Isolation and characterization of Group B Streptococci and other pathogens among pregnant women in Ibadan, Southwestern Nigeria

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ABSTRACT

Objective: The detection of Group B streptococci (GBS) in pregnant women allows intrapartum administration of antibiotic prophylaxis to prevent perinatal infection. This study aimed to determine the incidence and antibiotic susceptibility profiles of group B streptococci and other pathogens isolated from pregnant women in Ibadan, Nigeria.

Methods and results: One hundred antenatal clinic attendees at Adeoyo Maternity Hospital, Ibadan, were recruited for this study. The age range of the participants was 16 – 41 years, with ages 21–30 years constituting 63%. Participants were interviewed using a questionnaire to gather demographic data. Two samples of high vaginal swab (HVS) were collected per participant and processed using standard bacteriological methods. The results showed that a total of 120 microbes were isolated and 10 (8.3%) of these isolates were GBS. Other organisms include Staphylococcus aureus [22(18.3%)], Candida albicans [17(14.2%)], Candida species [9(7.5%)], Proteus species [8(6.7%)], Escherichia coli [6(5.0%)], Trichomonas vaginalis [6(5.0%)], Streptococcus pyogenes [5(4.2%)], Enterococcus species [3(2.5%)], and non-pathogens [34(28.3%)]. The pattern of GBS isolated from different age ranges showed that, the incidence was not age-dependent (P=0.05). The antibiotic susceptibility profiles of the bacterial pathogens were studied using disc-diffusion techniques. All the GBS isolates were sensitive to Penicillin G and Erythromycin; with 80% sensitive to Ampicillin, Vancomycin and Augmentin.

Conclusion and application of findings: The relatively high incidence (8.3%) of GBS colonization among the pregnant women emphasizes the importance of correct diagnosis and prevention of neonatal infections and of detecting colonization at the end of pregnancy. Therefore, a thorough medical examination and culture of HVS is highly recommended for pregnant women to ensure detection of GBS infection among these immunosuppressed persons. The recovery of antibiotic resistant strains of other bacterial pathogens, especially those recommended in cases of penicillin allergy, also indicates the importance of evaluating microbial susceptibility to antibiotics.

Key words: Antibiotics, GBS colonization, HVS, pregnant women, susceptibility, Nigeria
INTRODUCTION

The adverse effects of infectious diseases in many developing countries, in particular, in sub-Saharan Africa is considerable and, within those countries, economically disadvantaged persons are most likely to contract communicable diseases and least likely to access appropriate treatment. Group B streptococci (*Streptococcus agalactiae*) constitute one of the many microorganisms that grow and multiply in human beings. GBS is the most frequent pathogen isolated from neonates with invasive bacterial disease and responsible for serious infections in newborns such as pneumonia, septicemia and meningitis (Artz *et al*., 2003; Motlová *et al*., 2004; Tor-Udom *et al*., 2006). GBS are also associated with significant maternal peripartal disease including bacteremia, endocarditis, chorioamnionitis, endometritis, urinary tract infections, arthritis, and responsible for serious bacterial illness and deaths in nonpregnant women with underlying diseases and in elderly adults (Dzowela *et al*., 2005; Tazi *et al*., 2008; Phares *et al*., 2008; Sendi *et al*., 2009). GBS can also pass through the cervix without causing serious cervicitis, and cross-intact amniotic fluid causing amnionitis thereby infecting the foetus in the uterus (Dzowela *et al*., 2005).

GBS are species of the normal flora of the female urogenital tract and rectum. Its chief clinical importance is that it can be transferred to a neonate passing through the birth canal and can cause serious infection with a high mortality rate (Wikipedia, 2009). It has been recognized since the late 1960’s as important group of opportunistic pathogens, representing a common cause of neonatal sepsis and meningitis as well as perinatal maternal infections (Artz *et al*., 2003; Motlová *et al*., 2004; Tor-Udom *et al*., 2006). GBS, even when it is asymptomatic, has been associated with adverse pregnancy outcomes such as low birth weight, pre-term delivery, premature rupture of the membranes (Baker & Edwards, 1995; Dzowela *et al*., 2005).

