group Mean= 27.69 (SD 5.54)			0.147
18 - 25 years	75 (37.5%)	15 (7.5%)	
26 - 35 years	109 (54.5%)	21 (10.5%)	
36 - 45 years	16 (8%)	0 (0%)	
Total	**200 (100%)**	**36 (18%)**	
Educational level			
No formal education	31 (15.5%)	2 (1%)	0.078
Primary Education	14 (7%)	3 (1.5%)	0.729
Secondary Education	143 (71.5%)	25 (12.5%)	0.763
Tertiary Education	12 (6%)	6 (3%)	0.003
Total	**200 (100%)**	**36 (18%)**	
Employment			0.735
Yes	36 (18%)	7 (3.5%)	
No	164 (82%)	29 (14.5%)	
Total	**200 (100%)**	**36 (18%)**	
Weight in kg Mean= 67.25 (SD 5.31)			
51-55	1 (0.5%)	5 (2.5%)	0.906
56-60	3 (1.5%)	20 (10%)	0.713
61-65	8 (4%)	37 (18.5%)	0.803
66-70	20 (10%)	71 (35.5%)	0.002
>70	4 (2%)	67 (33.5%)	0.002
Total	**36 (18%)**	**200 (100%)**	

4.3. GBS colonisation and maternal medical history

4.3.1. Diseases of lifestyle

Of the 200 cases, 13 mothers reported hypertension and seven reported diabetes (Table 4.3). Three of the 13 who reported hypertension, were GBS positive and none of the diabetics were colonised with GBS. Chi Square test showed that GBS colonisation was not significantly associated with hypertension ($p = 0.622$) and diabetes ($p = 0.209$).

4.3.2. Current symptoms of infection

Most of the mothers, 115 (57.5%) had vaginal discharge of whom 21 (10.5%) were colonised with GBS. Of the 111 (55.5%) who declared urinary tract infection, 17 (8.5%) had GBS colonisation. Chi Square test showed no significant association between vaginal discharge and UTI with GBS colonisation (Table 4.3).

Table 4.3. GBS colonisation and medical history.

Variables	Frequency (%)	GBS positive	*p* value
Hypertension			$p = 0.622$
Yes	13(6.5%)	3 (1.5%)	
No	187(93.5%)	33 (16.5%)	
Total	200 (100%)	36 (18%)	
Diabetes			$p = 0.209$
Yes	7 (3.5%)	0 (0%)	
No	193(96.5%)	36 (18%)	
Total	200 (100%)	36 (18%)	
Current symptoms of infection			
Vaginal discharge			$p = 0.911$
Yes	115 (57.5%)	21 (10.5%)	
No	85 (42.5%)	15 (7.5%)	
Total	200 (100%)	36 (18%)	
Urinary tract infection			$p = 0.463$
Yes	111 (55.5%)	17 (8.5%)	
No	89 (44.5%)	19 (9.5%)	
Total	**200 (100%)**	**36 (18%)**	

4.4. GBS colonisation and maternal reproductive history

4.4.1. Parity and Gravidity

Most of the mothers, (69.5%) reported a parity of 0–3. This group also had the highest GBS colonisation rate (12%), followed by the 4-5 parity group (25.5%) with a GBS colonisation rate of 6%. No GBS colonisation was found in 5% of mothers in the > 6 parity group. Chi-squared showed no significant difference between the different parity groups and GBS colonisation.

Significant differences in GBS colonisation were observed between the different gravidity groups (p = 0.048), with the highest colonisation rate reported in the 0-3 group (Table 4.4).

4.4.2. History of abortion

Thirty-four (17%) of the mothers had a history of abortion, of whom 6 (3%) had GBS colonisation as shown in Table 4.4. No significant association was noted between abortion history and GBS colonisation (Table 4.4).

4.4.3. Previous pregnancy outcomes

Previous pregnancy outcomes included 72% full term deliveries (FTD) and 28% preterm deliveries (PTD). There was no significant difference noted between GBS colonisation of mothers who previously delivered FTD or PTD (Table 4.4).

