MATERNAL CARRIAGE AND NEONATAL COLONISATION WITH GROUP B STREPTOCOCCUS AT MUHIMBILI NATIONAL HOSPITAL DAR ES SALAAM

By

Agricola Joachim, MD (UDSM)

A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine in Microbiology and Immunology of the Muhimbili University of Health and Allied Sciences.

Muhimbili University of Health and Allied Sciences

August 2009

CERTIFICATION

The undersigned certify that he has read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled: ***Maternal carriage and neonatal colonisation with group B streptococcus at Muhimbili National Hospital Dar es Salaam, Tanzania,*** in partial fulfillment of the requirements for the degree of Master of Medicine (Microbiology /Immunology)

.....

Prof. Eligius F. Lyamuya

Muhimbili University of Health and Allied Sciences

(Supervisor)

Date 02.11.2009

DECLARATION AND COPYRIGHT

I, Agricola Joachim, hereby declare that this dissertation is original work and that it has not been presented to any other University for a similar or any other degree award.

Signature.....

This dissertation is copyright material protected under the Bern convention, copyright Act 1966 and other international and national enactments, on that behalf, on intellectual property. It may not be reproduced by any means, in full or in part, except for short extracts in fair dealing, for research or private study, critical scholarly review or discourse with an acknowledgement, without written permission of the directorate of postgraduate studies, on behalf of both the author and Muhimbili University of Health and Allied Sciences.

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere gratitude to my supervisor Prof. Eligius F. Lyamuya for his guidance, encouragements and support throughout the different stages of this work. Special thanks go to the head of department Prof. Mecky I. Matee and other members of the Department of Microbiology and Immunology, including Prof. S. Y Maselle, Prof. F. S.Mhalu, Dr. D. Mwakagile, Dr S. Aboud and Dr S. Moyo for their valuable contribution during my training in the field of Microbiology and Immunology.

I extend my sincere thanks to all study participants, the sister in charge and the entire nursing staff of the antenatal clinic and labour ward, Muhimbili National Hospital. I would also like to acknowledge with thanks, the assistance of the laboratory technicians of the Department of Microbiology and Immunology of both MUHAS and MNH. I would also like to thank the MNH authorities for allowing me to conduct the study at MNH and providing me with necessary support.

Special thanks goes to my parents, my cousin Sophia and all good friends who had faith on me and encouraged me constantly throughout my scholarly pursuit.

Lastly I thank Almighty God and Jesus Christ for their mercy and guidance through the various stages of my academic career.

DEDICATION

I dedicate this dissertation to my parents Joachim Mkumbara and Angela J. Shirima.

ABSTRACT

Background: Group B streptococcus (GBS), which is a commensal bacterium that asymptomatically colonises the vaginal and rectal areas of about 10% to 30% of pregnant women, is the leading cause of perinatal bacterial infection responsible for septicemia, meningitis and pneumonia in neonates. In Tanzania no studies have been done on GBS colonisation or infection in pregnant women and neonates and associated risk factors as well as antimicrobial susceptibility pattern. This missing information is essential in formulating a policy for treatment, prevention and control of perinatal GBS disease in the country.

Objective: To determine the prevalence, risk factors of maternal carriage and neonatal colonisation of GBS and antimicrobial susceptibility pattern of the bacterium at Muhimbili National Hospital (MNH) in Dar es Salaam, Tanzania.

Study design and population: A cross sectional study involving 300 pregnant women attending antenatal clinic and their newborns delivered at MNH during the study period (October 2008 and March 2009).

Methodology: Specimens (high vaginal swab, rectal, nasal/ear and umbilical swabs) were examined for GBS by growth on Todd Hewitt Broth and subculture in 5% sheep blood agar. Isolated were identified by their colonial morphology, gram staining, and biochemical tests and confirmed by Lancefield's grouping (Streptex agglutination) test. Antimicrobial susceptibility testing was done using Kirby-Bauer method.

Results: Prevalence of GBS was 23% among pregnant women and 8.9% in neonates. The isolation rate of GBS was higher in sample taken from vagina (12.3%) as compared

to rectum (5%). Prolonged duration of labour (>12 hrs) was significantly shown to influence GBS colonisation in neonates P<0.05. However putative risk factors such as premature rupture of membrane, intrapartum fever, low birth weight and HIV infection did not correlate with GBS colonisation. All (100%) isolates were sensitive to vancomycin and ampicillin but resistance to clindamycin (17.6%), erythromycin (13%) and penicillin G (9.4%).

Conclusion and recommendation: Our findings seem to suggest that a quarter of pregnant women attending ANC clinic at MNH and approximately 10% of their newborns are colonised with GBS. The high isolation frequency of GBS among pregnant women and their neonate's calls for further study to elucidate extent of vertical transmission and follow up of neonates with GBS colonisation in order to establish extent of disease development. There is a need for continuous antibiotics surveillance of GBS to monitor trend of resistance thus guiding treatment and control strategies of GBS disease in the country.

TABLE OF CONTENTS

Content	Page
Certification	ii
Declaration and copyright	iii
Acknowledgements	iv
Dedication	v
Abstract	vi
Table of contents	viii
List of tables	xii
List of abbreviations	ix
1.0 Introduction and literature review	1
1.1 General introduction	1
1.2 GBS Infection and Diseases	2
1.2.1 GBS in pregnancy	2
1.2.2 GBS in Non-pregnant adults	4
1.2.3 GBS in neonates	5
1.2.3.1 Early-onset GBS disease	5
1.2.3.2 Late-onset GBS disease	7
1.3 GBS and HIV/AIDS	7
1.4 Bacteriology of group B streptococcus	8
1.5 Pathogenesis and virulent factors of GBS	9
1.5.1 Adherence	9

1.5.2 Invasion 10

1.5.3 Direct tissue injury 11

1.5.4 Avoidance of immune clearance 11

1.6 Surface Markers GBS 11

1.6.1 Capsular polysaccharide 12

1.6.2 Surface anchored protein 13

1.7 Detection of GBS colonisation and diagnosis of infection 13

1.8 Antimicrobial susceptibility of GBS 14

1.9 Prevention of neonatal GBS 16

2.0 Statement of the problem 18

3.0 Rationale of the study 19

4.0 Objectives of the study 20

4.1 Broad objective 20

4.2 Specific objectives 20

5.0 Hypothesis of the study 20

6.0 Materials and methods 21

6.1 Study design, participants and enrolment criteria 21

6.2 Sample size 21

6.3 Data and specimen collection 22

6.3.1 Data collection 22

6.3.2 Specimen collection and transportation 22

6.4 Laboratory procedures 23

6.4.1 Isolation of bacteria 23

6.4.2 Identification of bacteria isolates 24

6.4.2.1 Identification of GBS 24

6.4.2.2 Identification of *Neisseria gonorrhoeae* 24

6.4.2.3 Identification of *Trichomonas vaginalis* 24

6.4.3 Antimicrobial susceptibility testing 25

6.4.3.1 Inoculum preparation.... 25

6.4.3.2 Inoculation of test plates 26

6.4.3.3 Application of discs to inoculated agar plates 26

6.4.3.4 Reading plates and interpreting results 27

6.4.3.5 Verification of the results of antibiotic sensitivity testing 27

6.4.4 HIV serology 28

6.5 Quality assurance 29

6.6 Ethical consideration 29

6.7 Data analysis 30

6.8 Dissemination of the results 30

6.9 Limitation of the study 31

7.0 Results.... 32

8.0 Discussion 43

9.0 Conclusion and recommendations 50

10.0 References 52

Appendix I 62

Appendix II 65

Appendix III 69

LIST OF TABLES

I. Social-demographic characteristics of the study participants 33

II. Colonisation rate in relation to site of isolation 34

III. Association between social demographic factors and GBS colonisation among pregnant women 35

IV. Association between obstetric factors and GBS colonisation among pregnant women 37

V. Association between obstetric factors and GBS colonization rate in neonates 39

VI. Association between GBS colonisation among pregnant women and HIV 40

VII. Relationship between GBS colonisation and absolute CD4 counts among pregnant women with HIV 41

VIII. Antimicrobial Sensitivity pattern of GBS isolated from pregnant women and neonates 42

LIST OF ABBREVIATIONS

BA	Blood agar
CAMP	Christie, Atkins and Munch-Peterson
CA	Chocolate agar
CPA	Capsular polysaccharide antigen
CDC	Centres for disease control and prevention
CSF	Cerebrospinal fluid
CPL	Central Pathology Laboratory
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
GBS	Group B streptococci
HIV	Human immunodeficiency virus
HVS	High vaginal swab
MNH	Muhimbili National Hospital
MOHSW	Ministry of Health and Social Welfare
MUHAS	Muhimbili University of Health and Allied Sciences
MTM	Modified Thayer Martin medium
THB	Todd- Hewitt Broth
IPPM	Intramural Private Practice

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

Group B streptococcus (GBS) is a commensal bacterium that asymptomatically colonises the vaginal and rectal areas of women [1]. There is significant geographic variation in the proportion of women colonised with GBS; the range of colonisation reported from developing countries is somewhat different from industrialized countries [1, 2]. Low carriage rates have been reported from some developing countries such as Saudi Arabia (13.9%), India (5.8%) and Libya (5%) [3, 4]. This is in contrast to observations made in other developing countries where higher rates have been documented such as Nigeria (19.5%) [5], Gambia (22%) [6], Jordan (30.4%) [7], Zimbabwe (31.69%) [8] and Trinidad (32.9%) [4]. GBS infections tend to occur more commonly among adults than in neonates, but the overall mortality is higher in neonates. The risk of disease is greater in pregnant women than in non-pregnant women and men [9]. Approximately 60% of infants born to colonised mother become colonised with their mother's organisms. The likelihood of neonatal colonisation at birth is higher if the mother is heavily colonised [9].

GBS infection is the leading cause of perinatal bacterial infection [10], being commonly responsible for septicemia, meningitis and pneumonia in neonates. At birth, infants who are born to colonised mothers may also become colonised on their mucosal surfaces such as oral, nasopharynx, vaginal and anal mucosa and skin. Newborns acquire the organism intrapartum from their mothers, who are colonised in the genital tract or from the

ascending spread of the organism into the amniotic fluid. Perinatal transmission can occur across intact membranes [11], the incidence of neonatal infection varying between 0.3 and 5 per 1000 live births in developed countries [12, 13].

