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Research Article

A study of aerobic bacterial pathogens associated with vaginitis in reproductive age group women (15-45 years) and their sensitivity pattern

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ABSTRACT

Background: Aerobic vaginitis (AV) is caused by a displacement of the healthy vaginal Lactobacillus species with aerobic pathogens such as Enterococcus faecalis, Escherichia coli and Staphylococcus aureus that triggers a localized vaginal inflammatory immune response. AV if it is not diagnosed and treated early, especially during pregnancy can place the health of both the mother and the foetus at risk as it is associated with preterm birth, premature rupture of membranes (PROM) and chorioamnionitis. Hence the present study aims at analysing the prevalence of aerobic vaginitis in females in the reproductive age group (15-45 years) with special reference to pregnancy, pathogens involved and their antibiogram.

Methods: Over one year period, high vaginal swabs were collected from 125 women with clinical suspicion of vaginitis. They were then subjected to Gram' staining and culture was made onto blood agar, chocolate agar and MacConkey agar and incubated aerobically at 37 0c for 24 hours. The AV score was determined and the organisms were then identified and antibiotic sensitivity test of isolates were performed.

Results: The prevalence of Aerobic vaginitis in this study was 20.8% (26/125) and the most common age group affected was between 26-30 years. Out of the 26 positive cases, 21 samples yielded monobacterial growth and 5 cases polybacterial growth. The most common organism isolated was Enterococcus faecalis (32.26%), followed by Escherichia coli (25.8%), Staphylococcus aureus (19.35%) and β-hemolytic streptococci (9.68%). Antibiotics like β–lactams/ β–lactamase inhibitor combinations, vancomycin and linezolid were found to be more effective against all Gram positive isolates whereas the Gram negative isolates were more sensitive towards β–lactams/ β–lactamase inhibitor combination, aminoglycosides and meropenem.

Conclusions: This study emphasized on the need to identify the aerobic vaginal pathogens associated with vaginitis especially in reproductive age group women which can go a long way in preventing the adverse outcomes associated with pregnancy and also ensures the necessity to determine the antibiotic sensitivity pattern of the pathogens which can aid in making a suitable therapeutic choice for 'aerobic vaginitis' by considering an antibiotic that is characterized by an intrinsic activity against the majority of bacteria of faecal origin, bactericidal effect and without any interference with the vaginal microbiota.

Keywords: Aerobic vaginitis, Lactobacillus, *Enterococcus faecalis*, *Escherichia coli*

INTRODUCTION

The vaginal microflora constitutes a complex micro-ecological environment composed of different microbiological species in variable quantities and relative proportions.[1]

The term vaginitis is the diagnosis given to women who present complaining of abnormal vaginal discharge with vulval burning, irritation or itching.[2] Inflammation of the

vaginal mucosa, called vaginitis, is one of the most frequent complaints in women attending gynaecological clinics accounting for 10 million office visits each year.[3]

The leading causes of symptomatic vaginal discharge are bacterial vaginosis, candidiasis, trichomoniasis.[2] Although the clinical features and treatment of some common forms of vaginitis are well defined, such as trichomonal vaginitis (TV), vulvovaginal candidiasis (VVC), and bacterial vaginosis (BV), other abnormal vaginal conditions have yet to be defined.[1] Between 7% and 70% of women who have vaginal discharge complaints will have no definitive diagnosis.[2] The problem is that some forms of abnormal vaginal micro-flora are neither normal, nor can they be called bacterial vaginosis. Such forms of abnormal flora have been termed 'intermediate flora' in some studies, or been included with full-blown bacterial vaginosis in others.[4-6] This type of undefined abnormal flora may be of crucial importance in pregnant women at risk of preterm delivery.[4,6]

Therefore, now it has been clear that the classifications of Candida vaginitis, Trichomonas vaginitis, and Bacterial vaginosis are insufficient to explain all clinical symptoms, therapy failures and the surprising outcomes of some studies on the link between some forms of bacterial flora and preterm birth rate. Such forms of abnormal vaginal flora have been termed as 'intermediate flora' and this type of abnormality as "Aerobic vaginitis".[7]

These infections if not treated or ignored could debilitate the patient and could become a source of infection for the neonates especially in case of reproductive age group women. Hence the present study is designed to isolate and identify the aerobic bacterial pathogens associated with vaginitis in the reproductive age group women and to study their latest antibiotic sensitivity patterns.

