DETERMINATION OF GROUP B *Streptococcus* (GBS) SEROTYPES, ITS VIRULENECE GENES AND PATIENT'S CLINICAL PRESENTATION AMONG NON-PREGNANT ADULTS IN KELANTAN

DR FARIDAH BTE SULUNG @ AB HADI

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PATHOLOGY (MEDICAL MICROBIOLOGY)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCLATURE

Symbols/ abbreviations	Meaning
<	Less than
>	More than
C	Degree Celsius
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standard Institute
CPS	Capsular polysaccharide
DM	Diabetes mellitus
et.al.	Et alia (and others)
GBS	Group B *Streptococcus*
GI	Gastrointestinal
GPC	Gram positive coccus
IPC	Intrapartum chemoprophylaxis
n	Number
neg	Negative
NT	Non-typeable
PCR	Polymerase chain reaction
pos	Positive
PRGBS	Reduced penicillin susceptibility GBS
SSTI	Skin and soft tissue infection
US	United State
UTI	Urinary tract infection
β-hemolytic	Beta-hemolytic
µl	Microlitre
µm	Micrometre

ABSTRAK

Group B *Streptococcus* (GBS) serotip dan gen virulensi yang diisolasi dalam kalangan pesakit dewasa yang tidak mengandung di Pantai Timur, Malaysia dan perkaitannya dengan diagnosis klinikal.

Latar belakang: Penyakit jangkitan kuman GBS di kalangan orang dewasa yang tidak hamil kini semakin meningkat. Keseriusan penyakit yang disebabkan oleh jangkitan GBS dipercayai bergantung kepada serotip dan kehadiran gen virulensi pada *strain* tertentu. Kajian ini bertujuan untuk menentukan taburan serotip GBS dan gen virulensi dan untuk mengetahui perkaitan serotip GBS dengan diagnosis klinikal pesakit.

Kaedah: Kajian keratan rentas ini melibatkan pemeriksaan rekod retrospektif telah dilakukan terhadap sejumlah 75 isolat GBS yang dikumpulkan dari Oktober 2018 hingga Oktober 2019 di dua hospital besar di Kelantan (Pantai Timur, Malaysia). Pengasingan tersebut berasal dari pelbagai klinikal sampel orang dewasa berumur > 18 tahun yang tidak hamil (tidak termasuk sampel sapuan faraj). Semua isolat diserotip (serotip Ia, Ib, II hingga IX), dan tujuh gen virulensi (*scpB, lmb*, *bca*, *bac*, *rib*, *cylE,* and *hylE*) dikenalpasti menggunakan ujian PCR konvensional. Ujian antibiotik sensitif diambil dari data *The BIOMIC V3, Microbiology System.* Maklumat klinikal pesakit diperolehi dari rekod perubatan dianalisa dan dibentangkan dalm bentuk graf dan jadual.

Keputusan : GBS lebih banyak diasingkan di kalangan orang dewasa berusia antara 41-60 tahun (66.7%, n = 50). Dari semua isolat yang diuji, serotip III (21.3%, n = 16) adalah yang paling tinggi diikuti oleh serotip Ia (18.7%, n = 14), II (12%, n = 12), dan IV (13.3%, n = 10). Hasil PCR menunjukan gen virulensi *cylE, scpB, lmb, hylE*, dan *bca* ditemui dikebanyakan isolat manakala gen *rib* dan *bac* dikesan pada 10.7% (n = 8) dan 2.7% (n = 2) isolat. Diagnosis klinikal yang paling tinggi direkodkan adalah jangkitan kulit dan tisu lembut (66.7%, n = 50); dengan perkaitan sangat yang tinggi dengan serotaip Ia (22%, n = 11). Kencing manis adalah faktor risiko utama penyakit GBS (76%, n = 57). Sebilangan besar isolat (98.7%, n = 73) sensitif terhadap antibiotik *penicillin*. Satu isolat serotip IV mempunyai kurang kerintangan terhadap penicillin. Kadar rintangan antibiotik *clindamycin* dan *erythromycin* masing-masing adalah 9.3% (n = 7) dan 6.7% (n = 5). Kadar kematian adalah 2.7% (n = 2). Tiada perkaitan diperolehi di antara serotip GBS yang paling banyak, dan diagnosis klinikal.

Kesimpulan: Serotype III adalah serotip yang paling banyak dikesan di kalangan orang dewasa yang tidak hamil yang dijangkiti kuman GBS. GBS pada umumnya menjangkiti orang dewasa yang menghidap kencing manis dan menghidap jangkitan di kulit dan tisu lembut.

Kata kunci: GBS, tidak hamil, serotip, gen virulensi

ABSTRACT

Serotype and virulence genes of Group B *Streptococcus* (GBS) isolated among non-pregnant adults in East Coast Malaysia and its association with patient's clinical diagnosis.

Background: GBS infection among non-pregnant adults is emerging nowadays. The severity of the disease caused by GBS depends on the serotype and presence of virulence genes in the particular strains. This study aimed to determine the distribution of GBS serotypes and virulence genes and to know the association of GBS serotype with the clinical disease.

Methods: A cross-sectional study involving retrospective record review was done involving a total of 75 GBS isolates collected from October 2018 till October 2019 in two major hospitals in Kelantan (East coast of peninsula Malaysia). Those isolates were from various clinical samples of non-pregnant adults > 18 years old (excluding vaginal swab). Identification of all isolates serotype (serotype Ia, Ib, II until IX) and their virulence genes (*scpB, lmb, bca*, *bac*, *rib*, *cylE,* and *hylE*) were done using conventional PCR. The antibiotic susceptibility testing was traced from The BIOMIC V3, Microbiology System. Clinical information of patient was assessed from medical records and were analysed and presented as tables and figures.