GBS is present in up to one-third of women of childbearing age, and one in every thousand live births will be affected by group B streptococcal infection. The vaginal carriage isolated immediately before delivery is in the range of 5-12% and approximately 20% of pregnant women are asymptotically colonized with GBS (Artz *et al*., 2003). The incidence of early-onset neonatal disease (EOD) and premature rupture of membranes is highly correlated with heavy GBS colonization of the vagina, cervix, and rectum of pregnant women (Baker & Edwards, 1995). Vertical transmission of GBS from mother to infant is the most common mode of transmission, although other means of transmission have been described, including nosocomial or community acquisition (Romero *et al*., 1993).

The diagnostic standard is the culture of anal and genital specimens obtained at 35 to 37 weeks of gestation or at delivery when at least one risk factor associated with neonatal infection is present. In order to detect GBS in vaginal specimens, efficient standard culture and a rapid screening method is required to identify carriage of GBS in pregnant women at the time of delivery (Artz *et al*., 2003). Many studies have been carried out on streptococcal infection of the female genital tract with emphasis on Group B streptococci. Approximately 10 to 30 % of pregnant women are colonized with GBS in the vaginal or rectal area. Of all infants born of these women, 1 to 2 % will develop early onset invasive disease (EOD) (Motlová *et al*., 2004).

In an effort to reduce the incidence of EOD, Centers for Disease Control and Prevention (CDC) issued revised guidelines for the use of intrapartum antibiotic prophylaxis in 2002 (Schrag *et al*., 2002). Intrapartum chemoprophylaxis decreases the incidence of early onset from 1.7 to 0.6 per 1,000 live births (Schrag *et al*., 2002). The recommended agents are intravenous penicillin G or ampicillin (Siegel, 1998). However, the most reliable way of preventing the disease is currently unclear. Vaccination of adolescent women is considered an ideal solution, but a good candidate vaccine is yet to be identified. In the meantime, prophylactic treatment with antibiotics (typically...
intravenous ampicillin) during delivery is the prevention measure most commonly used.

The emergence of antibiotic resistant bacterial pathogens has become a major public health concern (Cheng et al., 2004; Mafu et al., 2009). Many bacterial and parasitic diseases could, until recently, be treated with inexpensive antimicrobial agents, but treatment has recently been made more expensive and less successful by the emergence and spread of resistant organisms (Okeke et al., 2007). Drug resistance is a large and growing problem in infections that account for most of Africa’s disease burden, including malaria, tuberculosis (TB), HIV infection, and respiratory and diarrheal diseases (Okeke et al., 2007).

The prevalence of GBS among black pregnant women both in Africa and the United States has been shown to be higher than in women of other racial group and in general terms GBS among pregnant women worldwide ranges between 10-30% (Moyo, 2002). Most data on GBS epidemiology over the years has come from the Europe and North America and to date only Zimbabwe and Malawi in Africa has an active research programme on GBS colonization and the burden of disease (Moyo, 2002; Dzowela et al., 2005). Adequate treatment and control of these conditions requires a good knowledge of the Streptococcus species involved and their susceptibility to antimicrobial agents (Adedeji & Abdulkadir, 2009). Since majority of the treatments begin or are done completely empirically, the knowledge of the organisms, their epidemiological characteristics, and their antibacterial susceptibility is mandatory. Obtaining data is essential to optimize treatment and minimize the emergence of bacterial resistance, which is responsible for the increasing number of therapeutic failure (Adedeji & Abdulkadir, 2009).

The aim of this study was to isolate, identify and determine the antibiotic sensitivity profiles of GBS (Streptococci agalactiae) and other pathogens in the genital tract of pregnant women in Ibadan, Southwestern, Nigeria.

MATERIALS AND METHODS

Study area: The study was carried out in the municipal area of Ibadan, which is made up of five local government areas. Ibadan city lies 3°5’ E and 7°23’ N. The city is characterized by low level of environmental sanitation, poor housing, and lack of potable water and improper management of wastes especially in the indigenous core areas characterized by high density and low income populations.

Study population: One hundred pregnant women of different ages and socioeconomic status attending antenatal clinic at Adeoyo maternity hospital, Ibadan, were enrolled in this study. Their samples were collected over a period of six months from March to August, 2000. Questionnaires were prepared and subjects gave detailed information relating to their present pregnancies and previous ones, if any.

