

# Vaginal Carriage of *Group B Streptococcus* in Pregnant Women in Rural Areas in Senegal

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#### Abstract

Vaginal carriage of Group B Streptococcus (GBS) is a maternal and child health issue. Our objective was to determine the prevalence of GBS carriage; identify the factors associated with this carriage and determine the antibiotic sensitivity of the isolated strains. We conducted a cross-sectional and prospective study in rural Senegal (in the health district of Sokone). Socio-demographic, clinical and gynaeco-obstetrical data were collected. Vaginal swabs were taken by the midwives on specific settings in order to test for GBS and other High Risk Vaginal Bacteria (HRVB). Antibiotic susceptibility testing was done according to the recommendations of the CA SFM/EUCAST 2020. In total, 100 pregnant women were targeted and 97 pregnant women were included. Their age ranged from 18 to 40 years with 64.9% (63/97) of participants belonging to the "20 - 30" age group. The overall prevalence of Group B Streptococcus carriage was 15.5% (15/97). However, the proportion of women with at least one high risk infectious bacteria was 29.89% (29/97). No statistically significant differences were found between GBS carriage and the potential factors studied. However, the study also looked for the presence of other high-risk bacteria and coinfections were indeed found between GBS and E. coli and Staphylococcus aureus. Antibiotic susceptibility testing shows that GBS strains were fully susceptible to penicillin G, erythromycin, clindamycin, chloramphenicol, rifampicin and vancomycin. Sensitivities to norfloxacin and gentamycin were 73.3% and 86.7% respectively. In contrast, high resistance to tetracycline (86.7%) was observed. GBS carriage remains a major public health issue because of its consequences for the mother and the newborn. Correct screening and proper monitoring of strain susceptibility remain one of the most effective means of patient management and care.

#### **Keywords**

*Group B Streptococcus (Streptococcus agalactiae*), Vaginal Carriage, Antibiotic Susceptibility, Senegal

#### **1. Introduction**

The carriage of "High Risk Vaginal Bacteria" (HRVB) is an issue public health issue problem affecting exclusively pregnant women because of the serious maternal-fetal and neonatal complications that can occur during the rupture of the membranes or the opening of the cervix before term or at delivery [1] [2].

Among the pathogens named HRVB for the mother or the newborn, the best documented is *Streptococcus agalactiae* or *Group B Streptococcus* (GBS) which is a commensal bacterium of the gastrointestinal and urogenital tract of women [2]. *Group B Streptococcus* bacterial meningitis and sepsis are the major complications in new born [3].

The prevalence of *GBS* vaginal carriage varies from one geographical area to another. However, a meta-analysis by Russell in 2017 estimated the prevalence of *GBS* vaginal carriage at 15% worldwide [4]. In Senegal, a study conducted among pregnant women from October 2013 to December 2018 (rural and urban areas) showed a *GBS* carriage's prevalence of 16.1% [5].

GBS colonisation can be intermittent throughout pregnancy. However, 17% to 28% of women with vaginal or rectal colonisation in the first trimester have sustained colonisation in the second and third trimester or at term [4].

For the preventive management of these complications, guidelines have been proposed recommending systematic screening of all pregnant women between 35 and 37 weeks of amenorrhoea [6]. The implementation of this recommendation for Senegal requires first an updated estimate of the prevalence of *GBS* carriage in pregnant women.

To this end, we conducted a study among pregnant women in rural Senegal in order to determine the prevalence of *GBS* carriage, the factors associated with this carriage and the antibiotic susceptibility of isolated strains.

#### 2. Materials and Methodology

This cross-sectional and prospective study was conducted in April 2021 at the health posts of Nemanding and Keur Samba Gueye in the health district of So-kone located in the medical region of Fatick.

Initially, the project targeted pregnant women in the villages of Dielmo and NDIOP. The population survey carried out in the villages of Dielmo and Niop at the start of the study already found 30 pregnant women. At the same time, the

project provided 100 vaginal swab kits (to test for high-risk vaginal bacteria). So in order to reach a wider target group, the study was transferred to the health posts of Keur Samba gueye and Nemanding which are the health posts overseeing the health needs of the villages of Dielmo and NDIOP. The test was then offered to a further 70 pregnant women attending the Keur Samba Gueye and Némanding health posts.