Results*:* GBS was commonly isolated among adults aged between 41-60 (66.7%, n=50). Of all the isolates tested, serotype III (21.3%, n=16) was the most common followed by serotype Ia (18.7%, n=14), II (12%, n=12), and IV (13.3%, n=10). Virulence gene PCR showed *cylE*, *scpB*, *lmb*, *hylE*, and *bca* were discovered in most isolates while *rib* and *bac* were detected in 10.7% (n=8) and 2.7% (n=2) of the isolates. The most common clinical diagnosis was skin and soft tissue infection (66.7%, n=50); it was mainly associated with serotype Ia (22%, n=11). Diabetes mellitus (DM) was the leading risk factor for GBS disease (76%, n=57). The majority of the isolates (98.7%, n=74) were penicillin-sensitive. One of the serotype IV isolates had reduced susceptibility to penicillin. The resistance rate to clindamycin and erythromycin were 9.3% (n=7) and 6.7% (n=5), respectively. The mortality rate was 2.7% (n=2). There was no association between the most common GBS serotypes, and clinical diagnosis.

Conclusion: Serotype III was the most common serotype detected among non-pregnant adults infected with GBS. GBS generally infected adults with underlying DM who had skin and soft tissue infections.

Keywords: GBS, non-pregnant, serotype, virulence gene

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Group B *Streptococcus* (GBS) or also known as *Streptococcus agalactiae*, is a gram-positive bacterium, one of the leading causes of severe infections in neonates and pregnant women in the 1970s. Since the implementation of intrapartum chemoprophylaxis (IPC) in the 1990s, the incidence rate of early-onset neonatal GBS has been reduced (Skoff et al., 2009). There was an increase in GBS infections among non-pregnant adults in the subsequent era, particularly in patients > 65 years of age with existing underlying medical diseases.

The incidence rate in the United States (US) has risen from 8.1 to 10.9 cases per 100,000 population over 8years of period (Watkins et al., 2019). The fatality rate was 6.5% (Watkins et al., 2019). The epidemiology of GBS disease in non-pregnant adults has not been extensively studied in Malaysia. A study in Malaysia reported that GBS infection which was non-pregnancy related was higher among two major ethnic groups; Malay and Indian races. This finding correlates with the diabetes status among this ethnicity (Karunakaran et al., 2009).

Sepsis, meningitis, and pneumonia are the primary clinical syndromes in neonatal GBS infection. However, this clinical presentation is less seen in non-pregnant adults. The most common clinical entities are skin and soft tissue infection (SSTI) and bacteremia without a focus (Chaiwarith et al., 2011; High et al., 2005; Raabe and Shane, 2019). DM is the main risk factor for GBS infection (Watkins et al., 2019; High et al., 2005; Karunakaran et al., 2009; Lopes et al., 2018a; Paveenkittiporn et al., 2020).

To date, ten GBS serotypes have been identified: Ia, Ib, II to IX. Determining GBS serotype distribution in a particular population and geographical area is crucial for vaccine development strategies. The most prevalent GBS serotypes in the US were type V, Ia, II, and III. However, serotype VI was higher in Malaysia, followed by VII, III, and Ia (Eskandarian et al., 2015). The serotype distribution continues to vary depending on the geographical area, time, source of the isolates, and population.

Penicillin is the primary antibiotic for IPC and treatment of GBS infection. For patients with penicillin allergy, clindamycin or erythromycin is the substitute treatment (Watkins et al., 2019; Hanna and Noor, 2020; Karunakaran et al., 2009). GBS is generally sensitive to penicillin. Penicillin resistance is extremely rare. However, since 2008, isolates with reduced susceptibility to penicillin have been discovered.

1.2 Literature Review

1.3 Group B *Streptococcus* (GBS) taxonomy

GBS is among the most common pathogen isolated from daily culture specimens taken, particularly from high vaginal swabs of pregnant women. This streptococci species belong to the Lancefield group B. It is a gram-positive coccus (GPC) in chain bacterium, facultative anaerobe, and exhibit beta-hemolytic (ß-hemolytic) appearance on blood agar.

Taxonomically, the genus *Streptococcus* belongs to the bacteria family of Streptococcacea with the two less common genera; Lactococcus and Lactovum. The details taxonomic classification of *Streptococcus agalactiae* is shown in the hierarchy as below:

Domain: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Lactobacillales

Family: Streptococcacea

Genus: *Streptococcus*

Species: *Streptococcus agalactiae*

1.4 History of GBS infection

GBS was first isolated as a causative agent of bovine mastitis due to agalactia (Shabayek and Spellerberg, 2018). Agalactia means lack of milk production in Latin. It was identified in 1887 by Edmond Nocard, a French veterinarian and microbiologist (Hanna and Noor, 2020). Initially, this pathogen was known as *Streptococcus mastitis* until 1933, Rebecca Lancefield produced serological identification of streptococci. She identified this pathogen as Group B *Streptococcus*. In 1938, Fry reported the first case of

fatal human GBS infection occurred in a pregnant woman (Shabayek and Spellerberg, 2018). Since then, GBS was recognized as a significant human pathogen, especially in pregnant adults and newborns. GBS emerged as the most important causative agent of invasive neonatal infection in the 1970s, and it was considered rare in non-pregnant adults (Hanna and Noor, 2020; Rodríguez et al., 2010; Shabayek and Spellerberg, 2018). The first case of GBS disease in non-pregnant adults was reported in 1940 by Ranz. The patient was a diabetic elderly with septic arthritis (Rodríguez et al., 2010). For the past three decades, this trend subsequently shifted worldwide after the implementation of the antenatal screening program, and IPC in GBS carrier mother. Cases among pregnant women and neonates reduced in trend and increased trend seen in cases of GBS disease among non-pregnant adults.

1.5 Epidemiology of GBS among non-pregnant adults

The incidence rate of GBS disease in non-pregnant adults has risen two to four times over the past three decades. In the US, the incidence rate rose from 8.1 in 2008 to 10.9 cases per 100,000 population in 2016 (Francois Watkins et al., 2019). The GBS incidence varied by age, sex, and race. Age > 65 years had a higher incidence of GBS relative to the younger age group. The incidence was higher among men than women. Overall, blacks had a greater incidence than whites, with a fatality rate of 7.4% in blacks and 6.3% in whites (Francois Watkins et al., 2019). A comparison study done by Chaiwarith et al. reported the mortality rate range from 3 to 30% (Chaiwarith et al., 2011). This variation depends on the severity of the infection, whether it was associated with bloodstream infection, hypotension, concurrent infection, virulence strain, and older age.

