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Bacterial Distribution and Antibiotic Susceptibility Pattern of *group B Streptococcus β hemolytic* (GBS) in Vaginal Infections at Cotonou in Benin

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Abstract No orthodox practices disrupt the vaginal flora and expose it to pathogenic microorganisms including group B Streptococcus β hemolytic (GBS). The study aims to describe the bacterial profil and resistance of GBS to antibiotics. Retrospective study included 640 women at Cotonou suspected of vaginal infections or vaginal discharge during 1st January 2004 to 31st December 2015. Three swabs were collected and analyzed to identify the bacteria by standard biochemical reactions, diagnosis of bacterial vaginosis and identification of *Trichomonas vaginalis*, yeasts and leukocyte count. Antibiogram was performed according to the CA-SFM. Among 640 samples, 502 (78.4%) were positive. The most encountered microorganisms were *Candida albicans* (37.45%) and GBS (19.92%). GBS sensitivity ranged from 80-100% for augmentin, pefloxacin and nitrofuran. But resistance was observed to netilmicin, tetracyclin, cefoxitin, cephalotin, thiamphenicol, trimethoprim / sulfamethnoxazole between 80-100%. A high percentage of resistance is the result of uncontrolled access to antibiotics and improper antibiotic policy. Routine susceptibility testing will allow to take appropriate treatment of GBS in Benin.

Keywords: *vaginal infections, GBS, antibiotics, Cotonou*

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1. Introduction

The presence of lactobacillus ensuring generally acid vaginal pH [1,2] notifies the normal state of the cervicovaginal environment which greatly participates in the defense against the growth of pathogens [3]. The use of antibiotics, intrauterine devices, contraceptive pills, pregnancy and sexually transmitted infections are factors causing an imbalance and profound modification of the commensal flora of the vagina promoting the growth by bacterial vaginosis [4,5] including *Candida albicans* (CA) and *Group B streptococcus β hemolytic* (GBS). Bacterial vaginosis (BV) misdiagnosed and untreated can lead to severe complications including pelvis inflammatory disease[6], infertility, preterm birth, low birth weight [7], chorioamnionitis and premature rupture of membranes [3]. In fact, it has been reported that women carrying *Candida albicans* were likely to develop GBS [8]. GBS may be carried by pregnant and non-pregnant women. Alp *et al.* reported in Turkey that 16.5% of non-pregnant women were GBS carriers compared to 9.8% of pregnant women[9]. Since 1970, GBS were recognized as a major cause neonatal mortality and morbidity in the United States [10]. A GBS transmission rate of 60% to their newborn was reported by Namavar *et al.* in pregnant women infected with GBS [11]. GBS have also been identified as responsible of stillbirth [12]. The GBS infections are early-onset infections and intra-partum antibiotic prophylaxis treatment is the way to prevent it [13]. According to the Centre for Disease Control and prevention (CDC), all pregnant women should be screened at 35-37 weeks of gestation and providing intra-partum antibiotic prophylaxis [13].

In Benin, none study did not document GBS resistance isolated in vaginal. Hence, the presence study is designed to identify the vagina bacterial infections

and susceptibility of GBS towards currently used antibiotics.

2. Methodology

2.1. Study Design

It was retrospective cross sectional study conducted on samples collected during a period of twelve year from women at Cotonou between 1st January 2004 and 31st December 2015. For each woman (n= 640) between 2 and 60 years of age, we extracted from the laboratory records, age, sex, vaginal sample culture results, identification of strain responsible of vagina bacterial infections and the corresponding antimicrobial susceptibility test (AST) results.

2.2. Ethical Committee

This study was approved by the Research Ethics Committee for Applied Biomedical Sciences (CER-ISBA) of Abomey Calavi University of Benin.

2.3. Sample Collection

Vaginal and cervical swabs samples (Three) were obtained from each woman as described by Onderdonk *et al*. [14]

2.4. Processing of Samples

The first swab was inoculated onto blood agar, MacConkey agar and chocolate agar (with 20% CO_2 moist atmosphere) and incubated aerobically at 37°C for 48 hours [15,16]. Bacterial growth was identified by standard biochemical reactions [17]. The second was used to prepare a gram smears for diagnosis of BV by the Nugent scoring system [18].

