Vaginal Carriage of Group B *Streptococcus* (GBS) in Pregnant Women, Antibiotic Sensitivity and Associated Risk Factors in Dakar, Senegal

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Ndeye Safietou Ngom[1], Omar Gassama[2], Assane Dieng[1], Elhadji Bambo Diakhaby[1], Serigne Mbaye Lo Ndiaye[1], Alioune Tine[1], Farba Karam[1], Gora Lo[1], Awa Ba-Diallo[1], Cheikh Saad Bouh Boye[1], Coumba Toure-Kane[1], Abdoulaye Seck[3], Halimatou Diop-Ndiaye[1] and Makhtar Camara[1]

[1]Bacteriology-Virology Laboratory, National University Hospital Center Aristide Le Dantec, Dakar, Senegal. [2]Gynecological and Obstetrical Clinic, National University Hospital Center Aristide Le Dantec, Dakar, Senegal. [3]Laboratory of Medical Biology, Pasteur Institute of Dakar, Senegal.

ABSTRACT: The eradication of neonatal Group B *Streptococcus* (GBS) infections, considered as a major public health priority, necessarily requires a mastery of the data on vaginal carriage in pregnant women. The aims of this study were to determine the prevalence of vaginal carriage of GBS in pregnant women, antibiotic susceptibility, and associated risk factors. This was a cross-sectional, descriptive study conducted over a period of 9 months (July 2020 to March 2021) in pregnant women between 34 and 38 weeks of gestation (WG) followed at the Nabil Choucair health center in Dakar. Identification and antibiotic susceptibility of GBS isolates were performed on the Vitek 2 from vaginal swabs cultured on Granada medium. Demographic and obstetric interview data were collected and analyzed on SPSS (version 25). The level of significance for all statistical tests was set at $P < .05$. The search of GBS vaginal carriage had involved 279 women aged 16 to 46 years, with a median pregnancy age of 34 (34-37) weeks' gestation. GBS was found in 43 women, for a vaginal carriage rate of 15.4%. In 27.9% (12/43) of volunteers screened, this carriage was monomicrobial, while in 72.1% (31/43) of women, GBS was associated with other pathogens such as *Candida* spp. (60.5%), *Trichomonas vaginalis* (2.3%), *Gardnerella vaginalis* (34.9%) and/or *Mobiluncus* spp. (11.6%). The level of resistance was 27.9% (12/43) for penicillin G, 53.5% (23/43) for erythromycin, 25.6% (11/43) for clindamycin and 100% for tetracycline. However, the strains had retained fully susceptible to vancomycin and teicoplanin. The main risk factor associated with maternal GBS carriage were ectocervical inflammation associated with contact bleeding (OR = 3.55; $P = .005$). The high rate of maternal vaginal GBS carriage and the levels of resistance to the various antibiotics tested confirm the importance of continuous GBS surveillance in our resource-limited countries.

KEYWORDS: Group B *Streptococcus*, pregnant women, epidemiology, antibiotic resistance, associated risk factors, Senegal

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CORRESPONDING AUTHOR: Ndeye Safietou Ngom, Bacteriology-Virology Laboratory, National University Hospital Center Aristide Le Dantec, 30 Avenue Pasteur, Dakar BP: 7325-Dakar, Senegal. E-mail: safietou11@hotmail.com

Introduction

Group B *Streptococcus* (GBS) or *Streptococcus agalactiae* is responsible for severe neonatal infections that can lead to early illness in the form of pneumonia, sepsis, or life-threatening meningitis.[1] It causes lethality ranging from 1% to 8.4% in term infants and 5% to 20% in preterm infants. High early mortality rates of up to 27% have been observed in Africa.[2] GBS is also responsible for 1% of adverse pregnancy outcomes worldwide and 4% in Africa.

A commensal bacterium of the gastrointestinal and genitourinary tracts of approximately 20% to 30% of healthy women,[3] some strains of GBS have increased pathogenic potential due to the evolutionary acquisition of specific virulence factors that increase efficiency of dissemination, immune evasion, and tissue damage. The rate of maternal colonization is variable (ranging from 1.6% to 36% depending on the study), intermittent and/or transient.[4,5] A small proportion of those colonized with GBS suffer invasive disease. Cases of endometritis, chorioamniotitis, bacteriuria, and maternal bacteremia have been reported.[6] This suggests that host-specific factors potentially play a role in determining susceptibility among individuals.