1.2 GBS Infection and Diseases

1.2.1 GBS in pregnancy

GBS are present in 15% to more than 30% of pregnant women [8]. GBS carriage rates among black pregnant women have been reported to be higher than those among women in other racial groups [14]. The reason is not clearly known but could be related to their genetic makeup. Anorectal site has been implicated as the source of GBS, resulting in vaginal colonisation. During pregnancy, the foetus is protected from most bacterial infections by the chorioamniotic membrane and the placenta. The amniotic fluid in which the foetus is bathed also has inhibitory properties preventing bacterial growth thus the foetus or newborn infant acquires GBS infection commonly during labour or delivery. Upon colonisation there is rapid proliferation of micro organisms in the infant's respiratory and gastrointestinal tract, conjunctiva, skin and umbilical stump [15]. The mechanisms by which bacterial colonisation converts to bacterial invasion and disease remains poorly understood but likely reflect both host immunological factors and virulent factors of the micro-organism. There is an association between asymptomatic GBS colonisation and adverse pregnancy outcome. This includes premature delivery, premature rupture of membranes and low birth weight. Women with heavy GBS colonisation at 23 to 26 weeks of gestation have an increased risk at delivery of preterm

low birth weight infants [14]. The risk of intra-amniotic infection increases with duration of rupture of membrane and the duration of labour. Duration of labour longer than 12 hours increases the risk of transmission. GBS attack rate increases by tenfold in infants delivered to colonised women who also have chorio-amnionitis, which may present with fever, uterine tenderness and increased heart rate in the baby. Some infants have bacteremia due to GBS at the time of delivery, which suggests that invasive infection occurred perinataly either by invasion through the placenta or by fetal aspiration of infected amniotic fluid. It is also known that GBS can cause uterine infection before or after delivery. The most common manifestations of GBS in pregnant women are urinary tract infections, amnionitis, endometritis, and post-surgical wound infections (post cesarean section and post episiotomy) [16].

Women at high risk for passing on the infection to their newborn include those who have premature delivery, prolonged rupture of the membranes (>18 hours), intrapartum fever (temperature >38°C) or multiple pregnancy [4]. Evidence from some studies suggests that the risk of maternal colonisation with GBS increase with number of sexual partners. For example, a study done in Mexico among pregnant women reported that the risk of GBS colonisation increases by four-fold with a history of three or more sexual partners [17]. Surveillance conducted in Atlanta in 1990 identified a total of 600 invasive GBS cases, 67 (11%) of which occurred in pregnant women [18]. The outcome of pregnancy was known for 43 women with invasive GBS disease; 21 (49%) delivered infants with no apparent illness, 15 (35%) delivered infants who survived neonatal GBS disease, 4 (9%)

women had infants who died of neonatal GBS disease, and 7% had pregnancies that resulted in stillbirth [18]. Surveillance for invasive GBS disease among pregnant women may underestimate the burden of perinatal GBS infections in areas where collection of blood cultures from women with amnionitis or intrapartum fever is not performed routinely. GBS is clearly a cause of stillbirth in some instances [19]. In 1996 Moyo and co-workers reported GBS to be among the common cause of intrauterine infection, accounting for chorioamnionitis in 79% of stillbirths and 30% of live births in Zimbabwe [20].

1.2.2 GBS in Non-pregnant Adults

Invasive GBS disease occurring outside the perinatal period has been recognized practically since the advent of the serologic classification system for streptococci. However, recent efforts to define the magnitude of all invasive disease due to GBS demonstrated that a substantial burden of GBS-related illness occurs outside the better recognized risk periods of pregnancy and early infancy [16]. Group B streptococcal disease in non-pregnant adults is increasing, particularly in elderly persons and those with significant underlying diseases. Conditions such as diabetes, breast cancer, neurological impairment, and cirrhosis have been associated with increased risk for invasive GBS disease [21]. Schwartz and co-workers estimated that persons with diabetes mellitus had nearly 14 times the risk of invasive GBS disease compared with other adults [22]. The clinical presentations of invasive GBS disease among non-pregnant adults most often take the form of primary bacteremia however; skin, soft-tissue, and osteoarticular

infections, pneumonia, and urosepsis are also common manifestation. Meningitis and endocarditis are less common but associated with serious morbidity and mortality [23, 24]. Disease is frequently nosocomial and may be related to the placement of an intravenous catheter. Reports on series of adult patients with invasive GBS disease from individual hospitals gave the impression that this pathogen predominantly affects debilitated hosts, supporting Lancefield's initial view that sero-group B streptococci were saprophytic organisms [16].

1.2.3 GBS in neonates

GBS disease in neonates can occur in two clinical forms: a) early-onset disease, defined by the development of disease in newborn infants up to the seventh day of life, accounting for about 85% of the neonatal infections caused by this agent. b) late-onset disease, characterized by occurrence between the eighth day and the third month of life; colonised neonates have an estimated risk of 1-2% in developing the disease [14]. The following sections describe the early-onset and late-onset diseases in neonates.

1.2.3.1 Early-onset GBS disease

Early-onset GBS disease is defined as the development of symptoms during the first seven days of life, with mean age at onset of 20 hours [1]. The majority (> 90%) of early-onset disease and almost all fatal infections occur within the first day of life with a median age at onset of one hour [14]. The most important risk factor for early-onset GBS infection in the neonates is the presence of the organism in the maternal

genitourinary tract at delivery. Early-onset GBS disease is often due to aascending bacteria from the maternal genital tract, infecting the amniotic membranes usually after they are ruptured [25]. Alternatively, the newborn may become contaminated with GBS during vaginal delivery. When the foetus aspirates contaminated amniotic fluid, group B streptococci reach the lower respiratory tract and damage pulmonary epithelial cells, resulting in pneumonia and respiratory distress usually within the first few hours after birth. Severe GBS sepsis occurs with intravascular invasion of bacteria and infection of the membranes, with subsequent events occurring in utero, resulting in stillbirths or death within hours after birth [25]. Perinatal transmission can occur across intact membranes [11]. Main clinical syndromes of early-onset GBS disease include septicemia, meningitis and pneumonia. Bloodstream infection, with or without pneumonia, is the main manifestation of GBS disease in infancy and manifests in 89% of cases [26]. Meningitis is less common, occurring in approximately 10% of neonatal infections [26]. The presenting signs and symptoms of early- onset GBS infection are lethargy, cyanosis, apnea, poor feeding, jaundice, abnormal temperature, pallor and hypotension [15]. The predisposing factors for early-onset GBS are pre-maturity, low birth weight, prolonged rupture of membranes, amnionitis, intrapartum fever, maternal vaginorectal colonisation with GBS, heavy (dense) genital colonisation with GBS, urinary tract infection with GBS, previous still birth, multiple pregnancy and maternal age <20 years [16]. The reason for high risk of GBS colonization in this age group is not well defined, but could probably be due to the fact that they are highly sexually active group of women and this may suggest sexual transmission. This, however, remains to be ascertained.

1.2.3.2 Late-onset GBS disease

Late-onset GBS infection has an onset from seven days to three months of age with mean age of onset of 24 days [1]. The pathogenesis of late-onset disease is less well understood, usually there is no history of obstetric complications and the disease is unrelated to vaginal colonisation of the mother. Nosocomial and community sources are probably involved in some cases of late-onset disease [27], but the risk factors are not well understood. Even for infants with late-onset infections whose mothers have the same serotype, the precise mechanism of apparent mother to infant transmission has not been identified. Bacteremia with concomitant meningitis is the most common manifestation [8], but septic arthritis, osteomyelitis, conjunctivitis, sinusitis, otitis media, endocarditis and peritonitis may occur [28]. Additional clinical findings that have been associated with fatal outcome or permanent neurological sequelae include prolonged seizures, neutropenia at admission and high concentration of type III polysaccharide antigen in cerebrospinal (CSF) specimen drawn on admission. It has been shown that a significant number (25 to 50%) of survivors of group B streptococcal meningitis irrespective of whether early or late onset type will have permanent neurological sequelae [1]. Purulent meningitis is the prototype of late-onset infection and this can occur in other healthy full-term infants born to mothers who are not GBS carriers [15].

1.3 GBS and HIV/AIDS

There is paucity of information regarding the relationship between GBS and HIV infection. The burden of bacterial infection among patients with HIV has been noted to

be high since the onset of the HIV and AIDS pandemic. HIV infection has been associated with other bacterial pathogens causing severe disease including *Streptococcus pneumoniae*, *Haemophilus Influenzae* and some of the enteric bacterial pathogens. Among the few studies done on GBS and HIV infection, some have reported prevalence of GBS colonisation to be higher in HIV infected pregnant women (19.8%) than HIV uninfected pregnant women (14.1%) although the difference was not statistically significant [29]. However, there was no association between maternal colonisation and their immunological status [29]. In a population based study it was reported that, the rate of invasive GBS infection is 30 times higher in HIV infected patients than in HIV negative subjects of comparable age [30]. An observational study involving 101 pregnant women with HIV infection in Brazil, reported a higher risk of GBS colonisation among non-white (black) pregnant women and among women with concomitant diabetes mellitus [31]. Findings from these few reported studies indicate that HIV infection is an important risk factor for GBS colonisation and invasive GBS disease. Since the extent to which GBS colonisation and infection occurs in Tanzania where the prevalence of HIV remains high is not clearly known, it is of interest to investigate, among other aspects of GBS, the association between HIV infection and GBS colonisation.