The third was taken to prepare the smear on a sterile glass slide for the detection of *Trichomonas vaginalis*, yeast cells, clue cells, number of leukocytes, type of vaginal flora, and parabasal epithelial cells [19].

Antibiotics' susceptibility was performed by disk diffusion method in solid middle according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) [20]. Antibiotic discs were obtained from Bio-Rad, Marne la Coquette, France.

2.5. Statistical Analysis

The statistical test EpiData 3.1 was used to check-in results and EpiData Analysis V2.2.2.182 for data analysis. The chi-2 test was used to compare proportions.

3. Results

3.1. Distribution of Organisms Isolated from Vaginal Infections

Overall 640 vaginal cultures were performed during 2004-2015 period. The rate of positive cultures was (n= 502, 78.4%) of which 506 microorganism (BV, candidiasis, gonorrhoeae, trichomoniasis and others aerobic microorganisms) have been isolated. The distribution of vaginal samples and vaginosis based on age ranges are showed in Figure 1. The mean age of the women in this study was 29.8 years [95% Confidence Interval (CI) 29.00-30.57] ranging from 2 to 60 years (Table 1). Figure 2 illustrates the types and proportions of microorganisms isolated in vaginal from 502 women with vaginal infections or symptomatic vaginal discharge attending the National Laboratory (NL) of Benin Heath Ministry.

The most common microorganisms encountered was *Candida albicans* (n= 182, 37.45%) followed by *Group B streptococcus β hemolytic* (n= 100, 19.92%), *Staphylococcus aureus* (n= 60, 11.95%), *Staphylococcus dore* (n= 46, 9.16%).