Specimen collection: Duplicate samples of High Vaginal Swabs were collected under aseptic condition from 100 pregnant women following their informed consent and the ethical approval of the study. Samples were collected under the supervision of a gynaecologist with a commercially available collection and transport system for aerobes and anaerobes (ampoules containing 0.5ml of modified Stuart bacterial transport medium), BBL Culture Swab Plus (Becton Dickinson, Heidelberg, Germany).

Culture isolation and identification: The High vaginal swabs collected were cultured in sheep blood agar, Todd-Hewitt broth containing colistin (10 micrograms/ml) and nalidixic acid (15 micrograms/ml), and Neomycin-Nalidixic tryptic-soy blood agar. This was followed by a confirmatory test as described by García et al. (2003), El Beitunea et al. (2006) and Strus et al. (2009). Sterility test was done by incubating the last poured plate overnight at 37ºC. Known Streptococcus agalactiae, Staphylococcus aureus and E. coli strains (Oxford Strain NCTC 6571, 6591 and 10418, respectively) were used as positive control and while Enterococcus served as negative control. All media were prepared according to the manufacturer’s specification. All cultures were incubated at 37ºC in 5-10% CO₂ for 36-48 hours in an incubator. The plates were examined for growth of Beta hemolytic colonies, while negative plates were re-incubated for 48 hours and examined accordingly.

For identification of GBS, the specimens were incubated on standard agar medium (β-streptococcus selective agar base, used according to the
manufacturer’s specification), and colonies of streptococci suspected of being beta-hemolytic were subcultured for serological grouping. Finally, a minimum of 36 h was required for GBS identification. Positive cultures were graded as negative (no growth of GBS), + (slight colonization, less than 100 CFU/agar plate), ++ (moderate colonization, between 100 and 300 CFU/agar plate), or +++ (heavy colonization, more than 300 CFU/agar plate) as described by Artz et al. (2003).

All isolates were further confirmed, characterized and identified by observing colony morphology and beta-hemolysis type on blood-agar, catalase, Sodium Hippurate, Sugar-serum agar fermentation, litmus milk, bacitracin disc, Germ tube, cAMP, and serological tests (GBS were confirmed by specific-group (Streptococcal grouping kit, Oxoid) and specific-type antigen detection (Denka Seiken Co LTD, Japan) as described by Cheesbrough (2004) and Borger et al. (2005). The results were analyzed using the χ2-test, with the level of significance set at p < 0.05.

Antibiotics susceptibility testing: The pure colonies of the pathogens recovered from pregnant women were subcultured on Neomycin-Nalidixic tryptic-soy blood agar, Sheep blood agar and Todd-Hewitt agar plates and the antibiotic sensitivity discs were placed on them (Cheesbrough, 2004; Coyle, 2005). The antibiotics used for Gram positive organisms were Erythromycin (15µg), Ampicillin (10µg), Penicillin G (10U), Augmentin (15µg), Tetracycline (30µg) and Vancomycin (30µg). The antibiotics used for the Gram negative organism were Ampicillin (10µg), Augmentin (15µg), Vancomycin (30µg) and Gentamycin (10µg) and were evaluated according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002) guidelines. Sterility test was done by incubating the last poured plate overnight at 37°C; control plates using known Streptococcus agalactiae as positive and Enterococcus as negative control were included.

RESULTS

Of the 100 pregnant women participating in this study, 10% (n = 100) had positive growth of Streptococcus agalactiae in their HVS. Ninety-two percent (92.0%) of the samples yielded microbial growth, while there was no microbial growth in 8% of the samples (Table 1). The number of organisms isolated from the HVS was 120 (100%), of which 24 (21.0%) were polymicrobial growth, 50 (44.0%) were monomicrobial growth of pathogens and 16 (14.0%) were pure growth of normal flora.

Table 1: General growth pattern of microbial isolates from pregnant women in Ibadan, Nigeria (duplicate High Vaginal Swab samples).

