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Original Research Article

Aerobic Gram Negative Bacterial Profile in High Vaginal Swab

T. Geetanjali[1], C. N. Soumya[2], A. Surekha[3], B. Naga Srilatha[4]

[1]Associate Professor, Department of Microbiology, Government Medical College, Kadapa, Andhra Pradesh, India.
[2]Assistant Professor, Department of Microbiology, Government Medical College, Kadapa, Andhra Pradesh, India.
[3]Professor, Department of Microbiology, Government Medical College, Kadapa, Andhra Pradesh, India.
[4]Professor, Department of Microbiology, Government Medical College, Kadapa, Andhra Pradesh, India.

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Corresponding Author: Dr. T. Geetanjali
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Abstract:

Introduction: Bacterial vaginosis is a polymicrobial condition involving facultative and anaerobic organisms in addition to *Gardenerallvaginalis*. Patients present with a clinical condition when there is an overgrown of the bacteria or by acquiring an exogenous flora. Gram negative organisms also cause bacterial vaginosis and their susceptibility pattern is required to manage the clinical condition. The aim of the present study is to know the gram negative bacterial infections predominance in bacterial vaginosis and its antibiotic susceptibility pattern.

Materials and Methods: A Prospective cross sectional study was undertaken in the Department of Microbiology, ACSR Medical College, Nellore during the study period from February 2022 to March 2023. A total of 200 patients between 15-55 years presenting with complaints of vaginal discharge at the outpatient department of OBG were included in the present study. Culture swabs were collected and processed according to the standard guidelines.

Results: Among 78 pathogens, (22 out of 78) 28.2% *Escherichia coli*, (18 out of 78) 23.07% *Klebseillaspp*, (15 out of 78) 19.2% *S. aureus*, (14 out of 78) 17.9% *Candida spp*, (4 out of 78) 5.1% *Enterococcus spp* (3 out of 78) 3.8% *CoNS*, and (2 out of 78) 2.5% *Proteus spp*. Gram negative bacilli are highly susceptible to colistin (100%), tigecycline (100%), amikacin (95.2%), meropenem (90.4%), ertapenem (85.7%), followed by levofloxacin (66.6%), Piperacillin-tazobactam (59.5%), Ceftazidime-clavulanic acid (54.7%), cefipime (54.7%), ceftazidime(47.6%), ceftriaxone (42.8%) and they were least sensitive to amoxyclav (42.8%) and amoxicillin (16.6%).

Conclusion: The resistance bugs are in rise in many communities and the relapses are common in bacterial vaginosis, so it is better to track the pathogen by utilizing the laboratory services and start antibiotic after choosing suitable drug.

Keywords: Bacterial Vaginosis, Gram Negative Bacteria, Infections.

Introduction

The vagina has a diverse range of microorganisms forming a complex ecosystem [1]. It is the most frequent reason why women seek medical attention for vaginal problems. The lower portion of the vagina contains hundreds of different gram-positive and gram-negative organisms. Bacterial vaginosis is a polymicrobial condition involving facultative and anaerobic organisms in addition to *Gardenerellavaginalis* [2]. Patients present with a clinical condition when there is an overgrown of the bacteria or by acquiring an exogenous flora.

One of the main causes of bacterial vaginosis is overgrowth of organisms and another unavoidable cause is presence of the small urethra and its functional and anatomical closeness to the anal canal. In women the most common presentation of bacterial vaginosis is vaginal candidiasis. Other three common bacteria responsible for bacterial vaginosis are *group B streptococci, E. coli, enterococci,* and *trichomoniasis.* Although the precise cause of bacterial vaginosis (BV) is unknown, it is thought to be linked to a decrease in lactobacilli and hydrogen peroxide generation, an increase in vaginal pH, and an overgrowth of organisms associated with BV.

The pathophysiology of BV appears to be influenced by the synergistic action of several anaerobic organisms, such as *G. vaginalis, Prevotella spp., Porphyromonas spp., Bacteroides spp., Peptostreptococcus spp., Mobiluncus spp., and Mycoplasma spp* [3]. It affects millions of women annually and is significantly connected with various adverse health outcomes including premature labour and delivery, pelvic inflammatory

disease, postpartum and post abortal endometriosis [4,5]. There is also a higher risk of acquiring HIV and moreover it can relapse often which leads to mental health problems [6].

