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Vaginal colonization and resistance profile of group B *Streptococcus* among pregnant women in Yaoundé Gynecology, Obstetric and Pediatric Hospital in Cameroon

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In order to obtain reliable data on vaginal carriage of *Streptococcus agalactiae* in pregnant women and to formulate a prevention program of neonatal Group B *Streptococcus* (GBS) disease, we carried out a prospective cross sectional study for 6 months. The general objective of the study was to evaluate the prevalence of vaginal carriage and the resistance profile of GBS. The study involved 142 pregnant women presenting for antenatal care in Yaoundé Gynecology-Obstetric and Pediatric Hospital (YGOPH). Participants were interviewed using a standard structure questionnaire. Low vaginal swabs were collected and cultured on specific media. A presumptive identification of isolates was made using standard bacteriological methods. Confirmative identification of Group B *Streptococcus* was done and antimicrobial sensitivity testing was performed. Among the 142 pregnant women GBS colonization was confirmed in 11 (7.7%). The rate of carriage was 3.8% in the first trimester, 7% in the second trimester and 11.1% in the third trimester. The predominant germ was *Candida albicans* with a frequency of 45.2% among the germs found in monomicrobial culture and Gardnerella vaginalis (77.8%) among the germs in polymicrobial culture, followed by *Candida* spp (11.8%), *S. agalactiae* (8.6%) and *Escherichia coli* (4.3%). The result of antimicrobial sensitivity testing showed that all the GBS strains were sensitive to major antibiotics drugs tested. The highest rates of resistance were found with gentamycin (100%) and Cefuroxim (81.8%). The vaginal carriage of GBS among pregnant women is still high. Thus, well-planned, prospective studies will be necessary to fully appreciate the magnitude of the problem of GBS in our hospitals.

Key words: Group B *Streptococcus*, neonatal infections, antibiotic, low vaginal swabs.

INTRODUCTION

Streptococcus agalactiae is a normal flora of the female genital tract and an important cause of neonatal sepsis, meningitis and pneumonia (Altay et al., 2011). Group B *Streptococcus* (GBS) are facultative, Gram positive cocci.

Some strains of GBS are β- hemolytic and produces zones of hemolysis that are slightly larger than the colonies (1 to 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 (Salah and Abouzeid, 2009). GBS is a bacteria found in 25% among pregnant women and infection with GBS can cause serious illness and sometimes death especially in newborn infants, the elderly, and patients with compromised immune systems (Schrag et al., 2002). GBS are also prominent veterinary pathogen, because they can cause bovine mastitis (inflammation of the udder) in dairy cows: it is a part of the normal flora gut and genital tract. It may be harmful to both mother and the baby itself. Infection of this organism may result in neonatal death due to severe neonatal infections such as septicemia, meningitis and pneumonia with a mortality rate of 20% (Quiroga et al., 2008). The carriage of the organism is asymptomatic (Quiroga et al., 2008).

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 others (Center for Disease Control (CDC), 2010). However, efforts are being made to reduce the incidence of early onset disease. It was reported that intrapartum chemo-phrophylaxis decreases the incidence of early onset disease from 1.7 to 0.6 per 1000 life birth (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 (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 erythro-mycin and clindamycin (Priscila et al., 2011). Resistance rates are reported to vary with geographical location (Priscila et al., 2011). Most data on GBS epidemiology
In Cameroon, Bernard Bonnin and Tetanye Ekoe showed that from 1982 to 1983, GBS constituted the third group of germs after *Pneumococcus* and *Haemophilus* in the pediatric unit of the Yaoundé Central Hospital, Cameroon (Bernard et al., 1985). Kago et al. (1990) demonstrated that from 1985 to 1988, GBS was responsible for 26.66% of infant meningitis from 0 to 2 months and in 1990 it represented 31.25% meningitis cases in the neonatal period at Yaoundé Central Hospital, Cameroon (Kago et al., 1990). Foumane et al. (2009) in a retrospective and descriptive study carried out in the Yaoundé General Hospital from June, 2001 to May, 2002 showed that GBS carriage in pregnant women was 6.7% among 194 pregnant women.

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 (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 erythro-mycin and clindamycin (Priscila et al., 2011). Resistance rates are reported to vary with geographical location (Priscila et al., 2011).

Knowing the prevalence of GBS infection is very important if a strategy is to be developed to manage in Cameroon. Obtaining data on antibacterial susceptibility is essential to optimize treatment and minimize the emergence of bacterial resistance, which is responsible for the increasing number of therapeutic failure. With this understanding in mind, our objective as we embarked on this study was to prospectively evaluate the prevalence of vaginal carriage and the resistance profile of GBS in pregnant women attending antenatal clinics.

METHODOLOGY

A prospective cross sectional study was carried out involving pregnant women who were present for antenatal care in Yaoundé Gynecology, Obstetric and Pediatric Hospital (YGOPH), regardless the gestational age. Participants who later signed the informed consent to participate in the study were interviewed using a standard structure questionnaire to gather demographic data and other relevant information such as maternal age, gestational age, previous gyneco-obstetric history, parity and administration of antibiotics. Different criteria were necessary before the sample collection, each participant is free of antibiotic treatment, no personal hygiene in the morning of collection and no sexual haemolysis, catalase test and Gram staining. Confirmative identification of Group B *Streptococcus* was done.using latex agglutination test with specific antiserum using the slidex Strepto

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Table 1. GBS colonization and demographics.

