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RESEARCH

Group B streptococcus colonisation in pregnant women at Dr. George Mukhari Hospital, South Africa

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The aim of the study was to estimate group B streptococcus (GBS) colonisation in pregnant mothers using selective enrichment broth and solid media for culturing GBS. Vaginal and rectal swabs were collected from 413 pregnant women for GBS culture at recruitment stage. Direct plating and enrichment broth culture methods were compared by using the same swab samples. The swabs were cultured on colistin nalidixic agar (CNA) plate and incubated at 37°C and examined after 18-24 h. The samples which were culture negative on a CNA agar plate were then inoculated into a Todd-Hewitt enrichment broth to recover any GBS present that was not recovered on the solid agar. With the CNA agar plate, the samples were cultured separately to enable identification of colonised sites such as vaginal sites or rectal sites. Rectal and vaginal swabs were inoculated into Todd-Hewitt enrichment broth at the same time in the same tube. The GBS colonisation rate in pregnant women was 30.9% (128/413). The CNA agar plate recovered 45.3% (58/128) of the GBS isolates, whereas 54.7% (70/128) isolates were recovered from Todd-Hewitt broth. Pregnant women of various ages were found to be at risk of GBS colonisation. The colonisation rate was however highest among women of 25–29 age groups as compared with other age groups. Detection of group B streptococcus improved when both rectal and vaginal swabs were collected for laboratory analysis. The simultaneous use of Todd-Hewitt broth and CNA plate also improved the yield of group B streptococcus.

Keywords: colonisation,detection methods, Group B streptococcus, pregnant women

Introduction

Streptococcus agalactiae (Group B streptococcus; GBS) is an encapsulated organism capable of infecting newborn babies, and can produce severe disease in immunocompromised hosts.[1] Infection is rare in immunocompetent patients except among those with underlying conditions.[2] The major colonised sites for GBS are reported to be the vagina and the rectum. The colonisation of these regions is a risk factor for subsequent infection in pregnant women and newborns.[3,4] Ethnicity, maternal age, parity, marital status, education and smoking have been reported not to influence the prevalence of colonisation of GBS.[5]

The study done by Dangor et al. confirmed the high incidence of invasive GBS disease observed in the last two decades in parts of Africa.[6] Maternal screening for GBS recto-vaginal colonisation during pregnancy in South Africa is still not routinely performed in public health facilities.[7] The high incidence might be attributed to the prevalence of HIV as an underlying condition predisposing patients to GBS infection.

The screening-based strategy can reduce early onset of neonatal GBS disease. The strategy's effectiveness depends on the specimen sampling site, the timing of collection and sensitivity of the microbiological methods used.[8] The centre for Disease Control and Prevention (CDC) recommends that isolation of group B streptococcus from vaginal and rectal swabs should be done in a selective broth medium (Todd-Hewitt broth with gentamicin, colistin and nalidixic acid) followed by subculture on blood agar or selective media.[9] Sensitivity of selective broth medium for GBS was reported to be 82 to 99%.[8,10,11] Different strategies for identification of high risk mothers and infants and provision of intrapartum prophylaxis may reduce the rate of neonatal sepsis, though they are unlikely to eliminate the problem.[12] Limitations to the use of intrapartum antibiotics include the emergence of antibiotic-resistant GBS and ineffectiveness in preventing late onset disease due to absence of protective antibodies in mothers.[13]

The aim of this study was to assess the colonisation rate of GBS and the associated risk factors in pregnant women at Dr. George Mukhari Academic Hospital.

Method

Study area

The study was conducted at an antenatal clinic at Dr. George Mukhari Academic Hospital in Ga-Rankuwa, Pretoria, which services the city of Tshwane municipality in Gauteng, South Africa. The hospital is a teaching hospital for the Sefako Makgatho Health Sciences University (SMU), formerly known as the University of Limpopo (Medunsa campus). Ga-Rankuwa has a mixed population comprising various black African tribal groups. The hospital's antenatal clinic and paediatric outpatient department serve the immediate surrounding areas and distant areas such as Limpopo and North West provinces.