Diagnosis of bacterial vaginosis is by assessing clinical examination in most of the clinical scenarios. Microbiological diagnosis like wet mount, gram stain and culture help for accurate diagnosis and the susceptibility pattern of yeast and bacteria. Gram negative organisms also cause bacterial vaginosis and their susceptibility pattern is required to manage the clinical condition. This can help clinicians to treat the patient accurately. The aim of the present study is to know the gram negative bacterial infections predominance in bacterial vaginosis and its antibiotic susceptibility pattern.

Materials and Methods

A Prospective cross sectional study was undertaken in the Department of Microbiology, ACSR Medical College, Nellore during the study period from February 2022 to March 2023. Informed consent from patients was taken before doing the study. A pre structured questionnaire was planned and used to tabulate the results findings and analyze. A total of 200 patients presenting with complaints of vaginal discharge at the outpatient department of OBG were included in the present study.

Inclusion criteria: Women between 15 to 55 years presenting with vaginal discharge.

Procedure

Samples were collected in the OBG department and were sent to the Microbiology lab and the samples were processed without delay. If there is any delay then the samples are stored at 4-6˚c.Three high vaginal swabs were collected for Wet mount, Gram staining and Culture purposes accordingly.

Wet mount preparation: Examined for presence of any pus cells, clue cells, organisms and its motility.

Gram staining – To differentiate gram positive and gram negative bacteria, presence of yeast cells, hyphae or pseudohyphae, presence of pus cells, squamous cells and clue cells. Nugent's score was noted.

Culture: the swabs were streaked on nutrient agar, blood agar, mac conkey agar, chocolate agar and incubated at 370c for 24 hrs if no growth is seen in 1 day the plates are incubated further for 48 hrs.

Biochemical reactions: identification of the isolates were further proceeded by biochemical properties like catalase, oxidase, coagulase, bile esculin hydrolysis, indole, citrate utilization, urease hydrolysis and triple sugar iron agar tests.

Antibiotic susceptibility test: Antibiotic sensitivity test was performed by Kirby Bauer disk diffusion method by adjusting the turbidity of the suspension according to McFarland's standard scale. Gram negative isolates antibiotics were: amoxyclav (30 µg), piperacillin+tazobactum (100/10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), Ceftazidime+clavulanic acid (30/10 µg), piperacillin+tazobactum (30/6 µg), levofloxacin (5 µg), meropenem (10 µg), amikacin (30 µg), tigecycline (15 µg) and colistin (50 µg). Standard Quality Control strains were used as a part of testing. Multi Drug testing was done for all strains isolated according to CLSI guidelines.

Statistical Analysis: Data was collected and entered into a spread excel sheet. All descriptive variables were calculated as numbers or percentages.

Results

Out of200 samples, the majority of the patients were in reproductive age group years, it was 91%.

Table 1. Age distribution of bacterial vaginosis patients

Age group in years	No. of patients	Percentage
15-35	182	91%
36-55	18	9%
Total	200	100%

Culture positivity was seen in 78 samples (39%) and the remaining 122 samples were culture negative (61%).

Among various bacterial isolates from vaginosis patients, Escherichia coli and Klebsiella spp were predominant isolates. Out of 78 isolates, 28.2% (22 out of 78) were gram positive, 53.8% (42 out of 78) were gram negative and remaining 17.9% (14 out of 78) were Candida species.

Among 78 pathogens, (22 out of 78) 28.2% Escherichia coli, (18 out of 78) 23.07% Klebseilla spp, (15 out of 78) 19.2% S. aureus, (14 out of 78) 17.9% Candida spp, (4 out of 78) 5.1% Enterococcus spp, (3 out of 78) 3.8% CoNS, and (2 out of 78) 2.5% Proteus spp.