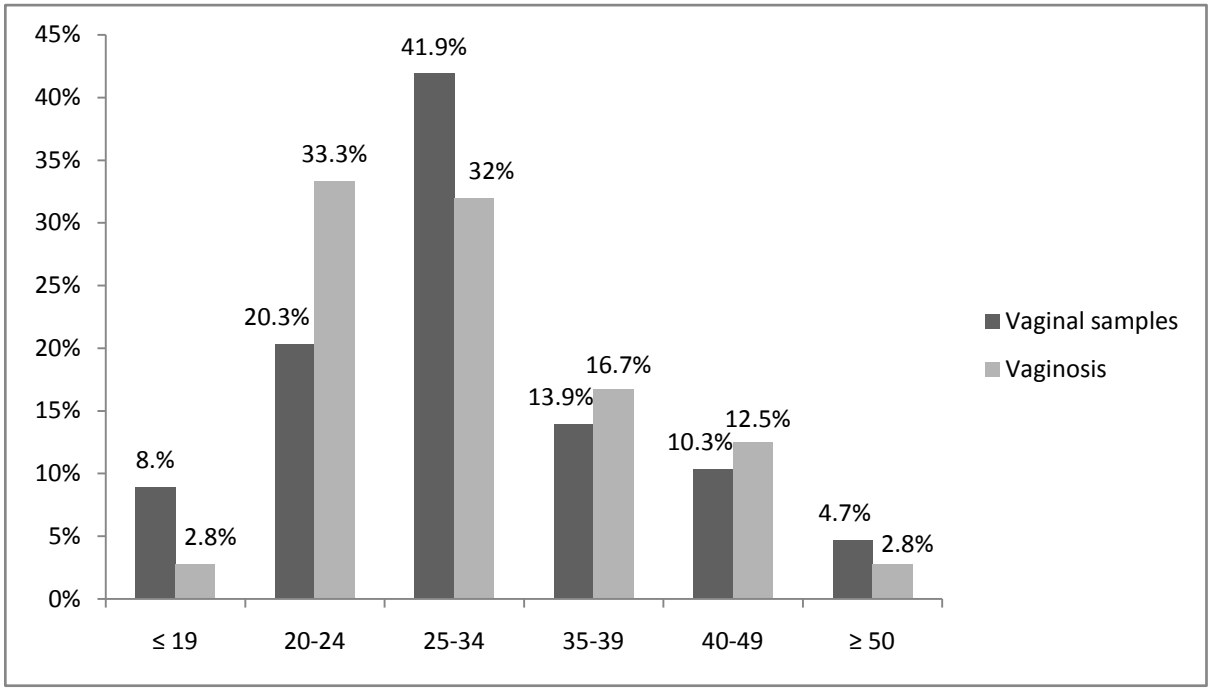

Figure 1. The distribution of vaginal samples and vaginosis based on age ranges

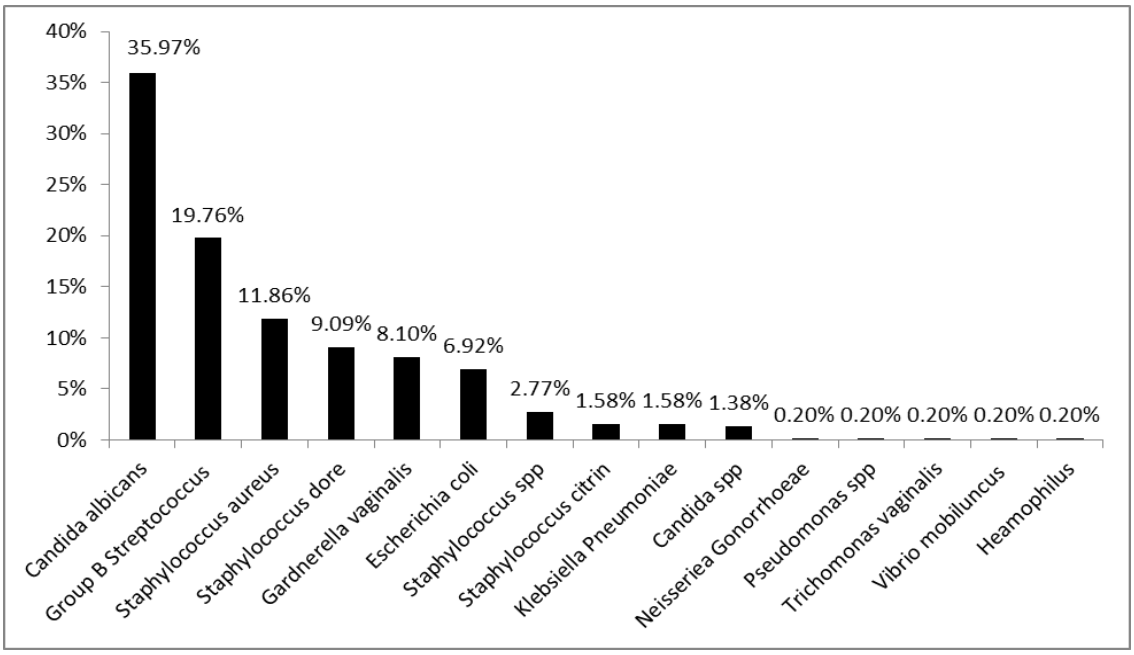

Figure 2. Types and proportion of microorganisms isolated in vaginal from women attending NL at Cotonou, Benin

Table 1. Characteristics of patients included in the study

Characteristic	All patients (N= 640)
Age (years)	
Minimum	2
Maximum	60
Mean	29.8
Cultures	
Positive	502 (78.4%)
Negative	138 (21.6%)

Table 2. Antimicrobial susceptibility pattern of GBS isolated from women attending NL at Cotonou, Benin

