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Gardnerella Vaginalis- Associated Bacterial Vaginosis in Nigerian Women in Parts of Enugu State, Nigeria

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Abstract:
Bacterial vaginosis is the overgrowth of bacteria, Gardnerella vaginalis in the reproductive tract of women often caused by disruption of vaginal pH levels, a drop in immune system response, use of douches, some contraceptive devices and sponges, diaphragms and unattended tampons. Some antibiotics or substances containing the molecule nonoxynol-9 can also kill other healthy bacteria resident in the vagina. Although any woman can develop bacterial vaginosis, it is more commonly associated with women who are sexually active. The purpose of this study was to determine the isolation rates of Gardnerella vaginalis among pregnant and non-pregnant women in Enugu State, Nigeria and to determine the accuracy of clinical diagnosis. A total of 500 high vaginal samples gotten from 250 pregnant and 250 non-pregnant women were examined. Cultures were done on chocolate, schaedler, blood agar, sabourand's dextrose agar and MacConkey agar. G. vaginalis was isolated from 43.2% of pregnant women and 56.8% from non-pregnant women respectively. 50% isolates of Gardnerella vaginalis fermented starch, were inhibited by 3% hydrogen peroxide, showed negative catalase reaction, showed haemolysis on human blood agar, hippurate hydrolysis, resistance to sulphonaide and sensitivity to bacitracin and 50µg metronidazole. Other organisms isolated include Staphylococcus species with highest prevalence at 65 (13%) followed by Escherichia coli at 30(6%), Klebsiella species at 15(3%), Proteus species at 7(1.4%), Pseudomonas species at 4(0.8%), Trichomonas vaginalis at 3(0.6%) and Candida species at 100(20%). Both pregnant and non-pregnant women showed concomitant infection with other microorganisms. Candida infection was higher in pregnant women than in the non-pregnant group. By the use of clinical signs in the diagnosis of bacteria vaginosis, a strong correlation was found between G. vaginalis isolation (99.2%) and bacteria vaginosis ($p < 0.05$). Clinical criteria and scoring of Gram staining of vaginal smear were sensitive in the prediction of bacterial vaginosis, is easy to perform and while this infection may be predominantly asymptomatic there is need for inclusion of screening during antenatal care and routine check in treatment of vaginitis.

Keywords*: Bacteria vaginosis, G. vaginalis, Amsel's criteria, Nugent's score*

1. Introduction

Gardnerella vaginalis, initially known as Haemophilus vaginalis, is affiliated to the family Bifidobacteriaceae and primarily, identified as the sole cause of bacterial vaginosis. It is a fastidious organism and requires complex medium for growth, as well as a 10% CO_2 atmosphere, because they are facultative anaerobic bacteria [1,2]. The essential participants in pathological polymicrobial associations, which could be used as markers for bacterial vaginosis, are Gardnerella vaginosis and Atopobium vaginae [3,4]. In relation to virulence factors G. vaginalis is responsible for the hemolysin and vaginolysin that can be associated for its capability for biofilm formation. G. vaginalis has the capability of forming an adherent biofilm on the vaginal epithelium of women with BV. The aggregation ability of G. vaginalis is considered a virulence factor that enhances the bacterial attachment to epithelial surfaces. The aggregation of bacteria in monolayer prevents the access of antimicrobial agents against them, that are usually dormant, and confers resistance to the host's immune defenses [5,6]. The formation of biofilm confers resistance on the bacteria to lactic acid and hydrogen peroxide which are by products of lactobacilli normally present in the vagina [6,2]. The bacterium produces hemolysin, a 59kd spore-forming cytolysin and aids as a virulence factor. It is very selective on human erythrocytes and after the formation of spore on the target membrane, induces cell lysis through a colloid osmotic mechanism [7]. Gardnerella vaginalis hemolysin could be associated to the alteration of epithelial cells forming so- called clue cells [7]. Some studies also associate the elevation of immunoglobin A levels in the vaginal fluid of many patients with acute BV with the production of perforin-like protein [7]. Vaginolysin is a cholesterol dependent cytolysin which increases the availability of the cellular contents, like a substrate to bacterial growth [2,7]. This cytolisin is a pore-forming protein and utilizes the complement regulatory molecules CD59 to activate on human epithelial cells, the epithelial

p38-mitogen-actived protein kinase, leading to the cell death. The mucosa responds to the process by increasing the level of immunoglobinA [6]. Gardnerella vaginalis is important not just for its role in bacterial vaginosis but also for its involvement in sexual complications affecting organs both in pregnant and non-pregnant women and even in males. It has been encountered in preterm labour, premature rupture of membranes chorioaminionities [8,9] and neonatal meningities [10,11] following hysterectomy and also following prostatectomy [12,13].

