Incidence and anti-microbial resistance profile of Group B Streptococcus (GBS) infection in pregnant women in Nsukka, Enugu State, Nigeria

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This study was performed to determine the incidence and antimicrobial resistance profile of Group B Streptococcus in pregnant women in Nsukka, Enugu State. From January 2010 to July 2011, vaginal swab and rectal swab samples were obtained from 200 pregnant women of gestational age from 24 to 37 weeks that were attending antenatal clinic at Bishop Shanahan Memorial Hospital and Nsukka District Hospital. Vaginal and rectal cultures for the isolation and identification of Group B Streptococcus (GBS) were carried out according to standard microbiological methods. The Kirby-Bauer disk-diffusion method was employed to determine antibiograms of GBS isolates. Samples were also obtained from 200 non-pregnant women. The carriage rate of GBS among pregnant women was 18.00%, while in non-pregnant women it was 5.5%. Statistical analyses proved the difference to be significant (P<0.05). A total of 58 GBS isolates were used for in vitro susceptibility test to ampicillin and cloxacillin, amoxycillin, cefuroxine, ceftriaxone, erythromycin, ciprofloxacin, streptomycin, Pefloxacin, sulphamethoxazole and trimethoprim and gentamycin. No resistance to Ampicillin and Cloxacillin, amoxycillin, cefuroxine and ceftriaxone was found. Of the isolates examined, 6.70, 8.62, 8.62, 18.95, 32.75, 50.00% were resistance to ciprofloxacin, erythromycin, pefloxacin, streptomycin, sulphamethoxazole and trimethoprim and gentamycin, respectively.

Key words: Group B Streptococcus (GBS), incidence, anti-microbial resistance profiles, pregnant women, Nsukka.

INTRODUCTION

Streptococcus agalactiae is a normal flora of the female genital tract and an important cause of neonatal sepsis, meningitis and pneumonia (Tor-Udon et al., 2006; Altay et al., 2011). Group B Streptococci (GBS) are facultative, Gram positive cocci. Some strains of GBS are β-hemolytic and produce zones of hemolysis that are slightly larger than the colonies (1-2 mm in diameter).

GBS is present in up to one-third of women of child bearing age, and one in every thousand live births is affected by group B Streptococcal infection. Maternal GBS colonization is the most important risk factor for developing disease in the newborn (Baker and Edwards, 2001; Salah and Abouzeid, 2009). Maternal colonization can initiate the process of labour and cause preterm labour. Group B Streptococcus can ascend via lower genital organs to the uterus and may cause fetal membrane inflammation which leads to preterm rupture of membrane and labour (MacGregor and French, 2000; Quiroga et al., 2008). This vertical transmission of GBS from mother to infant is the most common mode of transmission.

In pregnant women that are highly colonized GBS can
cause bladder infection, womb infection and still birth. The baby may develop symptoms of GBS disease in the first week of life. The symptoms include respiratory distress, sepsis among other (CDC, 2010). However, efforts are being made to reduce the incidence of early onset disease. It was reported that intrapartum chemophrophylaxis decreases the incidence of early onset disease from 1.7 to 0.6 per 1000 life birth (Schrag et al., 2002; CDC, 2010; Onipede et al., 2012). Adequate treatment and control required a good knowledge of the species involved and their susceptibility to antimicrobial agents.

The increased use of antimicrobials for prophylaxis has raised concerns regarding the emergence of resistance (Joyce et al., 2001). Penicillin has been the drug of choice for prophylaxis and treatment of GBS disease and as at 1995, resistance to this drug had not been reported (Andrew et al., 2000; CDC, 2010; Altay et al., 2011). However, macrolides are the recommended second line agents and the first alternative in mothers with penicillin allergy. In recent times, GBS strains that are resistant to macrolides have emerged and these strains have also shown resistance to other antibiotics such as erythromycin and clindamycin (Priscila et al., 2011). Resistance rates are reported to vary with geographical location (Joyce et al., 2001).

This study aimed at determining the incidence of GBS in pregnant women in Nsukka and studying the antimicrobial resistance patterns of the isolates.

**MATERIALS AND METHODS**

**Study population and sampling**

From January 2010 to July 2011, vaginal and rectal swabs samples were collected from 200 pregnant women of gestational age from 24 weeks to 37 weeks that were attending antenatal clinic at Bishop Shanahan Memorial Hospital and Nsukka District Hospital. Also, two hundred non-pregnant women were sampled from female hostels in University of Nigeria, Nsukka and some laboratories within Nsukka.