The data collection was carried out within the framework of the CAADE project (Community Engagement to Improve Access to Essential Diagnostic Services) funded by the NGO FIND (Foundation for Innovative New Diagnostics) and conducted by the Pasteur Institute of Dakar.

All pregnant women aged 18 and over consulted at the health posts and having agreed to take part in the study (signed consent) were included. The support used is a data collection form designed by the research team to collect data from the health post consultation registers. Socio-demographic, clinical and gynaeco-obstetrical data (gestity, parity, age of pregnancy, contraceptive methods, obstetrical history, etc.) were collected. Vaginal swabs were taken by the midwives to test for GBS and other HRVB.

The samples were transported to the laboratory of the Pasteur Institute of Dakar using the PORTAGERM<sup>TM</sup> Amies Agar transport medium from Bio Mérieux. The Granada chromogenic mediumfrom BD (Beckton Dickinson) was inoculated to test for *Streptococcus B* carriage. The BD Chocolate or Cooked Blood Agar (Beckton Dickinson) was inoculated for the detection of other germs such as *Escherichia coli, Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumonia.* The seeded culture media were incubated in an aerobic atmosphere in an oven at  $35^{\circ}C \pm 2^{\circ}C$  for  $20 \pm 4$  hours.

Antibiotic susceptibility testing was performed on *GBS* strains using the disc diffusion method (Biorad, France) according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA SFM) based on the EUCAST, 2020 reference. Antibiotics tested were: penicillin G (1 IU), erythromycin (15  $\mu$ g), clindamycin (2  $\mu$ g), gentamicin (500  $\mu$ g), norfloxacin (10  $\mu$ g), chloramphenicol (30  $\mu$ g), vancomycin (5  $\mu$ g), tetracycline (30  $\mu$ g) and rifampicin (5  $\mu$ g).

From a pure culture of 18 to 24 hours on agar medium, a bacterial suspension was prepared by taking 2 to 3 colonies mixed with 1 ml of sterile saline (0.9% *NaCl*), then adjusted to 0.5 McFarland. This bacterial suspension was plated onto Mueller-Hinton agar plates by swabbing. The antibiotic discs were placed on the agar surface with a minimum distance of 15 mm between the edge of the plate and the peripheral discs and 30 mm between the discs. The plates were then incubated in an oven at  $35^{\circ}C \pm 2^{\circ}C$  for  $20 \pm 4$  hours. The results were read using the Bio-Rad ADAGIO automaton according to the recommendations of the CA SFM/EUCAST 2020.

#### 2.1. Data Analysis

The data collection was done on a physical data collection form. The data was

entered into Microsoft Excel 2010 and analysed using Rstudio version 1.4. A descriptive analysis was performed on all data including the antibiotic susceptibility profile of the strains.

To describe the degree of association between GBS positive or negative status and other variables (socio-demographic, clinical and biological) a bivariate analysis and generalized linear modelling were performed. The Fisher test was used with a significance level of 5%.

# 2.2. Ethical Considerations

The CAADE protocol was approved by the National Health Research Ethics Committee of Senegal (CN SN20/71). Participants were included after receiving information about the project. They agreed to provide biological samples and signed an informed consent form. Each participant was identified by a unique numerical code respecting confidentiality.

# 3. Results

# **3.1. Socio-Demographic Characteristics**

Our study targeted 100 pregnant women, however 3 of these women were under 18 years of age and were excluded from the study because they did not have the consent of their legal guardian. This study therefore included 97 participants for whom the average age was 26.5 years with a standard deviation of 5.3 years. The minimum age was 18 years and the maximum 40 years. Participants aged from 20 to 30 years were the most represented with 64.9% (63/97).

Almost of all participants were housewives 97.9% (95/97). More than half of participants 64.9% (63/97) lived in a monogamous household. The majority of participants 60.8% (59/97) had attended Koranic school or primary school (Table 1).

# **3.2. Obstetrical Characteristics**

The majority of women 86.6% (84/97) were multigestant. The results also showed that 21.6% (21/97) of participants were carrying a pregnancy for 35 weeks or more (**Table 2**). The proportion of women who had a history of obstetrics was 7.2% (7/97). Contraceptive use was reported in 32.9% (32/97) of the women and in most cases the injectable method was used 71.9% (23/32).