	Antibiotics	*group B Streptococcus β hemolytic* (n=100)	
		R (%)	S (%)
Penicillin	Ampicillin (30 ug)	66.7	33.3
	Amoxicillin(25 ug)	32.7	67.3
	Amoxicillin-clavulanic acid (20/10 ug)	20.0	80.0
	Methicillin (5 ug)	55.6	44.4
	Carbénicillin (100 ug)	33.3	66.7
	Oxacillin (5 ug)	6.1	37.9
1st G cephalosporin	Céphalotin (30 ug)	100.0	0.0
2nd G Cephalosporin	Céfoxitin (30 ug)	100.0	0.0
	Cefuroxim (30 ug)	66.7	33.3
3rd G Cephalosporin	Céfotaxim (30 ug)	50.0	50.0
	Ceftriaxon (30 ug)	41.4	58.6
Aminoglycosides	Nétilmicin (30 ug)	80.0	20.0
	Gentamicin (30 ug)	61.5	38.5
Tetracyclin	Tétracyclin (30 UI)	91.7	8.3
	Minocyclin (30 UI)	60.0	40.0
	Erythromycin (15 UI)	50.5	49.5
Macrolides	Lincomycin (15 ug)	52.9	47.1
	Spiramycin (100 ug)	75.0	25.0
Phenicoles	Chloramphenicol(30 ug)	32.1	67.9
	Thiamphenicol	100.0	0.0
	Ciprofloxacin (10 ug)	64.9	35.1
Quinolones	Pefloxacin (5 ug)	0.0	100.0
	Ofloxacin (5 ug)	50.9	49.1
Trmethoprim+sulfonamides	Trimethoprim+Sulfonamides(1.25/23.75ug)	69.7	30.3
Nitrofurans	Nitrofurantoin (300 ug)	0.0	100.0

3.2. Percentage of Sensitivity GBS Isolated to Various Antibiotics

Table 2 summarizes the antibiotic susceptibility of vagina bacterial isolates from women attending of NL.

Among penicillin, amoxicillin, amoxicillin/clavulanic acid and carbenicillin were the most active against GBS with rates 67%, 80% and 67% respectively. The resistance rates of 67%, 66% and 62% were observed towards ampicillin, methicillin and oxacillin respectively.

Regarding cephalosporin, third generation antibiotics (cefotaxim, ceftriaxon) were most effective (50% and 59% respectively) while second generation antibiotics (cefuroxim, cefoxitin) and first generation cephalothin were lost effectiveness with resistance rates of 67%, 100% and 100% respectively.

GBS were resistance of all antibiotics class tested among aminoglycosides, tetracyclin and Macrolides. About aminoglycosides, the resistance rates were 80% and 61% for netilmicin and gentamycin respectively while 92% and 60% resistance rates were observed for tetracyclin and minocyclin respectively in tetracyclin group. As macrolide, resistance rates were observed in 50%, 53% and 75% respectively for erythromycin, lincomycin and spiramycin.

GBS were 100% resistant to thiamphenicol in phenicol group while chloramphenicol was effective at 68%.

In quinolone group, 65% and 51% of GBS were resistant to ciprofloxacin and ofloxacin respectively while pefloxacin was effective at 100%.

Nitrofuran was more effective at 100% against GBS while 70% of GBS were resistant to trimethoprim/sulfamethoxazole.

4. Discussion

To our knowledge, it is the first study conducted in Benin to determine the distribution of vagina bacterial infections and GBS susceptibility in patients attending NL of health Ministry of Benin.

The overall prevalence of vagina bacterial infections was 78.4% in our study. This high rate could be explained by intravaginal pratices that are common in Africa such as cleaning inside the vagina beyond the introitus or insertion of substances into the vagina to dry or tighten the vagina [21]. In addition, Benin is a country neighboring West Africa Nigeria which comes many counterfeit products as gynecological solutions and other pharmaceutical products. Some rates of 49.5% [22], 44% [23] and 27.6% [24] were reported in Vietnam, Thandalam and Iran respectively. Our result was fivefold higher (15.4%) that observed Wondemagegn *et al* in Ethiopia (p = 0.001) [25]. These differences reported rates could also be explained by economic status, methods of identification of microorganisms, geographical racial differences as highlighted by Wondemagegn *et al.*

In this study, *Candida albicans* (n= 182, 37.45%) was the most microorganism isolated followed by GBS (n= 100, 19.92%). CA is responsible of 80-92% of yeast infections [26]. Several studies have shown association between *Candida albicans* and GBS [8,27]. CA is the normal microflora of oral cavity, vagina and gastrointestinal tract [28], but can become pathogenic under certain conditions such as pregnancy [29]. It is the third most prevalent of pediatric health care-associated bloodstream fungal infection [28]. A rate of 46.5% has been reported in Iran [28]. Our result was significantly different that observed Giraldo *et al*. in preterm labor women (p= 0.01) but not significantly different in full term labor women (p= 0.25) in the same study [3].

