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Organisms isolated in aerobic vaginitis and it's antibiotic sensitivity pattern in patients presenting with vaginal discharge in a tertiary care hospital

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Abstract: ***Introduction:*** Bacterial vaginosis is the most common outpatient clinical condition in gynecology department; globally Diagnosis of bacterial vaginosis is by amsel's criteria during clinical examination, nugents criteria of gram stain and culture analysis can help to detect the exact pathogen and to start the accurate treatment. The aim of this study is to know the aerobic bacteriology of bacterial vaginosis and their antibiotic susceptibility pattern.***Materials and Methods:*** A total of 363 patients participated in this descriptive cross sectional study was between the age group of 15 to 55 years. Their samples were collected and processed as per guidelines by performing gram stain and culture & sensitivity testing method. Patient details such as age, socioeconomic status, relapse of bacterial vaginosis, number of partners, type of discharge, associated symptoms and signs, and microbiological details were tabulated in an excel sheet to evaluate further. ***Results:*** Gram positive cocci are highly susceptible to gentamicin(100%), high level gentam icin(100%), vancomycin(82.8%) followed by linezolid(71.4%), clindamycin(60%), amoxiclav(48.6%) and least sensitive to azithromycin(28.6%). Gram negative bacilli are highly susceptible to amikacin(88.9%), meropenem(85.7%), cefipime(69.2%), followed by piperacillin tazobactam(60%), cotrimoxazole (53.5%), ciprofloxacin (50%), ofloxacin (44.4%), cefotaxime (40.7%), doxycycline (33.3%) and they were least sensitive to ceftriaxone (27.8%). Between the age group of 15 – 28 years, E.coli and Staphylococcus aureus were the most common organism isolated, followed by klebsiella pneumonia, Enterococci, CONS, Candida, Acinetobacter and Klebsiella oxytoca. Between the age group of 29 – 55 years, E.coli was the most common organism isolated, followed by Klebsiella pneumoniae , Enterococci and Staphylococci aureus. ***Conclusion:*** This study emphasizes the need to understand the aerobic pathogens associated with vaginitis especially in the reproductive age group to prevent adverse complications seen during pregnancy and labour.

Keywords:
Bacterial vaginosis, aerobic bacteria

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INTRODUCTION

Bacterial vaginosis is the most common outpatient clinical condition in gynecology department; globally it is affecting millions of women mostly in the reproductive age group [1]. Many research works have been underway since many decades to find out the pathophysiology behind the etiology and relapse of condition but still it is difficult to track down.

Bacterial vaginosis is the most common cause of vaginitis, presenting with vaginal discharge. The evaluated root cause behind the vaginosis is reduction in the number of healthy bacteria Lactobacillus and a simultaneous overgrowth of

pathogenic bacteria, such as *Gardnerella vaginalis, Atopobium vaginae, Megasphaera spp., Prevotella spp. and Sneathia spp [2].*

Bacterial vaginosis mode of occurrence is due to the imbalance of microbial flora in vagina. Risk factors of bacterial vaginosis are vaginal infections, multiple sexual partners, recent antibiotic use, smoking, and contraceptive use [3]. The clinical presentation can be vaginal discharge, burning micturition, dyspareunia, perineal itching, redness, edema etc. Most of the women are facing problems with relapse of bacterial vaginosis, the reason behind is elusive. Preventive strategies target the risk factors or behaviors for a disease including age, marital status, employment status, occupation, recent antibiotic use, decreased estrogen production of the host, douching, sexual activity, lower age of first intercourse, more frequent episodes of receptive oral sex, spermicide use, STDs, working as a sex worker, smoking, alcohol intake, stress, contraceptives used, frequency of vaginal intercourse, and race/ethnicity [4].

Diagnosis of bacterial vaginosis is by amsel's criteria during clinical examination, nugents criteria of gram stain and culture analysis can help to detect the exact pathogen and to start the accurate treatment.

But in recent years, there is an increase in the aerobic organisms which cause vaginal infections, so in this study we are concerned about the aerobic vaginitis and their antibiotic susceptibility pattern. Since there is very minimal study on this topic it is necessary to understand the pattern of aerobic vaginitis in order to provide appropriate treatment, and to avoid inappropriate drug usage.