METHODS

Study design

It was a prospective type of study, conducted on samples collected during a period of one year between November 2011 and November 2012.

Inclusion criteria:

Vaginal swab specimens were collected from females in the reproductive age group of 15-45yrs with symptomatic vaginal discharge, attending the Gynaecology clinic of Dr. B.R. Ambedkar Medical College Hospital.

Exclusion criteria:

Patients treated with oral or parenteral or with local application of antibiotics for at least one month before attendance to the hospital were excluded and patients with diagnosis of bacterial vaginosis, candidiasis and trichomoniasis were excluded from the study.

Institutional ethical committee clearance was taken. The samples were collected after obtaining informed consent from the patients. They were explained the purpose of the study and the procedures involved.

Processing of sample

Two high vaginal swabs (HVS) were collected using sterile cotton swabs and were then immediately brought to the laboratory for processing. The first swab was used for Gram stain examination under 400x (for determining AV score) and 1000x magnification (for identification of organism). The AV score was calculated by determining the presence or absence of healthy Lactobacilli, number of leukocytes, type of vaginal flora, and parabasal epithelial cells under 400x magnification, according to a modified Donder's score,[7] without considering the variable "proportion of toxic leukocytes", as it could not be assessed after Gram-staining.[8] An AV score of <3 was taken as 'no signs of aerobic vaginitis', 3 – 4 as 'light AV', 5 to 6 as moderate AV, and any score >6 as 'severe AV'. Aerobic vaginitis (AV) was diagnosed if smears were deficient in lactobacilli, positive for cocci or coarse bacilli, positive for parabasal epithelial cells, and positive for vaginal leukocytes.[7]

The second swab was inoculated onto MacConkey's agar, blood agar and chocolate agar.[9,10] The aerobically incubated bacterial growth was identified by standard biochemical reactions.[11] The antibiotic sensitivity of aerobic bacterial isolates was performed by standardized Kirby Bauer disc diffusion technique as per the CLSI guidelines.[12] The antimicrobial discs were obtained from Hi Media Laboratories Private Limited, Mumbai.

Statistical analysis:

The results were expressed as percentages for the analysis of various data. Microsoft excel was used for the interpretation of these results.

RESULTS

A total of 125 vaginal swabs collected from patients with suspicion of vaginitis were sent from the Obstetrics and gynaecology department to the laboratory for culture, out of which 26 samples yielded growth under aerobic conditions. Hence, the prevalence of aerobic vaginitis in this study was 20.8%.

The study group included women in the reproductive age group i.e. between 15-45 years. The maximum number of aerobic vaginitis (AV) cases fell in the age group of 26-30 years (30%) followed by 31-35 years (26.08%) [Table 1]. The prevalence of AV cases was higher among non-pregnant (27.71%) compared to pregnant cases (7.14%) [Table 2].

Table 1: Age wise distribution of AV cases.

Age group (years)	No. of cases studied (n=125)	No. of positive cases(n=26)
15-20	8	1 (12.5%)
21-25	24	5 (20.83%)
26-30	40	12 (30%)
31-35	23	6 (26.08%)
36-40	11	1 (9.09%)
41-45	19	1 (5.26%)

Table 2: Distribution of AV cases among pregnant and non-pregnant women.

Category	Total no. of women (n=125)	No. of positive cases (n=26)	Percentage positivity
Pregnant	42	3	7.14%
Non-pregnant	83	23	27.71%

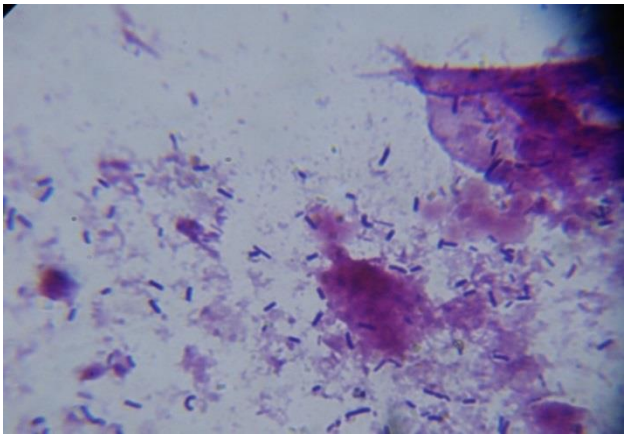

Figure 1: Gram's staining (1000x magnification): normal vaginal flora.