However, maternal colonization with *S. agalactiae* is known to be the cause of neonatal colonization and infection. Neonatal acquisition of GBS occurs in 40% to 75% of births to colonized mothers and can occur in utero; through placental membranes, or most commonly intra amniotically, during labor through ingestion or inhalation of contaminated secretions by the newborn.[7] The only clinical intervention to prevent early-onset disease in newborns recommended by the Centers for Disease Control and Prevention (CDC) is screening during pregnancy for GBS in pregnant women between 34 and 38 weeks of amenorrhea (WA), and administration of intravenous antibiotics to colonized pregnant women during delivery.[8] Implementing strategies to identify women at risk who should receive antibiotics during delivery is a major public health challenge. Recently, significant effort has been devoted to measuring global rates of GBS colonization and associated risk factors.

In the absence of health policies aimed at reducing neonatal GBS infection, in resource-limited settings, screening of pregnant women carrying the bacteria and antibiotic susceptibility testing of GBS are not systematic. In Senegal, few data on GBS carriage exist and need to be updated. It is in this context that this study was undertaken. The objective was to determine the prevalence and associated risk factors of vaginal GBS carriage in pregnant women and to study the susceptibility of the strains to commonly used antibiotics.

Methodology

Design, study population and sampling procedure

The study obtained the approval of the National Ethics Committee for Health Research (CNERS) of the Cheikh Anta Diop University of Dakar and the management of the health centre Nabil Choucair. This was a cross-sectional, analytical, monocentric study conducted over a period of 09 months (July 2020 to March 2021) among pregnant women attending the Nabil Choucair health centre in Dakar. The study included all pregnant women between 34 and 38 weeks of gestation, without any associated pathology, who came to the Nabil Choucair health center for a prenatal consultation and for whom informed consent had been obtained. Have not been included were parturients who had received antibiotic therapy of any kind and duration in the 15 days preceding the consultation, women with pregnancy complications such as placenta previa, vaginal bleeding or an underlying pathology; hypertension, diabetes, HIV and women in labor.

Socio-demographic characteristics such as age, marital status, education level and gyneco-obstetric characteristics namely gestational age, history of current pregnancy, associated pathologies and local symptoms were collected through a well-structured questionnaire during face-to-face interviews and recorded on a specific coded information sheet for each participant. Each pregnant woman was placed in the gynecological position and underwent a clinical and obstetrical examination. The physicians assessed and recorded the progress of the pregnancy. Afterward, the sampling was performed. Two sterile swabs were taken, one from the posterior vaginal cul-de-sac, kept in 1 ml of physiological water, and the other from the endocervix. The pH of the vaginal secretions was determined using pH indicator paper. Other macroscopic characteristics were noted, including the color, appearance and odor of the discharge. The samples were sent within 3 hours to the bacteriology laboratory at Aristide Le Dantec Hospital in Dakar.

Treatment of samples

Microscopic examination and bacterial isolation. All collected samples were processed immediately for possible microscopic identification and isolation of pathogenic microorganisms according to standard laboratory protocols. A saline wet preparation of a drop of vaginal secretion was used for direct examination of leukocytes, red blood cells, epithelial cells, yeast and *Trichomonas vaginalis*.

A Gram stain was performed to assess the state status of the vaginal flora, the microbial flora, typing and Nugent scoring.[9] Culturing on specific media for the detection of pathogenic microorganisms, in particular GBS, was done on fresh blood agar made selective by the addition of nalidixic acid GSN (Bio-Rad, Marnes-la-Coquette, France) and on Granada medium (Becton Dickinson GmbH, Heidelberg, Germany).[10] Yeasts responsible for vulvovaginal candidiasis were isolated on Sabouraud chloramphenicol medium.[11] Anaerobic bacteria were grown on Thioglycolate broth. *Escherichia coli* strains were isolated on Eosine Methylene Blue (EMB) medium (Merck, Darmstadt, Germany). *Neisseria gonorrhoeae* was tested from the endocervical swab on selective VCN agar supplemented vancomycin, colistin and nystatin.[12] These culture media were incubated at 37°C for 24 to 48 hours. The blood agar and Granada media were incubated in an atmosphere enriched with 5% CO_2.