MATERIALS AND METHODS

Study Design & Settings:

The present study is a descriptive cross sectional study conducted in the department of Microbiology, Government Medical College, Anantapur. Most of the patients hail from low socioeconomic status or from rural background to this hospital. Women patients attending the OBG OPD complaining of vaginal discharge were considered for this study, those who gave consent to this study were included. A total of 363 patients were willing to give the specimens to study about the bacteriological profile and between the age group of 15 to 55 years during the study period of March 2023 to January 2024 were included in this study.

Specimen collection and Transport:

Two high vaginal swab specimens and one slide were collected in the OBG department of outpatients and sent to the microbiology lab immediately for processing of wet mount, gram stain and culture & sensitivity.

Specimen processing:

Wet mount was performed with one swab for the examination of pus cells or any motile organisms. One slide was used for gram stains to study about clue cells and organisms. The other swab was streaked on nutrient agar, blood agar, mac conkey agar, chocolate agar and incubated at 37 ˚c for 24 hrs if no growth is seen in 1 day the plates were incubated further for 48 hrs. Identification of the isolates was further preceded by biochemical properties like catalase, oxidase, coagulase, bile esculin hydrolysis, indole, citrate utilization, urease hydrolysis and triple sugar iron agar tests.

Antibiotic susceptibility testing (AST):

AST is done by modified Kirby bauer disc diffusion method on Mueller Hinton agar based on CLSI guidelines [7,8]. The quality check done with the quality control strains. – Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and S.aureus ATCC 25923.

Antibiotic disks used for Gram positive organisms testing were penicillin (10U), gentamicin (10µg), amoxyclav (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), erythromycin (5µg), clindamycin (2µg), cotrimoxazole (1.25 µg/23.75 µg), cefoxitin (30 µg), linezolid (30 µg), vancomycin (30µg) and teicoplanin (30µg).

Gram negative isolates antibiotics were: amoxyclav (30 µg), piperacillin+tazobactum (100/10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefipime (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.

Data Collection: Patient details such as age, socioeconomic status, relapse of bacterial vaginosis, number of partners, type of discharge, associated symptoms and signs, and microbiological details were tabulated in an excel sheet to evaluate further. All descriptive quantitative variables were expressed as numbers and percentages.

RESULTS

Out of 363 samples, 307 were between 15 – 28 years (84.6%) and 56 were between 29 – 55 years (15.4 %).

Table 1. Age distribution of the patients

Age group in years	Frequency	Percentage (%)
15-28	307	84.6
29-55	56	15.4
Total	363	100

Culture positivity was seen in 124 samples (34.2%) and the remaining 239 samples were culture negative (65.8%). Among the 124 samples 103 samples were between 15- 28 years (33.6%) and 21 samples were between 29 – 55 years (37.5%).

Table 2. Culture positive and negative percentage in different age groups.

Age group in years	Culture positive	Culture negative
15-28 years	103 (33.6%)	204 (66.4%)
29-55 years	21 (37.5%)	35 (62.5%)

Staphylococcus aureus, CONS, Enterococci, E.coli, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus, Acinetobacter, Candida are the organisms isolated from the growth plates.

Between the age group of 15 – 28 years, E.coli and Staphylococcus aureus were the most common organism isolated, followed by klebsiella pneumonia, Enterococci, CONS, Candida, Acinetobacter and Klebsiella oxytoca.

Between the age group of 29 – 55 years, E.coli was the most common organism isolated, followed by Klebsiella pneumoniae , Enterococci and Staphylococci aureus.

TABLE 3- DISTRIBUTION OF MICROORGANISMS WITH RESPECT TO AGE.