1.4 Bacteriology of GBS

Group B streptococci (also called *Streptococcus agalactiae*) belong to Lancefield group B. The serological differentiation of haemolytic streptococci by group was described by Rebecca Lancefield in 1933. It was based on capillary precipitation reaction between the

group-specific carbohydrate antigens in the cell wall, which was solubilised by acid treatment of the organism with hyperimmune antisera that were prepared by immunization of rabbits with formalin- killed whole bacterial cells [32]. GBS are gram positive cocci, facultative anaerobic bacteria that form chain of variable length that grow on a variety of bacteriologic media. They grow well on nutritional enriched media. Colonies of GBS are grayish whitish, mucoid, large (2-3mm) and form narrow zone of β-hemolysis when grown on sheep blood agar. Some strains 1-2% are non- hemolytic although in some culture hemolysis is of the α-type. Carbohydrates are fermented, resulting in the production of lactic acid. They are catalase negative and hydrolyze sodium hippurate. GBS produces thermostable extracellular diffusible protein referred to Christie, Atkins and Munch Peterson (CAMP) factor that results in synergistic haemolysis of sheep erythrocytes with the β-lysin of *Staphylococcus aureus. S. aureus* produces sphingomyelinase C, which bind to erythrocyte membranes and when exposed to the group B CAMP factor, the cell undergoes hemolysis [9].

1.5 Pathogenesis and virulence factors of GBS

1.5.1 Adherence

GBS bind efficiently to human vaginal cells, with maximum adherence at the acidic pH of vaginal mucosa [33]. The bacterium also adheres efficiently to human cells from a variety of foetal, infant and adult tissues including placenta membranes, buccal or pharyngeal mucosa, alveolar epithelium and endothelium. However, the underlying mechanisms are poorly understood. Adherence of this bacterium to such epithelial cell is

an important step for host tissue invasion. Like many other pathogens, GBS can attach to host cell surfaces by binding to different host cell proteins. Binding of GBS to human laminin is mediated by the lipoprotein lmb (laminin-binding protein). C5a peptidase from GBS mediates binding of the bacteria to fibronectin and this plays a role in the invasion of host cells [34]. Recent studies have shown that, the Fibrinogen Receptors FbsA and FbsB promote adherence of *Streptococcus agalactiae* to human epithelial cells [35,36]. FbsA and FbsB proteins are involved in fibrinogen binding. They are unrelated to each other on amino acid level but both have a surface exposed localization in the cell wall of the bacteria.

1.5.2 Invasion

GBS can penetrate and survive within several human cell types. This property may be attributed to the ability of GBS to transverse a number of host cellular barriers including placenta membrane, the alveoli of the infant lung and neonatal blood brain barrier. This provides opportunity for GBS to reach blood stream, the meninges and when in utero, to reach the foetus. GBS can also penetrate amniotic fluid through intact placental membrane. GBS invasion to human epithelial cells are also mediated by fibrinogen-binding protein FbsB and C5a peptidase [36].

1.5.3 Direct tissue injury

GBS causes disruption of the epithelial and endothelial cell barriers through direct cell injury. This can facilitate placental penetration and systemic spread of the organism in the infected foetus [20].

1.5.4 Avoidance of immune clearance

The penetration of GBS into tissue or blood steam of a human host triggers mechanisms to clear the organism. Host phagocytic cells such as neutrophils and to some extent monocytes and macrophages are responsible. Like most pathogenic bacteria, effective phagocytosis of GBS by neutrophils and monocytes requires opsonization. Without participation of specific antibodies (especially the IgG class) and serum complement, phagocytosis of GBS is insufficient and without participation of opsonins, the polysaccharide capsular substance and perhaps other surface- localized components will impede effective phagocytosis. The vulnerability of certain neonates to invasive GBS disease may thus reflect quantitative or qualitative deficits in phagocytic cell function, opsonizing antibodies and some of the cleavage products of complement activation especially C3b. In addition to these host factors, GBS posses' fibrinogen-binding FbsA protein which protects this bacterium from opsonophagocytosis [36].

1.6 Surface Markers of GBS

GBS has important surface markers of the most important of which are briefly described hereunder:

1.6.1 Capsular polysaccharide

Group B streptococci are divided into 9 distinct serotypes based on their capsular polysaccharide antigen which are immunologically distinct from the common group determinant. Capsular polysaccharide antigen types Ia, Ib, II,III, IV, V, VI, VII and VIII strains all have specific carbohydrate determinant. Capsular polysaccharide antigen of GBS extracted with hot hydrochloric acid by the Lancefield procedure [9] contains galactose, glucose, N- acetyl-glucosamine and sialic acid. These serotypes are important epidemiologically with serotype Ia, III and V being most commonly associated with colonization and disease.

Recent studies in the United States have demonstrated that serotypes Ia, III, and V accounted for 78-87 % of early-onset invasive disease in newborn infants and parturient women [37]. Late-onset GBS disease is dominated by serotype III, followed by serotypes Ia and V [38]. However serotypes VI and VIII have been identified frequently from pregnant women in Japan [39]. Tracking the serotypes associated with GBS cases is important for vaccine development. Serological tests such as fluorescent antibody technique, slide agglutination or immuno-electron microscopy [40] suggest that the serotype-specific capsular polysaccharide antigens are located peripherally to the serogroup B carbohydrate antigen, and are often referred to as capsular antigens.

1.6.2 Surface anchored protein

Surface anchored proteins of GBS include beta antigen of C-protein complex (cβ), alpha antigen of C-protein (Cα), R-protein (R1-R4), protein rib, rib-like protein, alpha-like protein and sip protein. Recent investigations focusing on these surface proteins suggest that these proteins are common to multiple capsular types and that they also play a role in both virulence and protective immunity [41,42].

1.7 Detection of GBS colonisation and diagnosis of infection

Important factors that influence the accuracy of detecting GBS maternal colonisation are the choice of bacteriological media, the body sites sampled, and the timing of the sampling. GBS resides in the genitourinary and gastrointestinal tracts where large numbers of gram negative bacteria are also present. The optimal time for performing antenatal cultures is between 35 to 37 weeks gestation [43]. The highest culture yield is obtained when both the lower vaginal area and anorectal sites are sampled [44]. To enhance the accurate detection of even low numbers of GBS from sites such as genital or gastrointestinal tract a number of selective media have been employed. The most widely used selective medium is Todd-Hewitt broth with gentamycin (8 µg/ ml) or colistin (10 µg /ml) and nalidixic acid (15 µg/ ml) [15]. Swabs are inoculated into selective broth medium, followed by overnight incubation and then sub cultured onto solid blood agar medium. This selective broth media inhibits the growth of gram negative enteric bacilli and other normal flora hence increases culture sensitivity for GBS [45]. Identification of GBS is based on detection of group B specific cell wall antigens common to all strains

[46, 47]. These antigens can be detected by various rapid diagnostic methods including counter-current immunoelectrophoresis, enzyme-linked immunosorbent assay (ELISA), fluorescent antibody tests, staphylococcal co-agglutination and latex agglutination. Latex agglutination is the most widely used method due to commercial availability and simplicity.

Identification of GBS is also done by the CAMP test. GBS produces CAMP factor, which is a diffusible thermostable extra cellular protein that results in synergistic haemolysis of sheep erythrocytes with the β-lysin of *Staphylococcus aureus* and this is usually observed in 98 to 100% of GBS strains [15]. Hydrolysis of sodium hippurate is another identification test. The combination of the CAMP test with bacitracin sensitivity, and esculin reaction has been suggested as an adequate method for presumptive differentiation of GBS from other serogroups of β- hemolytic streptococci [46]. Identification of GBS from culture has limitation of time because 24 to 48 hrs is required for a result, making it less useful in situations where immediate action is required like when a patient presents in labor [38]. The diagnosis of invasive GBS infection is established by isolation of the organism from culture of blood, cerebrospinal fluid, amniotic fluid or specific site of infection such as bone or joint fluid.

1.8 Antimicrobial susceptibility of GBS

GBS is an important cause of neonatal and adult infection worldwide which require treatment with antibiotics. For this reason a continuous surveillance of antimicrobial

susceptibility testing should be done routinely to select suitable antibiotics for patient treatment as well as to monitor emergence of resistance. Sensitivity tests can be done by either diffusion technique or dilution technique [48]. Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial sensitivity and the methods used are Stokes agar disc diffusion or Kirby-Bauer method. GBS are generally susceptible to penicillin (particular penicillin G and ampicilin) which is the drug of choice for both treatment and prophylaxis. Erythromycin and clindamycin are used as alternative therapy for patients allergic to penicillin. However, the proportion of isolates with in vitro resistance to clindamycin and erythromycin has increased since 1996 [49]. The prevalence of resistance among invasive GBS isolates ranges from 7 to 25% for erythromycin and from 3 to 15% for clindamycin [50] and resistance might be associated with certain serotypes. Most studies have reported a higher erythromycin than clindamycin resistance thereby favouring clindamycin more as alternative treatment for penicillin intolerant patients. GBS are sensitive to vancomycin and if GBS resistance to clindamycin or erythromycin is known or suspected, the recommended treatment is vancomycin [43]. Quinolones may have efficacy for GBS, but little clinical experience exists. Despite consistent susceptibility of the majority of GBS strains to penicillin and ampicilin [51,52] there are several reports of poor clinical responses to appropriate therapy and relapses in GBS infection in newborn infants and adults [53,54]. These failures have led to changes in the treatment of GBS infections. Increased dosage or combination with aminoglycoside has been recommended for treatment of serious GBS infections in adults and neonates [55]. Some investigators have demonstrated penicillin

tolerance in some strains of this organism and have suggested that this contributes to treatment failure [51].

1.9 Prevention of neonatal GBS

Group B streptococcus disease in neonates is potentially preventable. The recognition of risk factors, early maternal prenatal screening for GBS, followed by maternal (intrapartum chemoprophylaxis to carriers) and fetal prophylaxis with antibiotics, and immunization have been reported to reduced the incidence of GBS in neonates and its sequelae. Hospital policies of intrapartum administration of antibiotics have also been shown to produce 30-fold reduction in the incidence of GBS disease in neonates [18]. The most effective way to prevent neonatal early-onset infection is maternal antibiotic administration during labor. However, there is no evidence that chemoprophylaxis prevents late-onset disease. The Centers for Disease Control and Prevention (CDC) consensus recommendations for group B streptococcal prophylaxis were originally put into practice in 1996, and by the end of 1999, the incidence of early-onset GBS disease in the US decreased by a remarkable 70% [56]. Two preventative approaches were used: a culture screening-based and a risk-based approach. The first approach involved universal screening for GBS colonisation of all pregnant women between 35 and 37 weeks gestation using vaginal and rectal cultures to detect GBS colonisation. Properly obtained and processed antenatal cultures correctly will identify most women colonized with GBS at the time of labor. Intrapartum antibiotics are administered to all those with a positive GBS culture regardless of risk factors. The risk-based approach involves administration

of antibiotics based solely on the presence of antenatal or intrapartum risk factors. Maternal risk factors for group B streptococcal neonatal sepsis include preterm labour, premature rupture of membranes (< 37 wks' gestation), prolonged rupture of membranes (≥18 h), intrapartum fever ≥100.4° F (≥38.0° C), history of a previous newborn with GBS disease, and group B Streptococcus bacteruria during pregnancy [43]. In 2002, CDC issued revised guidelines for prevention of perinatal GBS disease. These guidelines recommend universal prenatal culture based screening for vaginal and rectal colonisation in all pregnant women at 35-37 weeks of gestation and use of intrapartum antibiotic prophylaxis for GBS carriers [43].