Microbiological identification. Inflammation was indicated when the mean number of leukocytes was greater than 10/field at high magnification. Observation of *Trichomonas vaginalis* by its typical morphology and mobility under a wet preparation was the control for also the identification of T. vaginitis (TV).[13] The diagnosis of vulvovaginal candidiasis (VVC) was retained when yeasts or spores were found associated with a growth on Sabouraud medium.[14] The typing of the flora was performed according to the recommendations of Aly Abbara and the Nugent et al score was adopted to diagnose the bacterial vaginoses (BV); (7-10: BV; 4-6: intermediate BV; 1-3: normal).[15] Germ-positive cultures on EMB and Sabouraud were identified by conventional methods, including Gram stain, colonic morphology, filamentation test, classical gallery, and by biochemical tests.[16] The identification of pink colonies on Granada medium representing GBS strains according to the manufacturer's instructions was confirmed by conventional methods by studying morphological, biochemical, cultural and antigenic characters, and then confirmed on Vitek 2 (bioMerieux, France) with GP (Gram-positive) cards.[17] Antimicrobial susceptibility testing of *S. agalactiae* isolates was performed by microdilution on an automated instrument the Vitek 2 with AST ST-03 cards.[18]

The interpretation of the results was done according to the EUCAST 2021 recommendations.[19]

Statistical analysis

The study data were entered on Filemaker pro (version 16) software and analyzed on Excel and SPSS version 25 (Statistical Package for Social Sciences; Chicago, IL, USA). Categorical variables were presented as frequency and percentage, whereas continuous variables were expressed as median. Differences in continuous and categorical variables

Table 1. Socio-demographic characteristics of the study participants.

PARAMETERS	PREGNANT WOMEN N (%)				
	CLASSIFICATION	GBS (+) (N=43)	GBS (–) (N=236)	PARTIAL PREVALENCE	*P*
Age (years)	Median (IQR)	25 (22.5-29.5)	26 (22-30)		.410
Age group (years)	16-24	19 (44.2)	93 (39.4)	16.9	**.046**
	25-33	24 (55.8)	107 (45.3)	18.3	
	34-46	0	36 (15.3)	0	
Marital status					.193
	Married	43 (100)	227 (96.2)	15.9	
	Single	00 (0.0)	09 (3.8)	0	
Marital regime					.253
	Monogamy	33 (76.7)	198 (83.9)	14.3	
	Polygamy	10 (23.3)	38 (16.1)	20.8	
Level of education					.174
	Not educated	4 (9.3)	36 (15.3)	10.0	
	Primary	10 (23.3)	64 (27.1)	13.5	
	Secondary	16 (37.2)	85 (36.0)	15.8	
	higher	4 (9.3)	30 (12.7)	11.8	
	Arabic	9 (20.9)	21 (8.9)	30.0	

GBS (+), GBS positive; GBS (–), GBS negative; *P*, *P*-value; IQR, interquartile range.
Bold values indicate the age ranges thus distributed were significantly associated with vaginal carriage of GBS.

between groups were analyzed using non-parametric Mann-Whitney U and chi-squared tests, respectively. Logistic regression was used to assess the associated risk factors. The level of significance for all statistical tests was set at $P < .05$.

Results

In the present study, 279 pregnant women with a median age of 26 years (16-46), a median gestational age of 34 (IQR) [34-37] weeks' gestation and a median gestation of 2 [1-3] pregnancies per woman were examined. Bacteriological testing identified 43 strains of GBS, corresponding to a vaginal carriage rate of 15.4% (43/279).

Socio-demographic characteristics

The socio-demographic characteristics of pregnant women in relation to maternal vaginal GBS colonization are summarized in Table 1.

The detailed results of the statistical analysis showed that there was no significant difference between the median age of GBS colonized women and that of uninfected women. However, the estimated prevalence of GBS carriage was higher in women aged [25-33] years (55.8%) than in those aged [16-24] years (44.2%). Of the pregnant women included in the study, 96.7% (270/279), were married of which 82.8% (231/279) were in a monogamous relationship. Interestingly, GBS was only detected in married women (43/279) and mainly in monogamous women (76.7%; 33/43). However, there was no correlation between vaginal carriage of GBS and marital status ($P = .19$) on the one hand and marital regime ($P = .25$) on the other. Women with Arabic education had the highest rate of GBS vaginal colonization, estimated at 30.0% (9/30), although there was no significant relationship between GBS vaginal carriage and education level ($P = .17$).

Clinical characteristics of the study participants

Clinical characteristics consisting of gyneco-obstetrical data and clinical symptoms experienced by pregnant women in relation to the presence of GBS in the vagina are described in Table 2.