#### **3.3. Prevalence of HRVB**

The overall prevalence of *Group B Streptococcus* carriage was 15.5% (15/97). However, the proportion of women carrying at least one HRVB was 29.89% (29/97). Bacteria such as *Escherichia coli* and *Staphylococcus aureus* were present in 12.4% (12/97) and 13.4% (13/97) respectively of participants. Co-infections were observed at 7.2% (7/97) and 4.1% (4/97) for GBS-*E. coli* and GBS-*S. aureus* respectively.

Label	N (97)	%	
Agegroup			
[18 - 20]	18	18.6	
[20 - 30]	63	64.9	
[30 - 40]	16	16.5	
Levelofstudy			
None	18	18.6	
koranic/primary	59	60.8	
University	2	2.1	
NA	2	2.1	
Maritalstatus			
Monogamy	63	64.9	
Polygamy	34	35.1	
Occupation			
university student	2	2.1	
Housewife	95	97.9	

 Table 1. Socio-demographic characteristics of participants.

# Table 2. Obstetrical characteristics of participants.

Label	N (97)	%	
Parity			
Multiparous	64	66.0	
Nulliparous/primiparous	33	34.0	
Gestation			
Multigestant	84	86.6	
Primigest	13	13.4	
Contraceptive method			
NA	2	2.1%	
No-injectable	9	9.2	
Injectable	23	23.7	
None	63	64.9	
Week of amenorrhea			
NA	2	2.1	
<35	74	76.3	
≥35	21	21.6	
History of obstetrics			
NA	12	12.4	
Yes	7	7.2	
No	78	80.4	

#### 3.4. Search for GBS Risk Factors

Age group between 30 and 40 years was the most affected by GBS with a prevalence of 25% (4/16), followed by those aged between 20 and 30 years (14.3% (9/63). However, the difference observed was not statistically significant (p = 0.487). The prevalence of *GBS* in monogamous women 15.9% (10/63) was higher than in polygamous women 14.7% (5/34), but a statistically significant difference was not observed between the two groups (p = 0.879). It was substantially the same in the primigeste and multigeste groups with proportions of 15.4% (2/13) and 15.5% (13/84) respectively with p = 0.993.

The presence of *GBS* was less frequent in women beyond the thirty-fifth week of amenorrhoea, *i.e.* a proportion of 14.3% (3/21) compared with a proportion of 16.2% (12/74) in those with less than thirty-five weeks of amenorrhoea without any statistically significant difference (p = 0.830).

The prevalence was higher in those using non-injectable methods 22.2% (2/9). However, in our study the use of contraceptive methods was not associated with Streptococcus B carriage (p = 0.691).

Streptococcus B carriage was higher in those with a previous obstetrical history 42.9% (3/7) without any statistically significant difference (p = 0.068).

These socio-demographic and obstetric variables retained for this study do not explain the fact of being infected or not with Streptococcus B because the bivariate analysis does not show any significance (**Table 3**). In addition, the generalized linear model and the stepwise verification method used, confirm that no variable was retained to explain Streptococcus B infection.

# 3.5. Antibiotic Susceptibility Profile of *Group B Streptococcus* Strains

**Table 4** below shows that all Streptococcus B strains were fullysensitive to penicillin G, erythromycin, clindamycin, chloramphenicol, rifampicin and vancomycin. Sensitivities to norfloxacin and gentamycin were 73.3% and 86.7% respectively. In contrast, the strains showed high resistance to tetracycline (R = 86.7%).

# 4. Discussion

The investigation of vaginal *GBS* carriage is of particular importance in the prevention of neonatal meningitis which is a major contributor to infant morbidity and mortality in developing countries.

Indeed, approximately 21% of neonatal deaths are attributed to infectious causes with or without underlying prematurity [7]. Understanding the transmission of these infections remains important for achieving the sustainable development goal of reducing the under-five mortality to 25 per 1000 by 2030 [7].

In this context, our study investigated the vaginal carriage of *GBS* in pregnant women in rural Senegal. We found a prevalence of *GBS* carriage of 15.5%.