2.0. STATEMENT OF THE PROBLEM

Group B Streptococcus has been reported as an important cause of maternal and neonatal morbidity and mortality in many parts of the world. Estimates of maternal GBS carriage varies with geographical distribution and ranges from 5% and 35 % whereas invasive neonatal disease ranges between 0.2 and 3.0 per 1000 live births in developed countries [57]. Data have shown that at birth, 50- 65% of infants who are born to colonised mothers have positive GBS cultures from mucus membranes and skin [43]. Of these neonates colonized with GBS one to two per cent develops invasive GBS infection whereas over 20% of survivors of neonates who suffer from GBS meningitis have permanent neurological sequelae, including sensorineural hearing loss, mental retardation, cortical blindness and seizures [58]. A review on maternal carriage of group B Streptococci in developing countries reported varying percentages ranging from 1.8% in Mozambique to 22%in Gambia [59]. In the year 2000 a study was done in Zimbabwe and reported a high prevalence (31.7%) of GBS colonisation in pregnancy [8]. A recent study conducted in Eastern Turkey among pregnant women and their neonates reported the prevalence to be 32% and 17.3%, respectively and the overall rate of GBS vertical transmission was 54.2% [60]. From the studies mentioned above it is clear that GBS colonisation in pregnant women varies with geographical distribution. In Tanzania the rate of GBS colonisation among pregnant women and neonates has not been studied and therefore to date no strategies have been formulated to prevent neonatal GBS infection in the country. In 2000- Blomberg and co-workers conducted a study on bloodstream infections among children aged less than 5 years and reported the prevalence of GBS

infection to be 1.2% in neonates [61]. However one of the limitations of Blomberg study was that two thirds of the study population had received antimicrobial therapy prior to blood culture. Thus, results of that study may not reflect the true picture of GBS infection among neonates in Tanzania.

3.0 RATIONALE OF THE STUDY

In Tanzania no studies have been done on GBS colonisation or infection in pregnant women and neonates. Thus there are no data for GBS infection and disease in the country. This study will provide data regarding the prevalence and risk factors of GBS colonisation in pregnant women and neonates. The study will also provide information on antimicrobial susceptibility pattern of GBS which will guide the treatment and management of GBS infection and disease. In addition to that, information on prevalence of GBS and risk factors for colonisation together with their antimicrobial susceptibility pattern will assist in formulating a policy for treatment, prevention and control of perinatal GBS disease in the country.

4.0 OBJECTIVES OF THE STUDY

4.1 Broad objective

To determine the prevalence, antimicrobial susceptibility pattern and risk factors of maternal carriage and neonatal colonisation with GBS at MNH, Dar es Salaam Tanzania.

4.2 Specific objectives:

1. To determine the prevalence of GBS carriage among pregnant women at MNH, Dar es Salaam.
2. To determine the neonatal colonisation rate of GBS at MNH, Dar es Salaam.
3. To determine the risk factors for GBS colonisation among pregnant women, and their neonates at MNH Dar es Salaam.
4. To determine the association between GBS colonisation and HIV infection among pregnant women in MNH, Dar es Salaam.
5. To determine the association between GBS colonisation and level of CD4 counts among HIV infected pregnant women in MNH, Dar es Salaam
6. To determine the antimicrobial susceptibility pattern of the isolated GBS at MNH, Dar es Salaam

.

5.0 NULL HYPOTHESES

1. There is no GBS resistance to commonly used antimicrobial agents.
2. There is no difference in antibiotic susceptibility pattern to GBS between HIV infected and uninfected pregnant women.

MUHIMBILI MEDICAL UNIVERSITY COLLEGE LIBRARY DSM

3. There is no difference in GBS colonisation rate between HIV seropositive pregnant women and HIV seronegative pregnant women.

6.0 MATERIALS AND METHODS

6.1 Study design, participants and enrolment criteria

This was a cross-sectional study conducted at MNH in Dar es Salaam, Tanzania between October 2008 and March 2009. The study included 300 third trimester pregnant women (from 37 weeks of gestation) attending the antenatal clinics at MNH for routine antenatal visits and their neonates (180) delivered at MNH labor ward. Participants were enrolled in the study after obtaining an informed written consent (appendix II).

Inclusion criteria

- Consenting clients
- Gestational age ≥37 weeks

Exclusion criteria

- Participants who did not consent
- Clients with history of using antibiotic(s) within two weeks prior to recruitment
- Gestational age <37 weeks

MUHIMBILI MEDICAL UNIVERSITY COLLEGE LIBRARY DSM

6.2 Sample size

Sample size was calculated from the following formula

$n=z2p\ (100-p)/e^2$

Where as

n=sample size

z = standard normal deviate corresponding to a two sided specified significance level.

p was set at 16%, being the median prevalence of GBS carriage among pregnant women in African countries [8, 59].

e=margin of error on p

After substituting the values p=16%, e=5% and z =1.96 yielded a minimum sample size is 210.

For the purpose of increasing the power of the study, the sample size was raised to 300.

6.3 Data and specimen collection

6.3.1 Data collection

Data was collected after obtaining written informed consent using a standard structured questionnaire designed to obtain social demographic data and other relevant information such as maternal age, gestational age, previous obstetric history, history of current pregnancy, parity, marital status, mode of delivery, administration of antibiotics during labor and neonate's birth weight. Pregnant women were interviewed at 37 weeks of gestation, during labour and delivery.

6.3.2 Specimen collection and transport

High vaginal swabs (HVS) were collected at 37 weeks of gestation using a Cusco's speculum and rectal swab using sterile swab sticks. Umbilical swabs, ear canal and nasal

swabs were collected from neonates after delivery. The swabs were placed in Stuart's transport medium (Oxoid Ltd) and transported to Central pathology laboratory of the MNH and processed within 2 hours of collection. For HVS two specimens were taken, one for GBS isolation and the other for isolation of sexual transmitted pathogens. HVS for GBS isolation and rectal swab were inoculated direct into Todd Hewitt broth after collection.

About three milliliters of venous blood were collected into sterile vacutainer tubes without anticoagulant and left to clot to obtain serum for detection of IgG antibodies against HIV. For those who tested positive three milliliters of whole blood were collected in EDTA vacutainer tubes for CD4+ measurements.

6.4 Laboratory Procedures

6.4.1 Isolation of bacteria

The swabs were inoculated in Todd–Hewitt broth (Oxoid Ltd) containing nalidixic acid (15 mg/L) and gentamicin (8 mg/L) and incubated at 37°C in 5% co_2. After 24 h of incubation from culture broth, subcultures were performed in 5% sheep blood agar for isolation of GBS. Broth cultures showing no visible turbidity after overnight incubation were further re-incubated and then sub cultured after 48 hours on sheep blood agar. Wet preparation were performed on the second HVS and then inoculated in Chocolate agar (CA) and Modified Thayer Martin medium (MTM) and incubated at 37°C in 5-10% co_2 for 48hrs for isolation of *N. gonorrhoeae*. Culture broth/cultures were prepared according to manufacturer's instructions (Oxoid Ltd)

6.4.2 Identification of the bacteria

Isolates were identified by observing the colonial morphology, gram staining, biochemical tests and serological tests as described below.

6.4.2.1 Identification of GBS

After observing the colonial morphology (typical GBS hemolytic colonies or non hemolytic colonies) on sheep blood agar, gram staining from each isolate looking for gram positive cocci, catalase test negative, hippurate hydrolysis and CAMP test were performed. Lancefield's grouping (streptex- agglutination) test was performed to confirm all positive isolates as GBS. GBS isolates were subjected to antimicrobial susceptibility testing and also stored at -80°C for genotyping and virulence studies in future.

6.4.2.2 Identification of *N. gonorrhoeae*

Identification of *N. gonorrhoeae* was based on their growth on CA and selective media (MTM), colonial morphology and gram-negative diplococci on gram staining, oxidase test positive reaction, CHO utilization pattern which was guided by detection of acid produced aerobically from glucose but not maltose and confirmed by phadebact agglutination test.

6.4.2.3 Identification of T. vaginalis.

Wet preparation using sterile saline was used to detect motile *T. vaginalis*.

6.4.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility of all GBS isolates was determined by Kirby-Bauer disk diffusion method according to clinical and laboratory standard institute (CLSI) (formerly NCCLS). The following antimicrobial disks were tested: penicillin G (10 U), ampicillin (10μg), clindamycin (2μg.), erythromycin (15μg), vancomycin (30μg), ciprofloxacin (5μg), cefriaxone (30μg) and co-trimoxazole 25μg. Procedure for performing the disk diffusion test (Kirby-Bauer disk diffusion) was as follows:

6.4.3.1 Inoculum preparation

At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a sterile wire loop, and the growth transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as sterile peptone water or saline. The broth culture was incubated at 35°C until it achieved or exceeded the turbidity of the 0.5 McFarland standard (usually after 2 to 6 hours). The turbidity of the actively growing broth culture was adjusted with sterile saline to obtain turbidity optically comparable to that of the 0.5 McFarland standard which results in a suspension containing approximately 1 to 2 x 10^8 cfu / ml for *S. Aureus* ATCC 25923. Using similar inoculums preparation, inoculums of the control organism standard *S. agalactiae* ATCC 27956 was prepared.