Table 4.4. GBS colonisation with maternal reproductive history

	Frequency (%)	**GBS positive**	***p* value**
Parity Mean 1.76 (SD 1.84)			*p*= 0.192
0-3	139 (69.5%)	24 (12%)	
4-5	51 (25.5%)	12 (6%)	
>6	10 (5%)	0 (0%)	
Total	**200 (100%)**	**36 (18%)**	
Gravidity Mean 3.30 (SD 1.84)			*p*= 0.048
0-3	97 (48.5%)	21 (10.5%)	
4-5	70 (35%)	14 (7%)	
>6	33 (16.5%)	1 (0.5%)	
Total	**200 (100%)**	**36 (18%)**	
Abortion			*p*= 0.953
Yes	34 (17%)	6 (3%)	
No	166 (83%)	30 (15%)	
Total	**200 (100%)**	**36 (18%)**	
Previous Pregnancy outcomes			*p*= 0.431
FTD	144 (72%)	24 (12%)	
PTD	56 (28%)	12 (6%)	
Total	**200 (100%)**	**36 (18%)**	

Abbreviations: SD, standard deviation; FTD, full-term delivery; PTD, preterm delivery

4.5 Effect of GBS on current pregnancy outcomes

Of the 36 mothers with GBS, 18 delivered FTD and 18 delivered PTD (Table 4.5). There was no significant difference between PTD and FTD delivery when compared for GBS colonisation.

Table 4.5. GBS and current pregnancy outcomes

GBS +	**Frequency**	**FTD** **n (%)**	**PTD** **n (%)**	***p*-value**
Yes	36	18 (18%)	18 (18%)	p =1.000
No	164	82 (82%)	82 (82%)	
Total	**200 (100%)**	**100 (100%)**	**100 (100%)**	

4.6. Maternal details, GBS colonisation and current pregnancy outcomes

Two way ANOVA was applied to determine a significant association between GBS colonisation, maternal demography and current pregnancy outcomes with GBS colonisation as the dependent variable and risk factors and pregnancy outcomes as independent variables (Table 4.6).

4.6.1. Association with Age

GBS colonisation was not significantly associated with age and pregnancy outcomes (Table 4.6).

4.6.2. Association with weight

Most mothers (67%) weighed > 66 kg and they also showed the highest rates of GBS colonisation (12%) and PTD (85%). Weight and pregnancy outcomes were significantly associated with GBS colonisation pregnancy outcomes (p = 0.003).

4.6.3. Association with Education

Of the mothers with secondary educational levels, 69% delivered PTD and 74% delivered FTD. Although this group showed the highest rates of GBS colonisation, education levels and pregnancy outcomes were not significantly associated with GBS colonisation (p = 0.07) (Table 4.6).

4.6.4. Association with Employment

Only 18 % of mothers had employment and although GBS and PTD were higher in the unemployed (Table 4.6), this finding was not statistically significant (p = 0.992).

4.6.5. Association with Hypertension

Only three of the 13 mothers (6.5%) who reported hypertension were GBS-positive and all delivered FTD (Table 4.6). GBS colonisation and PTD were significantly higher in mothers who did not report hypertension (Table 4.6).

4.6.6. Association with Diabetes

Seven of 200 mothers (3.5%) reported diabetes, of whom six delivered PT (Table 4.6). GBS was not significantly associated with diabetes and PTD (p = 0.982).

4.6.7. Association with Infection (vaginal discharge and urinary tract infection)

Of the 115 women (57.5%) who presented with a vaginal discharge, 21 (10.5%) were positive for GBS and 99% delivered PT (Table 4.6). Of the 85 who reported no discharge, 15 were GBS-positive and only one delivered PT. Of the 111 (55.5%) mothers who had UTI, 99 delivered PTD. GBS colonisation was not significantly associated with infection nor pregnancy outcomes (Table 4.6).