<table>
<thead>
<tr>
<th>Growth pattern</th>
<th>No. tested (%)</th>
<th>No. positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total growth</td>
<td>200*</td>
<td>184 (92.0)</td>
</tr>
<tr>
<td>No growth</td>
<td>200</td>
<td>16 (8.0)</td>
</tr>
<tr>
<td>Polymicrobial</td>
<td>200</td>
<td>24 (21.1)</td>
</tr>
<tr>
<td>Monomicrobial</td>
<td>200</td>
<td>50 (44.0)</td>
</tr>
<tr>
<td>Non pathogen (Normal flora)</td>
<td>200</td>
<td>16 (14.0)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>120 (60.0)</td>
</tr>
</tbody>
</table>

*100 women participated, each with duplicate samples = 200 samples.

A total of 120 microbial isolates were obtained from the 200 samples cultured in this study (Table 1), of which 8.3% were Streptococcus agalactiae (Table 2). Results in table 2 show that one in every 10 pregnant women is a vaginal carrier of Streptococcus agalactiae [10 (8.3%)]. Other organisms isolated include Staphylococcus aureus [22 (18.3%)]; Candida albicans [9 (7.5%)]; Proteus species [8 (6.7%)]; Escherichia coli [6 (5.0%)]; Streptococcus pyogenes [5 (4.2%)]; Enterococcus species [3 (2.5%)]; and non-pathogens [34 (28.3%)].

The subjects participating in this study were between ages 16 and 41 years, with 21-30 years range constituting the majority (Table 3). Teenagers between the age group 16 – 20 years constituted 17% of the study population. Ninety-three percent (93%) of the subjects were in their third trimester, which was very suitable for this study. Nearly half (45%) of the women were traders.
Table 2: Frequency and distribution of Microbial Isolates from pregnant women in Ibadan, Nigeria.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>22 (18.3)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>17 (14.2)</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>10 (08.3)</td>
</tr>
<tr>
<td>Candida species</td>
<td>09 (07.5)</td>
</tr>
<tr>
<td>Proteus species</td>
<td>08 (06.7)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>06 (05.0)</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>06 (05.0)</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>05 (04.2)</td>
</tr>
<tr>
<td>Enterococcus species</td>
<td>03 (02.5)</td>
</tr>
<tr>
<td>Non-pathogens (Normal flora)</td>
<td>34 (28.3)</td>
</tr>
<tr>
<td>Total</td>
<td>120 (100.0)</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of Group B Streptococci in relation to demographic characteristics of pregnant women in Ibadan, Nigeria.

<table>
<thead>
<tr>
<th>Age Group (years)</th>
<th>No. Tested</th>
<th>Trimester</th>
<th>Occupation</th>
<th>No.</th>
<th>No. Positive for GBS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-20</td>
<td>17</td>
<td>0 5 12</td>
<td>Trader</td>
<td>45</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>21-25</td>
<td>38</td>
<td>0 2 36</td>
<td>Teacher</td>
<td>20</td>
<td>2 (05.7)</td>
</tr>
<tr>
<td>26-30</td>
<td>25</td>
<td>0 1 24</td>
<td>Artisan</td>
<td>15</td>
<td>2 (08.0)</td>
</tr>
<tr>
<td>31-35</td>
<td>13</td>
<td>0 1 12</td>
<td>Civil servant</td>
<td>10</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td>36-40</td>
<td>05</td>
<td>0 1 4</td>
<td>Student</td>
<td>07</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>&gt;40</td>
<td>02</td>
<td>0 0 2</td>
<td>House wife</td>
<td>03</td>
<td>0 (00.0)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>0 10 90</td>
<td></td>
<td>100</td>
<td>10 (10.0)</td>
</tr>
</tbody>
</table>

Table 4 shows the antibiotics susceptibility profiles of the bacterial isolates obtained from pregnant women in Ibadan, Southwestern Nigeria. The sensitivity pattern of Streptococcus agalactiae showed that 100% were sensitive to Penicillin G and Erythromycin. Eighty percent (80%) were sensitive to Ampicillin, Vancomycin and Augmentin, while Tetracycline was markedly resisted with a sensitivity of 30% (Table 4).