Label	Negative (N = 82)	Positive (N = 15)	Total (N = 97)	p value
Agegroup				0.487
[18 - 20]	16 (88.9%)	2 (11.1%)	18 (100.0%)	
[20 - 30]	54 (85.7%)	9 (14.3%)	63 (100.0%)	
[30 - 40]	12 (75.0%)	4 (25.0%)	16 (100.0%)	
Marital status				0.879
Monogamy	53 (84.1%)	10 (15.9%)	63 (100.0%)	
Polygamy	29 (85.3%)	5 (14.7%)	34 (100.0%)	
Occupation				0.541
University student	2 (100.0%)	0 (0.0%)	2 (100.0%)	
Housewife	80 (84.2%)	15 (15.8%)	95 (100.0%)	
Gestation				0.993
Multigestant	71 (84.5%)	13 (15.5%)	84 (100.0%)	
Primigestant	11 (84.6%)	2 (15.4%)	13 (100.0%)	
Parity				0.951
Multiparous	54 (84.4%)	10 (15.6%)	64 (100.0%)	
Nulliparous/primiparous	28 (84.8%)	5 (15.2%)	33 (100.0%)	
Week of amenorrhea				0.830
NA	2	0	2	
<35	62 (83.8%)	12 (16.2%)	74 (100.0%)	
≥35	18 (85.7%)	3 (14.3%)	21 (100.0%)	
Contraceptive method				0.691
NA	1	1	2	
injectable	19 (82.6%)	4 (17.4%)	23 (100.0%)	
none	55 (87.3%)	8 (12.7%)	63 (100.0%)	
non-injectable	7 (77.8%)	2 (22.2%)	9 (100.0%)	
History of obstetrics				0.068
NA	12	0	12	
No	66 (84.6%)	12 (15.4%)	78 (100.0%)	
Yes	4 (57.1%)	3 (42.9%)	7 (100.0%)	

**Table 3.** Distribution of participants according to streptococcal B test result with sociodemographic and obstetric characteristics.

Antibiotics	Susceptibility % Sensitivity	Resistance	
Chloramphenicol	100.0	0.0	
Clindamycin	100.0	0.0	
Erythromycin	100.0	0.0	
Penicillin G	100.0	0.0	
Rifampicin	100.0	0.0	
Vancomycin	100.0	0.0	
Gentamycin	86.7	13.3	
Norfloxacin	73.3	26.7	
Tetracycline	13.4	86.7	

**Table 4.** Sensitivity profile of the isolated strains.

Previously, a study conducted in Senegal in 2018 showed a prevalence of 16.1% [5]. The same trends were found in other African countries such as Ethiopia 16.3% [8], Egypt 17.8% [9] and Morocco 20.2% [10].

This prevalence appears to be higher in Africa compared to other countries in the world. Indeed, a meta-analysis of data from 37 countries in 2016 showed that the average prevalence of recto vaginal carriage of *Group B streptococcus* was highest in Africa (22.4%, 18.1 - 26.7) [11].

Nevertheless, high prevalences have been found in Michigan (US) and in Italy with respectively 25% and 33.9% [12] [13].

In our study, we found a 12.4% carriage of *Escherichia coli*. A quite similar prevalence of 16.3% was observed in a study in Mozambique between 2014 and 2015 [14]. Rare studies, focused on other high-risk vaginal bacteria observed prevalences of 25.0% and 27.1% for *E. coli* in pregnant women in Kenya and in South Africa respectively [15].

In our study, *Staphylococcus aureus* presented a prevalence of 13.4%. A similar prevalence (14.5%) was observed in the United States [14] [16]. However in France, a low rate of *S. aureus* carriage (5.9%) was observed [17].

The prevalence of HRVB vaginal carriage remains variable from one geographical area to another with rare differences that may often be due to the study population or the type of sample taken. Indeed, there is a difference depending on whether the sample taken is a vaginal or rectal swab [8]. It is necessary to emphasize the importance of introducing the swab without a speculum technique (scanning the inner vagina to the vestibule and vulva).

We found no statistically significant difference between *group B Streptococcus* carriage and any of the potential factors identified. Although the literature on *GBS* screening is extensive, most studies of *GBS* carriage have not found associations with the identified factors neither [10]. Similarly, studies anterior to ours conducted in Senegal did not find a statistically significant difference [18] [19]. However, few studies have reported an association between *GBS* carriage and prematurity and urinary tract infections [9].