Table 4.6. Maternal details and pregnancy outcomes

	Frequency (%)	**GBS-positive (%)**	**FTD (n = 100) (%)**	**PTD (n = 100) (%)**	***p*-value**
AGE (years) Mean= 27.69 (SD 5.54)			**Mean 27.22 (SD 5.95)**	**Mean= 28.16 (SD 5.85)**	$p = 0.163$
18 - 25 years	75 (37.5%)	15 (7.5%)	39 (39%)	36 (36%)	
26 - 35 years	109 (54.5%)	21 (10.5%)	56 (56%)	53 (53%)	
36 - 45 years	16 (8%)	0 (0%)	5 (5%)	11(11%)	
Total	**200 (100%)**	**36 (18%)**	**100 (100%)**	**100 (100%)**	
WEIGHT (kg) Mean= 67.25 (SD 5.31)			**Mean 65.03 (SD 5.97)=**	**Mean= 69.46 (SD 3.32)**	$p = 0.003$
51 – 55	5 (2.5%)	1 (0.5%)	5 (5%)	0 (0%)	
56 – 60	20 (10%)	3 (1.5%)	20 (20%)	0 (0%)	
61 – 65	37 (18.5%)	8 (4%)	22 (22%)	15 (15%)	
>66	138 (67%)	24 (12%)	53 (53%)	85 (85%)	
Total	**200 (100%)**	**36 (18%)**	**100 (100%)**	**100 (100%)**	
EDUCATIONAL LEVEL Mean					$p = 0.070$
No formal education	31 (15.5%)	2 (1%)	13 (13%)	18 (18%)	
Primary education	14 (7%)	3 (1.5%)	8 (8%)	6 (6%)	
Secondary education	143 (71.5%)	25 (12.5%)	74 (74%)	69 (69%)	
Tertiary education	12 (6%)	6 (3%)	5 (5%)	7 (7%)	
Total	**200 (100%)**	**36 (18%)**	**100 (100%)**	**100 (100%)**	
Employment Mean					$p = 0.992$
Yes	36 (18%)	7 (3.5%)	20 (20%)	16 (16%)	
No	164 (82%)	29 (14.5%)	80 (80%)	84 (84%)	
Total	**200 (100%)**	**36 (18%)**	**100 (100%)**	**100 (100%)**	
Hypertension					$p = 0.000$

Yes No **Total**	13 (6.5%) 187 (93.5%) **200 (100%)**	3 (1.5%) 33 (16.5%) **36 (18%)**	13 (13%) 87 (87%) **100 (100%)**	0 (0%) 100 (100%) **100 (100%)**	
Diabetes					$p = 0.982$
Yes No **Total**	7 (3.5%) 193 (96.5%) **200 (100%)**	0 (0%) 36 (18%) **36 (18%)**	1 (1%) 99 (99%) **100 (100%)**	6 (6%) 94 (94%) **100 (100%)**	
Infection					
Vaginal discharge					$p = 0.669$
Yes No **Total**	115 (57.5%) 85 (42.5%) **200 (100%)**	21 (10.5%) 15 (7.5%) **36 (18%)**	16 (16%) 84 (84%) **100 (100%)**	99 (99%) 1 (1%) **100 (100%)**	
Urinary tract infection					$p = 0.341$
Yes No **Total**	111 (55.5%) 89 (44.5%) **200 (100%)**	17 (8.5%) 19 (9.5%) **36 (18%)**	12 (12%) 88 (88%) **100 (100%)**	99 (99%) 1 (1%) **100 (100%)**	

4.7. Maternal reproductive history and pregnancy outcomes

Two way ANOVA was applied to determine a significant association between GBS colonisation, reproductive history and current pregnancy outcomes.

4.7.1. Association with Parity

Most of the mothers (69 %) who delivered PTD reported a parity of 0 – 3, followed by 24 % in the 4-5 group and 7% of mothers in the >6 group. Similarly, most of the mothers in the FTD group (70 %) fell within the 0-3 parity group, followed by 27 % in the 4-5 parity group and only 3 % in the >6 group. Parity and pregnancy outcomes were not significantly associated with GBS (Table 4.7).

4.7.2. Association with Gravidity

Here again, the most frequently reported gravidity was 0-3 (48.5%). In this category, 53% delivered PTD, followed by 25% in the 4-5 group and only 22% in the >6 group. In the FTD group, 44 % of mothers were in the 0-3 group, with 45% in the 4-5 group and 11% in the >6 group. No significant association was found between gravidity or pregnancy outcomes and GBS (Table 4.7).