6.4.3.2 Inoculation of test plates

Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was then be rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The dried surface of a Mueller-Hinton agar plate supplemented with 5% defibrinated sheep blood was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums. As a final step, the rim of the agar was swabbed. The lid was left for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

6.4.3.3 Application of discs to inoculated agar plates

The predetermined battery of antimicrobial disks was dispensed onto the surface of the inoculated agar plate. Each disk was pressed down to ensure complete contact with the agar surface. Whether the disks were placed individually or with a dispensing apparatus, they were distributed evenly so that they were no closer than 24 mm from center to center. Ordinarily, up to five disks were placed on one 100 mm plate. Because some of the drugs diffuse almost instantaneously, discs were not relocated once they had come into contact with the agar surface. Instead, a new disc was placed in another location on the agar. The plates were inverted and placed in an incubator set to 35°C within 15 minutes after the disks application.

6.4.3.4 Reading plates and interpreting results

After 20 to 24 hours of incubation, each plate was examined. Zones were measured to the nearest whole millimetre, using sliding calliper which was held on the back of the inverted Petri dish. The plate was held a few inches above a black, non reflecting background and illuminated with reflected light. The zone margins were taken as the area showing no obvious, visible growth that could be detected with the unaided eye. Faint growth of tiny colonies, which could be detected only with a magnifying lens at the edge of the zone of inhibited growth was ignored. However, discrete colonies growing within a clear zone of inhibition were sub cultured, re-identified, and retested. The sizes of the zones of inhibition of the organisms were reported as sensitive, intermediate, or resistant to the agents that were been tested according to standard tables for the Kirby Bauer method (CLSI). For convenience, an interpretation of "intermediate sensitivity" was considered as sensitive.

6.4.3.5. Verification of results of antibiotic sensitivity testing.

The standard control organism was used to monitor the accuracy and precision of the susceptibility testing procedures, antibiotic disk used as well as the performance of the person carrying out the test and read the results. The following were done: Standard control organism *S. agalactiae* ATCC 27956 was inoculated in prepared medium/broth and incubated at 37°C for 24hrs to determine the ability of such media to support growth. Regarding antimicrobial sensitivity testing, inoculums suspension of *S. agalactiae* using colonies taken from an overnight (18 to 20 hrs) sheep blood agar plate was prepared to achieve turbidity equivalent to a 0.5 McFarland standard. Plate inoculation was

performed using the inoculums suspension within 15min after adjusting the turbidity. Four antibiotic disks were placed on a 100mm plate and then incubated at 35°C in 5% co_2 for 20 to 24hrs before measuring the zone of inhibition. Zone of inhibition was measured in nearest millimetres and interpreted according to standard tables for the Kirby Bauer method (CLSI). When significant change in the acceptable zone diameter limit stated in Kirby Bauer tables was noted, a fresh culture control strain was obtained for retesting. This quality control test was perfomed on each test day for susceptibility testing. All the results obtained from drug tested on a patient's isolate were reviewed in line with quality control procedures. Accuracy of results was accepted when antimicrobial susceptibility results were consistent with isolate identified, the isolate was susceptible to agent for which resistance has not been documented (e.g. vancomycin), proper disk placement and accurate zone measurements.

6.4.4 HIV Serology

Screening was done by rapid test, SD-Bioline HIV 1 / 2 (SD Standard Diagnostic, Inc Korea) and reactive samples were confirmed by Determine TM (Abbott Laboratory, Tokyo Japan). A sample was considered seronegative if it was non reactive at screening and seropositive when reactive on both assays. There were no discordant samples. This testing strategy is in line with National algorithm for HIV testing [62,63]. Results of pregnant women who had already been tested for HIV under the Prevention of mother to child transmission (PMTCT) programme at MNH were accepted as they were, because the testing was also based on the national HIV rapid test algorithm. CD4+ measurements

were performed using BD FACSCalibar flow cytometer to all participants who tested HIV positive.

6.5 Quality assurance

Quality assurance was achieved by assessing the quality of the specimens, source of culture media, preparation of culture media, culture procedures, biochemical tests, and staining procedures. Dehydrated or commercially available media were used and prepared according to the directions of the manufacturers. The pH of each medium was checked using pH meter. For sterility check three plates of the respective batch were incubated at 37°C for 48 hrs and the ability to support growth of the suspected organism was determined by inoculating the medium with a typical stock culture. Negative and positive controls such as streptococci species, staphylococci species and those provided by the manufacturers were used to determine the biochemical response of the reagents/test kits.

6.6 Ethical consideration

The study was carried out in accordance with existing ethical guidelines. Ethical and research clearance was obtained from the Muhimbili University of Health and Allied Sciences Senate Research and Publication Committee. Permission to conduct this study was obtained from MNH authority. A written consent was obtained from each participant prior to enrolment. Information about the study was given to the participants to ensure that they have the information needed to make an informed choice; a complete

description of the aims of the study, organism to be screened including STIs, blood collection procedures and assurance of confidentiality of any information given as well as test results. HIV- pre and post test counselling were provided to the participants according to the national guidelines for HIV testing and counselling [64]. Participants who were found to be HIV positive were referred to HIV care and treatment clinic within MNH for further management, treatment and care in line with the National guidelines for Care and Treatment, Ministry of Health, Tanzania. Appropriate treatment was given to neonates, who were found to be colonised with GBS by their respective doctors after being advised on participant's results. Clients who did not consent for participation were assured of the same quality of care as any other clinic attendees. All participants' results were kept confidentially.

6.7 Data analysis

The data obtained was analysed on SPSS for windows version 13.0 [65]. Assuming the data follows a normal distribution, comparison of proportions of maternal and neonatal colonisation of GBS and statistical significance of HIV infected and non HIV infected pregnant women were tested by using the Chi-squared test or Fishers Exact Test. A p-value less than 0.05 were considered statistically significant.

6.8 Dissemination of the study findings

Findings emanating from this study will be disseminated to the scientific community through a dissertation report and publication in scientific journals. Dissemination will

also be made through presentations in national and international fora whenever an opportunity arises. A popular version of the key findings will also be written for communication to the public.

6.9 Limitations of the study

This study had several limitations.

1. Due to financial constraints it was not possible to do the following :

   (a) DNA finger printing for the GBS isolated and link the neonatal isolates to those of the mothers. Thus it was not possible to analyse the rate of vertical transmission.

   (b) Serotyping

2. There was also limitation of diagnostic kits for some of the sexually transmitted pathogens found in Tanzania such as *C. trachomatis*.

7.0 RESULTS

Between October 2008 and March 2009 a total of 300 pregnant women (from 37 weeks of gestation) and 180 neonates were enrolled in the study. There was loss of follow up of 120 neonates, due to the fact that not all pregnant women delivered at MNH.

7.1 Socio-demographic characteristics of the study group

Table I shows socio-demographic characteristics of the study group. The age of pregnant women ranged from 16-44 years with mean age of 26.6 years (SD ±5.1). Women aged 25 to 29 years constituted the majority of the study population (35.7%) while those aged more than 35 years were very few (8.3%). Regarding marital status, more than three quarters (81.3%) of the study population were either married or cohabiting, 17.3% were single, while 1.4% were either divorced or widowed. Nearly half (48.3%) of the study group had completed primary education while a small proportion (2.4%) had no formal education. Of the 300 women investigated, the majority (93%) reported having one sexual partner while 21(7%) had multiple sexual partners in the last one year preceding the study. About two thirds (66.3%) of the women were attending public antenatal clinic (where payment of consultation and other fees is exempted) while one third (33.7%) were attending Intramural private practice (IPPM) antenatal clinic (where they pay for the services provided).

Table I: Social-demographic characteristics of the study participants

Parameter	Number	Percentages
Age		
<20	26	8.7
20-24	86	28.7
25-29	107	35.7
30-34	56	18.7
35 +	25	8.3
Marital status		
Single	52	17.3
Married/Cohabiting	244	81.3
Divorced/Widow	4	1.4
Education level		
None	7	2.3
Primary	145	48.3
Secondary	105	35
Post secondary	43	14.3
Number of sexual partner		
One	279	93
Multiple	21	7
ANC categories		
Public	199	66.3
IPPM	101	33.7

7.2 Prevalence of GBS carriage among pregnant women

As shown in Table II Group B Streptococci were isolated from 69(23%) pregnant women. The relative distribution of GBS isolation from different sites from the pregnant women is summarized in Table II where it is obvious that most isolates were from the vaginal swabs.

Table II: Colonisation rate in relation to site of isolation

Variable	Number of GBS	Percentage
Vaginal swab only	37	12.3
Rectal swab only	15	5
Both vaginal and rectal	17	5.7
Total	**69**	**23**

7.3 Neonatal colonisation rate of GBS

A total of 180 neonates were screened and GBS colonisation was found in 16 (8.9%) samples from the babies. Vertical transmission was examined in forty three women out of sixty nine pregnant women with GBS colonisation and was found in 16(37.2%) neonates.

7.4 Risk factors for GBS colonisation among pregnant women, and their neonates

Table III summarises the prevalence of GBS colonisation by social demographic characteristics. The colonisation rate was high among the age group 30-34 years (32.1%) but lower (15.4%) in women aged less than 20 years, however this difference was not statistically significant ($p>0.05$). GBS was more prevalent among women with no formal education (37.5%) when compared with those with secondary education (26%), post secondary (20.9%) and primary education (20.7%). GBS colonisation did not differ significantly with marital status, being 25% among divorced women, 23% among married and 19.3% among single women ($p>0.05$).