It should be noted that this vaginal carriage of *GBS* is intermittent in pregnant women. This is an important element in their follow-up. In addition, it is important to screen or confirm carriage before initiating treatment intrapartum as recommended by the CDC.

These recommendations for universal screening for maternal *GBS* colonisation at 35 - 37 weeks' gestation and the use of intrapartum antibiotic prophylaxis have significantly reduced the burden of early-onset *GBS* in newborns [20].

Indeed, about one third of children born to mothers who are carriers of *Group B Streptococcus* are colonised at birth. *GBS* colonisation rarely affects the health of the newborn but, within the first 7 days of life, about 3% of colonised infants develop a serious early-onset infection, particularly *meningitis*, which can be fatal or life-threatening. The infections (after 7 days of life) do not appear to be related to intrauterine's colonisation. The risk of early neonatal *GBS* infection increases with preterm delivery, maternal fever during delivery and rupture of membranes more than 18 hours before delivery.

We studied the susceptibility profile of *GBS* strains. The strains showed full sensitivity to Penicillin G. This is in agreement with the literature [8]. This sensitivity is the basis for the CDC's recommendations for antibiotic treatment of pregnant women with *GBS*.

In addition to Penicillin G, we found full sensitivity to *erythromycin*, *chloramphenicol*, *vancomycin* and *clindamycin*. This partly corroborates the results obtained in Nigeria in 2019 by Idih EE [18] and those obtained by Musa Mohammed in Ethiopia in 2010 [9].

CDC guidelines recommend that patients who are not allergic to penicillin should receive penicillin or ampicillin. In case of minor allergy, cefazolin is recommended and in case of major allergy, clindamycin or vancomycin is recommended as *GBS* is known to be sensitive to these antibiotics [19] [20].

However, fairly recent studies have found strains resistant to Penicillin G and clindamycin [21] [22] [23]. Resistance to vancomycin is now empirical and has been reported by some authors such as Gizachew M and Park C in 2018 and 2014 respectively [21] [24].

Furthermore, we observed a high level of resistance to tetracycline (86.7%). This level of resistance has been reported by several authors, notably in the meta-analysis by Gizachew M who found 82.6% resistance to tetracycline [21]. This has been reported since 2001 in France (88.1%) and in Tunisia in 2012 (97.3%) [25] [26]. Tetracycline resistance in GBS is high (usually >80%), and multiple resistance determinants can be found in *GBS* strains [27]. Most GBS strains isolated from humans are resistant to tetracycline, and in particular the acquisition of *tetO* and *tetM* resistant elements by a subset of *GBS* clones has led to their selection and expansion [28].

It is therefore important to maintain the surveillance of the sensitivity of

strains to the various antibiotics used, especially in countries with limited resources where poorly conducted antibiotic therapy is the main factor in the emergence of antibiotic resistance. In practice, other molecules of the cyclin family (minocycline or tigecycline) should be tested separately to investigate the sensitivity of strains to these antibiotics.

*Streptococcus B* carriage remains a serious issue for maternal and child health. The prevalence of *GBS* carriage in rural pregnant women in Senegal is quite high, while none of the identified potential factors seem to favour it, which means that a wider range of variables will have to be explored to hope to find risk factors. In addition, the molecules recommended for treatment generally have a good sensitivity to the strains. Nevertheless, there is an increasing emergence of antibiotic resistance as found in the study with tetracycline, an antibiotic to which the bacteria are 86.7% resistant. It is therefore important to implement a good screening strategy and monitor the sensitivity of the strains in order to orientate the treatment in the best way.

This study was limited by the sample size, as this study was a pilot phase to assess the health affections of mothers and children in rural areas. The results thus obtained may enable us to envisage subsequent studies with a larger sample size in order to gain a better understanding of the problem of vaginal carriage of high-risk infectious vaginal bacteria, with a view to better inform public policies regarding this issue.

#### **5.** Conclusions

*Group B Streptococcus* infections are a significant public health issue. Our study showed an important level of carriage among women in rural area, sometimes associated with other HRVB.

However, the antibiotics used in the treatment kept their sensitivity. A surveillance program is necessary as a first step of a strategy to reduce the burden of infections due to *GBS*.

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#### **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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