4.7.3. Association with previous pregnancy outcomes

Thirty-four of 200 mothers (17%) had a previous abortion of whom six were GBS-positive and six delivered preterm (Table 4.7). Previous abortion and current pregnancy outcome were not significantly associated with GBS colonisation (p = 0.947). Fifty-six mothers (28%) reported a previous PTD, of whom 12 were colonised with GBS. Although 53 had a current PTD, these findings were not statistically significant (Table 4.7).

Table 4.7. Maternal reproductive history and pregnancy outcomes

	Frequency (%)	**GBS positive**	**FTD N (%)**	**PTD N (%)**	***p* value**
Parity Mean= 1.76) (SD 1.84)			**Mean= 1.65 (SD 1.82)**	**Mean=1.87 (SD 1.86)**	$p = 0.810$
0-3	139 (69.5%)	24 (12%)	70 (70%)	69 (69%)	
4-5	51 (25.5%)	12 (6%)	27 (27%)	24 (24%)	
>6	10 (5%)	0 (0%)	3 (3%)	7 (7%)	
Total	**200 (100%)**	**36 (18%)**	**100 (100%)**	**100(100%)**	
Gravidity Mean= 3.30) (SD 1.84)			**Mean 3.41 (SD 1.57)**	**Mean= 3.19 (SD 2.09)**	$p = 0.280$
0-3	97 (48.5%)	21 (10.5%)	44 (44%)	53 (53%)	
4-5	70 (35%)	14 (7%)	45 (45%)	25 (25%)	
>6	33 (16.5%)	1 (0.5%)	11 (11%)	22 (22%)	
Total	**200 (100%)**	**36 (18%)**	**100 (100%)**	**100(100%)**	
Previous Abortion					$p = 0.947$
Yes	34 (17%)	6 (3%)	28 (28%)	6 (6%)	
No	166 (83%)	30 (15%)	72 (72%)	94 (94%)	
Total	**200 (100%)**	**36 (18%)**	**100 (100%)**	**100 (100%)**	
Previous term of delivery					$p = 0.236$
FTD	144 (72%)	24 (12%)	97 (97%)	47 (47%)	
PTD	56 (28%)	12 (6%)	3 (3%)	53 (53%)	
Total	**200 (100%)**	**36 (18%)**	**100 (100%)**	**100 (100%)**	

Abbreviations: SD, standard deviation; FTD, full-term delivery: PTD, preterm delivery

4.8. Serotypes and pregnancy outcomes of mothers colonised with GBS

In the maternal samples, serotype VI was the predominant serotype, followed by serotypes III, IV and V (Table 4.8).

Eighteen mothers were GBS-positive in each of the preterm (PTD) and full term (FTD) groups and although serotype VI predominated in both FTD and PTD groups, serotypes III and V were more frequently found in mothers who delivered FTD, while serotypes IV and VI were more frequently detected in PTD. Maternal GBS serotypes were not significantly associated with pregnancy outcomes (Table 4.8).

Table 4.8. Serotypes of GBS in maternal samples

Maternal GBS serotypes	**N (%)**	**Delivery method**		***p*-value**
		FTD (%)	**PTD (%)**	
III	6 (3%)	4 (4%)	2 (2%)	$p = 0.683$
IV	6 (3%)	2 (2%)	4 (4%)	$p = 0.683$
V	6 (3%)	4 (4%)	2 (2%)	$p = 0.683$
VI	18 (9%)	8 (8%)	10 (10%)	$p = 0.621$
GBS-negative	164 (82%)	82 (82%)	82 (82%)	
Total	**200 (100%)**	**100 (100)**	**100 (100%)**	

4.9. Neonatal data.

4.9.1. Neonatal GBS colonisation and birth weight.

Of the 200 recorded neonatal weights, 49% had normal birth weight (NBW), while 47.5% had low birth weight (LBW), very low birth weight (VLBW) and extremely low birth weight (ELBW). Above normal birth weight (ANBW) was recorded for 3.5% (Table 4.9). LBW was significantly associated with GBS colonisation ($p = 0.001$) using the Chi-squared test.