DISCUSSION

The present study has shown the prevalence of GBS (Streptococcus agalactiae) colonization in Ibadan is 8.3% which is low compared to 16.5% in Malawi (Dzowela et al., 2005), 19.0% in Ivory Coast, 20.0% in Nigeria, 22.0% in Gambia (Stoll & Schuchat, 1998), and 20 - 32% in Zimbabwe (Moyo, 2002). The relatively low GBS colonization rate reported in this study corresponds to the 9.5% reported by Jang et al. (1997) in Korea, 1.0% and 4.0% reported by (Stoll & Schuchat, 1998) in Mozambique and Togo respectively. The differences could be due to geographic, ethnic, and socioeconomic factors, as well as differences in sampling and culturing techniques (Hansen et al., 2004; Busetti et al., 2007). Variations may also reflect differences in sexual practice and environmental factors such as hygiene and nutrition.

Also, we reported higher percentage for Streptococcus pyogenes (4.2%) and Staphylococcus aureus (18.3%) compared to what was reported by other authors elsewhere in the world (CDC, 2004, 2005; Tyrrell et al., 2005; Ekelund et al., 2005a,b; Hollm-Delgado et al., 2005; Laupland et al., 2006; Lamagni et al., 2005, 2007, 2008; Wong et al., 2009). Group A and B Streptococcus increased substantially during the 1980s (Farley et al., 1993; Wong et al., 2009). Strus et al. (2009) found neonates born by colonized mothers with complicated pregnancies to be more often colonized with GBS than those from mothers with a normal pregnancy (35 versus 26.7 %). The sensitivity of cultures increased when selective broths were used as the primary detection method as reported by Garcia et al. (2003).
The pattern of GBS isolated from different age ranges showed that the incidence was not age dependent. In 1997, Baker et al. (cited in Dzowela et al., 2005) reported that the distribution of isolates from asymptotically colonized pregnant was even irrespective of age. However, irrespective of the source, GBS colonization rate of the vagina appears to decrease with age (Dzowela et al., 2005). The poor socio-economic status of women is usually implicated (Moyo, 2002; Dzowela et al., 2005) as one of the risk factors for GBS colonization but in this study one marker of socio-economic status i.e. occupation level was not significantly related to colonization (P > 0.05).

Sexual contact does not seem to be the principal way of transmitting GBS (Strus et al. 2009) and in our study women in their 2nd and 3rd trimester were more often colonized than those in their 1st trimester. Neonatal GBS sepsis can be prevented by identifying and treating pregnant women who carry GBS and have a risk of transmitting the bacteria to their newborns (Artz et al., 2003). The current recommendation is to screen pregnant women using a culture of vaginal and anal secretions obtained at 35 to 37 weeks of gestation (Artz et al., 2003).

The United States uses the most aggressive strategy in which all pregnant women are screened for S. agalactiae (Apgar et al., 2005) and prophylactic antibiotics are given to all positive cases. Because of this strategy, America has seen a marked reduction in babies born with early-onset infection. Most European countries do not generally screen, but use a risk-based strategy applied at the time of delivery. In agreement with our observations, Baker et al. (1975) found that the colonization rate almost doubles between the second trimester and delivery. In contrast, Hansen et al. (2004) did not find any significant variation in the prevalence of GBS during pregnancy and in the intrapartum and postpartum periods.

Varying results of GBS susceptibility to antibiotics have been reported by Moyo et al. (2000), Lin et al. (2000), Garcia et al. (2003), Motlová et al. (2004), Borger et al. (2005), Dzowela et al. (2005), Torres-Udom et al. (2006), Gray et al. (2007), and Tazi et al. (2008). It is believed that the differences in antimicrobial use, prophylaxis practice and serotype frequency may result in regional differences in the susceptibility of GBS to antibiotics (Uh et al., 2001).

In this study, no resistance to erythromycin was reported for GBS, which deviates from 20% erythromycin resistance reported by Lin et al. (2000), 14% by Moyo et al. (2001), 21.4% by De Mouy et al. (2001), 4% by Weisner et al. (2004), 9.4% by Borger et al. (2005), 21% by Gray et al. (2007) and 15% by Pinheiro et al. (2009). This also compared favourably with what was reported by Tazi et al. (2008). Generally, there was a high level of resistance to tetracycline in this study. According to Nkang et al. (2009, 2010), this could be attributed to indiscriminate use of antimicrobial drugs (ampicillin, penicillin, erythromycin, rifampicin, tetracycline, vancomycin).