Study design and population

This was a descriptive, prospective study, and the participants were pregnant women who were able to provide informed consent for participation. The women were at least 18 years of age and were assessed to be at 16 weeks gestational age and above. Women who had received antibiotic treatment in the

2 weeks prior to recruitment, and who were regarded as critically ill and admitted by the attending physician, were excluded from the study. A convenient sampling method was used. Vaginal (lower and higher vaginal) and rectal swabs were collected from 413 pregnant women between February 2012 and October 2012 by a trained research nurse. Group B streptococcus colonisation was defined as isolation of GBS from at least one type of sample.

Data on socio-economic status (level of education, employment, and marital status), previous and current obstetric history (parity, miscarriages, stillbirths) and current health status were collected using a questionnaire at recruitment.

Sample collection and processing

Specimens were collected using a cotton wool swab and placed in Amies transport medium without charcoal (Rochelle Chemicals & Lab equipment, South Africa). All samples were accurately labelled and placed in a cooler box filled with ice blocks and transported from the antenatal clinic to Dr. George Mukhari Academic Hospital National Health Laboratory Science (NHLS) Medical Microbiology laboratory for processing within 2h.

All vaginal and rectal swab specimens were cultured on colistin nalidixic agar (CNA) plates, and into Todd-Hewitt broth supplemented with 8 µg/ml colistin and 15 µg/ml gentamicin to inhibit other normal flora and recover GBS that may not have been detected on CNA.[14] Low vaginal swabs were those that were inserted into the vagina with a depth less than 2 cm, whereas high vaginal swabs were those that were inserted with a depth greater than 2 cm. The plates and tubes were incubated overnight at 37°C in the presence of 5% carbon dioxide. After 18-24 hours of incubation, primary plates were examined for β-haemolytic streptococcal colonies typical of GBS. A single colony from each isolate was picked and subcultured onto blood agar. Plates showing no growth were incubated for another 24h. Todd Hewitt broth cultures were inoculated onto 5% sheep blood agar plates after overnight incubation. Two CNA agar plates were used to culture the swabs, one plate was divided into two to culture high vaginal and lower vaginal swabs and another plate was used for rectal swab culture. Isolates were identified as GBS by the following criteria: β-haemolysis on blood agar, Gram staining showing Gram-positive cocci in pairs or short chains, negative reaction with catalase reagent, bile esculin agar and Lancefield grouping with group B antisera (Streptex; Biomereux and Remel Ltd, France). If the results were not clear, CAMP (Christie Atkins Munch-Petersen) test was employed to confirm group B streptococcus isolates. Data analysis of socio-demographic factors was done using the Chi-squared test at a significance level of $p < 0.05$.

Ethical approval

Informed consent was obtained from the patients and the human experimentation guidelines of the South African Medical Research Council (MRC) were followed in the conduct of the research. The study was approved by Higher Degrees Ethics Committee and SMU/Medunsa Research and Ethics Committee, Dr. George Mukhari Academic Hospital Management (MREC/P/02/2011: IR) and UNISA College of Agriculture and Environmental Sciences.

Results

GBS colonisation was detected in 30.9% (128/413) of the pregnant women. All the women enrolled in the study were of the same racial group (black Africans) with an age range of 18–45 years and mean age of 30. The study participants were from different geographic areas (urban, semi-urban and rural) surrounding Dr. George Mukhari Academic Hospital. The majority of the participants were single, widowed or divorced. The highest proportion of GBS colonised women was among those of the age group between 25 and 29 (Table 1). The *p*-values were computed after comparative analysis of the risk factors for each socio-demographic characteristic.