For the Malaysian population, local data showed that higher incidence was seen among Indian and Malay ethnicities at 44.9% and 40.8%, respectively. The incidence were lower in Chinese (14.3%) (Karunakaran et al., 2009). It correlates with diabetes status among the ethnicities in Malaysia, which were higher in Malays and Indians. The 41-60 years old age group was more affected in Malaysia compared to worldwide incidence, which more likely occurred in elderly > 65years old (Karunakaran et al., 2009).

1.6 Colonization and transmission of GBS

GBS typically inhibits the human gastrointestinal (GI) tract and genitourinary tract. Occasionally, it colonizes the upper respiratory tract. Up to 15-35 % of males and female GI tract have been colonized with GBS. About 20-30% of healthy women have GBS colonization of the vaginal tract. Urinary tract colonization is less frequent compared to the genital area. GBS vaginal colonization is likely from the GI area, while an ascending route from the vagina causes GBS urinary tract colonization (Shabayek and Spellerberg, 2018; Ulett et al., 2009).

Asymptomatic healthy women with GBS colonization is usually harmless. However, colonization in pregnant women may lead to harmful consequences. It causes early-onset neonatal GBS either *in utero* through ascending infection or intrapartum caused by neonatal aspiration of contaminated amniotic or vaginal fluids (Ulett et al., 2009). For neonates, neonatal colonization occurs in 50-70% of vaginal-delivery from GBS positive mother. GBS invasive disease is seen in 1-2% of cases. (Spencer et al., 2019).

GBS colonization in pregnancy can be intermittent and transient. Colonization may be present in early or mid-pregnancy but absent during delivery. The rate of vagina and rectum colonization among pregnant women is comparable worldwide (Shabayek and Spellerberg, 2018). The factors that induce colonization include African American origin, obesity, promiscuity, fellatio, sexually active, tampon user, and irregular handwashing (Hanna and Noor, 2020; Sendi et al., 2008).

A study of the colonization rate in healthy young men and women reported that 34% of women and 20% of men have GBS carriage at one or more sites (urine, vaginal, anal orifice, and throat). A higher rate of GBS colonization was seen in the sexually experienced compare to inexperienced students. This situation indicates GBS can be transmitted through intimate contact (Manning et al., 2004). It was supported by an analysis of 86 % of co-colonized sexual couples that had similar GBS strains (Sendi et al., 2008).

A study by Sarah Shabayek et al. reported serotype Ia, Ib, II, III, and V were the predominant colonizers in the US, Europe, and North America (Shabayek and Spellerberg, 2018). In contrast to Malaysia, serotype Ia and VI were the most prevalent colonizing strain in adults (Karunakaran et al., 2009).

1.7 Pathogenesis of GBS disease in non-pregnant adults.

The severity of GBS infection depends on virulence factors. The more virulence factors in the particular GBS isolate, the more virulence it has. Polysaccharide capsule is the main element responsible for virulence in GBS. The other GBS virulence factors are surface proteins, hydrolytic enzymes, and toxins. The component of surface protein and enzyme are Rib protein (*rib*), alpha and beta-antigens of C protein (*bca* and *bac*), laminin-binding protein (*lmb*), and C5a peptidase (*ScpB*), which function as an adhesin (Eskandarian et al., 2015). β-hemolysin/cytolysin (*cylE*), hyaluronidase (*hylE*) and the CAMP factor (*cfb*) are the toxin produced by GBS. These toxins promote the entry of the pathogen into the cells. GBS virulence factors and its specific function are summarized in Table 1.1

A study by Kevin et al. suggested that the pathogenesis of invasive GBS disease in non-pregnant adults, mainly in the elderly were caused by several factors. The proposed pathogenesis is shown in Figure 1.1. The factors involved in the pathogenesis of GBS disease are an underlying chronic condition, altered integrity of anatomical barrier, immunity status, delay in diagnosis, and reduction in physical capacity (High et al., 2005).

Chronic disease like DM is associated with phagocyte function abnormalities. Hyperglycemic state and the presence of glycation end products will reduce the neutrophil response. Patients with underlying diabetes may have complications such as peripheral neuropathy and peripheral vascular disease (High et al., 2005). Injury to the lower extremities alters the skin and mucous membrane integrity, which allows the colonising GBS to penetrate the skin or mucous membrane and cause skin and soft tissue infection.

Bedridden and hospitalized patients with poor oral hygiene, swallowing, and speech disabilities have a higher tendency for GBS oral colonizers (High et al., 2005). ß-hemolysin/ cytolysin secreted by GBS induce damage to lung epithelial cells and cause

GBS pneumonia. Strict oral hygiene may theoretically minimize the risk for GBS pneumonia.

Immune status is compromised with advanced age. However, the degree of impaired T-cell proliferation and decreased T-cell mediated Th1 cytokine reactions that facilitate GBS virulence has not been studied (High et al., 2005). A weakened immune process may delay in diagnosis of GBS infection. The elderly patients usually present with vague symptoms, and they cannot express specific symptoms due to dementia. Besides that, the febrile response is blunted in the elderly, even in bacteremia. These factors further promote GBS infection.