All GBS isolates in this study were also susceptible to the recommended penicillin-G (100.0%) as observed in previous studies (Moyo et al., 2000, 2001; Dzowela et al., 2005), even though in other settings there have been reports of resistance to penicillin-G and erythromycin of up to 35.0% (Lin et al., 2000; Uh et al., 2001; Dzowela et al., 2005). Our finding also compared favourably with Gray et al. (2007), who in their study, found all isolate to be susceptible to the β-lactam antimicrobial drugs and 96% to be resistant to tetracycline, as would be expected. This deviates from what was reported by Tazi et al. (2008) who reported GBS susceptibility to all other antimicrobial drugs (penicillin, erythromycin, clindamycin, tetracycline, rifampicin, vancomycin) and low-level resistance against aminoglycosides. Susceptibility to gentamycin by E. coli and Proteus sp in this study was 67.0 and 75.0% respectively. Though with slight variations, this compared favourably with what was reported by Nwadioha et al. (2010).

GBS isolates with confirmed resistance to β-lactams (penicillin or ampicillin) have not been observed to date (Motlová et al., 2004; Hayes & Meyer, 2007). The proportion of other isolates with in vitro resistance to clindamycin and erythromycin has increased in recent years. The prevalence of resistance among invasive GBS isolates ranged from 7 to 25 per cent for erythromycin and might be associated with certain serotypes (Andrews et al., 2000). Tazi et al. (2008) described to their knowledge, the first GBS clinical isolate in France resistant to fluoroquinolones and no other relevant respiratory bacterial pathogens present in their samples.

Indeed, the problem of antibiotic resistance is global since once a resistant organism is introduced into a population, it is rapidly disseminated. For susceptible strains, β-lactams, which still constitute the first-line recommended antimicrobial drugs, should be used for treatment of these patients (Hayes & Meyer, 2007). Physicians worldwide have been encouraged to
join public health authorities, the infection-control community, and the pharmaceutical industry to curb the inappropriate use of antibiotics and promote responsible prescribing (Hsu et al., 2007). This will greatly help to improve prevention and control of drug resistant organisms in communities.

In Nigeria, prenatal screening and a prevention programme of early onset invasive disease (EOD) caused by GBS have been carried out only partially and in a non-standardized way. Prenatal screening is not based on the CDC recommended criteria (Schrag et al., 2002). Data needed for formulation of an effective prevention programme are not available (Mottová et al., 2004). Our data on GBS carriage, susceptibility to antimicrobials and active surveillance of EOD due to GBS would be useful as background information for nationwide implementation of GBS prenatal screening and formulation of a prevention program in Nigeria. According to Hsu et al. (2007), continued surveillance would serve as an impartial feedback on the efforts of infection control programs for the future. Such surveillance of clinical microbiology isolates is a critical first step towards controlling the growing worldwide threat of antimicrobial drug resistance, and WHONET is a useful tool in this respect (Wenzel & Edmond, 2000). More so, further extensive work should be done to ascertain the extent of drug resistant pathogens in our environment.

CONCLUSION

In conclusion, GBS is an unusual cause of acute bacterial exacerbation of chronic bronchitis compared with other respiratory pathogens such as S. pneumoniae, but pathologies associated with this bacterium are changing. Clinical microbiologists should be aware of these changes and test isolates of Streptococcus spp. for susceptibility to antimicrobial drugs (Tazi et al., 2008). We have demonstrated a pattern of pregnant women GBS disease similar in scale and serotype distribution to reports from the industrialized world but with a significantly worse outcome. Our results clearly indicate that rates of GBS colonization among pregnant women in Ibadan have reached levels comparable to those reported in other countries. GBS has been a leading cause of neonatal illness and death in many parts of the world, especially industrialized countries (Poland and other European clinical centres etc., for several decades (Schrag et al., 2000; Weisner et al., 2004; Shet & Ferrieri, 2004; Schrag & Schuchat, 2004; Strus et al. 2009). In contrast, until recently GBS was infrequently reported in the developing world (Gray et al., 2007). According to Gray et al. (2007), a World Health Organization multicenter study of the bacterial etiology of serious infections in young infants of <3 months of age reported in 1999 that the "virtual absence of GBS was striking" (WHO, 1999). Yet the prevalence of maternal carriage of GBS in developing countries, including populations in tropical Africa, is also similar to that identified in populations in the United States (Dawodu et al., 1983; Suara et al., 1994; Stoll & Schuchat, 1998). Recent studies from Kenya (Berkley et al., 2005), South Africa (Madhi et al., 2003), Zimbabwe and Malawi (Milledge et al., 2005; Gray et al., 2007) suggest that GBS is emerging as an important cause of neonatal sepsis in Africa. The largest of these studies reported that 136 of 801 bacterial isolates from 784 Malawian neonates were GBS (Milledge et al., 2005; Gray et al., 2007).