Most participants had an educational level below matric (56.8%), with the fewest participants having a tertiary level education (16.6%). The difference in colonisation rates were significant

Table 1: Socio-demographic characteristics of pregnant women investigated for GBS colonisation

Characteristics	Category	GBS colonised (*n* = 128)	GBS non-colonised (*n* = 285)	*p*-value
		Frequency (%)	Frequency (%)	
Age in years	< 20	2 (1.6)	10 (3.5)	0.07
	20–24	28 (21.8)	47 (16.5)	
	25–29	39 (30.5)	71 (24.9)	
	30–34	24 (18.8)	95 (33.3)	
	35–39	24 (18.8)	57 (20.0)	
	≥ 40	10 (7.8)	20 (7.0)	
Geographical areas	Urban	113 (88.8)	246 (86.3)	0.08
	Urban-rural	8 (1.9)	28 (9.8)	
	Rural	4 (1.0)	2 (0.7)	
Marital status	Married	27 (21. 1)	71 (17.2)	0.56
	Cohabiting	31 (24.2)	58 (14.0)	
	Single/widowed /divorced	67 (52.3)	147 (35.6)	
Educational level	Below matric	71 (55.5)	107(37.5)	<0.0001
	Matric	35 (27.3)	153(53.7)	
	Tertiary	15 (11.7)	15 (5.2)	
Employment	Unemployed	46(35.9)	179 (62.8)	0.003
	Employed	51(39.8)	97(34.0)	

among the women at different educational levels ($p < 0.05$). There was also a significant difference in colonisation rates between unemployed women compared with employed ones ($p < 0.05$).

The age range of the GBS colonised pregnant women was 18–45 and the age categories were compared. The GBS colonisation rate in pregnant women was highest in the 25-29 year age group, and lowest in the < 20 year age category. Adverse pregnancy outcomes were categorised into miscarriages, stillbirths and HIV-positive as an underlying condition. Pregnant women who were currently colonised with GBS and had miscarriages previously comprised 25.0% (32/128) while 9.4% (12/128) had stillbirths (Table 2). N/A means the number of women who were not categorised based on trimester as this data was not available. The *p*-values were computed after comparative analysis of the risk factors for each variable. None of the risk factors were significantly associated with GBS colonisation.

Comparison between media and site used for diagnosis

The importance of selective enrichment broth and selective plating is shown in Table 3. Slightly more than half of GBS-positive samples were isolated using Todd-Hewitt broth, whereas less than half were isolated from CNA agar plate. Of 58 colonised pregnant women, 12 had strains cultured only from vaginal swabs, while 22 had strains isolated only from the rectal swabs and another 24 had strains isolated simultaneously from both the vaginal and rectal swabs when cultured on a CNA agar plate.

Discussion

Group B streptococcus is prevalent worldwide and its colonisation rate varies depending on geographical areas and socio-demographic factors. Of the GBS positive cases studied, 52.3% were women of 20–29 of age. This is an age where sexual activity is increased and GBS is often regarded as sexually transmitted.[15] The factors that were investigated in this study included reproductive history and HIV status. The high prevalence of HIV infection might be considered to aggravate the burden of GBS disease in South Africa, but maternal HIV infection has not been associated with a high prevalence of GBS colonisation.[6] No association of HIV status and GBS colonisation was found in the current study. Women with $CD4^+$ lymphocyte counts of > 500 cells/mm^3 have, however, been reported as an exception with regards to the association.[7]

GBS was previously reported as being associated with preterm delivery, miscarriages, still birth and parity. In various studies, there were no significant differences in colonisation rates noted on the basis of age or parity, though some studies report that increasing age and parity can be associated with lower rates of GBS carriage.[16,17] Regan et al.[18] described GBS carriage as being

Table 2: Variables analysed for association with GBS colonization in pregnant women