2. Materials and Methods

Population: Pregnant women involved in the study were aged between 21-40 years and were attending ante-natal care at Enugu State University of Science and Technology teaching hospital, Enugu during the period from March to February, 2018. Non-pregnant women were female students of Enugu State University of Science and Technology. A total of 500 women comprising of 250 each for the pregnant and non-pregnant women were used.

2.1. Collection of Specimens

Dual swabs were used to collect vaginal discharge from each woman. One swab was used for Gram-stain and wet mount in search of epithelial 'clue cells', Candida and Trichomonas. Amine odour test was performed by adding a drop of 10% KOH on some vaginal discharge placed on a clean slide, the slide was sniffed for fishy odour and recorded positive. pH was conducted on G. vaginalis culture isolates using universal pH indicator with colour code ranging from 1 to 11. The 2nd swab was used for culture.

2.2. Culture and Identification

Vaginal swabs were collected and transported to the laboratory in stuart's transport medium and the 2nd swab was cultured on chocolate agar, Columbia agar base (oxoid) with 10% horse blood heated to 80°C, Schaedler agar (oxoid) with horse blood added (5%), MacConkey agar, blood agar and sabouraud's dextrose agar. Cultures on chocolate and blood agar were incubated in 7% CO_2 in the incubator for 48 h. Cultures on schaedler agar were incubated in an anaerobic condition for 48h while cultures on MacConkey and Sabouraud's dextrose agar were incubated aerobically at 37°C for 48h.
Isolates positive for G. vaginalis were Gram-positive, negative and variable small pleomorphic bacilli, showing β haemolysis on human blood agar. G. vaginalis isolates and mixed bacterial flora were subcultured on starch serum agar and incubated at 37°C, after 3 days the plate was flooded with lugol's iodine solution, positive results showed presence of clear colourless zone. Acid production from carbohydrates was checked using the method of Green-wood and Pickett. Separate culture bottles containing dextrose, galactose, glycerol, lactose, starch, sucrose, dextrose, maltose and arabionose medium were heavily inoculated by stabbing with 48 hours cultures of G. vaginalis. G. vaginalis were grown on schaedler agar with lysed horse blood and incubated aerobically for 5 days. Positive isolates showed starch fermentation. Isolates were picked from the starch serum agar using the end of a capillary tube filled with 3% hydrogen peroxide, positive isolates showed evolution of bubbles indicating inhibition by 3% hydrogen perioxide. The rapid method of Hwang was used to test for hippirate hydrolysis and positive results indicated G. vaginalis isolates. 48 hour broth culture of the organism was inoculated on schaedler agar (with lysed horse blood) with bacitracin 5(µg) and 50 (µg) metronidazole and sulphonamide (100µg) and incubated anaerobically for 24h. G. vaginalis positive isolates showed resistance to sulphonamide and sensitivity to bacitracin and metronidazole. Positive isolates showed acid production indicating presence of a yellow colour. The isolation and identification of other microorganisms together were done on MacConkey and sabourand agar and were incubated aerobically at 37°C for 24h. Haemophilus vaginalis NCTC 10287 was used as a control organism.

2.3. Gram's Staining

Smears were prepared from the vaginal samples and classified into three grades, according to the Nugent et al., 1991and Hay/Ison criteria. In this scale, a score of 0-10 was generated. Grade 1 were comprised of samples with normal vaginal flora having lactobacillus morphotypes predominating (Nugent score 0-3; Ison/Hay score 0-1). Grade 2 comprised of samples with mixed flora with some lactobacilli spp present, but Gardnerella or mobiluncus morphotypes also present. They were classified as intermediate vaginal flora (Nugent score 4-6; Ison/Hay score II). Grade 3 comprised of samples with predominately Gardnerella and or mobiluncus morphotypes present. Lactobacilli spp were few or absent in the samples (Nugent score 7-10; Ison/Hay score III). Grade 3 were classified indicative of Bacteria vaginosis.