GBS infections are important health problems that can be treated or preventable. Some variable rates have been reported in Wordwide. Several studies have reported GBS infections rates ranging 16%-30% [30,31,32]. A 19.92% rate reported in our study was significantly high (p=0.007) that reported (13.6%) by Alp *et al*. in Turkey [9]. Our rate was also significantly high that described by Alp *et al*. in pregnant women (9.8%) (p= 0.03) and not pregnant women (16.5%) (p=0.001) [9]. The study conducted in Ethiopia by Mengist *et al*. among pregnant women has shown 10.4% rate of GBS, that is lower (p=0.01) compared in our study [33]. The high sample size may explain the significant difference of GBS rate observed in our study compared to others studies.

Vaginal GBS infections are treated with administration of proper antibiotics. Thus, GBS susceptibility and resistance have been identified in this study. GBS were susceptible to amoxicillin, amoxicillin/clavulanic acid and carbenicillin with 67%, 80% and 67% rates respectively. Susceptible similar rates were also reported in Pakistan [34] with amoxicillin (81.3%) and amoxicillin/clavulanic acid (100%) [35]. GBS resistance rate to ampicillin was 62%, similar that observed in Ethiopia [25]. In contrary, some studies reported susceptibility to this antibiotic at 62.8% in Iran [36], 96 % in Pakistan [34], 100% in Ethiopia [33].

GBS isolates were susceptible to third generation cephalosporin (cefotaxim and ceftriaxon) at 50% and 59% respectively. Nasri *et al*. [36] and Mumtaz *et al*. [34] reported 54.2% and 90% GBS susceptible rates to cefotaxim while resistant to ceftriaxon has been described in Iran [37] and 9.7% were found in Ethiopia [33].

All GBS isolates were resistant to second and first generation cephalosporin (cephalothin), also aminoglycosides, tetracyclin, Macrolides, thiamphenicol, ciprofloxacin and ofloxacin, trimethoprim/sulfamethoxazole tested in our study, implying their impossible use for GBS infections treatment in Benin. These resistances to antibiotics might be attributed to the wide use of antimicrobial drugs. As above highlighted, in Benin, people easily take care at Dantokpa market with antibiotics without physician prescription.

The 50% resistant GBS rate to erythromycin is not surprisingly and has been already reported ranging from 0.7% to 51.3% [38,39]. Also, 6.5% resistance rate has been reported in Ethiopia [33]. These data suggest that routine susceptibility testing is recommended to ensure proper therapy during erythromycin use as second-choice drugs in individuals with penicillin allergy [40]. In addition, this antibiotic cannot longer use intrapartum prophylaxis of early-onset GBS neonatal sepsis as highlighted in study performed in Geneva [41].

We found 100% of GBS resistance to cefoxitin that has also been described in use for broad-spectrum treatment of endometritis or chorioamnionitis [42]. Resistance to tetracyclin

(92%), gentamycin (61%), trimethoprim/sulfamethoxazole (70%) and ciprofloxacin (65%) reported in our study has previously described. Those findings are consistent with findings from another studies that showed 97%, 50%, 82.5% and 9.7% of resistance to tetracyclin, gentamycin, trimethoprim/sulfamethoxazole and ciprofloxacin respectively [33,34,40]. Respectively, resistance rates of 29% and 45.2% to trimethoprim/sulfamethoxazole and tetracyclin have been also reported in Ethiopia [33]. Most of the GBS have been found resistant towards lincomycin (53%), while study performed in Parkistan showed susceptibility rate of 66.7% [34].

Nitrofuran was 100% effective against all GBS isolates in our study. But Ghiasi *et al*. describes resistance to this antibiotic in Iran [37].

This study was not without limitations because bacterial identification by culture method use may be origin introduce bias highlight the limitations of laboratory setups in Benin. All information was obtained in the laboratory records, hence the possibility data mistakes results. In addition, vaginal infections and antibiotic susceptibility may not be representative of the community because the study is based in NL.

5. Conclusion

A high percentage of resistance is the result of uncontrolled access to antibiotics and improper antibiotic policy. Routine susceptibility testing will allow to take appropriate treatment of GBS in Benin.

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