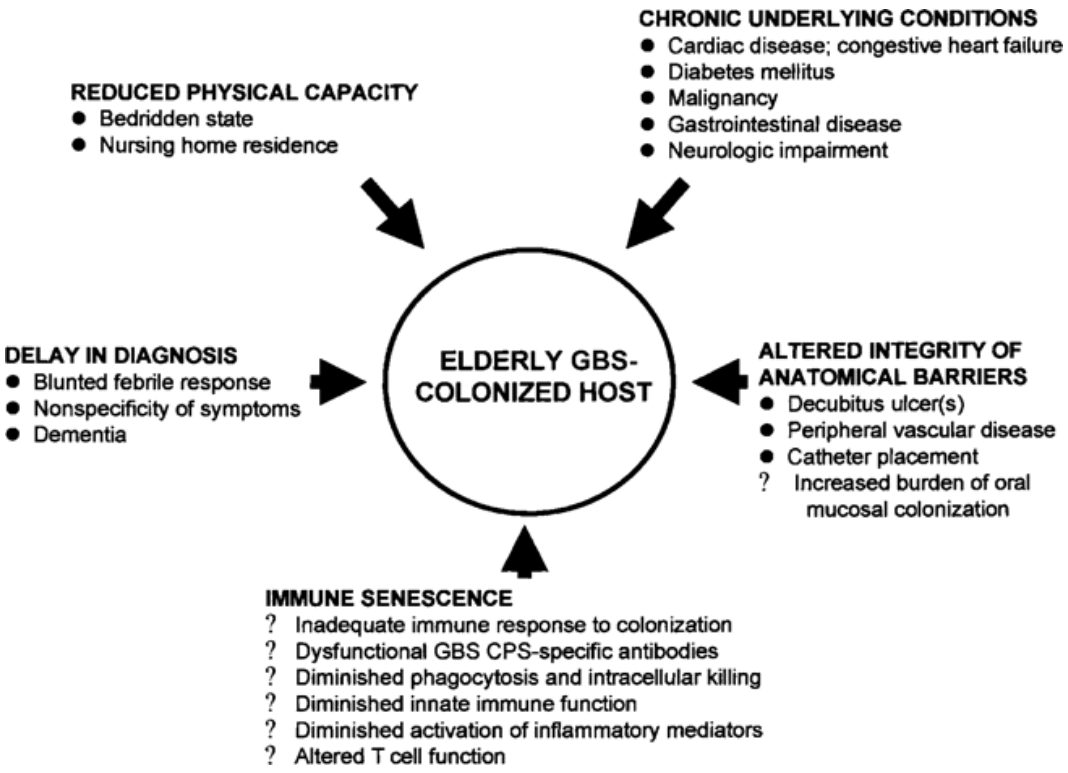

Figure 1.1: Proposed pathogenesis of invasive GBS infections in elderly. [Adapted from (High et al., 2005)]

Table 1.1: Virulence factors of Group B *Streptococcus* (adapted from George)

Virulence factor	Genes	Molecular or cellular action	Proposed contribution to disease pathogenesis
Beta-hemolysin/ Cytolysin	c*yl*E	– Forms pores in cell membranes induces apoptosis – Promotes cellular invasion triggers iNOS, cytokine release	– Direct tissue injury penetration of epithelial barriers induction of sepsis syndrome
Hyaluronate lyase	h*yl*B	– Cleaves hyaluronan and chondroitin sulfate	– Spread through host tissues
C5a peptidase	*scp*B	– Cleaves human C5a binds fibronectin	– Inhibit PMN recruitment reduce opsonophagocytosis host cell attachment/invasion
CAMP factor	*cfb*	– CAMP reaction (co-hemolysin) – Binds to Fc portion of IgG, IgM	– Direct tissue injury – Impairment of antibody function
C protein (α and β components)	*bca* (α) *bac* (β)	– Binds epithelial cells – Blocks intracellular killing by neutrophils – Non-immune binding of IgA	– Epithelial cell adherence Epithelial cell invasion – Resistance to phagocytic clearance
Alpha-like protein (Alp) family	*bca, eps, rib, alp*2, *alp*3	– Binds epithelial cells – Suffers antigenic variation as evasion mechanism of antibody detection	– Epithelial cell adherence Epithelial cell invasion
Fibrinogen binding proteins A and B	*fbs*A *fbs*B	– Binds ECM fibrinogen through repetitive structure motifs	– ECM attachment – Epithelial adherence – Promotes entry of GBS into host cells
Pili	PI-1 PI-2a PI-2b	– Promotes resistance to antimicrobial peptides by an unknown mechanism	– Promotes adherence of GBS to host cells

1.8 Risk factor for GBS disease in non-pregnant adults

95% of the cases had at least one underlying medical condition (Watkins et al., 2019). The important predisposing condition includes DM, malignancy, liver cirrhosis, stroke, congestive heart failure, chronic kidney disease, bedridden, and residence in the nursing home (Eskandarian et al., 2013; Hanna and Noor, 2020; Sendi et al., 2008). Those with DM, older age > 65years, cancer, and immunocompromised are at risk for acquiring invasive GBS disease (Karunakaran et al., 2009; Ruppen et al., 2018; Sendi et al., 2008).

1.9 GBS disease in non-pregnant adults

Group B streptococcus is a significant causative agent in neonates and pregnant adults, but now the cases have emerged among non-pregnant adults. Patients with GBS infection unrelated to pregnancy may present with primary bacteremia without focus (39.3%), 25.5% was SSTI, pneumonia about 12.5%, bone (9.4%), or joint infection (7.8%) and urinary tract infection (UTI). The least common clinical diagnosis was endocarditis (3.0%) and meningitis (1.6%) (Skoff et al., 2009).

1.9.1 Primary bacteremia without primary focus

Primary bacteremia without primary focus was found in 20% to 50% of GBS disease cases (Bennett et al., 2014). It was considered as primary bacteremia when this pathogen was isolated from the blood culture, but there was no evident site of infection. The patient may present with fever, chills, and alter in mental status. It was associated with a high fatality rate.

1.9.2 Skin and soft tissue infection (SSTI)

SSTI is the most common cause of GBS infection in non-pregnant adults (Eskandarian et al., 2013; Karunakaran et al., 2009; Ruppen et al., 2018). It usually manifests as infected ulcers (11.3%), cellulitis or erysipelas and wound infection accounts for 1.7% each while abscess and necrotizing fasciitis was about 1.1% (Paveenkittiporn et al., 2020). DM is the main risk factor for SSTI (Ruppen et al., 2018). Loss of skin integrity in diabetic patients as a consequence of peripheral neuropathy promote GBS to penetrate the skin and mucous membrane causing infection. Early wound debridement or drainage with adequate coverage of antimicrobial therapy results in a good outcome. In the US, GBS invasive disease was typically seen in SSTI, rising from 27.2% in 2008 to 34.0% in 2016 (Francois Watkins et al., 2019).