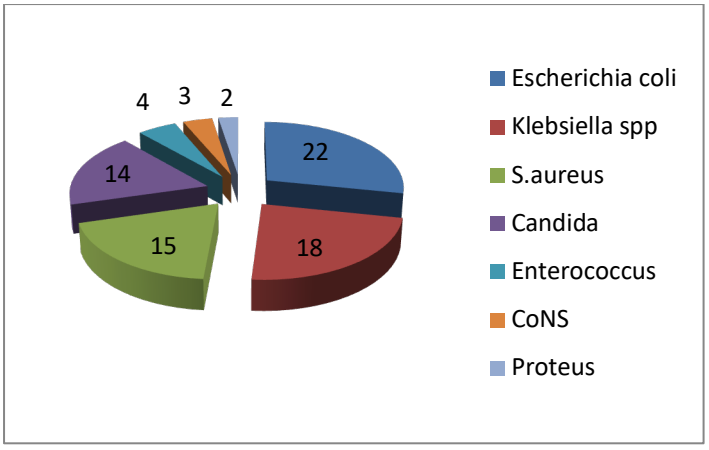

Figure 1. Distribution of pathogens among bacterial vaginosis

Gram negative bacilli are highly susceptible to colistin (100%), tigecycline (100%), amikacin (95.2%), meropenem (90.4%), ertapenem (85.7%), followed by levofloxacin (66.6%). Piperacillin-tazobactam (59.5%), Ceftazidime-clavulanic acid (54.7%), cefipime (54.7%), ceftazidime(47.6%), ceftriaxone (42.8%) and they were least sensitive to amoxyclav (42.8%) and amoxicillin (16.6%).

Table 2. Antibiotic susceptibility pattern of gram negative pathogens

Antibiotics	Sensitive	%	Intermediate	%	Resistant	%
Amoxicillin	7	16.6	0	0	35	83.3
Amoxyclav	18	42.8	2	4.7	22	52.3
Ceftazidime	20	47.6	3	7.14	19	45.2
Ceftriaxone	18	42.8	2	4.7	22	52.3
Cefipime	23	54.7	0	0	19	45.2
Ceftazidime-clavulanic acid	23	54.7	3	7.14	16	38.09
Piperacillin-tazobactum	25	59.5	3	7.14	14	33.3
Levofloxacin	28	66.6	6	14.2	8	19.04
Amikacin	40	95.2	0	0	2	4.7
Meropenem	38	90.4	2	4.7	2	4.7
Ertapenem	36	85.7	2	4.7	4	9.52
Tigecycline	42	100	0	0	0	0
Colistin	42	100	0	0	0	0

Discussion

Vaginitis is an inflammation of the vagina, usually characterized by any of the following: vaginal discharge containing many white blood cells (WBCs), vulvar itching, vulvar irritation, vaginal odor, vaginal erythema, dyspareunia, and dysuria [7]. The three most common causes of vulvovaginitis are bacterial vaginosis (BV), being the most prevalent one, followed by Candidiasis and Trichomoniasis [8].

Bacterial vaginosis is one of the most common lower genital tract conditions. The most severe form of aerobic vaginitis equals desquamative inflammatory vaginitis. A higher incidence of premature rupture of membranes (PROM) was significantly associated with AV. More attention should be given to vaginal microbiota evaluations during pregnancy. The diagnosis of vaginosis is a little difficult for laboratory physicians as it is of polymicrobial nature. A combined approach of diagnosis including provision of clinical details, examination of direct high vaginal smear and high vaginal swab culture aids in the accurate diagnosis of the pathogen.

In 1980s to 1990s research works stated there was a strong association between diagnosis of bacterial vaginosis and the concomitant occurrence of *Gardnerella vaginalis, Mobiluncus spp. and Bacteroides spp* [9]. *Bacteroides, G. vaginalis and Ureaplasma urealyticum* were found to be the most commonly occurring group in women with BV [10].

The prevalence rate of Bacterial vaginosis is 24% in India [11] and 33% in Egypt [12]. In this study out of200 samples, the majority of the patients were in reproductive age group years, it was 91%. Bacterial vaginosis presentation frequently noted in reproductive age group women [13, 14].

Out of 78 isolates, 28.2% (22 out of 78) were gram positive, 53.8% (42 out of 78) were gram negative and remaining 17.9% (14 out of 78) were *Candida species* as per this study. Ranjit E et al [15] documented that 32% were gram negative and

45.3% were gram positive. Other isolates including yeasts and parasites were 22.7%.

Among 78 pathogens of bacterial vaginosis, (22 out of 78) 28.2% isolates were Escherichia coli, (18 out of 78) 23.07% *Klebsiellaspp*, (15 out of 78) 19.2% *S. aureus*, (14 out of 78) 17.9% *Candida*spp, (4 out of 78) 5.1% *Enterococcus* (3 out of 78) 3.8% *CoNS*, and (2 out of 78) 2.5% *Proteus spp* in the present study. *E.coli* is the most common pathogen isolated in both the age groups. Comparable results were seen in a study done by Jahic et al [16], Li N et al [17].This could be due to the poor personal hygiene since *E.coli* is a commensal in the gastrointestinal tract. The closer proximity of the female genital tract and the anal opening makes it easy for these organisms to spread to the genitourinary tract with sexual intercourse, facilitating this transmission. But few studies have reported *Staphylococcus, Enterococcus* and *Klebsiella* as the most common organisms isolated as in Bitew A et al [18] and Mulu W et al [19].