Table III: Association between social demographic factors and GBS colonisation among pregnant women

Variable	Total	Number of GBS isolated	Percentage	P-value
Age group				0.43
<20	26	4	15.4	
20-24	86	19	22.1	
25-29	107	23	21.5	
30-34	56	18	32.1	
35+	25		20	
Marital status				0.74
Single	52	10	19.2	
Married/Cohabiting	244	58	23.8	
Divorced / widow	3	1	33.3	
Education level				0.54
None	8	3	37.5	
Primary	145	30	20.7	
Secondary	104	28	26.0	
Post secondary	43	8	20.9	
Number of sexual partner				0.65
One	279	65	23.3	
Multiple	21	4	19	
Husband/partner Circumcision status				0.61
No	31	6	19.4	
Yes	269	63	23.4	
ANC category				0.42
Public	199	43	21.6	
IPPM	101	26	25	

Table IV shows maternal obstetric factors in relation to GBS colonisation. Women with gestation age of between 41 and 42 weeks were found to have significantly higher colonisation rate (46.7%), than those with gestational ages of 37-38 weeks (23.6%) and 39-40 weeks (15.4%), $P< 0.05$. Colonisation rate was higher (50%) in women who had delivered four or more times and lower in women who had delivered once (19.8%). However, this difference was not statistically significant ($P>0.05$).

Women with previous history of stillbirth had higher rate (28.6%) of GBS colonisation followed by those with normal previous obstetric history (23.2%) and with history of spontaneous abortion (11.1 %) ($P>0.05$). No GBS colonisation was observed in women with previous history of premature labour.

Sixty six (23.4%) of the women with GBS colonisation had no vaginal discharge.

Table IV: Association between obstetric factors and GBS colonisation among pregnant women

Variables	Total	Number of GBS isolated	Percentage	P-value
Gestational age				0.03
37-38	220	52	23.6	
39-40	65	10	15.4	
41-42	15	7	46.7	
Gravidity				0.63
1	125	30	24	
2	94	18	19	
3	54	13	24.1	
≥4	23	8	29.6	
Parity				0.40
0	138	33	23.9	
1	91	18	19.8	
2	51	11	21.6	
3	12	3	25	
≥4	8	4	50	
Previous obstetric history				0.44
Normal healthy baby	137	33	23.2	
Premature	6	0	0	
Spontaneous abortion	18	2	11.1	
Stillbirth	14	4	28.6	
Dysuria				0.38
No	273	64	23.4	
Yes	27	5	18.5	
Vaginal discharge				0.67
No	284	66	23.2	
Yes	16	3	18.8	

*125 out of 300 pregnant women were primigravida and had no previous obstetric history.

Table V shows the association between GBS colonisation rate in neonates and obstetric factors. Prolonged duration of labour was significantly shown to influence GBS colonisation in neonates 6/17 (35.3%), P <0.001. Regarding mode of delivery, women who delivered by spontaneous vaginal delivery had 14/139 (10.1%) neonates with GBS colonisation compared to 2/41 (4.9%) among the neonates delivered through caesarean section, P>0.05.

Nearly all women (15/16) who had history of taking antibiotic during labour had neonates with no GBS colonisation. The time taken between antibiotic intake and delivery was approximately one to four hours for those who were given intravenous antibiotics (ampicillin, and ceftriaxone) at labour ward.

Table V: Association between obstetric factors and GBS colonisation rate in neonates (n=180)

Variables	Total	Number of GBS isolated	Percentage	P-value
Mode of delivery				0.61
SVD	139	14	10.1	
C/S	41	2	4.9	
Time of ROM				0.65
≤ 18hrs	178	16	9	
>18hrs	2	0	0	
Intrapartum fever				0.58
No	177	16	9	
Yes	3	0	0	
Antibiotic use before/ during labour				0.69
No	164	15	9.1	
Yes	16	1	6.3	
Duration of labour				<0.00
≤ 12hrs	163	10	6.1	
>12hrs	17	6	35.3	
Weight of baby				0.51
<2.5kg	20	1	5	
≥2.5kg	160	15	9.4	

7.5 Association between GBS colonisation and HIV infection among pregnant women

Table VI and Table VII show the rate of GBS colonisation among pregnant women with HIV and their immunological status, respectively. HIV infection was detected in 24/300 (8%) of the study population, among whom 2/24 (8.3%) had GBS colonisation (P>0.05). In this analysis Chi-square test and Fisher's Exact test were used where appropriate.

Table VI: Association between GBS colonisation among pregnant women and HIV

HIV status	Total	Colonised	Percentage
Negative	276	67	24.3
Positive	24	2	8.3
Total	**300**	**69**	**23**

$\chi^2 = 3.16$; P=0.075; Fisher's exact test 0.081

Regarding immunological status of the two women with GBS colonisation one had CD 4 count less than 200 cells/µl and the other had CD4 count above 200 cells/µl.

Table VII: Relationship between GBS colonisation and CD4 level among pregnant women with HIV (n=24)

CD4 counts (Cells/µl)	Number of GBS isolated N (%)	Number of GBS isolated N (%)	Total
< 200	1(50)	1(50)	2
200-350	0(0)	5(100)	5
351-499	1(12.5)	7(85.5)	8
>500	0(0)	9(100)	9

χ^2 =6.0; P=0.11; Fisher's exact test 0.134

7.6 Aantimicrobial susceptibility pattern of the isolated GBS

Table VIII summarises the antimicrobial susceptibility pattern of GBS isolated from different sites. GBS isolated from vaginal canal showed high sensitivity to most of the antibiotics tested including vancomycin (100%), ampicillin (100%), penicillin G (98.1%) and ciprofloxacin but resistance to clindamycin (17.6%), erythromycin (13%) and ceftriaxone (13%). GBS isolated from the rectum showed high sensitivity to vancomycin (100%), ampicillin (100%), ciprofloxacin (93.8%) and resistance to erythromycin (18.7%), ceftriaxone (15.6%), cotrimoxazole (15.6%) and penicillin G (9.4%). GBS isolated from neonates had similar pattern of antimicrobial susceptibility as GBS isolates from vagina, however, they appeared to be more resistant to erythromycin (18.7%).

Table VIII: Antimicrobial sensitivity pattern of GBS isolated from pregnant women and neonates

Antimicrobial agent	GBS isolation (% of sensitivity)		
	Vaginal	**Rectal**	**Neonates**
Penicillin G	98.1	90.6	100
Ampicillin	100	100	100
Vancomycin	100	100	100
Erythromycin	87.0	81.3	81.3
Clindamycin	83.3	84.3	87.5
Ciprofloxacin	94.4	93.8	100
Cotrimoxazole	90.7	84.4	87.5
Cefriaxone	87.0	84.4	93.8

Sexually transmitted pathogens like *N. gonorrhoeae* and *T. vaginalis* was not detected/isolated among the study participants

8.0 DISCUSSION

This is the first published report on GBS colonisation among pregnant women and neonates in Dar es Salaam, Tanzania. The overall prevalence of GBS among pregnant women in this study was found to be 23% and in neonates was 8.9%. These findings are consistent with reports from previous studies of GBS colonisation in some of the developing countries [5,6,59], but the rates are lower than those reported in studies conducted in Zimbabwe 31.6% and Trinidad 32.9% [4,6]. Indeed, the rate of GBS colonisation varies widely throughout the world with colonisation rates, ranging from 5-35% [8]. The possible reasons for these variations in these studies could be differences in culture methods used, the number and type of sites cultured and type of medium used as well as difference in the populations investigated [66]. Apart from the differences attributable to microbial detection techniques, geographical variation exists.

In this study 120 neonates of the expected 300 were lost follow up. This was due to the fact that not all pregnant women delivered at MNH, a tertiary facility which handles referrals from peripheral and upcountry hospitals. Some of the antenatal clinics attendees who are referred to MNH are normally sent back to deliver in the municipal hospitals if after evaluation they are confirmed to have no serious risk factors.

In this study vaginal carriage rate (12.3%) was higher than rectal colonisation rate (5%), a finding that concur with those reported by Moyo et al [8] who found that the vaginal and rectal colonisation rate in Zimbabwe was 12.6% and 6.3%, respectively. Previous studies

investigating the relationship between rectal and vaginal colonisation in pregnant women suggested that the gastro-intestinal tract may be the primary site of colonisation by GBS and that vaginal colonisation may represent contamination from this source [44, 67]. However the finding of this study showing higher isolation frequency of GBS in samples taken from the vagina do not appear to support the theory of GBS contamination of the vagina from gastro-intestinal tract. The fact that GBS was at times isolated from one and not the other site clearly indicate that it is important to sample both vaginal and rectum when screening for GBS carrier in pregnant women.

Samples cultured from mothers and neonates showed that 37% pairs had positive GBS culture results. However it is difficult to say whether this is vertical transmission or not since DNA finger printing was not performed to confirm the transmission. Notably, other studies have reported transmission rates that ranged 29% to 85% [2, 68].

This study attempted to investigate association between GBS carriage in pregnant women, social demographic factors and obstetric factors e.g. parity, number of sexual partners, and level of education and gestation age. Of all the factors, maternal colonisation was significantly higher in women of gestation age between 41 and 42weeks compared to women of gestation age between 39 and 40 weeks, indicating an increase GBS carriage with gestation age. The other maternal factors including social demographic factors were not significantly associated with GBS colonisation.

In this study the rate of GBS colonisation was higher among multiparous (4+) women but not statistically significant. In a previous study that showed an increased risk of GBS carriage among multipara women attributed the finding to sexual activity [8], being more prevalent in women who reported multiple sexual partners and frequent intercourse [17, 69]. However, in the present study history of multiple sexual partners during the last one year before the date of the interview did not appear to increase the risk of GBS colonisation.

In the present study GBS was isolated more frequently from women of age group 30-34 (32.1%) compared with women aged <20 years (15.4%), which is in contrast with reports from another study showing high isolation frequencies in women younger than 20 years [16], differences which are difficult to explain.

In this investigation women with no formal education (37.5%) were more likely to be colonised with GBS, a finding that is consistent with observations from a study conducted by Regan et al [69] that showed GBS colonisation to be less common among women with more education. The association between GBS colonisation and level of education could be explained, at least in part, by personal hygiene, which is more likely to be better among educated than the less educated women.

This study attempted to associate GBS colonisation in neonates with maternal factors such as duration of labour, prolonged rupture of membrane, intrapartum fever, mode of

delivery and low birth weight. Regarding these factors, GBS colonisation in neonates was associated with prolonged duration of labour P <0.001, an observation which is in keeping with an earlier study finding showing that duration of labour longer than 12 hrs increases the risk of GBS transmission from pregnant women to neonates[16]. The most plausible explanation for this observation is prolonged exposure of the neonate in the birth canal that increases the risk of GBS colonisation.