**Sample collection**

Vaginal swabs and rectal swabs were collected under aseptic condition from both pregnant and non-pregnant women following their informed consent and the ethical approval of the study. The vaginal and rectal swabs were collected using sterile cotton - tipped wooden swab sticks (Evepon industries Limited, NAFDAC REG. NO. 03-0482). These samples were put in a cold box with frozen ice packs and transported to the laboratory where they were cultured the same day.

**Isolation and identification of organism**

Vaginal and rectal swabs were inoculated on Todd-Hewitt broth (Oxoid, UK) which was supplemented with 8 μg/ml of gentamicin sulfate and 15 μg/ml of nalidixic acid and incubated for 18 - 24 hours at 37°C. The next day, 5 μl of the broth culture from each of the bijou bottles was inoculated onto a sterile selective GBS agar base medium (Oxoid U.K.) which was supplemented with 5% horse serum. A sterile glass rod was used to spread the inoculum over the entire surface of the GBS agar plate, to ensure an even distribution of the inoculum. The plates were then incubated at 35-37°C under anaerobic condition in an anaerobic jar with gas pack (Code No. AN0035A, from Oxoid) and read for the presence of orange-pigmented colonies after 18-24 h. Negative plates were re-incubated for an additional 24-48 h before being discarded.

Isolates having orange pigment were subcultured onto sterile Blood agar plates and incubated for 18-24 h at 37°C in an atmosphere containing 5% carbon dioxide. Following incubation, colonies were examined for the presence of Beta - hemolysis. Thereafter, β-hemolytic colonies from the blood agar were characterized using standard microbiological methods including colony morphology, Gram staining, catalase test, Christie, Atkins and Munch - Peterson (Camp test) and latex agglutination test using DRO587 latex grouping Reagent B (Oxoid Streptococcal Grouping Kit, RG 24 8pw U.K.), as described by (Cheesbrough, 2004).

**Antimicrobial susceptibility test**

A total of 58 GBS strains were used for in vitro susceptibility test. Susceptibility test to ten antibiotics namely- Ampicillin and Cloxacillin, Amoxyccillin, Cefuroxine, Ceftriaxone, Erythromycin, Ciprofloxacine, Streptomycin, Pefloxacin, Sulphamethoxzole, Trimethoprim and Gentamycin were carried out on Mueller-Hinton agar supplemented with 5% sheep blood, using the disc diffusion technique. The isolates were considered susceptible or resistant according the zones of inhibition recommended by the clinical and laboratory standard institute (CLSI) (2007).

**Data analysis**

The data were analyzed using statistical package for the social sciences (SPSS). The relationship between values was tested using analysis of variance, chi-square and student t-test. The level of significance was taken at p-value < 0.05.

**RESULTS**

The incidence of GBS was 18.00% in pregnant women, and 5.50% in non-pregnant women. Statistical analysis proved the difference to be significant (P < 0.05). The percentage occurrence of GBS from vaginal swabs and rectal swabs samples of pregnant women were 8.50 and 8.50%, respectively, while in non-pregnant women percentage occurrence of GBS isolates were: vaginal, 2.50% and rectal 2.00% (Figure 1). GBS colonization rates in pregnant women of different age groups were: 15-20 years, 19.05%; 21-25 years, 18.75%; 26-30 years, 15.58% and 31-45 years, 21.05% (Figure 2). There was no significant difference (P>0.05) in these values. In non-pregnant women, Group B Streptococcus colonization was highest in women of age between 21-25 years, 9.86% (Figure 2). Based on parity, the GBS colonization rates were higher in pregnant women within their second pregnancy (25.53%) and third pregnancy (21.74%) than in those within their first pregnancy (15.31%) and fourth pregnancy (5.85%) (Figure 3). However, these differences were not statistically significant. The susceptibilities of the GBS isolates to all the antibiotics tested...
Figure 1. Percentage occurrence of Group B Streptococcus according to sample source (pregnant and non-pregnant women). *The error bars indicate standard deviation.

Figure 2. Percentage occurrence of Group B Streptococcus carriage rates in pregnant and non-pregnant women within different age brackets. *The error bars indicate standard deviation.

Figure 3. Group B Streptococcus (GBS) carriage rates in different parity groups. *The error bars indicate standard deviation.
are summarized in Table 1. All GBS were susceptible to ampicillin and cloxacillin, amoxycillin, cefuroxine and ceftriaxone. 91.37, 93.10 and 91.37%, of the isolates were considered susceptible to erythromycin, ciprofloxacin and pefloxacin respectively. Resistance to erythromycin, ciprofloxacin, pefloxacin, streptomycin, sulphonmethoxazole and trimethoprim and gentamycin was found to be 8.6, 6.7, 8.6, 18.95, 32.75 and 50.00%, respectively.