Demographic factors		Patient tested No. (%)	GBS colonization No. (%)	P -Value
Age	<20	20 (14.08)	1 (5.0)	NS
	20-35	107 (75.35)	9 (8.41)	
	>35	15 (6.6)	1 (6.6)	
Occupation	Civil servant	27 (18.3)	2 (7.4)	Ns
	student	46 (35.0)	4 (8.7)	
	House wife	44 (33.3)	5 (11.4)	
	Trader	19 (12.5)	1 (5.2)	
	Farmer	6 (0.8)	0 (0.0)	

NS: Not statistically significant P > 0.05

Plus. Antimicrobial sensitivity testing was perform by the disk-diffusion (Kirby-Bauer) method. The sensitivity of the following reference strain (American Type Culture Collection (ATCC)): *S. aureus* ATCC 25923 was tested. The results were only validated when the diameters of the inhibitions zones of the reference strain were in accordance. Data were analyzed using Microsoft office Excel and EPI INFO (version 3.3.2) and the results were analyzed using the chi square test, with the level of significant set at P < 0.05.

RESULTS

From 1st March to 30th August, 2008, 142 pregnant women who were present for antenatal care in Yaoundé Gynecology, Obstetric and Pediatric Hospital (YGOPH) were included in the study. GBS colonization was confirmed in 11 (7.7%). The age of the women ranged from 17 to 41 years, with mean age of 27.03 years (standard deviation ± 5.53). Generally, GBS colonization did not appear to be influenced by maternal age and occupation. The study revealed a higher colonization rate among the age group 20 to 35 years (8.41%) but lower (5.0%) in women aged less than 20 years, however, the difference was not statistically significant (NS). GBS colonization was more prevalent among house wives when compared with students, civil servants, traders and farmers; but this proved not to be statistically significant (NS) (Table 1).

The association between GBS colonization rate and maternal obstetric factors is summarized in Table 3. The rate of carriage was 3.8% in the first trimester, 7% in the second trimester and 11.1% in the third trimester. Women with gestational age in the third trimester were found to have a higher colonization rate compared to the second and the first, but this proves not to be statistically significant (NS). Colonization rate was higher (9.3%) in women who had delivered more than two times (multiparity) and lower (6.6%) in women who had delivered once (primiparity). However, this was not statistically significant (NS). Women with previous history of neonatal deaths had a higher rate (31.25%) of GBS colonization followed by those with history of labour < 37 weeks (16.6%), history of neonatal infection (10.5%) and those with prolonged rupture of amniotic membrane (PROM) (5%). Statistical analysis only showed significant correlation between history of neonatal deaths and GBS colonization status (P < 0.05) (Table 2).

The predominant germ was *Candida albicans*, with a frequency of 45.2% among the germs found in monomicrobial culture and *Gardnerella vaginalis* (77.8%) among the germs in polymicrobial culture, followed by *Candida spp* (11.8%), *Streptococcus agalactiae* (7.7%) and *Escherichia coli* (4.3%). The result of antimicrobial sensitivity testing showed that all the GBS strains were sensitive to major antibiotics drugs tested. The highest rates of resistance were found with gentamycin (100%) and cefuroxim (81.8%).

DISCUSSION

The present study has shown the prevalence of GBS colonization to be 7.7% among pregnant women. This rate is lower compared to 11% in Canada(Seaward et al. , 1998) and 12% in Belgium (Schuchat, 2000). Our result is also lower compared to 10% found in France by Agence Nationale d'Accréditation et d'Evaluation en Santé (ANAES) (2001), with a rate of 9.6% reported by Joelle et al. (2003). In Nigeria, the result showed prevalence of 11.3% GBS vaginal colonization which increased with age (Onipede et al., 2012). In Tunisia, 13% in 2006 were found by Feriani et al. (2006). Some other researchers found a rate of GBS carriage which varies from 10 to 40% (Bevilacqua, 1999; Horvath et al., 1998) and others from 12 to 15%(Rosa et al., 1999; Claeys et al., 2001). These results indicate significant country variations which could be due to differences in sampling sites and techniques. For instance, previous

Table 2. Relationship between GBS colonization and obstetric characteristics.

Obstetric characteristics		Patient tested No. (%)	GBS colonization No. (%)	P value
Gestational age	First trimester	24(16.9)	1 (3.8)	NS
	Second trimester	70 (49.3)	5 (7.0)	
	Third trimester	48 (33.8)	5 (11.1)	
Parity	Nulli	48 (33.8)	3 (6.25)	NS
	Primi	30 (21.12)	2 (6.6)	
	Multi	64 (45.07)	6 (9.3)	
History of PROM	Yes	20 (14.08)	1 (5.0)	P < 0.05
	No	122 (86.00)	10 (8.2)	
History of labor < 37 weeks	Yes	18 (12.7)	3 (16.16)	NS
	No	124 (87.3)	8 (6.45)	
History of neonatal deaths	Yes	16 (11.3)	5 (31.25)	NS
	No	126 (88. 7)	6 (4.7)	
History of neonatal infection	Yes	19 (11.3)	2 (10.5)	NS
	No	123 (86.7)	9 (7.3)	

NS: Not statistically significant P > 0.05

studies used low vaginal swabs (Lamagni et al., 2008) while other investigators used high vaginal swabs. Other variations in isolation frequency could be due to differences in culture methods, and type of culture media used as well as population investigated.