1.9.3 Pneumonia

GBS pneumonia is common among adults with DM, nursing home, bedridden and neurological disease (e.g., cerebrovascular accident, and dementia) (Farley and Strasbaugh, 2001). I t was associated with GBS oral colonizer (High et al., 2005). The chest x-ray reveals bilateral or lobar infiltration in many patients. Most of the GBS pneumonia is associated with polymicrobial with GBS as the main organism. Patient GBS pneumonia may complicate with pleural empyema. Fatality rate is about 30% - 85%.

1.9.4 Meningitis

GBS meningitis is the usual presentation in neonates but uncommon among non-pregnant adults. GBS is the causative pathogen in 4% of adult bacterial meningitis (Farley and Strasbaugh, 2001). Mostly seen in postpartum women, elderly, and adults with underlying medical illness. Among the meningitis survival, 7% may complicate with

deafness. Those present with coma and shock had a poor outcome. The case fatality rate is about 27% to 34% (Farley and Strasbaugh, 2001).

1.9.5 Urinary tract infection

GBS are significant uropathogen in both pregnant and non-pregnant adults (Muñoz et al., 1992). UTI account for about 5% to 23% of invasive GBS disease in non-pregnant adults (Farley and Strasbaugh, 2001). GBS UTI manifests as asymptomatic bacteriuria, cystitis, pyelonephritis, urethritis, and urosepsis. Advanced age, underlying condition (e.g., DM, chronic renal failure), on an indwelling catheter, history of urinary tract infection, urinary tract structural anomalies either congenital or acquired (e.g., stone), and prostatic disease (e.g., benign prostate hyperplasia, prostate tumor), as well as neurogenic bladder, are among the risk factors for GBS UTI (Farley and Strasbaugh, 2001; Muñoz et al., 1992). The possible complications of GBS UTI are pyelonephritis and renal abscess. A study reported that GBS UTI among non-pregnancy related were higher among elderly > 70 years (39%) compared to patients aged 15 to 70 (Farley and Strasbaugh, 2001).

1.9.6 Osteomyelitis

GBS osteomyelitis is rarely reported. It occurs via hematogenous seeding, contiguous spread or direct inoculation. Adjacent focal of infection, vascular insufficiency, overlying ulcer or history of orthopaedic surgery are among the risk factors of GBS osteomyelitis (Farley and Strasbaugh, 2001). It mainly involves bones of vertebra, foot, hip, tibia, and toes. Those with diabetic foot ulcers have a high tendency to get GBS osteomyelitis of the foot.

1.9.7 Arthritis

GBS septic arthritis is an orthopaedic emergency that results in sepsis, limb loss, and a high mortality rate. In the past few years, GBS has been identified as a significant pathogen in adult septic arthritis after *Staphylococcus aureus.* Typically, it is monoarticular involvement in two-third of the patients. However, a recent study in 2018 reported that patients with GBS arthritis predominantly presented multiple joint involvements (Wang et al., 2018). Knee and shoulder is the common site for septic arthritis. It may affect any other joints, including small joints like sternoclavicular or wrist joints. Prosthetic joints are also at risk for GBS arthritis, and the majority of patients had the infection three months after the prosthesis surgery. GBS arthritis is usually associated with patients with underlying joint disease; rheumatoid arthritis, DM, and the elderly.

1.9.8 Endocarditis

Bacterial endocarditis infrequently caused by GBS (Baddour, 1998). It infects patient with underlying heart diseases like valvular disease and rheumatic heart disease or without heart illness (e.g., intravenous drug user, DM, and malignancy). The vegetations of GBS endocarditis usually large, friable, and easily embolize at an early stage (Farley and Strasbaugh, 2001). The most common site for vegetation was the mitral valve (48%), followed by aortic (29%), mitral and aortic (10%), and tricuspid valves (5%) (Bennett et al., 2014). The mortality rate is between 35% to 50%. Larry et al. reported a lower mortality rate (12.6%) due to fewer intravenous drug abuser (Baddour, 1998). Longer duration of medical therapy and aggressive surgical intervention also reduced the mortality rate.

1.10 Distribution of GBS serotypes

Many countries have done researches to identify the GBS strains implicated in GBS disease in non-pregnant adults. The distribution of serotypes is an essential target for vaccine development strategies (Dutra et al., 2014). GBS strains have been classified into serotypes based on structural variations between sialic acid-rich capsular polysaccharide (CPS) and surface protein antigens. CPS is part of the virulence factor. It plays a crucial role in immune invasion, disturbs the complement-dependent pathways, inhibits the phagocytic activity of neutrophil and influence biofilm formation of GBS (Shabayek and Spellerberg, 2018). Nine serotypes that have been discovered; serotype Ia, Ib, and II till VIII (Sendi et al., 2008). Recently, the classification has evolved, and the latest serotype was recognized worldwide was serotype IX. The serotypes were varied based on time, population, and geographical area.

Between 2005 to 2006, the most prevalent GBS serotypes in the US were type V (29.2%), followed by Ia, II, and III (24.3%, 13.5%, and 11.4%, respectively).

A prevalence study among non-pregnant adults in Tehran, Iran, showed that the prevalence of GBS among pregnant and non-pregnant was reliable and similar. Serotype III (36%) was the most common serotype identified in this study, followed by serotype II (32%), Ia (26%), and Ib (6%) (Goudarzi et al., 2019).

In southeast Asia, Malaysia's data in 2009 demonstrated that the majority of GBS isolates were from serotypes Ia (22.3%) followed by VI (17.8%) III and V (13.3% each) (Karunakaran et al., 2009). Five years later (2014), the trend was changed with serotypes VI was more prevalent (22.3%) followed by VII (21.4%), III (20.4%), and Ia (17.5%) (Eskandarian et al., 2015). The earliest GBS study done in Thailand reported that the most predominant serotypes were serotype III (87.15%), Ia (5.2%), and V (3.81%). The latest study published in 2020 showed serotype III and V still the most common serotype

isolated in non-pregnant adults in Thailand, 46.4%, and 21%, respectively (Paveenkittiporn et al., 2020).