3. Results

3.1. Prevalence of G. Vaginalis in Pregnant and Non-Pregnant Women

Five hundred women were included in the study and divided in two groups (Table1). Among the 250 pregnant women examined G. vaginalis isolates were recovered from 108 (43.2%) while in non-pregnant women, 142 (46.8%) were recovered respectively. 250 (50%) isolates were Gram-positive, negative and variable small pleomorphic bacilli and were subsequently identified as G. vaginalis (Table 2 and 3).

Group	Number	No Positive with G. Vaginalis
Pregnant	250	108 (43.2%)
Non-pregnant	250	142 (57%)
Total	500	250 (50%)

Table 1: Prevalence of G. Vaginalis in Pregnant and Non-Pregnant Women

3.2. Identification Patterns of G. Vaginalis Isolation

All G. vaginalis isolates were inhibited by 3% hydrogen peroxide and were catalase-negative with variation in their zone sizes. They showed starch fermentation, hippurate hydrolysis, haemolysis on human blood agar, sensitivity to metronidazole (50µg), bacitracin (5IU) and resistance to sulphonamide (100µg) (Table 2).

	Characteristics	Number Positive with G. Vaginalis	Growth Result
1	Catalase negative	250 (100%)	-ve
2	Inhibition by 3% hydrogen peroxide	250 (100%)	-ve
3	β haemolysis on blood agar	220 (88%)	+ve
4	Hippirrate hydrolysis	250 (100%)	+ve
5	Starch fermentation	250 (104%)	+ve
6	Resistance to sulphonamide (100ug)	220 (88%)	-ve
7	Sensitive to bacitracin (5IU)	231 (92.4%)	+ve
8	Sensitivity to metronidazole (50ug)	242 (97%)	+ve

Table 2: Identification Patterns of G. Vaginalis Isolation

3.3. Reaction of G. Vaginalis Isolates to Carbohydrate Fermentation Tests (N=250)

Fermentation tests were positive for maltose and starch at 100% respectively. There were variations in other fermentation tests and are shown in Table 3.

Carbohydrate	No of Isolates %	Growth Result
Lactose	20 (80%)	+ve
Starch	250 (100%)	+ve
Glycerol	0 (0%)	-ve
Galactose	200 (80%)	+ve
Sucrose	50 (20%)	+ve
Dextrose	232 (92.4%)	+ve
Mannitol	0 (0%)	-ve
Maltose	250 (100%)	+ve

Table 3: Reaction of G. Vaginalis Isolates to Carbohydrate Fermentation Tests (N=250)

3.4. Occurrence of Different Identification Techniques of G. Vaginalis

A comparison of the different methods of identification is shown in Table 4, G. vaginalis were identified at 100% on chocolate and schaedler agar.

Medium	No of Isolates Positive %
MacConkey agar	0 (0%)
Blood agar	220 (88%)
Chocolate agar	250 (100%)
Schaedler agar	250 (100%)
Sabourand dextrose agar	0 (0%)
Nutrient agar	0 (0%)

Table 4: Occurrence of Different Identification Techniques of G. Vaginalis

3.5. Prevalence of Microorganism from Pregnant and Non-Pregnant Women

The results of isolation of other isolates are shown in Table 5. Staphylococcus species were highest in prevalence at 65 (13%) followed by Escherichia coli at 30(6%), Klebsiella species at 15(3%), Proteus species at 7(1.4%), Pseudomonas species at 4(0.8%), Trichomonas vaginalis at 3(0.6%) and Candida species at 100(20%). Both pregnant and non-pregnant women showed concomitant infection with other microorganisms. Candida infection was higher in pregnant women than in the non-pregnant group.

Microorganisms	Number Positive (%)	Number Positive (%) in Pregnant	Number Positive (%) in Non-Pregnant Women
Gram Negative			
Gardnerella vaginalis	250(50%)	108 (43.2%)	142(56.8%)
Escherichia coli	30(6%)	18 (3.6%)	22 (4.4%)
Klebsiella species	15(3%)	5 (1%)	10 (2%)
Proteus species	7(1.4%)	4(0.8%)	3 (0.6%)
Pseudomonas species	4(0.8%)	1(0.2%)	3 (0.6%)
Gram Positive			
Staphylococcus species	65(13%)	25(5%)	40 (8%)
Others:			
Yeast candida species	100(20%)	68(14%)	32 (6.4%)
Trichomonas vaginalis	3(0.6%)	-	3 (0.6%)

Table 5: Prevalence of Microorganism from Pregnant and Non-Pregnant Women

Total number tested 500. G. vaginalis alone 128 (25.6%) and mixed in 130 (26%).