In our series, 41.9% (18/43) of the GBS colonized women had no GBS-related symptoms. The remaining 58.1% (25/43) reported the presence of clinical symptoms such as irritation felt in 44.2% (19/43), followed by burning sensations evoked in 30.2% (13/43), incriminating pelvic pain in 27.9% (11/43) and dyspareunia in 23.3% (10/43) of cases. All these clinical symptoms were not significantly associated with the presence of GBS in the vagina ($P = .77$).

Table 2. Clinical characteristics of the study participants.

PARAMETERS CHARACTERISTICS		PREGNANT WOMEN N (%)			
		GBS CARRIAGE (N = 43)	NO GBS CARRIAGE (N = 236)	PARTIAL PREVALENCE	*P*
Local clinical symptoms					
Symptoms	Present	25 (58.1)	132 (55.9)	15.9	.778
	None	18 (41.9)	104 (44.1)	14.8	
Dyspareunia	Present	10 (23.3)	37 (15.7)	21.3	.222
	Absent	33 (76.7)	199 (84.3)	14.2	
Pelvic pain	Present	12 (27.9)	55 (23.3)	17.9	.516
	Absent	31 (72.1)	181 (76.7)	14.6	
Burns	Present	13 (30.2)	81 (34.8)	13.8	.565
	Absent	30 (69.8)	152 (65.2)	16.5	
Obstetrical characterics					
Gestational age (WA)	Median (IQR)	34 (34-37)	34 (34-37)		.690
	34	23 (53.5)	122 (51.7)	15.9	
	35	04 (9.3)	19 (8.1)	17.4	
	36	02 (4.7)	26 (11.0)	7.1	.112
	37	10 (23.3)	26 (11.0)	27.8	
	38	04 (9.3)	43 (18.2)	8.5	
Number of pregnancy	Median (IQR)	2 (1-3)	2 (1-3)		.771
	Primigestes	16 (37.2)	105 (44.5)	13.2	.376
	Multi-gestures	27 (62.8)	131 (55.5)	17.1	

On the gynecological plan, GBS carriage was more common at 37 weeks' gestation with 27.7% (10/36) of cases, and at 34 weeks' gestation with 15.9% (23/145). GBS carriage was more common in multigestational women (62.8%; 27/43), than in primigravida women, with a frequency of 37.2% (16/43). However, there was no statistically significant relationship between the number of pregnancies ($P = .37$), stage of pregnancy and vaginal GBS carriage ($P = .11$).

Laboratory findings

Characteristics of vaginal discharge. In the present study, the macroscopic characteristics of vaginal secretions from GBS colonized women are summarized in Table 3. These vaginal discharges containing GBS were colored in the following proportions: white in 67.4% (29/43) of cases, yellow in 16.3% (7/43) or streaked with blood (14%; 6/43). In terms of consistency, it was thick (81.4%; 35/43) or rarely milky (9.3% 4/43). A foul odor was observed in 32.6% (14/43) of the cases, while in 67.4% (29/43) of the samples containing GBS, the vaginal discharge was odorless. With the exception of the thick consistency of the vaginal discharge, there was no correlation between color, other types of consistency, odor of the vaginal discharge and vaginal GBS carriage.

Rate of germ isolation. Table 4 lists the microorganisms found during the study. GBS as a single pathogen was isolated in 27.9% (12/43) of cases. According to the vaginal inflammatory response (leukocytes greater than 10/field), in these 12 pregnant women, the presence of GBS was defined as 06 cases of GBS vaginitis and 06 cases of simple carriage. In addition to the 43 GBS strains isolated 48 other pathogens were identified from the pregnant women. The rate of isolation of the other germs encountered was 60.5% (26/43) for *Candida spp*, 2.3% (1/43) for *T. vaginalis*, 34.9% (15/43) for *Gardnerella vaginalis*, and 11.6% (5/43) for *Mobiluncus spp*. No *Neisseria gonorrhoeae* strains were isolated. The presence of *Candida spp*. was statistically associated with vaginal GBS carriage ($P = .006$).

Co-infections. Table 5 details the distribution of germs in co-infection with GBS. The rate of GBS co-infection was 72.1% (31/43). GBS was associated in 32.5% (14/43) with

Table 3. Characteristics of vaginal discharge.