Table 4.9. Neonatal GBS colonisation and birth weight.

Birth weight	**Frequency**	**Neonatal GBS+**	***p*-value**
ELBW (≤0 .999g)	9 (4.5%)	0	-
VLBW (1.000g -1.999g)	30 (15%)	0	-
LBW (2.000g -2.999g)	56 (28%)	5 (2.5%)	0.001
NBW (3.000g - 3.999g)	98 (49%)	2 (1%)	0.229
ANBW (4.000g -4.999g)	7 (3.5%)	0	-
Total	**200 (100%)**	**7**	

ELBW= Extremely low birth weight; VLBW= Very low birth weight; LBW= Low birth weight; NBW= Normal birth weight; ANBW= above normal birth weight

4.9.2. Neonatal birth weight and pregnancy outcomes

The mean birth weight of the 200 infants was 3.32 g (SD 0.928). A comparison of the different birth weight categories within the 100 PT and the 100 FT cases showed that infants born PT had significantly lower birth weights than those born FT (Table 4.10). Of the 100 infants born PT, nine had ELBW, 27 were of VLBW and 37 had LBW. PTD was significantly associated with BW (*p*= 0.001).

Table 4.10. Neonatal birth weight and pregnancy outcomes

	N (%)	Delivery Period		*p*-value
Birth Weight		PT	FT	$p = 0.001$
ELBW (≤0 .999g)	9 (4.5%)	9	0	
VLBW (1.000g -1.999g)	30 (15%)	27	3	
LBW (2.000g -2.999g)	56 (28%)	37	19	
NBW (3.000g - 3.999g)	98 (49%)	24	74	
ANBW (4.000g - 4.999g)	7 (3.5%)	3	4	
Total	**200 (100%)**	**100**	**100**	

ELBW, extremely low birth weight; VLBW, very low birth weight; LBW, low birth weight; NBW, normal birth weight; ANBW, above normal birth weight; PT, preterm; FT, full-term

4.9.3. Vertical transmission of GBS

Only seven neonates had GBS (Table 4.11). Neonatal GBS was significantly associated with maternal GBS colonisation ($p = 0.001$) using the Chi-squared test.

Table 4.11. Neonatal GBS and maternal GBS colonization

Neonatal GBS	**N %**	**Maternal GBS**	***p*- value**
Yes	7 (3.5%)	36 (18%)	0.001
No	193 (96.5%)	164 (82%)	
Total	**200 (100%)**	**200 (100%)**	

4.9.4. Vertical transmission of GBS Serotypes.

Of the seven cases where vertical transmission of GBS occurred, serotypes III and V were the only serotypes transmitted (Table 4.12).

Table 4.12. Vertical transmission of GBS Serotypes.

GBS Serotype	N (%) Mother	Vertical transmission rate
III	6 (3%)	3 (8.3%)
IV	6 (3%)	0 (0%)
V	6 (3%)	4 (11.1%)
VI	18 (9%)	0 (0%)
Total	36 (18%)	7 (19.4%)

4.9.5. Neonatal GBS serotypes related to pregnancy outcomes and neonatal birth weight

Three of the seven neonates born FTD were positive for serotype III and four were positive for serotype V. None of the PTD cases had GBS. Table 4.13. shows a significant association between GBS serotypes and pregnancy outcomes (p = 0.027) as well as with LBW (p= 0.001).

