Antibiotic Resistance-Renewed Fear in Gardnerella Vaginalis and Its Role In Bacterial Vaginosis

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Abstract:
Background: Bacterial vaginosis is associated with adverse gynecological and pregnancy outcomes. Diagnosis is totally depending on molecular/culture methods with clinical co-relation. In appropriate interpretation might lead to complication associated with pregnancy.

Methods: In our study we have included culture and microscopy in association with clinical history. Culture and microscopy was performed as per standard tests. Nugent’s scoring system was used for microscopy along with culture on blood agar and mac-conkey agar. Antibiotic susceptibility was performed by Kirby- Bauer disc diffusion method.

Results: All the isolates were found to be sensitive to imipenem and meropenem. But efficacy of ampicillin has been reduced up to a very low level (9.37%). We also noted the increase drug resistance towards urinary antibiotics, which might be a topic of concern in treatment and outcome.

Conclusion: This study emphasizes the need of routine observation of drug resistance in bacteria so outcome of treatment will be better for clinicians and patients.

Keywords: Gardnerella vaginalis, Bacterial vaginosis, Nugent’s scoring system

I. Introduction

Bacterial vaginosis is the most common infection in females worldwide leading to vaginal disorders. A large population of African American women (30-50%) is infected particularly of reproductive age 20-35 years Patterson et al., 2010 [1]. It may lead to severe conditions such as preterm delivery, preterm labor, post abortion endometritis, post-partum endometritis and low birth weight (Srinivasan and Fredricks, 2008; Swidsinskiet al., 2008; Menard et al., 2012) [2,3]. It may be a polymicrobial syndrome but recent studies have shown Gardnerella vaginalis(G. vaginalis) also can be a primary pathogen in half of the cases of bacterial vaginosis. However, correlation of G. vaginalis becomes unclear due to lack of animal models Turovskiy et al., 2011 [4]. So it becomes important to look for the pathogen and study their presentation or pathogenesis. BV can be controlled by administration of antibiotics but cross-resistance between species or emerging resistance in primary pathogen becomes more important to look for. Therefore, this study was designed to access the G. vaginalis.

II. Methods And Materials

2.1 Study population, Exclusion and inclusion criteria for specimens-
A total of 160 G. vaginalis was isolated from September 2014 to January 2016 in District public health laboratory Swahidmukundakakati civil hospital, Nalbari, Assam. Patients presented with other complaints than vaginal discharge, no clinical sign and symptoms, use of contraception, with history of usage any antibiotics for the last two weeks was excluded from the study. Inclusion criteria for study was clinical presentation of lower abdominal pain, itching, history of vaginal discharge, fishy odor and age group from 10-60 years.

2.2 Aims of study-
A total of 160 G. vaginalis was isolated from September 2014 to January 2016. Patients presented with other complaints than vaginal discharge, no clinical sign, and symptoms, usage of contraception, with the history of usage any antibiotics for last two weeks was excluded from the study. Inclusion criteria for study were clinical presentation of lower abdominal pain, itching, history of vaginal discharge, fishy odor and age group from 10-60 years.

2.3 Identification and sensitivity of isolates-
Two high vaginal swabs were collated from each patient. One swab was proceeded for microscopy
gram stain following Nugent’s scoring system. (According to Nugent’s grading score of $\geq 7$ on a Gram stained vaginal smear indicates the presence of BV (Cauci et al., 1996; Srinivasan and Fredricks, 2008; Swidsinski et al., 2008) and another swab was cultured on Columbia blood agar and Mac-conkey agar.) pH of vaginal discharge was measured by indication paper (range from 1-14). In gram stain, Clue cells were observed along with other bacteria and Trichomonas vaginalis.

The patient presented with clinical symptoms (Amsel criteria) and a Nugent’s grading score of 7-10, and culture positive for *G. vaginalis* was labeled as *G. vaginalis* infection. Identification of *G. vaginalis* was confirmed, as per standard protocols culture, microscopy, and biochemical characterization.

For the culture of *G. vaginalis* Columbia blood agar for β-hemolysis and Mac-conkey agar for non-lactose forming colonies were used.

Antibiotic susceptibility was performed by Kirby-Bauer disc diffusion method. Antibiogram was observed for following antibiotics, ampicillin (AMP), cefuroxime (CF), gentamycin (GM), amikacin (AK), nitrofurantoin (NZ), nalidixic acid (NA), imipenem (IMP), and meropenem (MRP).

Data analysis was done by Microsoft excel program.

### III. Result

One hundred sixty samples were studied from female patients, presented with clinical history. The Mean age of this population was 30.88 while the range was 10-60. The isolates were cauterized against the age with their frequency in Table 1.

**Table 1** *G. vaginalis* distribution with respect to age.

Correlation between pH, microscopy and culture are presented in Table 2 with respect to their frequencies.