**DISCUSSION**

Group B *Streptococcus* (GBS) is an important cause of infection in pregnant women and their newborn in many countries, but there is a paucity of data available from Africa (Collins et al., 1998; Trujillo et al., 1990). Maternal Group B *Streptococcus* colonization continues to be the most important risk factor for developing disease in the newborn (Baker and Edward, 2001; Elbaradie et al., 2009). Most data on maternal Group B *Streptococcus* colonization over the years has come from Europe and North America and to date only Zimbabwe and Malawi in Africa have an active research programme on GBS colonization and the burden of the disease (Dzwela et al., 2005). The incidence of GBS among pregnant women in this study was 18.00% which is in agreement with 16.50% reported in Malawi (Dzwela et al., 2005), 19.00% in Ivory Coast, 22.00% in Gambia (Stoll and Schuchat, 1998), 20-32% in Zimbabwe (Moyo, 2002) and 11.3% in Ile-Ife, Nigeria (Onipede et al., 2012). However, some other investigators have reported lower values e.g. 9.50% in Korsa (Uh et al., 2001). In non pregnant women the incidence was 5.50%. This is also in agreement with reports of other authors that have shown higher incidence in pregnant than non-pregnant women (Farley, 2001; Blancas et al., 2004).

It was observed that the majority of pregnant women enrolled in this study were between the ages of 21 to 30 years. The incidence of Group B *Streptococcus* in pregnant women within these various age range was highest in women of age between 31 - 45 years followed by those between 15 - 20 years. The pattern of GBS isolated from different age range in this study showed that the incidence was not age dependent. This agrees with some authors who reported that the distribution of isolates from symptomatically colonized pregnant women was irrespective of age (Baker et al., 1997; Timothy et al., 1998). In non-pregnant women, Group B *Streptococcus* colonization was highest in women of age between 21-25 years. This may be attributed to the fact that young women in this age bracket are more sexually active than older women. It has been reported that GBS is more common in sexually active women including both frequent intercourse and multiple sex partners (Meyn et al., 2002).

GBS colonization was evaluated in relation to parity and the highest incidence was observed among group two (25.53%) and group three (21.74%) and the lowest incidence in group four (5.88%). This variation within the various parity groups can be explained on the basis of the difference in hygienic status of different populations.

In this study, we found no resistance to Ampicillin and Cloxacillin, Amoxycillin, Cefuroxine and Ceftriaxone. So, Penicillin or Ampicillin remains the drugs of choice for intrapartum antibiotic prophylaxis for GBS colonization in pregnant women.

Erythromycin is the drug of choice for women with serious penicillin allergy who are colonized with GBS (CDC, 2010). An increase in resistance of GBS to erythromycin has been reported (Dipersio, 2006; Samar et al., 2012). We found that 8.6% of the isolates were resistant to erythromycin. This rate of resistance to erythromycin observed among the GBS isolates in this study is consistent with reports from other authors (Di Bartolomeo et al., 2005; Mollerach et al., 2007; Aziz et al., 2011). The rate of erythromycin resistance in the GBS isolates strongly supports the current CDC recommendation that antibiotic susceptibility test should be performed if erythromycin therapy is needed to prevent neonatal GBS infection (Heclan et al., 2004).

**Table 1.** Antibiotic susceptibility profiles of group B *Streptococcus* isolates.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible (%)</th>
<th>Intermediate (%)</th>
<th>Resistance (%)</th>
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<tbody>
<tr>
<td>Cefuroxine</td>
<td>58 (100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>58 (100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>58 (100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ampicillin + cloxacillin</td>
<td>58 (100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>53 (91.37)</td>
<td>-</td>
<td>5 (8.60)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>54 (93.10)</td>
<td>-</td>
<td>4 (6.70)</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>53 (91.37)</td>
<td>1 (1.72)</td>
<td>4 (6.70)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>47 (81.08)</td>
<td>9 (15.51)</td>
<td>2 (3.40)</td>
</tr>
<tr>
<td>Sulphonmethoxazole + trimethoprim</td>
<td>39 (69.24)</td>
<td>9 (15.51)</td>
<td>10 (17.24)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>29 (50%)</td>
<td>6 (10.34)</td>
<td>23(39.66)</td>
</tr>
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</table>
Resistance to quinolones (pefloxacin and ciprofloxacin) has only recently been described for GBS (Quroga et al., 2008; Tazi et al., 2008). In our study, we identified 6.70 and 8.62% of the isolates resistant to pefloxacin and ciprofloxacin, respectively.

With more widespread use of antibiotics, selection of antibiotic resistant GBS may occur. If resistance continues to be identified and increase, surveillance of antibiotic resistance patterns among several antimicrobial classes will be important in determining optimal prophylaxis and treatment of GBS infections.

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REFERENCES