Analysis done by Paveenkittiporn et al. showed serotype III also be the most dominant serotype isolated among non-pregnant in other countries like Sweden, South Africa, Gabon, Japan, China, and Taiwan (Paveenkittiporn et al., 2020). Meanwhile, in Singapore, the Ministry of Health of Singapore has reported an outbreak of GBS bacteremia in 2015. It was related to the consumption of yusheng; a raw fish dish of the Chinese-style which was contaminated with GBS serotype III (Tan et al., 2016).

1.11 Laboratory detection of GBS

There are several methods available in most microbiology laboratories for the detection of GBS. The conventional approach has been used for many years in detecting GBS. This included colony appearance on blood agar, biochemical tests, and Lancefield grouping. Biochemical tests used for presumptive identification of GBS consist of CAMP (Christie, Atkinson, Munch, Peterson) test, bacitracin, or trimethoprim-sulfamethoxazole testing, Hippurate test, and PYR (pyrrolidonyl arylamidase) test. Novel diagnostic methods for GBS identification are matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), molecular techniques, and chromogenic culture media, including Granada-type media.

1.11.1 Colony morphology and culture methods.

GBS can be cultivated on enriched media; blood agar. After 18-24hours of incubation, it forms large (3 to 4 mm in diameter) whitish-grey colonies surrounded by a narrow or less pronounced zone of ß-haemolytic compare to other ß-haemolytic *streptococci* as shown in Figure 1.2. For some GBS strains, the haemolysis is generally

less obvious and can be seen when detached from the blood agar. Up to 11% of the GBS were non-haemolytic.

For Granada agar, GBS colonies will appear as brick or orange-red colonies. Typical ß-haemolysis will be seen on blood agar. However, if ß-haemolysis not seen, the plate should be reincubated overnight. The Lancefield grouping or CAMP test is used to confirm GBS-like colonies from blood agar or Granada agar. Further testing should be done to non-pigmented, and non-haemolysis GBS strains for confirmation (Rosa-Fraile and Spellerberg, 2017).

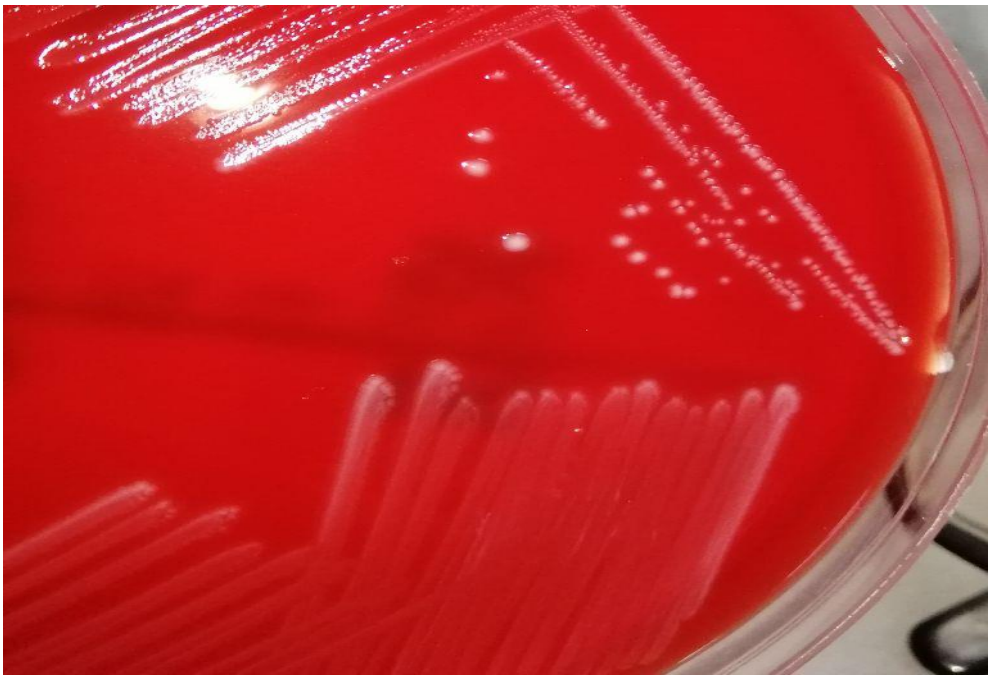

Figure 1.2: whitish-grey colonies surrounded by a narrow zone of ß-haemolytic. The hemolytic zone are less pronounced for this colonies

1.11.2 Biochemical test

There are several biochemical tests available to identify the GBS:

a. Hippurate test

Hippurate test is one of the presumptive identification of GBS. This test is used to differentiate ß-haemolytic GBS from other ß-haemolytic. The purpose of the Hippurate test is to detect the production of enzyme hippuricase. The test is positive when the solution appears deep purple and negative if colourless or slightly yellow pink. A false-positive result may occur if incubation with ninhydrin exceeds 30 minutes (Hall, 2013). The sensitivity of the Hippurate test was 77.78% while specificity was 50% (Nsagha et al., 2000).

b. CAMP test

The CAMP test is used to classify GBS from other species of ß-haemolytic *streptococcus* (Hall, 2013). GBS isolates produce a cytolytic toxin known as the CAMP factor. The CAMP factor is one GBS virulence factor encoded by the *cfb* gene. CAMP factor has a synergistic effect when it combines with the ß-lysin of *Staphylococcus aureus*. The GBS colonies are streaked at the right angle of *Staphylococcus aureus* streak on sheep blood agar and incubate overnight. The test is positive when an arrowhead zone of ß-haemolytic appears at two bacteria's intersection. This enhancement of ß- haemolytic activity is seen in both haemolytic and non-haemolytic GBS strains. In some laboratories in which Lancefield grouping is not available, the CAMP test is considered a confirmatory test for GBS identification. The CAMP test is also positive for *Listeria monocytogenes.*

c. PYR test

The PYR test is used to determine the ability of bacteria to produce pyrrolidonyl arylamidase enzymes. It is a rapid test for presumptive identification of Group A *Streptococci* and *Enterococci* species (Hall, 2013; Spellerberg and Brandt, 2016). The test is positive for both organisms but yields negative results for GBS.