3.6. Prevalence of Genital Symptoms in Pregnant and Non Pregnant Women

From the questionnaires, a total of 200 women comprising of 100 pregnant and non-pregnant women each indicated having symptoms of bacteria vaginosis. (Table 6). Offensive vaginal discharge at 44.4% and 50% and profuse vaginal discharge at 50% each were symptoms that occurred frequently for pregnant and non pregnant women respectively. Significant relationship at $p < 0.05$ (0.0013) was observed between presence of genital symptoms in pregnant and non- pregnant women and G.vaginalis.

Genital Symptoms	No Positive for Symptomatic Women (N=200)	Pregnant Symptomatic Women	Non-Pregnant Symptomatic Women	No Positive with G.Vaginalis
Offensive vaginal discharge	180*	80(44.4%)	100(50%)	180(100%)
Profuse vaginal discharge	200*	100(50%)	100(50%)	200(100%)
Pruritis	200	70(35%)	130(65%)	80(40%)
Dysuria	180	65(36.1%)	115(64%)	95(53%)

Table 6: Isolation of G.Vaginalis in Women with Genital Symptoms

3.7. Occurrence of G. Vaginalis in Pregnant and Non-Pregnant Women Using Amsel's Criteria

The results of Amsel's criteria for diagnosis of bacteria vaginosis are shown in Table 7. Between 100 to 106 pregnant women and 128 to 142 non-pregnant women were positive for various signs under the Amsel's criteria. Significant relationship at $p < 0.05$ (0.0012) was observed between presence of clinical signs in pregnant and non- pregnant women and G.vaginalis. By the use of clinical signs in the diagnosis of bacteria vaginosis, a strong correlation was found between G. vaginalis isolation (99.2%) and bacteria vaginosis ($p < 0.05$).

Amsel's Criteria	Total Positive Population (N=250)	Total Pregnant Population	Total Non-Pregnant Population	No Positive with G.Vaginalis
Homogeneous discharge	228(91.2%)	100(40%)	128(51.2%)	228(91.2%)
Positive amine test	244(91.6%)	104(41.6%)	140(56%)	201(80.4%)
pH >4.5	248(99.2%)	106(42.4%)	142(56.8%)	231(92.4%)
Clue cells	230(92%)	102(41.6%)	128(51.2%)	213(85.2%)

Table 7: Prevalence of Clinical Signs in Women with Bacteria Vaginosis

At least 3 out of 4 clinical signs confirm diagnosis

4. Discussion

Bacterial vaginosis is thought to arise as a result of an imbalance in the vaginal flora, when lactobacilli decrease in concentration and are replaced by anaerobic and facultative aerobic bacteria [13]. The results of this study showed that G. vaginalis isolates were recovered from 108 (43.2%) pregnant women and 142 (56.8%) non-pregnant women (Table I). This is

in agreement with the study of [21] who found G. vaginalis present at 40% in women with B.V. This study also match the results obtained by [17] who found out that (39.6%) of women with an abnormal vaginal discharge most commonly have G. vaginalis. This result is similar to that of [19] and is also in agreement with the study of [18] who found that G. vaginalis were responsible for 40-50% of all cases of vaginosis. G. vaginalis is a fastidious organism requiring complex medium for growth. The study employed a combination of methods in identification of G. vaginalis which included culture, fermentation tests, hippurate hydrolysis and B haemolysis. 250 (50%) isolates were Gram positive, negative, variable small pleomorphic bacilli which showed starch fermentation, inhibition by 3% hydrogen peroxide, negative catalase reaction, B haemolysis on human blood, sugar and hippurate hydrolysis, resistance to sulphonamide and sensitivity to bacitracin, and were identified as G. vaginalis (Tables 2, 3 and 4). This study matched the results obtained by [28; 29; 32; 27; 37]. This study is also in agreement with the study of [30] who found identification of G. vaginalis based on B haemolysis on human blood agar and use of hippurate hydrolysis specific for routine identification [30; 2].