CHARACTERISTICS	GBS CARRIAGE (N=43)	NO GBS CARRIAGE (N=236)	PARTIAL PREVALENCE	*P*
Color of vaginal discharge				
White	29 (67.4)	166 (70.3)	14.9	.41
Yellow	7 (16.3)	46 (19.5)	13.2	.40
Green	1 (2.3)	4 (1.7)	2.3	.57
Blood streaks	6 (14)	14 (5.9)	13.9	.06
graying	-	6 (2.5)	0	.25
Total	43	236		
Appearance of vaginal discharge				
Thick	35 (81.4)	216 (91.5)	13.9	.046
Fluid	1 (2.32)	6 (2.5)	14.2	.70
Glutinous	4 (9.3)	10 (4.2)	28.6	.185
Foamy	2 (4.6)	3 (1.3)	40	.171
Adherent	1 (2.3)	1 (0.4)	1	.285
total	43	236		
Odor of vaginal discharge				.350
Fetid	14 (32.6)	67 (28.4)	17.3	
Non fetid	29 (67.4)	169 (71.6)	14.6	

Table 4. Distribution of microorganisms isolated.

MICROORGANISMS ISOLATED	PREGNANT WOMEN N (%)			
	GBS CARRIAGE (N=43)	NO GBS CARRIAGE (N=236)	PARTIAL PREVALENCE	*P*
Simple GBS carriage	12 (27.90)	-	-	
GBS co-infection	31 (72.10)	-	-	
Candida spp.	26 (60.5)	87 (36.9)	23.3	**.006**
Gardnerella vaginalis	15 (34.9)	60 (25.4)	20.0	.198
Mobiluncus spp.	05 (11.6)	14 (5.9)	26.3	.173
Trichomonas vaginalis	1 (2.3)	81 (34.8)	13.8	.593
Neisseria gonorrhoeae	-	-	-	-

Bold values indicate vaginal *Candida* spp infection is statistically associated with maternal vaginal GBS carriage.

vulvovaginal candidiasis, in 2.3% (1/43) with Trichomonas Vaginalis, in 9.3% (4/43) with bacterial vaginosis (BV), and in 27.9% (12/43) with vulvovaginal candidiasis combined with bacterial vaginosis.

The pH. In simple GBS infections, the pH of vaginal secretions was equal to 4 if not impaired by the presence of blood. In 50% (n = 6) the vaginal pH was normal. In the other half, cases of inflammation of the exocervix associated with bleeding on contact with the cervix were observed.

In vaginal infections caused by yeast in association with GBS, the pH was normal at 4. In cases of BV associated with GBS carriage, the pH was elevated, usually above 5.

This finding shows that GBS is equally at home in both acidic and basic vaginal secretions and does not seem to influence the variation of vaginal pH.

Appearance of the ectocervix. Table 6 summarizes the results of visual examination of the ectocervix in relation to maternal vaginal colonization with GBS. Visual inspection of the cervix

Table 5. Distribution of germs in co-infection with GBS.

GBS CO-INFECTION		
INFECTIOUS DISEASE	N	%
Vulvovaginal candidiasis (VVC)	14	32.55
Vaginitis with TV	01	2.32
Bacterial vaginosis (BV)	04	9.30
BV with *Gardnerella vaginalis* (GV)	01	2.32
BV with GV-*Mobiluncus* spp. (Mob)	03	6.97
VVC associated with VB	12	27.90
VVC-GV	09	20.93
VVC-GV-Mob	03	6.97
Total	31	100

showed a normal appearance in 48.8% (21/43) and an abnormal appearance in 39.5% (17/43) of cases. In 11.6% (5/43) of pregnant women, the cervix was not visualized. The abnormal appearance of the ectocervix was constituted by cases of inflammation and/or bleeding in contact with the cervix. These cases of inflammation of the ectocervix associated or not with bleeding at the contact of the cervix were determining elements in the diagnosis of GBS carriage in pregnant women. There was a statistically significant relationship between inflammation, bleeding from the cervix and vaginal GBS carriage ($P = .01$).

Antibiotic susceptibility profile. Figure 1 summarized the results of antibiotic susceptibility testing of GBS strains from maternal vaginal carriage. All 43 GBS isolates were tested for minimum inhibitory concentration using the Vitek 2 compact automated system. AST ST-03 cards containing 11 antibiotics were used for the antimicrobial susceptibility study.