d. Bacitracin susceptibility test

Bacitracin susceptibility is a test used to differentiate between group A ß-haemolytic *Streptococci* from non-group A ß-haemolytic (Hall, 2013). Group A is susceptible to bacitracin while non-group A is resistant to bacitracin. Bacitracin is produced by *Bacillus licheniformis*. Its function to break the peptidoglycan. A negative test (no zone of inhibition) indicates GBS isolated, while a positive (zone of inhibition >10mm) test belongs to *Streptococcus pyogenes* (Hall, 2013). Bacitracin test and Lancefield grouping test are used together for better specificity in the identification of *Streptococcus pyogenes* and GBS.

e. Lancefield grouping

The Lancefield grouping system was established by Rebecca Lancefield and was used as a serological grouping for classifying ß-haemolytic streptococci. ß-haemolytic streptococci were categorized into six groups; Group A-G. ß-haemolytic streptococci strains are classified based on their cell surface carbohydrate antigens. This system detects antigens either at the cell wall polysaccharides for groups A, B, C, F, and G streptococci or cell wall lipoteichoic acid for group D streptococci. The original Lancefield grouping used the precipitin method, but it has been switched with enzymatic extraction techniques (Bannoehr et al., 2009). The marketed streptococcal grouping kits

now are either coagglutination or latex particle agglutination. A rapid and strong clumping of the latex particles indicates a positive reaction. Insufficient amounts of colonies or extraction reagents used may give false negative or false-positive reactions. *Streptococcus agalactiae* form agglutination to group B Lancefield grouping.

f. Group B *Streptococcus* serotyping

Several conventional methods available for serotyping, this include immunoprecipitation, enzyme immunoassay, coagglutination, latex agglutination, counterimmunoelectrophoresis and capillary precipitation, fluorescence microscopy, and inhibition enzyme-linked immunosorbent assay (Kong et al., 2002). Latex agglutination is the most widely used compared to other techniques. It is a serotype-specific antibody to the 10 identified GBS capsular polysaccharides (Ia, Ib, and II to IX).

Serological approaches have some drawbacks, as they are labour-intensive and costly as it requires high-titre of specific antibodies. Certain strains have a lack of detectable or low expression of capsular polysaccharide. These strains fail to be identified and considered as non-typeable (Kong et al., 2002; Yao et al., 2013).

The molecular serotype is an alternative to serotype GBS isolates, especially for non-typeable strain by latex agglutination. The benefits of PCR are good reproducibility, technological efficiency, availability equipment and reagents, and quick turnaround time.

1.12 Treatment of GBS

GBS isolates are generally considered susceptible to penicillin. Penicillin is the first-line antibiotic for intrapartum prophylaxis and GBS treatment unless the patient has penicillin allergy (Seki et al., 2015). Those with penicillin-allergy, clindamycin is the substitute treatment. Most of the isolates are susceptible to ampicillin, extended-spectrum penicillin, and first and second-generation cephalosporin.

GBS isolates with reduced susceptibility to penicillin are rarely reported. The emergence of penicillin resistance a few years back start to raising concern. Tomomi et al. reported the isolation rate of reduced penicillin susceptibility was increased from 2.3% in 2005 to 14.7% in 2013 (Seki et al., 2015). Penicillin resistance associated with a mutation in the penicillin-binding proteins (PBPs). Mutation in PBPs lead to resistance to other B-lactam antibiotic and tend to be non-susceptible to macrolides and fluoroquinolone, which results in multi-drug resistance GBS. (Seki et al., 2015).

Higher resistance rates to macrolide and lincosamide were also reported worldwide. A study in Portugal detected the resistance rate for erythromycin was 35.1%, and clindamycin was 33.9% (Lopes et al., 2018b). The resistance genes associated with macrolide-resistance are *erm*(B), *erm*(A), *erm* (T), and *mef* [*mef* (A) or *mef* (E)]. The patient infected with macrolide-resistant GBS, 39% of the isolates had *erm*A, and 27.6% was *erm*B (Lopes et al., 2018b).

The duration of treatment depends on the severity of the illness and clinical diagnosis. Proper guideline for the optimum course of antibiotic therapy is not established yet. Patients with invasive GBS disease, a minimum of two weeks of treatment is considered. Whereas, GBS endocarditis and osteomyelitis required longer antimicrobial therapy, at least four weeks (Farley and Strasbaugh, 2001).

1.13 GBS prevention strategies

GBS infection is preventable in non-pregnant adults. Unfortunately, there is no established preventive action for the prevention of GBS disease in this population. Control and prevention methods that are currently available primarily focused on neonates and pregnant mothers. The preventive measures recommended by the Centers for Disease Control and Prevention (CDC) for pregnant adults is the administration of IPC to GBS colonising mothers to avoid vertical transmission during childbirth (Horsley, 2011). For high-risk neonates, the CDC suggested empirical treatment with ampicillin and gentamicin. ß-lactams antibiotics (e.g. penicillin) are the antimicrobials of choice. However, the widespread use of these B-lactams contributes to the production of antibiotic resistance in GBS isolates. To date, there is no antibiotic prophylaxis that has been proposed for the prevention of GBS disease in non-pregnant adults at risk.

In general, to prevent serious GBS infection in high-risk patients (e.g., DM, bedridden, and elderly), we need to educate them to do proper skin care to prevent diabetic foot or pressure ulcers. The diabetic patient must be educated regarding foot care and foot ulcer treatment to avoid serious complications. Caretaker of the bedridden patient should know preventive measures to avert chronic pressure which may prevent the formation of decubitus ulcer (Farley and Strasbaugh, 2001).