This study employed the use of various selective agar media, and G. vaginalis were isolated satisfactorily at 100% (Table 4). This is in agreement with the studies of [18; 19; 30] who employed the use of selective media in the isolation of G. vaginalis. This study also employed the use of non-selective agar. These media supported the growth of other potential bacterial pathogens (Table 5). Staphylococcus species were highest in prevalence at 65 (13%) followed by Escherichia coli at 30(6%), Klebsiella species at 15(3%), Proteus species at 7(1.4%), Pseudomonas species at 4(0.8%), Trichomonas vaginalis at 3(0.6%) and Candida species at 100(20%). Both pregnant and non-pregnant women showed concomitant infection with other microorganisms.

This is in agreement with the study of [2; 19] who isolated other pathogens using selective and non selective media for G. vaginalis isolation. This study is also in agreement with the work of [18] who found Gram positive organism such as Corynebacterium sp (1.5%), Streptococcus sp 6.15%, Staphylococcus sp (99.9%), Gram negative organisms such as Eschericia coli (2.4%), Klebsiella sp (1.5%) and others such as Candida sp (20.4%) and Trichomonas vaginalis (2.7%) present in pregnant women with BV. This study found low prevalence rates of Candida spp at 14% and 6.4% for pregnant and non pregnant women respectively while isolation rates of Trichomonas vaginalis were 0.6% in non pregnant women. Angel Muller (2010)[19] found women with abnormal vaginal discharge positive with BV at 39.6% or less frequently, vulvovaginal candidiasis (11%) or trichomoniasis 0.8%. This study also used pre-experimental activity by obtaining information from the respondents in order to determine signs and symptoms often accompanying BV (Table 6). Significant relationship at $p < 0.05$ (0.0013) was observed between presence of genital symptoms in pregnant and non- pregnant women and G.vaginalis. Offensive vaginal discharge were rated at 44.4%, 50% for pregnant and non pregnant women respectively. Studies by [27; 22] indicate that the burden of BV is related not only to the prevalence of the condition, but also to a reduction in quality of life, as well as to anxiety and self conscious feelings relating to the fear of exclusion due to the foul smell associated with the infection. The results of this study also noted the presence of profuse vaginal discharge with prevalence of 50% for both groups. This is in line with the study of [22] who noted an increase in the amount of vaginal discharge with the presence of a genital tract infection. The Amsel's criteria and Gram staining are used to diagnose BV and Gram staining is considered the gold standard for this purpose [25]. The Nugent scoring system is applied to Gram stains of vaginal smears to visually estimate the numbers of lactobacilli and BV- associated organism, a Nugent score of 0 to 3 is considered healthy, 4 to 6 is intermediate and a score of 7 to 10 implies the presence of BV [19]. The result shows that the Amsel's criteria provide a good clinical tool for the diagnosis of BV. $pH > 4.5$ was observed at 42.4% and 56.8% in pregnant and non-pregnant women respectively. A pH greater than 4.5 is one of the universally accepted criteria for the diagnosis of BV [31]. This is in line with the work of Okwoli et al, 2002 who observed 71% of samples had pH between 6 and 7. 92% of the total positive population comprising of 41.6% and 51.2% pregnant and non-pregnant women respectively were positive for amine test. Clue cells were constantly found in the discharge of pregnant and non pregnant women. There was significant relationship between G. vaginalis and bacterial vaginosis diagnosed by clinical criteria at $p < 0.05$ (0.0012) (Table7). This is in agreement with [18; 24] who found strong association between G. vaginalis isolation and bacterial vaginosis. The comparative analysis of microscopic evaluation, culture and Amsel's criteria demonstrated greater concurrence between Gram staining and Amsel's criteria in detection of BV than culture (Table 8). These results are in agreement with the results of [18] who found Amsel's criteria sensitive and specific at 100% and 97.9% respectively for prediction of bacterial vaginosis. Gergova et al., 2013[19] also reported Gram staining a reliable test in detection of BV. BV is implicated as risk factors in preterm labour; ascending infection of the female genital tract; endometritis following caesarean section; vaginal cuff and neonatal sepsis [25; 30; 31; 22].

5. Conclusion

In relation to BV, clinical criteria and Gram staining is still the gold standard needed for prediction of BV. It is simple, less expensive, sensitive, easy to interpret and ideal for inclusion during routine ante-natal checks and treatment of vaginitis for a successful pregnancy outcome especially in under developed countries.

6. References

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