The results of the antibiotic susceptibility tests showed that all GBS isolates from pregnant women were mainly susceptible to vancomycin (100%), teicoplanin (100%), tigecycline (100%) and linezolid (100%). In contrast, GBS resistance to penicillin G was 27.9% (12/43). Resistance to levofloxacin and moxifloxacin was observed in 18.6% (08/43) and 16.3% (07/43) of cases, respectively. It was observed that 53.5% (23/43) of the strains were resistant to erythromycin and 25.5% (11/43) to clindamycin, while only one strain was resistant to chloramphenicol (1/43). It should be noted that all strains were resistant to tetracycline (100%).

Risk factors associed with GBS vaginal carriage

In Table 7, a summary of the multivariate analysis was performed on the association of vaginal GBS carriage in pregnant women with risk factors that were significantly associated with a P-value of $\leqslant .05$. The results showed that the confirmed risk factors associated with GBS carriage were ectocervical inflammation associated with cervical contact bleeding. Women with ectocervical bleeding were almost 3 times more likely to be GBS carriers than women with a healthy ectocervix (OR = 3.36; $P = .005$). Microbiologically, inflammatory smears were positively associated with the probability of isolating GBS (OR = 3.55; $P = .001$). While the probability of being colonized by GBS at the vaginal level was 2 times higher in pregnant women with vulvo-vaginal candidiasis (OR = 2.62; $P = .006$).

Discussion

The pathogenesis of GBS infection is studied in many Western countries. However, on the African continent, there are few studies reporting on GBS infections. In order to assess the incidence of GBS in Senegal, a survey of pregnant women allowed us to investigate the importance of vaginal carriage of *Streptococcus agalactiae*.

In this cross-sectional descriptive and analytical study conducted at the Nabil Choucair health center in Dakar, we reported an estimated prevalence of GBS colonization in pregnant women between 34 and 38 WG of 15.4% (43/279).

This prevalence rate is higher than the 14% observed in Dakar in 1979 in 100 mother-child pairs by F. Denis et al. [20] It is also known that GBS was the third most common cause of purulent neonatal meningitis in Senegal between 1983 and 1991 (12%).[21] In the meantime, these maternal GBS carriage rates has evolved. It is estimated at 16.1% in the Jung et al YJ, et al[22]. study in 2021 and at 25.7% in the Ndiaye et al study in 2022.[23] In the sub-region, particularly in West Africa, the overall prevalence of maternal GBS colonization was estimated at 15% in a meta-analysis conducted by Gizachew et al in 2019.[24] Also, some disparities had been noted in the prevalence rates of GBS colonization across countries. They were estimated at 12% in Kenya[2] in 2016; 19% in Gabon in 2015[25]; 14% in Cameroon in 2018,[26] compared to 19.7% in Nigeria[27] and 34% in Gambia in 2016.[28]

The causes of this disparity in GBS carriage rates between studies may be attributed to differences in methodology particularly due to variations in sampling methods, microbiological diagnostic techniques employed or study population size. In the Cools et al study, for example, only vaginal swab samples were collected, which they suggest may be responsible for the lower carriage rate.[29] Methods such as the use of blood agar may result in lower sensitivity if human blood was used instead of sheep or horse blood. Use of these non-selective media is a factor in under-identification of GBS and underestimation of prevalence.[30] Another feature that may explain a lower colonization rate was the collection of vaginal but not rectal swabs, which are known to have a higher yield for GBS.[31] The use of molecular biology, which are more sensitive PCR methods in the detection of GBS DNA especially in the Gambian study gives high rates of positivity.[28] And finally, using only a culture method, some pregnant women colonized with GBS could go

Table 6. Appearance of the ectocervix.

PARAMETERS CHARACTERISTICS		PREGNANT WOMEN N (%)			
		GBS (+) (N=43)	GBS (–) (N=236)	PARTIAL PREVALENCE	*P*
Ectocervix appearance	Normal	21 (48.8)	160 (67.8)	10.6	.002
	Abnormal	17 (39.5)	50 (21.2)	25.5	
	Not seen	05 (11.6)	26 (11.0)	21.2	
Inflamed	Present	15 (39.5)	35 (16.3)	30.0	.001
	Absent	23 (60.5)	180 (83.7)	11.3	
Bleeding on contact	Present	10 (26.3)	23 (10.9)	30.3	.010
	Absent	28 (73.7)	188 (89.1)	13.0	

GBS (+), GBS positive; GBS (–), GBS negative.