Variables	GBS colonised (*n* = 128)	GBS non-colonised (*n* = 285)	*p*-value
	Frequency (%)	Frequency (%)	
Gestational age (weeks)			0.12
First trimester	57 (44.5)	56 (19.6)	
Second trimester	100 (78.1)	108 (37.9)	
Third trimester	10 (7.8)	15 (5.3)	
N/A	35 (27.3)	20 (7.0)	
HIV Status			0.52
Positive	52 (40.6)	104 (36.5)	
Negative	72 (56.3)	170 (59.6)	
Parity			0.36
0	38 (29.7)	66 (23.2)	
1–2	68 (53.1)	160 (56.1)	
≥ 3	19(14.8)	50 (17.5)	
Stillbirths			0.24
0	113 (88.3)	258 (90.5)	
1–2	12 (9.4)	16 (5.6)	
≥ 3	0 (0.0)	2 (0.7)	
Miscarriages			0.33
0	93 (72.7)	223 (78.2)	
1–2	29 (22.7)	49 (17.2)	
≥ 3	3 (2.3)	4 (1.4)	

Table 3: Comparison between type of media and recovery sites

GBS colonised pregnant women	Type of a media Frequency (%)	GBS recovery sites Frequency (%)
128 (30.9)	CNA58 (45.3) Todd-Hewitt broth 70 (54.7)	Vaginal sites 12 (20.7)
		Rectal sites 22 (37.9)
		Both sites 24 (41.4)

more common among older women and women of lower parity. In this study, no association between colonisation and age or parity was found, similar to a study by Valkenburg-van den Berg, et al.[19] Ethnicity can explain differences in GBS colonisation rates. The GBS colonisation rate among, for instance, black pregnant women in some parts of Africa has been reported to be higher.[20] Comparisons between different ethnic groups were limited in the current study since all women were of the same ethnic group. Although the importance of infection as a cause of preterm delivery is gaining recognition, little is known about the role of group B streptococcus infection in miscarriages. Conflicting reports have been published to explain the role of GBS infection and miscarriage.[21–23] There was no association between pregnant women who had a history of miscarriages, stillbirth and group B streptococcus colonisation in the current study.

A comparison of GBS colonisation rate among different studies is often biased due to substantial methodological differences.[24] The maternal rate of GBS colonisation in this study was 30.9%, which is higher compared to other African countries, such as Mozambique where a colonisation rate of 1.8% was reported.[25] The difference in the colonisation rate reported from Mozambique and the present study may be due to the type of samples used for the isolation of GBS. In the Mozambican study they used blood for culturing but in this study only vaginal and rectal swabs were used. Geographic differences can also contribute to the different colonisation rates. Higher rates have been reported previously with 35% and 54% in Europe and 47% in Zimbabwe.[26–28] The reasons for these high colonisation rates in these studies might also be due to specimen collection and processing techniques used.

Selective broth medium with antibiotics, namely Todd-Hewitt broth with gentamicin and colistin or nalidixic acid, followed by subculture on blood agar was used in this study as recommended by the current Centres for Disease Control and Prevention guidelines for prenatal GBS screening.[9,29] There is limited data on the validity of this recommended method in low to middle income countries where there are differences in distribution of saprophytic organisms. In the current study, as with Manning et al.,[14] a combination of gentamicin and colistin was used for the recovery of GBS from pregnant South African women instead of combinations of nalidixic acid/colistin and nalidixic acid/gentamicin. This was done to improve the isolation rate of GBS strains. When direct agar plating was used instead of selective enrichment broth, as many as 50% of woman who are GBS carriers have false-negative culture results (CDC, 1999). Studies that used selective medium, reported slightly higher rates compared to those that do not.[30,31] A selective medium containing Todd-Hewitt broth, sheep blood, nalidixic acid and gentamicin was found to enhance the isolation of GBS significantly.[32] Different areas of specimen collection, may determine the sensitivity in detection of GBS colonisation.[4,33] Culture methods should not be neglected for the exclusive use of molecular assays in the detection of GBS.

In conclusion, this study revealed that pregnant women of all ages are at risk of group B streptococcus. The GBS colonisation rate of 30.9% among pregnant women at Dr. George Mukhari Academic Hospital was comparable to those reported in previous studies. The results confirmed that the recovery of GBS was improved when the use of Todd-Hewitt broth was combined with isolation on CNA plates.

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Conflict of Interest – None of the authors have a commercial or other association that might have posed a conflict of interest concerning the research presented.

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