**Table 2.** Correlation between Spiegel’s criteria with culture positivity of *Gardnerella*

<table>
<thead>
<tr>
<th>pH</th>
<th>Gram stain</th>
<th>Culture</th>
<th>Amines test</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;5</td>
<td>GNB + other commensal flora</td>
<td>+</td>
<td>+</td>
<td>102</td>
<td>63.75%</td>
</tr>
<tr>
<td>&lt;5</td>
<td>GNB + other commensal flora</td>
<td>+</td>
<td>-</td>
<td>58</td>
<td>36.25%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of organism</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2</td>
<td>1.25%</td>
</tr>
<tr>
<td>11-20</td>
<td>25</td>
<td>15.62%</td>
</tr>
<tr>
<td>21-30</td>
<td>58</td>
<td>36.25%</td>
</tr>
<tr>
<td>31-40</td>
<td>49</td>
<td>30.62%</td>
</tr>
<tr>
<td>41-50</td>
<td>13</td>
<td>8.12%</td>
</tr>
<tr>
<td>50-60</td>
<td>13</td>
<td>8.12%</td>
</tr>
<tr>
<td>Total Number of isolates</td>
<td>160</td>
<td>99.98%</td>
</tr>
</tbody>
</table>

**Fig. 1** consists the information about the recovered bacteria along with *G. vaginalis*. While fig. 2 shows sensitivity pattern of *G. vaginalis* isolates in our study.
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Figure 1. Various organisms isolated along with Gardnerella.

Figure 2. Drug sensitivity pattern of Gardnerella against tested antibiotic.

IV. Discussion

Bacterial vaginosis is the most common disease after urinary tract infection in female patients in which a shift occurs from a predominance of lactobacilli-dominated flora to a vaginal anaerobic environment, constituted by Gardnerella, Micrococcii, Streptococcii and Staphylococcii. Sign and symptoms for this are itching and discharge. A wide range of organisms can cause bacterial vaginosis. This study was primarily designed to study for antiibiogram, distribution of G. vaginalis according to age and compression of culture positive rate to microscopy.

In this study most common age group infected with G. vaginalis was 21-30 years standing for 36.25% cases followed by 31-40 years 30.62% [7]. The reason behind this high positivity rates is reproductive age. Age group below 20 and above 40 had fewer isolates in compression to reported by Dhall K et al 1990 [8]. Correlation of Spiegel’s criteria in our study was full filled and was in concordance with the study conducted by Dhall K et all 1990 and Schaaf VM et al 1990 [8-9]. Clue cells were present in 29% of the gram stain smears which correlates study[10].

Nugent’s score was 7-10 in 30.50% of the total cases which were higher than previously reports [11-13]. The reason behind this maybe person variability in scoring the smear. The commonest organism associated with G. vaginalis in our study was E. coli 30.19% similar data was presented by Dutta S et al., in Dhaka [14], followed by spore-forming bacteria or diphteroids 22%. The finding co-relates study conducted by Silamala Umadevi [15].

Candida is tolerant to acidic pH and hence it’s found in vagina. Our study showed higher prevalence rate of Candida and Coagulase negative staphylococcus 13%, and 12% respectively which was higher in compression to Nagalakshmi Narayana-Swamy [16].

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Mixed culture growth may be the result of contamination of the specimen while collecting. Antibiotic sensitivity pattern showed all isolates of G. vaginalis was sensitive to imipenem and meropenem. Amikacin, gentamicin was also effective in 91.25% and 87.76% followed by nitrofurantoin, cefotaxime, nalidixic acid 73.12%, 61.87% and 55.62% respectively. High drug resistance was observed in the case of ampicillin, only 9.37% isolates were susceptible to ampicillin. These results of our study co-relate to study conducted by Nagalakshmi Narayana-Swamy, expect in the case of fluoroquinolones (nalidixic acid) where we found more drug resistance in our isolates. We found more drug resistance to nalidixic acid in comparison to study of Nagalakshmi Narayana-Swamy [16]. The reason behind it maybe the use of this drug in routine treatment for urinary tract infection cases which might have led bacterias to acquire the drug resistance. So this becomes more important to look at the treatment options for bacterial vaginosis as there is the rise in drug resistance rate in urinary antibiotics which still stand a good and safe choice for treatment. It would be wise to use antimicrobials according to requirement and with the correlation of microbiological report.

V. Conclusion

Bacterial vaginosis is still considered a polymicrobial infection and is a very common infection of reproductive age. Due to lack of animal models, real etiology still remains doubtful. However, some studies have demonstrated G. vaginalis as a pathogen. So it will be the best option to utilize the microbiological report along with clinical symptoms and diagnosis to reach up to any conclusion for treatment and better outcome.

VI. Limitations of study

Further studies can be done to show the correlation between G. vaginalis and other commensal flora.

Reference