Table 4.13. Neonatal serotypes related to pregnancy outcomes and birth weight

Serotype	**Pregnancy outcome**			**Neonatal birth weight**					
	FTD	**PTD**	***p*-value**	**ELBW (≤ 0 .999g)**	**VLBW (1.000g - 1.999g)**	**LBW (2.000g - 2.999g)**	**NBW (3.000g - 3.999g)**	**ANBW (4.000g - 4.999g)**	***p*-value**
III (n = 3)	3 (3%)	0 (0%)	0.027	0 (0%)	0 (0%)	3 (1.5%)	0 (0%)	0 (0%)	0.001
V (n = 4)	4(4%)	0 (0%)		0 (0%)	0 (0%)	2 (1%)	2 (1%)	0 (0%)	
No GBS (n = 193)	93 (93%)	100 (100%)		9 (4.5%)	30 (15%)	51 (28%)	96 (49%)	7 (3.6%)	
Total (n = 200)	**100**	**100**		**9 (4.5%)**	**30 (15%)**	**56 (28%)**	**98 (49%)**	**7 (3.5%)**	

CHAPTER 5. DISCUSSION

Vertical transmission of GBS from the mother to the infant at birth increases the risk of neonatal GBS infection (Seale *et al*., 2017).

At the time of writing, this study is the first study to provide valuable knowledge regarding GBS carriage in a population of pregnant women in Libya and also the first study to use molecular genotyping to describe the GBS serotype distribution in Libya.

The aims and objectives of this study were to establish the GBS prevalence in Libyan women, examine for vertical transmission of GBS from mother to infant at birth, determine the predominant serotypes, and to determine whether previously reported risk factors were also risk factors associated with GBS colonisation of women in this study.

These objectives were achieved by employing the RT-PCR method to detect GBS colonization and GBS serotypes. Currently, there are many detection methods for GBS besides PCR, including culture and immunochromatographic detection and identification systems (Lee *et al*., 2019; Matsui *et al*., 2013). The limitations of these detection methods include reduced sensitivity and specificity of immunochromatographic methods as well as the time needed for cultural methods (Matsui *et al*., 2013; Helming *et al*., 2019). Compared with these, PCR has the advantage of a rapid turn-around time, yielding results within 1-2 hours with high specificity (84.6% to 100%) and sensitivity (62.5% to 100%) to reduce mortality (El Helali *et al*., 2012; Di Renzo *et al*., 2015). Similarly, for serotyping of GBS, latex agglutination kits may be used; however, non-molecular methods for GBS serotyping can be labour-intensive and expensive, and may need high-titre serotype-specific antisera (lmperi *et al*., 2010).

In the current study of 200 Libyan women, the prevalence of GBS colonisation was found to be 18%. This is comparable with other studies conducted in Africa and Middle Eastern countries such as Lebanon (18.4%, Ghaddar *et al*., 2014**),** Saudi Arabia (19%, Musleh and Al Qahtani, 2018), Kuwait (20.7%, Ghaddar *et al*, 2014) and South Africa (16.6%, Africa and Kaambo, 2018). However, a lower prevalence was reported in Sudan (0.5%, Abdullahi *et al*, 2017) and Turkey

(9.8%, Alp, 2016), Iran (11.8%, Darabi, 2017), Namibia (13.6%, Mukesi *et al*, 2019), Kenya (12%, Seale e*t al*., 2017) and Egypt (11.25%, Wassef *et al*., 2017) while a higher prevalence was reported in Israel (31.0%, Hakim, 2018), and Morocco (24.0%, Moraleda, 2018).

The above data shows that the prevalence of GBS colonization differs between countries and a wide variation can also be found in different regions of the same country. For example, in a study of different regions in Ethiopia, the colonisation rates differed from 9% to 19% (Mengist, 2016). Similarly, in a study from Saudi Arabia, the GBS colonisation was found to be 13.4% compared to 18.5% in Saudi women situated in the northern border with Kuwait (Khan *et al*., 2015).

There are several reports of higher GBS isolation rates from rectal swabs compared with vaginal swabs (Furfaro *et al*., 2019), supporting suggestions that the gastro-intestinal tract may be the primary site of colonisation by GBS (Khan *et al*., 2011) and that vaginal colonisation may occur through contamination from the rectum (Maghaddam, 2010). However, a study by Sadaka *et al*., (2018) found GBS vaginal colonisation to be 100%, while only four women (7.5%) had rectal colonisation. In this study, GBS was most frequently isolated from both vaginal-rectal samples with no significant difference between sample sites and GBS prevalence observed (p = 0.520), similar to a study by Nomura *et al.,* (2006), who found no significant difference in detection rates between vaginal and rectal samples. In similar studies, (Centres for Disease Control and Prevention, 2010; Sharmila *et al.,* 2011) reported that rectovaginal sampling yielded better results than vaginal or the rectal sampling alone.