AGE GROUP	NPO	S.aureus	CONS	Enterococci	Klebsiella pneumoniae	Klebsiella oxytoca	Acinetobacter	Proteus	Candida	E.coli
15 – 28 YRS	66.4 %	7.5%	2.6 %	5.5%	5.8%	0.6%	1.3%	0.3%	2.2%	7.5%
29 – 55YRS	62.5 %	5.3%	-	5.3%	10.7%	-	-	1.7%	-	14.2 %

TABLE 4 – FREQUENCY OF MICROORGANISMS DIFFERENT AGE GROUPS

AGE GROUP	NPO	S.aureus	CONS	Enterococci	Klebsiella pneumoniae	Klebsiella oxytoca	Acinetobacter	Proteus	Candida	E.coli
15 – 28 YRS	204(85.36%)	23(88.46%)	8(100%)	17 (85%)	18(75%)	2(100%)	4 (100%)	1(50%)	7(100%)	23(74.20%)
29 – 55YRS	35(14.64%)	03(11.54%)	•	03(15%)	06(25%)	•	-	1(50%)	-	08(25.80%)
Total	239 (100%)	26(100%)	8(100%)	20 (100%)	24(100%)	2(100%)	4(100%)	2(100%)	7(100%)	31(100%)

Based on the antibiotic susceptibility testing the gram positive cocci are highly susceptible to gentamicin(100%), high level gentamicin(100%), vancomycin(82.8%) followed by linezolid(71.4%), clindamycin(60%), amoxiclav(48.6%) and least sensitive to azithromycin(28.6%).

Gram negative bacilli are highly susceptible to amikacin(88.9%), meropenem(85.7%), cefipime(69.2%), followed by piperacillin tazobactam(60%), cotrimoxazole (53.5%), ciprofloxacin (50%), ofloxacin (44.4%), cefotaxime (40.7%), doxycycline (33.3%) and they were least sensitive to ceftriaxone (27.8%).

TABLE 5 - ANTIBIOTIC SENSITIVITY PATTERN IN GRAM POSITIVE BACTERIA

S.NO	ANTIBIOTICS	SENSITIVITY	RESISTANCE
1.	AMOXICLAV	48.6%	51.4%
2.	AZITHROMYCIN	28.6%	71.4
3.	GENTAMYCIN	100%	-
4.	HLG	100%	-
5.	CLINDAMYCIN	60%	40%
6.	VANCOMYCIN	82.8%	17.2%
7.	LINEZOLID	71.4%	28.6%

TABLE 6 – ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE BACTERIA

S.NO	ANTIBIOTICS	SENSITIVITY	RESISTANCE
1.	AMIKACIN	88.9%	11.1%
2.	COTRIMOXAZOLE	53.5%	46.5%
3.	CIPROFLOXACIN	50%	50%
4.	OFLOXACIN	44.4%	55.6%
5.	DOXYCYCLINE	33.3%	66.7%
6.	PIPERACILLIN TAZOBACTAM	60%	40%
7.	CEFOTAXIME	40.7%	59.3%
8.	CEFTRIAXONE	27.8%	72.2%
9.	CEFIPIME	69.2%	30.8%
10.	MEROPENEM	85.7%	17.3%

DISCUSSION

Aerobic vaginitis (AV) is a newly defined clinical entity which is distinct from candidiasis, trichomoniasis and bacterial vaginosis (BV). Because of its poor recognition of AV, this can lead to treatment failures and is associated with severe complications, such as pelvic inflammatory disease, infertility, preterm birth and fetal infections.vaginal infections can be prevented by understanding the vaginal microbiota. For many years, bacterial vaginosis received little attention as it is a common health problem and recurrence is common,but the scenario has been changed and raised awareness in many regions about the management of vaginosis to stop the serious medical consequences due to vaginosis.

Bacterial vaginosis is self limiting in 30% of cases, patients requiring treatment can be treated with antibiotics. Almost 10-15% of patients require additional antibiotic therapy due to relapse of infection [5]. This condition is not considered as STI, partners do not require treatment as there is no risk of passing infection back and forth between partners [6].

In the past years, the majority of pathogens causing of bacterial vaginosis were anaerobic bacteria such as *Gardnerella vaginalis*, anaerobic gram-negative rods belonging to the genera *Prevotella, Porphyromonas* and *Bacteroides, Peptostreptococcus* species, *Mycoplasma hominis, Ureaplasma urealyticum*, and often *Mobiluncus* species [7]. Nowadays community acquired bacterial infections are increasing due to various factors such as increase in population, unhygienic practices, misuse of antibiotics. Aerobic bacteria became a more common cause of vaginitis as per this study in bacterial vaginosis. Our study is supported by Gopalan Ushadevi et al[8].