In the future, vaccination will be an alternative for the prevention of GBS disease. However, none of the vaccines have been approved for use in clinical settings. It is still under development and clinical trials. It will be one of the promising methods for the prevention of both neonatal GBS and GBS in non-pregnant adults once it is available.

1.14 The rationale of the study

GBS is a well-known cause of neonatal sepsis and infections in pregnant women. However, the clinical significance of this organism among non-pregnant women has not been extensively studied in developed countries, including Malaysia. There are no prevalence studies done in Malaysia before. Local data for GBS disease in non-pregnant adults is limited. Study from various literature showed that particular serotype have cause invasive GBS disease. Worldwide epidemiological studies reported the serotypes vary depending on region, time and population. The most common serotype reported was serotype III. Though, a local study showed that the most common serotype was Ia (22.2%) (Karunakaran et al., 2009).

In most developed countries, GBS serotyping is not routinely done for surveillance purposes, probably due to expensive cost and lack of awareness regarding the significance of serotype to the clinical impact of GBS infection. Few studies did the correlation between serotypes and clinical diagnosis. Furthermore, the emergence of penicillin and macrolide resistance raise concerns among researchers. They began to correlate the severity of illness and antibiotic resistance strain with the serotypes. Besides that, serotype distribution is fundamental for vaccine formulation. GBS vaccine is still under development. The prevalence of GBS serotype aids vaccinology to make effective vaccine.

The findings of this study will determine the distribution of GBS serotypes, its infection characteristics that include risk factors, clinical diagnosis and association with clinical outcomes among non-pregnant adults. This study can provide baseline data for future reference and provide evidence to help the clinicians treat GBS infection in non-pregnant adults.

1.15 Objective of the study

1.15.1 General objective

To describe GBS serotypes distribution, its infection characteristics and association with clinical diagnosis in non-pregnant adults.

1.15.2 Specific objective

1. To describe the distribution of positive GBS isolates from various clinical samples and its serotypes
2. To describe the underlying medical condition and clinical diagnosis among non-pregnant adults infected with GBS
3. To determine the association between GBS serotypes and clinical diagnosis of infection.

1.16 Flow of the study

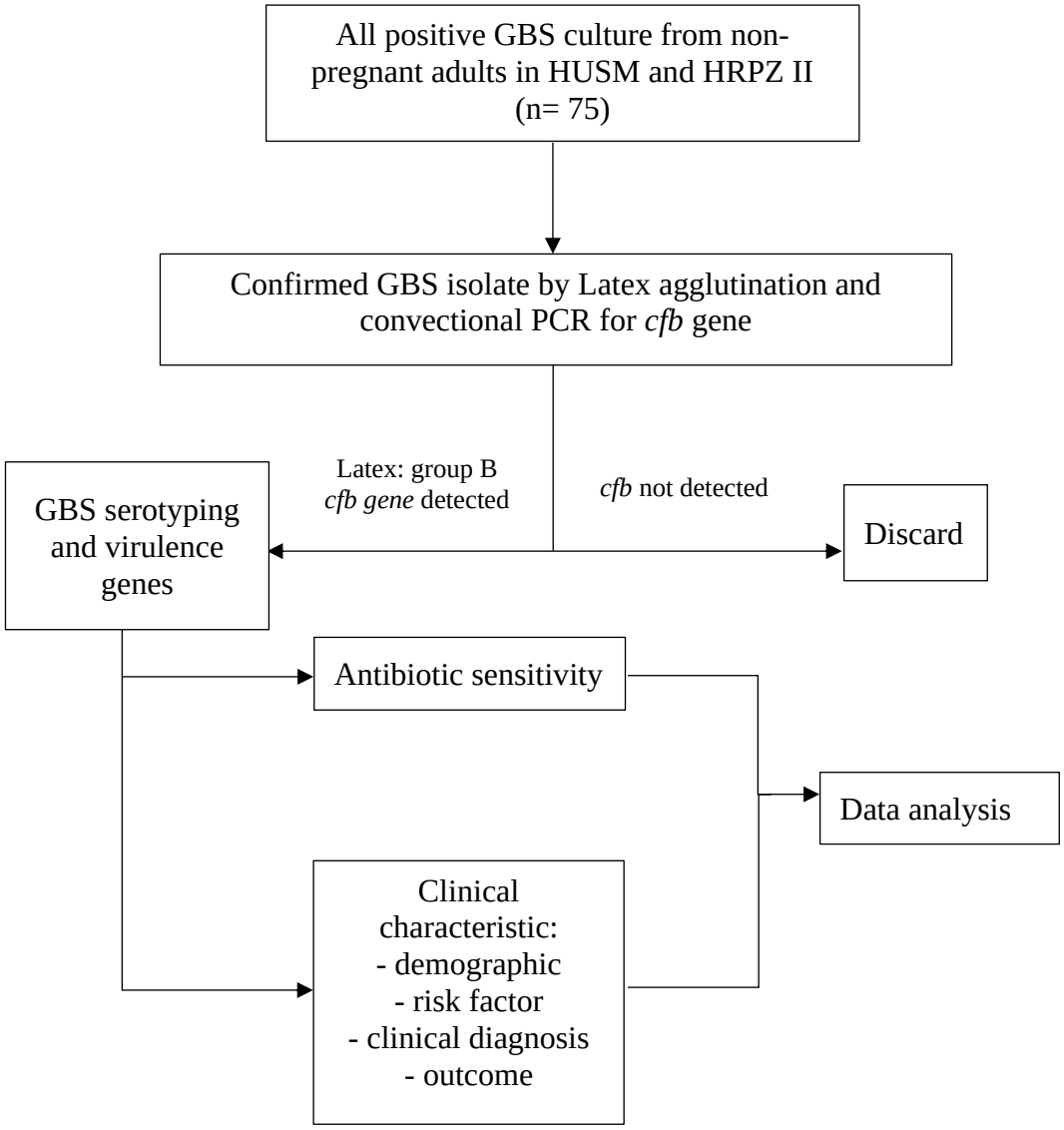

Figure 1.4 : Flow chart of GBS study