Many demographic risk factors such as maternal age, weight, socio - economic status, diabetes, and other medical history (Chawanpaiboon, 2011; Zhang *et al*., 2017; Berardi *et al*., 2014) are shown to be associated with higher prevalence rates of GBS.

In the current study, GBS was more frequently isolated from women aged 26 to 35 years, as in the study from Egypt where they found that about 31.6% women aged 20-30 years were carriers of

GBS (Sadaka *et al*., 2018). On other hand, studies from Nigeria and Iran reported an increase in GBS colonisation as the age increased (Onipede *et al*., 2012; Ghanbarzadeh *et al*., 2017). These variances are difficult to explain, but possibly highlight the fact that GBS colonisation might be related to multiple factors that differ from one geographical place to another.

In this study, GBS colonisation was significantly associated with maternal weight > 65 kg. A study by Shah *et al*., (2011) found a relationship between maternal weight and GBS colonisation. Also, a study conducted in USA (Kleweis *et al*., 2015) showed a relationship between GBS and increased BMI. The underlying aetiology of the association between GBS colonization and maternal weight is not clear.

This study showed an increased prevalence in mothers with secondary education unlike the study conducted by Namugongo *et al*., (2016) where the frequency of GBS colonisation was inversely proportional to higher educational status, thus implying that a higher GBS prevalence may be associated with a lack of knowledge of adequate hygiene practices among mothers with lower educational levels.

In the current study, diabetes and hypertension were not frequently found and showed no effect on GBS colonisation, as in the study in Saudi Arabia where no difference was observed between pregnant diabetic and non-diabetic women (Musleh and Al Qahtani., 2018). Previous studies (Dai *et al*., 2019; Edwards *et al*., 2019) found an association between chronic hypertension and gestational diabetes with GBS colonisation and it was concluded that this may be due to the immunologic impairment related to diabetes (Dai *et al*., 2019).

Although this study found a positive but not significant association between vaginal discharge and GBS carriage, previous studies (Kim *et al*., 2011; Munir *et al*., 2016) reported an increase in GBS colonisation in women with bacterial vaginosis compared with women without bacterial vaginosis. The fact that this study examined for the prevalence of GBS only without a diagnosis of AV or

BV may be perceived as a limitation of the study. Additional studies are needed to better comprehend the vaginal microbiome and GBS colonisation during pregnancy.

There are variable and unexplained outcomes on the influence of parity on GBS colonisation. In this study, a high but not significant prevalence of GBS colonisation was observed in women with reported parity of 0-3 similar to studies from Brazil and Nigeria (Adewumi *et al*., 2017; da Rocha *et al*., 2020). Another study reported an association between GBS colonisation and increasing parity (Musleh and Al Qahtani, 2018), but this was not observed in the current study.

In the present study, gravidity of 0 to 3 was significantly associated with GBS colonisation. Studies in Ethiopia and Nigeria (Mohammed *et al*., 2012; Onipede *et al*., 2012) observed that GBS colonisation was more often associated with primigravida women, while a significantly greater association was found with multigravida women in Korea (Kim *et al*., 2011) and India (Sharmila *et al*., 2011). These geographical differences highlight the need for epidemiologic studies of this nature.

In the present study, previous abortion was not associated with GBS colonisation, as in similar studies from Tanzania and India (Joachim *et al*., 2009; Sharmila *et al*., 2011). However, studies in Korea and Congo showed that a history of spontaneous abortion presented a significant association with GBS colonization (Kim *et al*., 2011; Mitima *et al*., 2014).

Unlike the studies of Patil *et al*., (2013) and Girma *et al*., (2020), this study showed no significant association between GBS carriage and PTD nor between GBS serotypes and PTD although specific serotypes appeared to be increased in FTD when compared with PTD.