Our study shows infection rate to be higher between the age group of 29-55 years followed by 15 – 28 years. The highest number of BV cases was seen among the 30–40 years' age group (8.8%) and least BV cases were seen in patients with the age group of 10–20 and 50–60 years (1.3%) [9]. Majority of the studies worldwide noted that the reproductive age group women were more prone for bacterial vaginosis. In Nigeria [10], 35.8% were in the 26-30 years age group with bacterial vaginosis. Highest prevalence in this age group could be due to various factors such as high sexual exposure, multiple sex partners, and unhygienic practices during sex.

In this study most common pathogens isolated were Escherichia coli (21.7), Klebisella pneumoniae (16.5%) and Staphylococcus aureus (12.8%). Among the isolates, 41 (32%) were Gram negative and 58 (45.3%) were Gram positive.

Other isolates including yeasts and parasites were 29 (22.7%). Of the Gram negative bacteria, *Pseudomonas* spp. (7.8%), *E.coli (6.3%),* and *Acinetobacter* spp. (6.3%) were predominant. Of the Gram positive bacteria, Staphylococcus aureus (5.5%) and Streptococcus agalactiae (5.5%) were predominant pathogens [11]. In Gram negative isolates Pseudomonas aeruginosa (6.3%) and Escherichia coli (6.3%) were observed predominantly and Lactobacillus spp was observed as the highest prevalent pathogen with the percentage of 27.3% [12]. But few studies have reported staphylococcus, group streptococcus and klebsiella as the most common organisms isolated as in Donders GG et al [13]. Ranjit E et al [14] observed the most common Gram positive cocci were Staphylococcus aureus and Streptococcus agalactiae in the study. The incidence was found to be 5.5% of total bacterial isolates each. This is consistent with the findings of Maghsoudi et al [15] in Pakistan, Tiyyagura et al [16] in India, and Al-Mousawi et al [17] in Iraq. A Study by AI-Kraety IAA et al [18] on bacterial vaginosis showed 43.4% of E.coli, 17.3% of Klebsiella spp.,17.3% of Staphylococcus spp., 6.5% of E.faecalis, 6.5% of R. ornithinolytica. The isolates of E.coli, Staphylococcus spp., Enterobacter spp., E.faecalis and R.ornithinolytica were found resistant to several antibiotics and considered multi-resistant.

In our study between the age group of 15 – 28 years *E.coli* and *Staphylococcus* are the most common organism isolated and between the age group of 29 – 55 years E.coli is the most common organism isolated followed by *Klebsiella pneumoniae*. *E.coli* is the most common pathogen isolated in both the age groups. Comparable results were seen in study done by Jahic et al [19], Li N et al [20]. This could be due to the poor personal hygiene since E.coli is a commensal in gastro intestinal tract.

The gram positive cocci are highly susceptible to gentamicin(100%), high level gentamicin(100%), vancomycin(82.8%) followed by linezolid(71.4%), clindamycin(60%), amoxiclav(48.6%) and least sensitive to azithromycin(28.6%). The Gram negative bacilli are highly susceptible to amikacin(88.9%), meropenem(85.7%), cefipime(69.2%), followed by piperacillin tazobactam(60%) , cotrimoxazole (53.5%), ciprofloxacin (50%), ofloxacin (44.4%), cefotaxime (40.7%), doxycycline (33.3%) and they were least sensitive to ceftriaxone (27.8%) in the present study.

In Uganda 29.6% women showed culture positivity when they studied a total of 361 women with abnormal vaginal discharge. The bacterial isolates were Staphylococcus aureus (48.6%), Klebsiella pneumoniae (29.9%), Enterococcus faecalis (15%), Escherichia coli (3.7%), and Streptococcus agalactiae (2.8%). According to their study and the antibiotic usage in their community observation of resistant patterns of bacterial isolates was highest with cefuroxime at 90.7% and ciprofloxacin at 81.3%. The highest resistance was observed with doxycycline at 86% and azithromycin (67.0%) [21].