In this study, gram negative bacilli are highly susceptible to colistin (100%), tigecycline (100%), amikacin (95.2%), meropenem (90.4%), ertapenem (85.7%), followed by levofloxacin (66.6%). Piperacillin-tazobactam (59.5%), Ceftazidime-clavulanic acid (54.7%), cefipime (54.7%), ceftazidime (47.6%), ceftriaxone (42.8%) and they were least sensitive to amoxyclav (42.8%) and amoxicillin (16.6%). In Gram negative isolates, common pathogen was *Pseudomonas spp*., accounting for 7.8% BV cases, followed by many other Gram negative bacteria, namely, *E. coli, Acinetobacter spp., Proteus spp., Klebsiella spp., N. gonorrhoeae, C. koseri, Enterobacter spp. and Lactobacillus spp* were observed as the highest prevalent pathogen with the percentage of 27.3% in Ranjit E et al study [15]. Razzak MS [20] observed the most common opportunistic bacterial isolates of vaginitis were *Staphylococcus aureus, Escherichia coli, Streptococcus agalactiae, and Klebsiella pneumoniae.*

The result showed that no *Citrobacter spp.* was resistant to nitrofurantoin and also no other gram-negative bacteria were resistant to amikacin. *Citrobacter spp.* recorded the highest resistance rate (>43%) with cephalexin, ceftazidime, nalidixic acid, ampicillin, gentamicin, and tetracycline. For *E. coli*, the highest resistance rates were recorded (>50%) with ampicillin, trimethoprim-sulfamethoxazole, and tetracycline. Furthermore, the lowest resistance rates (<9%) were recorded with amikacin, ceftazidime, chloramphenicol, and nitrofurantoin. In *Klebsiella spp*., the highest resistance rates (>47%) were recorded with ampicillin, nitrofurantoin, and tetracycline and the lowest resistance rates (<11%) for this strain were recorded with amikacin and ciprofloxacin. For *Proteus spp*., the resistance rates were more than 50% for almost all antibiotics tested. In the other gram-negative bacteria, high resistance rates (>57%) were recorded with cephalexin and ampicillin and the lowest resistance rates (<14%) were recorded with ceftriaxone, nalidixic acid, ciprofloxacin, and gentamicin. Of all isolated gram-negative bacteria, the highest resistance rates (>46%) were recorded with ampicillin and tetracycline. However, the lowest resistance rates (15%) with these bacteria were recorded with amikacin, ceftazidime, and ceftriaxone [21]. In similar to the present study *E. coli* was highly resistant to ampicillin, cefazolin, and trimethoprim-sulfamethoxazole [22], and *E. coli* were resistant to trimethoprim/sulfamethoxazole and ceftriaxone, but susceptible to ciprofloxacin, gentamicin, and nitrofurantoin [23]. Additionally, E. coli showed resistance to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole [24].

Acinetobacter showed 85.7% sensitivity to meropenem, cefixime and cefpodoxime; Citrobacter and other gram-ne gative bacteria showed sensitivity to imipenem (87.0%) and meropenem (82.6%). *Klebsiella pneumoniae* showed sensitivity to ceftazidime and cefpodoxime. Extended-spectrum beta-lactamase-producing *Escherichia coli* showed sensitivity to imipenem and cefixime, whereas *Gardnerellavaginalis* showed sensitivity to meropenem (100%) and imipenem (100%). *MRSA* strains were sensitive to ceftazidime (60.9%) and cefpodoxime (60.9%) [25].

Conclusion

Bacterial vaginosis aerobic organisms predominantly were gram negative followed by gram positive organisms and Candida in our community. Gram negative organism's susceptibility patterns vary from study to study, most of the pathogens are sensitive to penems and also beta lactam and beta lactam inhibitor combinations. It is strongly recommended to investigate for culture and sensitivity to choose proper antibiotic and treat effectively.

The resistance bugs are in rise in many communities and the relapses are common in bacterial vaginosis, so it is better to track the pathogen by utilizing the laboratory services and start antibiotic after choosing suitable drug.

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