In the current study VI was the most predominant serotype, followed III, IV, V. Mothers with the same serotypes as her baby showed that serotypes III and V were the only serotypes transmitted. Serotype III is reported to be the most common serotype in different geographic regions in Africa,

followed by serotypes Ia, Ib, II, and V (Edmonds *et al*., 2012) with serotype V more commonly reported from Egypt (Shabarek *et al*., 2014 and) Morocco (Moraleda *et al*., 2018).

In European countries and USA, serotypes Ia, Ib, III, V are the most commonly found GBS serotypes (Russell *et al*., 2017), while in Jorden the most predominant serotype is III (Clouse *et al*., 2019). Serotypes V and Ia more commonly reported from Kuwait (Boswihi *et al*., 2012) and Saudi Arabia (Mohamed *et al*., 2020) respectively.

A pentavalent Ia, Ib, II, III, and V conjugate vaccine developed by Pfizer (2017), was considered to be effective against GBS (Lin *et al*., 2018). Epidemiological literature confirms that the distribution of serotypes is not constant in all countries (Mukesi *et al.*, 2019) and no vaccine contains all GBS serotypes (Lin *et al*., 2018) with serotypes IV, VI, VII, VIII, and IX missing from the formulation. Therefore it is essential to recognise the serotype distribution in specific regions to apply appropriate control processes and to advance the improvement of specific vaccines against GBS serotypes (Lin *et al*., 2018).

Vertical transmission of GBS affects neonates as the most common infection accountable for sepsis in developing and developed countries; however screening and prophylactic treatments have helped decrease mortality rates, but a clear approximation of disease load in several developing nations remains unrecognized.

The rate of vertical transmission of GBS from mother to the newborn in the current study was (19.4%), much lower than reports from Bangladesh (38.0%, Saha *et al*., 2017) and Kuwait (35.5%, Sweih *et al*., 2005) but higher than that reported from China (7.6% to 16.7%, Chen *et al*., 2018), Germany (11.2%, Kunze *et al*., 2011) and Taiwan (15.1% and 16.7%, Yang *et al*., 2012). However, vertical transmission of GBS is avoidable and thus the use of intravenous intrapartum antibiotic prophylaxis (IAP) during labour may reduce early-onset GBS disease (Edmond *et al*., 2012). Since the focus of this study was on vertical transmission of GBS serotypes and did not include antibiotic sensitivity testing, no comment can be made regarding their antimicrobial profiles and this is viewed as a limitation of the study.

Other limitations of this study include that it only represents a small cohort of pregnant Libyan women within a confined geographical area, and that no follow-up of neonates was performed after the date of delivery, nor was GBS colonisation related to AV or BV diagnosis. A strength of this study is that this is the first of its kind conducted in Libya and it encourages the need for more studies with larger sample sizes and prolonged duration.

Conclusion

This study found that the prevalence of GBS in Libya was not significantly different from other Middle Eastern and African countries although the distribution of serotypes differed. Studies from multiple centres within Libya would provide an interesting comparison.

The application of RT-PCR affords a rapid and accurate detection of GBS serotypes and could inform the use of IAP to reduce neonatal infection.

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Data collection

Sample number: _____

Age: _____ Gravy: _____ Para: _____Wight……

Please specify: full delivery (FTD) ___ or preterm delivery (PTD) ___

Medical History:

Medical History	**tick (√) the correct box**
Hypertension	
Diabetes	

NEONATAL OUTCOME:

SEX	**tic (√) the correct box**
Male	
Female	

Weight birth	**tick (√) the correct box**
≤ 999	
1.000 - 1.999	
2.000 - 2.999	
3.000 - 3.999	
4.000 - 4.499	

If the neonatal has any Complication please specify: ___ ___ ___

DEMOGRAPHIC & LIFESTYLE FACTORS

EDUCATION: - YES ___ NO___

If yes, please which levels do you have? ___ ___ ___

EMPLOYMENT: - YES ___ NO___

Do you have vaginal discharge? YES ___ NO___

Do you have Urinary tract infection? YES ___ NO___

Previous Obstetric History:-

Have you ever been delivery at preterm PTD?

YES ___ NO___

Have you any abortion in pregnancy previous?

YES ___ NO___