The relatively high incidence (8.3%) of colonization by GBS emphasizes the need for strategies for correct prevention of neonatal infections, especially of detecting colonization at the end of pregnancy. Prevention strategies such as chemoprophylaxis are available for neonatal GBS but are difficult to apply in a resource-limited setting (Shet & Ferrieri, 2004; Schrag & Schuchat, 2004). Vaccination is an attractive option in this setting, and vaccines consisting of GBS capsular polysaccharide conjugated to a tetanus toxoid carrier protein have been under development (Baker et al., 2003a, b). The vaccines are immunogenic in women but of unproven clinical benefit (Gray et al., 2007). Chemoprophylaxis has been successful in reducing rates of EOD in many countries (Schrag & Schuchat, 2004; Gray et al., 2007). The findings of this study also confirm uniform susceptibility of GBS isolates from pregnant women to Penicillin G, Erythromycin and other beta-lactam antibiotics tested. GBS resistance to erythromycin was absent, but needs further surveillance, particularly in invasive GBS isolates. Treatment should be given to culture positive women in order to prevent subsequent infection of the neonate and secondary infection to the mother. Recommended drugs should include Penicillin G, Erythromycin, Augmentin and Vancomycin. The recovery of antimicrobial resistant strains of other bacterial pathogens, especially with resistance to antibiotics recommended in cases of penicillin allergy,
emphasize the importance of evaluating antimicrobial susceptibility.

Important information to support future preventive strategies includes estimate of rates of disease, timing of disease initial manifestations; and for vaccine development, description of serotype distribution in different populations (Schrag & Schuchat, 2004; Gray et al., 2007). However, this study adds to the growing evidence that GBS is an important cause of infectious illness in pregnant women and neonatal death in Africa. The incidence and outcome of disease support a more active approach for its prevention. These results provide a benchmark for future studies with what we believe to be reasonable minimum estimates of disease incidence, despite measurement limitations in both our denominator and numerator figures.

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Table 4: Antibiotic susceptibility profiles of microbial isolates from pregnant women in Ibadan, Nigeria.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>PEN</th>
<th>ERY</th>
<th>AMP</th>
<th>VAN</th>
<th>TET</th>
<th>AUG</th>
<th>GEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>S. aureus</td>
<td>17(77.0)</td>
<td>5(23.0)</td>
<td>14(64.0)</td>
<td>08(36.0)</td>
<td>14(64.0)</td>
<td>08(36.0)</td>
<td>14(64.0)</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>05(100.0)</td>
<td>0(00.0)</td>
<td>04(80.0)</td>
<td>01(20.0)</td>
<td>03(60.0)</td>
<td>02(40.0)</td>
<td>04(80.0)</td>
</tr>
<tr>
<td>S. agalactiae</td>
<td>10(100.0)</td>
<td>0(00.0)</td>
<td>10(100.0)</td>
<td>00(00.0)</td>
<td>08(80.0)</td>
<td>02(20.0)</td>
<td>08(80.0)</td>
</tr>
<tr>
<td>Enterococcus sp.</td>
<td>02(66.7)</td>
<td>1(33.3)</td>
<td>02(66.7)</td>
<td>01(33.3)</td>
<td>02(66.7)</td>
<td>01(33.3)</td>
<td>02(66.7)</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>05(63.0)</td>
<td>03(37.0)</td>
<td>NA</td>
</tr>
<tr>
<td>E. coli</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>04(67.0)</td>
<td>02(22.0)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Key: R = Resistant, S = Sensitive, NA = Not Applicable, %= Percentage; PEN = Penicillin; AUG = Augmentin; ERY = Erythromycin; GEN = Gentamycin; AMP = Ampicillin; VAN = Vancomycin.