According to a study conducted in Ethiopia, *S. aureus* was a highly prevalent bacteria and resistant to erythromycin (69.8%), trimethoprim/sulfamethoxazole (53.5%), and amoxicillin (39.5%), but susceptible to ciprofloxacin (93%), gentamicin (93%), and clindamycin (81.4%). Of the gram-negative bacteria, *E. coli* was a highly prevalent bacteria and resistant to trimethoprim/sulfamethoxazole (91.3%) and ceftriaxone (63.6%), but susceptible to ciprofloxacin (95.5%), gentamicin (93%), and nitrofurantoin (81.8%) [22]. *Staphylococcus aureus* was 100% sensitive to rifampicin and clindamycin and was highly resistant to penicillin G, trimethoprim-sulfamethoxazole, oxacillin, and tetracycline. This was consistent with other studies that *S. aureus* was highly resistant to tetracycline and trimethoprim-sulfamethoxazole [23] and *S. aureus* was resistant to erythromycin, trimethoprim/sulfamethoxazole, and amoxicillin.Staphylococcus has a high level of resistance to oxacillin, benzylpenicillin, levofloxacin, nitrofurantoin, trimethoprim-sulfamethoxazole, clindamycin, erythromycin, and tetracycline [18].

Microorganism with the highest frequency of infection was Escherichia coli (11.5%), followed by extended-spec trum beta-lactamase-producing *Escherichia coli* (6.5%), *diphtheroids* (6.0%), *Streptococcus agalactiae* (6.0%), *Pseudomonas aeruginosa* (5.0%), *Gard nerella vaginalis* (5.0%), *methicillin-resistant Staphylococcus aureus (MRSA)* (5.0%), *Citrobacter (5.0%),* and other gram-negative bacteria (5.0%). Candida albicans was isolated in 2.5% of the cases. Other organisms, such as *Proteus vulgaris, coagulase-negative Staphylococcus aureus, Acinetobacter, Klebsiella pneumoniae, Micrococci and Enterococci* [18]. *Escherichia coli* was the most sensitive to meropenem (100%) and imipenem (100%) and most resistant to amoxicillin (4.4%). Proteus vulgaris showed sensitivity to meropenem (100%), imipenem (100%), cefixime (87.5%), tigecycline (87.5%) and ciprofloxacin (87.0%). Diphtheroids showed sensitivity to ampicillin (95.7%), ceftria xone (91.3%), cefpodoxime (87.5%), ceftazidime (87.5%), and others. Streptococcus agalactiae showed sensitivity to meropenem (100%), imipenem (100%), amoxicillin (95.7%), ampicillin (91.7%) and cefixime (91.3%). Micrococci showed 100% sensitivity to meropenem and imipenem. Pseudomonas aeruginosa and Entero cocci were sensitive to meropenem and imipenem, Complete resistance to nalidixic acid was noted with all organisms.

Coagulase-negative Staphylococcus aureus showed sensitivity to meropenem (95.7%), imipenem (95.7%), cefpodoxime (87.5%), ceftazidime (87.5%), cefixime (62.5%) and tigecycline (62.5%). Acineto bacter showed 85.7% sensitivity to meropenem, cefixime and cefpodoxime; Citrobacter and other gram-negative bacteria showed sensitivity to imipenem (87.0%) and meropenem (82.6%). Klebsiella pneumoniae showed sensiti vity to ceftazidime and cefpodoxime. Extended-spectrum be ta-lactamase-producing Escherichia coli showed sensitivity to imipenem and cefixime, whereas Gardnerella vaginalis showed sensitivity to meropenem (100%) and imipenem (100%). MRSA strains were sensitive to ceftazidime (60.9%) and cefpodoxime (60.9%) [24].

These findings correlate well with study done by Sadiya shaik et al., where gram positive bacteria have high susceptibility to Vancomycin, linezolid, clindamycin and gram negative bacteria shows high susceptibility to amikacin, meropenem and shows least susceptibility to ceftriaxone.

CONCLUSION

This study concludie that the common organisms responsible for bacterial vaginosis are Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus. gram positive bacteria have high susceptibility to Vancomycin, linezolid, clindamycin and gram negative bacteria shows high susceptibility to amikacin, meropenem and shows least susceptibility to amoxycillin.

This study emphasizes the need to understand the aerobic pathogens associated with vaginitis especially in the reproductive age group to prevent adverse complications seen during pregnancy and labour. This also points out the need to determine the antibiotic sensitivity pattern which helps in suitable therapeutic choice for aerobic vaginitis. Thus appropriate diagnosis and correct therapy can prevent complications and inappropriate drug usage.

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