The other maternal risk factors including prolonged rupture of membrane, history of intrapartum fever and low birth weight did not correlate with the GBS colonisation in neonates. For example, although it is biologically plausible to argue that prolonged rupture of membranes would increase the risk of neonatal GBS colonisation especially among women harbouring GBS in the birth canal this association could not be found in the present study. The lack of association between these potential risk factors with neonatal GBS colonisation is in contrast with findings from a study done by Fitzroy et al which associated such factors with an increased risk of vertical transmission [4]. The lack of association with these factors can possibly be explained by the fact that the numbers of participants in this study with such risk factors were small (4%) and maybe have decreased the power of the study.

In this study neonates delivered by spontaneous vaginal delivery (10.1%) had higher rate of GBS colonisation compared to those who delivered by caesarean section (4.9%) although this finding was not statistically significant. Failure to demonstrate the

association between these two modes of delivery and the rate of GBS colonisation in neonates may be due to small sample size investigated in this study.

In the present study fifteen out of sixteen (~ 94%) women who were given parenteral antibiotics (usually penicillin or ampicillin) during labour had neonates with no GBS colonisation. This finding vindicates the USA Centers for Disease Control and Prevention (CDC) guidelines that recommend use of antibiotic prophylaxis to pregnant women with GBS colonisation as preventive measure for GBS transmission to neonates [43]. Previous studies have shown that therapeutic antibiotic concentration is achieved in amniotic fluid two to four hours after parenteral administration, which may guide the timing of antibiotic administration intended for minimising the risk of vertical transmission [43, 70,71].

GBS colonisation in studied population was not associated with either HIV infection or immunological status (CD4), which is in contrast to results of a previous study [30] which showed that invasive group B streptococcal disease occurs more frequently in HIV infected individuals than uninfected ones. Lack of association in the present study could partly be attributed to the low percent (8%) of HIV infected pregnant women among the studied population and the fact that only one woman had CD4 counts < 200 cells/μl.

Notably all GBS isolated during this study were sensitive to ampicillin and vancomycin, which imply that these two antibiotics could be used for empiric prophylaxis. However

use of vancomycin is not recommended for routine use in order to prevent emergence of resistance to this drug [72]. Most of isolates were sensitive (90% to 95%) to ciprofloxacin and penicillin G and (80% to 90%) sensitive to clindamycin, erythromycin, and ceftriaxone. Use of these antibiotics in treatment and prophylaxis of GBS carriage/infection should be guided by sensitivity testing.

Resistance to penicillin was found in 9.4% of the isolates. These findings differ from previous studies [8, 49,73,74] where resistance to penicillin has not been detected and provide an early warning sign to the problem of growing resistance of bacteria to commonly used antibiotics in our settings. The difference may suggest that there may be a change of susceptibility pattern to this antibiotic. The observed resistance to penicillin, though of a lower rate than that of clindamycin and erythromycin is of great concern because penicillins are the most widely used and comparatively more affordable antibiotics in our resource-constrained settings. Resistance observed from antibiotics commonly used in prophylaxis against GBS in pregnant women and neonates underscores the need for continuous surveillance of antibiotic sensitivity in order to monitor antimicrobial resistance pattern of this bacterium.

In this study it was generally noted that there were slight differences in sensitivity pattern of isolates from vagina compared to the isolates from the rectum to the investigated antibiotics. The possible reason for this site difference could be, some of the strains from these two sites may differ from each other, but this suspicion could not be substantiated

in the present study since finger printing was not performed. Another reason may be due to difference in level of normal flora from these different sites [75].

Besides GBS colonisation, the present study also investigated occurrence of STI agents in the pregnant women. Sexually transmitted pathogens like *N. gonorrhoeae* and *T. vaginalis* was not detected/found in the current study. The possible explanation for this finding is that these women may have been treated for STI early as they had attended antenatal clinic several times before being recruited in our study. Use of HVS and not endocervical swab could also have affected the yielding rate of *N. gonorrheae*. These results may also indicate decrease of these infections in the community as a result of increasing use of health facilities for health care and extensive training programme of health workers on syndromic STI management. There was also limitation of diagnostic kits for detection of other sexually transmitted pathogens like *C. trachomatis*.

9.0 CONCLUSION AND RECOMMENDATIONS

9.1 CONCLUSION

The overall prevalence of GBS among pregnant women was 23% with vaginal carriage rate (12.3%) higher than rectal colonisation rate (5%) and in neonates 8.9%. In this study, GBS colonisation in neonates was associated with prolonged duration of labour (>12). Other risk factors for maternal and neonates colonisation such as maternal age, premature rupture of membranes, intrapartum fever, low birth weight and HIV infection were not associated with GBS colonisation. All (100%) GBS isolates were sensitive to ampicillin and vancomycin and sensitive to erythromycin, clindamycin and ceftriaxone ranged 80% to90%.

9.2 RECOMMENDATIONS

Based on the results of this investigation the following recommendations seem pertinent:

1. It is recommended to conduct further studies to characterise GBS strains in order to further elucidate extent of mother to child transmission (vertical transmission) by performing DNA finger printing.
2. There is need to conduct a follow up study of neonates with GBS colonisation in order to determine the clinical outcome.
3. It is desirable to conduct larger study in order to further determine the potential risk factors for GBS colonisation that have been frequently cited in the literature such as prolonged rupture of membrane, intrapartum fever, mode of delivery and

low birth weight which could not be established in this study probably due to small sample size.

4. For the time-being ampicillin should be the preferred choice for treatment of GBS infections, however continuous antimicrobial surveillance is required to monitor trends of resistance.

10. REFERENCES

1. Edwards MS, Baker CJ. *Streptococcus agalactiae* (Group B Streptococcus). In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Disease, 5th edn. Philadelphia: Churchill Livingstone, 2000: 2156–67.
2. Schuchat A. Group B Streptococcus. *Lancet* 1999; 353: 51–6.
3. Baker CJ, Edwards MS. Group B streptococcal infections. In: Klein, JO and Marcy SM (eds). *Infectious Diseases of the Fetus and Newborn Infant*, 4th edn. WB Saunders, Philadelphia, 1994; 835-890.
4. Orrett FA. Colonization with Group B streptococci in pregnancy and outcome of infected neonates in Trinidad. *Pediatrics International* 2003; 45**:** 319–323.
5. Onile BA. Group B streptococcal carriage in Nigeria. *Trans Roy Soc Trop Med Hyg* 1980;74: 367–70.
6. Suara RO, Adegbola RA, Baker CJ, Secka O, Mulholland EK, Greenwood BM. Carriage of Group B streptococci in pregnant Gambian mothers and their infants. *Infect Dis* 1994; 170: 1316–19.
7. Sunna E, El-Daher N, Bustami K, Na'was T. A study of group B streptococcal carrier state during late pregnancy. *Trop Geog Med* 1991; 43: 161–4.
8. Moyo SR, Mudzori J, Tswana, Maeland JA. Prevalence, capsular type distribution, anthropometric and obstetric factors of group B streptococcus *(Streptococcus agalactiae*) colonization in pregnancy. *Central Afr J Med* 2000; 46(5):115-20

9. Patrick RM, Ken SR, Kobayashi GS, Michael AP: Medical Microbiology, fourth edition. Philadelphia: Churchill Livingstone. 2002; 23: 217-235.

10. Committee on Infectious Diseases and Committee on Foetus and Newborn. Guidelines for prevention of group B streptococcal (GBS) infection by chemoprophylaxis. *Pediatr* 1992; 90: 775-8.

11. Katz V and Bowes WA. Perinatal group B streptococcal infections across intact amniotic membranes. *J Reprod Med* 1988; 33: 445–449.

12. Ramos E, Gaudier FL, Hearing LR, Del Valle GO, Jenkins S, Briones D. Group B streptococcus colonization in pregnant diabetic women. *Obstet Gynecol* 1997; 89: 257–60.

13. Blumberg RM, Feldman RG. Neonatal group B streptococcal infection. *Current Pediatr* 1996; 6: 34–7.

14. Perovic O. Group B streptococcal infections. *S Afr J Epidemiol Infect* 1998; 13(1):26-33.

15. Mandell GL, Bennett JE, Dolin R. Principles and Practice of Infectious Disease, 5th edn. Philadelphia: Churchill Livingstone, 1995; 180: 1835-1845.

16. Schuchat A. Epidemiology of Group B Streptococcal Disease in the United States: Shifting Paradigms. *Clin Microbiol Rev* 1998; 11(3): 497–513.

17. Hernandez TM, Soriano BD. High prevalence of group B Streptococci colonisation in Mexico pregnant women. *Ginecol Obstet Mex* 2006; 74(3): 139-43.

18. Zangwill KM, Schuchat A, Wenger JD. Group B streptococcal disease in the United States, 1990: Report from a multistage active surveillance system. *MMWR* 1992; 41:25-32.

19. Daugaard HO, Thomsen AC, Henriques U, Ostergaard A. Group B streptococci in the lower urogenital tract and late abortions. *Am J Obstet Gynecol* 1988; 158:28–31.

20. Moyo SR, Tswana SA, Hagerstrand I, Nystrom L, Tswana AS, Blomberg J, et al. Stillbirth and intrauterine infection. A comparison between histological chorioamnionitis and microbiological finding. *Int J Obstet Gynecol* 1996; 54:115-123.

21. Farley MM. Group B Streptococcal Disease in Non-pregnant Adults. *Clin Infect Dis* 2001; 33:556–61.

22. Schwartz B, Anne S, Oxtoby MJ, Cochi SL, Hightower A, Broome CV. Invasive group B streptococcal disease in adults: a Population-based study in metropolitan Atlanta. *JAMA* 1991; 266: 1112–1114.

23. Baddour LM. The Infectious Diseases Society of America's Emerging Infections Network. Infective endocarditis caused by b-hemolytic streptococci. *Clin Infect Dis* 1998; 26:66–71

24. Domingo P, Barquet N, Alvarez M, *et al*. Group B streptococcal meningitis in adults: report of twelve cases and review. *Clin Infect Dis*1997; 25:1180–7.