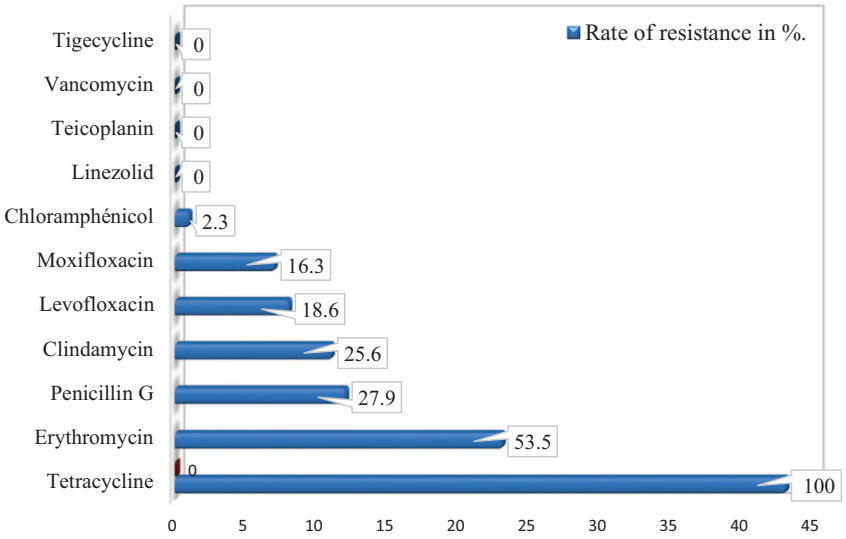

Figure 1. Antibiotic resistance profile of GBS strains.

undetected, especially in health centres where there are high rates of uncontrolled prescribing and abuse of antibiotics.[32]

Our results revealed that most of the participants colonized with GBS, were between 25 and 33 years old (55.8%), had completed at least elementary (23.3%) and secondary (37.2%) levels, were all married and had a gestational age of 37 weeks of gestation (27.8%). These results could be explained by the fact that our study took place in an urban setting where women had acquired a certain level of education before getting married and becoming pregnant. Currently, there is no vaccine that can prevent GBS infection. Screening and treatment of vaginal carriage during pregnancy remains the only way to prevent maternal-fetal GBS infection. This screening reveals the presence, or not, of the bacteria in the vaginal flora of the pregnant woman. It is performed as close as possible to the time when the mother can potentially transmit the bacteria to her newborn, that is, between 34 and 38 weeks of gestation.[7]

According to Shabayek and Spellerberg in 2017, low pH (pH to ±4), would promote epithelial adherence of GBS as well as biofilm production of the bacteria by increasing its density and resistance.[33] In our study, GBS was found in both normal and basic pH vaginal secretions. The pH variation was more related to the presence or absence of Döderlein bacilli and the occurrence of bacterial vaginosis. GBS did not appear to influence the change in vaginal pH. However, this result deserves special attention. Several studies including Patras et al (2013) suggest that translocation of GBS from acidic to neutral niches activates virulence-related genes, promoting the transition from commensal to invasive bacterial pathogen.[34] Would it be the most virulent strains that are able to survive and maintain themselves in a high pH vaginal ecosystem? This is a line of thought to be developed with the serotyping of strains.

An imbalance of the vaginal flora with or without BV has been associated with increased levels of GBS colonization.

Table 7. Risk factors associated with GBS carriage.

	GBS CARRIAGE N (%)	NO GBS CARRIAGE N (%)	OR	95% CI	*P*
	43 (15.4)	236 (84.6)			
Inflammation of the ectocervix	15 (34.9)	35 (14.8)	3.36	1.59-7.06	.005
Bleeding on contact	10 (23.3)	23 (9.7)	2.91	1.25-6.77	.042
Inflammatory reaction	19 (44.2)	43 (18.2)	3.55	1.78-7.06	.001
VVC	26 (60.5)	87 (36.9)	2.62	1.34-5.09	.006

The typing of the flora according to the Nugent criteria allowed us to find an imbalance of the vaginal flora in only 37.2% (16/43). Our results contradict this observation and support a predominance of GBS carriage in a healthy vaginal environment. Moreover, almost all cases of simple GBS vaginitis (11/12) were detected in the presence of lactobacillus, in a balanced vaginal flora (type 2).

According to Meyn et al 2009,[35] vaginal mycoses including a higher vaginal presence of *C. albicans*; and were associated with high levels of GBS colonization. Our culture results on Sabouraud do reveal a 60.5% (26/43) presence of *Candida* spp. in GBS positive samples and are in agreement with this statement.