Variations may also reflect differences in sexual practice and environment factors such as hygiene and nutrition (Schrag et al., 2002). However, our result is similar to 6.70% found by Foumane in General Hospital of Yaoundé (Foumane et al., 2009) and 7.3% showed by Kacou et al. (1988) in Abidjan. These results can also be similar to the one of Schmidt et al. (1989) in Ethiopia (9%), Bagnani et al. (1995) (8.2%). In 2003, a multicentric study on GBS in France reported a prevalence between 5.1 to 22.5% with some prevalence in centres 6, 7 and 8 similar to our result: 7% (among 127 pregnant women), 7.6% (among 52 pregnant women) and 7.7%(among 195 pregnant women) (Joelle et al., 2003). In this study, GBS was isolated more frequently from women of age group 20 to 35 (88.2%) compared with women aged ≤20 years (2.9%),which is in contrast with reports from another study showing high isolation frequencies in women younger than 20 years (Schuchat, 2000).

These differences are difficult to explain but possibly underscore the fact that GBS colonization might be influenced by multiple factors which may vary from one geographical location to another. The poor socio-economic status of women is usually implicated (Schuchat, 2000) as one of the risk factors for GBS colonization but in this study, one marker of socio-economic status that is, occupation level was not significantly related to colonization (NS). Of all possible factors for GBS colonization in neonates that were investigated, only neonatal death showed significant association, P≤0.05. This is consistent with other studies (Schuchat, 2000). The strong association between neonatal deaths and GBS colonization calls for routine antibiotic prophylaxis in such women in order to decrease the chances of subsequent neonatal deaths.

Maternal colonization was significantly higher in women in the third trimester compared to women in the second and first trimester, indicating an

increase GBS carriage with gestation age. Other risk factors such as PROM, history of neonatal infection and history of labour ≤37 weeks did not influence GBS colonization in neonates. The lack of association with these factors can possibly be explained by the fact that this is not a follow upstudy and the investigator only relied on the information given by the participants. The predominant germ was *Candida albicans* with a frequency of 45.2% among the germs found in monomicrobial culture and *Gardnerella vaginalis* (77.8%) among the germs in polymicrobial culture, followed by *Candida spp* (11.8%), *Streptococcus agalactiae* (7.7%) and *E. coli* (4.3%).

Fari (1998) found that this germ is the most predominant in women (Fari, 1998). The frequency of *Candida albicans* found in this study is lower than the result found by Maniatis et al. (1996) (54.1%) among 6,226 pregnant women (Maniatis et al., 1996). Our finding is also similar to 48.45% reported by Foumane in Yaoundé General Hospital (Foumane et al., 2009). Among 192 pregnant women in Togo, 33.3% were found less in our result (Balaka et al., 2003).

The result of antimicrobial sensitivity testing showed that all the GBS strains were sensitive to major antibiotics drugs tested (pénicillin G, amoxicillin, ampicillin, cefotaxim, erythromycin, lincomycin and clindamycin). The highest rates of resistance were found with gentamycin (100%) and cefuroxim (81.8%). Our results are similar to the finding of Joelle et al. (2003) and Sahnoun et al. (2007) who found a sensitivity of 100% to penicillin G, Amoxicillin, cefotaxim and Pristinamycin. Regarding clindamycin and erythromycin (Joelle et al., 2003; Sahnoun et al., 2007) found a resistance of 38.5% to erythromycin**.** Others authors confirmed that GBS was susceptible to penicillin G, erythromycin and clindamycin (Samar et al., 2012). All isolates that were resistant to gentamycin, showed a resistance of 0 .6% to gentamicin, this can be due to the fact that gentamycin used was less concentrated and also to natural resistance (Aziz et al., 2011).

Varying results of GBS susceptibility to antibiotics have been reported (Schuchat, 2000). It is believed that the differences in antimicrobial use, prophylaxis practice and serotypes frequency may result to regional differences in the susceptibility of GBS to antibiotics (Schuchat, 2000). In Cameroon, prenatal screening and a prevention program of early onset invasive disease 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 program is not available. Our data on GBS carriage and susceptibility to antimicrobials would be useful as background information for nationwide implementation of GBS prenatal screening and formulation of a prevention program in Cameroon.

Conclusion

The vaginal carriage of GBS among pregnant women is still high. Data on the prevalence of GBS neonatal disease, preventative measures and outcome of infected infants are greatly needed in our country to allow the most appropriate preventive strategy to be selected. Thus, well-planned, prospective studies which include more than 1,000 pregnant women will be necessary to fully appreciate the magnitude of the problem of GBS in our hospitals.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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