25. Galask RP, Varner MW, Petzold CR, Wilbur SL. Bacterial attachment to the chorioamniotic membranes. *Am J Obstet Gynecol* 1984; 148: 915-28.

MUHIMBILI MEDICAL UNIVERSITY COLLEGE
LIBRARY
DSM

26. Centers for Disease Control and Prevention. Decreasing incidence of perinatal group B streptococcal disease United States. *MMWR* 1997; 46: 473–477.

27. Trager JD, Martin JM, Barbadora K, Green M, Wald ER. Probable community acquisition of group B streptococcus in an infant with late-onset disease: demonstration using field inversion gel electrophoresis. *Arch Pediatr Adol Med* 1996; 150: 766–768.

28. Greenwood D, Richard C and John F. Medical microbiology. A guide to microbial infections: Pathogenesis, immunity, laboratory diagnosis and control. 16th edn Philadelphia: Churchill Livingstone. 2002; 16:180-188.

29. El Beitune P, Duarte G, Maffei CM, Quintana SM, Silva AC, Nogueira AA. Group B streptococcus carriers among HIV-1 infected pregnant women: prevalence and risk factors. *Eur J Obstet Gynecol Reprod Biol* 2006; 128 (2): 54-8.

30. Farley MM, Harvey RC, Stull T, Smith JD, Schuchat A, Wenger JD, et al. A Population-Based Assessment of Invasive Disease due to Group Streptococci non pregnant Adults. *N Engl J Med* 1993; 328: 1807-1811.

31. El Beitune P, DuarteG, Maffei CM. Pregnancy outcome for HIV -1-infected women with streptococcus agalactiae. *Int J Gynaecol Obstet* 2007; 96(1):35-6.

32. Lancefield RC. A serological differentiation of human and other groups of haemolytic streptococci. *J Exp Med* 1933; 57:571.

33. Tamura GS, Kuypers JM, Smith S, Raff H and Rubens CE. Adherence of group B streptococci to cultured epithelial cells: Roles of environmental factors and bacterial surface components. *Infect Immun* 1995; 62: 2450-2458.

34. Beckmann C, Waggoner JD, Harris TO, Tamura GS and Rubens CE. Identification of novel adhesions from group B streptococci by phage display reveals that C5a peptidase mediates fibronectin binding. *Infect Immun* 2002; 70: 2869-2876.

35. Axel S, Katherina Z, Giampiero P, Andreas M, pietro S, Bernhard JK, et al. The fibrinogen receptor FbsA promotes adherence of *Streptococcus agalactiae* to human epithelial cells. *Infect. Immun* 2004; 72: 6197-6205

36. Heike G, Bernhard JE and Dieter JR. The novel fibrinogen-binding FbsB promotes *Streptococcus agalactiae* invasion into epithelial cells. *Infect Immun* 2004; 72: 3495-3504.

37. Zaleznik DF, Rench MA, Hillier S, Krohn MA, Platt R, Lee ML, et al. Invasive disease due to group B Streptococcus in pregnant women and neonates from diverse population groups. *Clin Infect Dis* 2000; 30: 276-81.

38. Anita S, Patricia F: Neonatal & maternal group B streptococcal infections: A comprehensive review: *Indian J Med Res* 2004; 120: 141-150.

39. Lachenauer CS, Kasper DL, Shimada J, et al. Serotypes VI and VIII predominate among group B streptococci isolated from pregnant Japanese women. *J Infect Dis* 1999; 179:1030-3.

40. Kasper DL, Baker CJ. Electron microscopy definition of surface antigen of group B streptococcus. *J Infect Dis*1984; 139: 147-151.

41. Kasper DL, FM Ausubel, JL Michel, LC Madoff, and DE. Kling. Cloned alpha and beta C protein antigens of group B streptococci elicit protective immunity. *Infect Immun* 1991; 59: 2023–2038.

42. Stalhammar-Carlemalm M, Stenberg L and G Lindahl G. Protein Rib: a novel group B streptococcal cell surface protein that confers protective immunity and is expressed by most strains causing invasive infections. *J Exp Med 1993*; 177**:** 1593–1603.

43. Centers for Disease Control and Prevention (CDC). Prevention of Perinatal Group B Streptococcal Disease: Revised Guidelines from CDC. *MMWR 2002*; 51: 1-24.

44. Dillon HC, Gray E, Pass MA, and Gray BM. Anorectal and Vaginal carriage of group B streptococci during pregnancy. *J Infect Dis* 1982; 145: 794-9.

45. Persson KM and Forsgren A. Evaluation of culture methods for isolation of group B streptococci. *Diagn Microbiol Infect Dis* 1987; 6: 175-7.

46. Facklam RR, Padula JR, Thacker LG et al. Presumptive identification of group A, B, and D streptococci on agar plate medium. *J Clin Microbiol* 1979; 9: 665-72.

47. Washington JA, Laboratory procedures in clinical microbiology, second edition. 1985; 4:131-156.

48. WHO, Basic laboratory procedures in clinical bacteriology 1997.

49. Motlova J, Strakova L, Urbaskova P, Sak P, and Sever T. Vaginal & rectal carriage of *Streptococcus agalactiae* in the Czech Republic: incidence, serotypes distribution & susceptibility to antibiotics. *Indian J Med Res* 2004; 119: 84-7.

50. Andrews JI, Diekema DJ, Hunter SK, et al. Group B streptococci causing neonatal bloodstream infection: antimicrobial susceptibility. *Am J Obs Gyn* 2000; 183:859-862.

51. Betriu C, Gomez M, Sanchez A, Cruceyra A, Romero J and Picazo JJ. Antibiotic resistance and penicillin tolerance in clinical isolates of group streptococci. *Antimicrob Agents Chemother* 1994; 38:2183-86.

52. Berkowitz K, Regan JA, and Greenberg E. Antibiotic resistance pattern of group B streptococci in pregnant women. *J Clin Microbiol* 1990; 28:5-7.

53. Dorand RD and Adam G. Relapse during penicillin treatment of group B streptococcal meningitis. *J Pediatr* 1976; 89:188-190.

54. Franciosi RA, Knostman JD and Zimmerman RA. Group B streptococcal neonatal and infants infections. *J Pediatr* 1973; 82: 707-718.

55. McCracken GH Jr. New concepts in the management of infants and children with meningitis. *Pediatr Infect Dis* 1983; 2: 51-55.

56. Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease: a public health perspective. *MMWR* 1996; 45: 1–24.

57. Naiel B, Nicola J, Dror M, Colin B, Rosalind MH, Pablo Y, et al. Population structure of group B streptococcus from a low-incidence region for invasive neonatal disease. *J Clin Microbiol* 2005; 151: 1875–1881.

58. Edwards MS, Rench MA, Haffar AA, Murphy MA, Desmond MM, Baker CJ. Long-term sequelae of group B streptococcal meningitis in infants. *J Pediatr 1985*; 106: 717-22.

59. Stoll BJ, Schuchat A. Maternal carriage of group B streptococci in developing countries. *Pediatr Infect Dis J* 1998; 17: 499-503.

60. Kadanali A, Altoparlak U, Kadanali S. Maternal carriage and neonatal colonisation of group B streptococcus in eastern Turkey: prevalence, risk factors and antimicrobial resistance. *Int J Clin Pract 2005*; 59: 437–440.

61. Blomberg B, Karim PM, Urassa WK, Tamim BS, Mwakagile DS, Roland J, et al: Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: *BMC Infect Dis* 2007; 7: 43.

62. Tanzania National HIV Rapid Testing Algorithm 2007

63. Lyamuya EF, Aboud S, Urassa WK, Sufi J, Mbwana J, Ndugulile F, Massambu C. Evaluation of simple rapid HIV assays and development of national rapid HIV test algorithms in Dar es Salaam, Tanzania. *BMC Infect Dis* 2009; 18: 9-19.

64. Ministry of Health and Social Welfare, National AIDS Control Programme, Guidelines for HIV testing and counselling in clinical setting (PITC). April 2008.

65. SPSS Inc version 13.0. The statistical Package for the Social Sciences. Chicago II: SPSS Inc, 2004.

66. Schuchat A, Wenger JD, Epidemiology of group B streptococcal disease. *Epidemiol Rev* 1994; 16: 374-402.

67. Badri MS, Zawaneh S, CrusAC, Mantilla G, Baer H, Spellacy WN, et al. Rectal colonisation with group B streptococcus in relation to vaginal colonisation of pregnant women. *J Infect Dis* 1977; 135(2):308-12.

68. Uh. Y, Jang IH,Yoon KJ, Lee CH, Kwon JY, Kim MC. Colonisation rates and serotypes of groupB streptococci isolated from pregnant women in a Korean tertiay hospital. *Eur J Microbiol Infect Dis* 1997; 16: 753-6.

69. Regan JA, Klebanoff MA, Nugent RP. Vaginal Infection and Prematurity study Group.The epidemiology of group B streptococcal colonisation in pregnancy. *Obstet Gynecol* 1991; 77:604-610.

70. Baker CJ, Group B streptococcal infections. *Clin Perinatol* 1977; 24:59-70.

71. De Cueto M, Sanchez MJ, Sampedro A, MirandaJA, Herruzo AJ,Rosa-Fraile M. Timing of intrapartum ampicillin and prevention of vertical transmission of group B streptococcus. *Obstet Gynecol* 1998; 91(1):112-4.

72. Center for disease control and prevention. Recommendations for Preventing the Spread of Vancomycin Resistance. Recommendations of the Hospital Infection Control Practices Advisory Commtee (HICPAC). *MMWR* 1995; 44(12):1-13.

73. Lubna C, Lisa M, Kaaren B, Charles CK. Pattern of antibiotic resistance among group B streptococcus isolates. *Obstet Gynecol* 2006; 57492:1-4.

74. Costal AL, Lamy FilhoF, CheinMB, Brito LM, Lamy ZC, Andrade KL. Prevalence of colonisation by group B streptococcus in pregnant women from a public maternity of Northwest region of Brazil. *Rev Bras Ginecol Obstet* 2008; 30(6):274-80.

75. Antoine A. Commensal flora may play key role in spreading antibiotic resistance. *ASM news* 2003; 69(12):602.