In our study, 58.1% (25/43) of the women with GBS had irritation in 44.2% (19/43) of cases, burning sensation in 30.2% (13/43) and pelvic pain in 27.9% (12/43) of cases. These symptoms associated with GBS are probably due to concomitant yeast infection, BV or *T. vaginalis* infection, as we noted several cases of co-infection including 60.5% with *Candida* spp. as VVC, 34.88% with *G. vaginalis* as BV and 01 case with *T. vaginalis* (2.32%).

The results of the antibiotic susceptibility testing revealed that all the strains were susceptible to vancomycin, teicoplanin, tigecycline and linezolid. On the other hand, they showed an overall resistance of 100% to tetracycline. The resistance rate to third generation quinolones were 18.6% and 16.3%, respectively for levofloxacin and moxifloxacin. On the other hand, the resistance of GBS to penicillin G was 27.9% (12/43). These results showed that the beta-lactams commonly recommended as prophylactic regimen in first and second line could be ineffective on some isolated GBS strains.[36] Also, clindamycin, which is the recommended therapeutic alternative in case of allergy to beta-lactams, was not active on some strains (25.6%; 11/43).

Reduced susceptibility GBS strains to penicillin were already observed in 27% of the strains tested in Dakar in 2003.[37] This phenomenon had been previously reported in 1981 and was responsible for 5% of relapses of severe *S. agalactiae* infections in Senegal.[38] In the same year, the percentages of resistance obtained with erythromycin and lincomycin were 9 and 5%, much higher than those obtained in 1980 (0.75 and 0.4% respectively).[39] Currently a high level of resistance has been observed in this group with 53.5% (23/43) of strains resistant to erythromycin and 25.6% (11/43) resistant to clindamycin. This shows a clear progression of macrolide resistance in GBS strains isolated in Dakar. In the latest CDC recommendations of 2010[8], erythromycin is no longer considered an acceptable alternative for intrapartum GBS prophylaxis in women allergic to penicillin. Rightly so, even in our study, 56.5% of resistance had been observed against this molecule.

In our study, a systematic examination under speculum seemed desirable, in particular to appreciate the physical signs consisting by the ulcerations of the vagina and the ectocervix and possibly the inflammatory anomalies which can only be demonstrated with the help of a speculum examination. But also to detect bacterial vaginosis, the treatment of which could reduce the risk of pregnancy complications. We hope that these arguments and the resulting results will provide a definitive answer to the controversy about the use of the speculum in the search for GBS carriage.

During the endocervical sampling, we noticed cases of bleeding from the ectocervix on contact with the swab. This result was suspected to be related to the positive culture result on GBS specific media before it was later confirmed by statistical tests. We hope that this result will be further investigated in future studies on a larger cohort by including the search for HPV causing cervical cancer, of which inflammation of the ectocervix and abnormal bleeding in pregnant women is a major predictive factor.[40] Studies have previously demonstrated that Streptococcus endopeptidases promote HPV infection in vitro.[41] The opportunity should be taken to explore the role of *S. agalactiae* in HPV persistence and cervical cancer occurrence. But also that in the absence of GBS universal screening of pregnant women in low-income countries, the sign of inflammation associated with extra-cervical bleeding could be used in the identification of pregnant women between 34 and 38 WG who do not have access to the specialized laboratory in order to receive intrapartum antibiotic prophylaxis (IAP).

Conclusion

These data obtained in our study showing the high level of vaginal carriage and antibiotic resistance of GBS, as well as its involvement in possible inflammatory abnormalities in the ectocervix, deserve to be taken into consideration and important efforts should be undertaken for the control of GBS infections.

These very important findings from this study will hopefully have important implications for public health policy.

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Author Contributions

NSN, AD, OG, and MC designed the experiments. SMN and AT, analyzed the data. GL and FK wrote the first draft of the manuscript and jointly developed the structure and arguments of the article. ABD, AS and HDN contributed to the drafting of the manuscript. CSB, CTK, and MC made critical reviews and approved the final version.All authors agree with the results and conclusions of the manuscript and have reviewed and approved the final manuscript.

Ethical Considerations

All ethical approvals and requirements were addressed. The study obtained the approval of the National Ethics Committee for Health Research (CNERS) of the Cheikh Anta Diop University of Dakar and the management of the health centre. The purpose of the study was explained to the participants who gave verbal and written consent. Patients were free not to participate in the study without affecting their follow-up. Confidential information obtained from participants was coded and strictly maintained. All test results were sent to the patients. Those with a positive test for a pathogen were informed by the health care providers and received appropriate treatment.

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