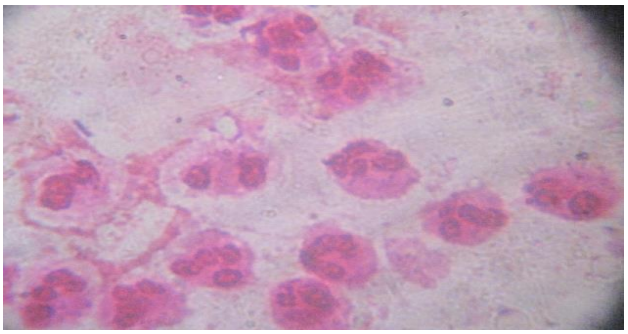

Figure 2: Gram's staining (1000x magnification): severe AV.

In this study, out of 125 cases, 99 cases (79.2%) had normal vaginal flora [Figure 1] and remaining 23 cases (88.46%) were observed as light AV, 2 cases (7.69%) as moderate AV and only 1 case (3.85%) with severe AV were detected. [Table 3] [Figure 2].

Table 3: Degree of aerobic vaginitis.

Degree of AV	No. of positive cases (n=26)	Percentage (%)
Light AV	23	88.46%
Moderate AV	2	7.69%
Severe AV	1	3.85%

In this study of 26 culture positive samples, 21 samples (80.77%) yielded single organism on culture and 5 (19.23%) yielded dual organisms (mixed) [Figure 3].

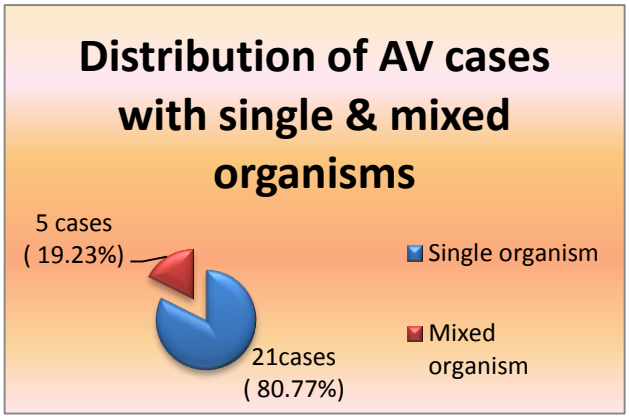

Figure 3: Distribution of AV cases with single and mixed organisms.

The most common aetiological agent of Aerobic vaginitis in this study was *Enterococcus faecalis* (32.26%), followed by *Escherichia coli* (25.8%), *Staphylococcus aureus* (19.35%) and *β-hemolytic streptococci* (9.68%) [Table 4].

Table 4: Distribution of organisms isolated from AV cases.

Organisms	Total no. of isolates	Percentage (%)
Enterococcus faecalis	10	32.26
Escherichia coli	8	25.8
Staphylococcus aureus	6	19.35
β-hemolytic streptococci	3	9.68
CONS	2	6.45
Klebsiella pneumonia	1	3.23
Pseudomonas aeruginosa	1	3.23
Total	31	100

There were 2 (40%) AV cases of mixed infections with *Enterococcus faecalis* and *E.coli* followed by 1 (20%) case each of *E.coli* + *Pseudomonas Aeruginosa*, *Staphylococcus aureus* + *E.coli* and *Klebsiella pneumoniae* + *CONS* [Figure 4]. There was a greater

predominance of gram positive organisms in this study which seemed to show more resistance to penicillin and ampicillin except for β-haemolytic streptococci, which showed 100% sensitivity. Among the *Enterococcus faecalis* isolates, only 1 (10%) of them showed high level resistance to gentamicin (120µg) and streptomycin (300 µg). Among the *Staphylococcus aureus* strains, 2 cases of MRSA (Methicillin resistant staphylococcus aureus) were isolated whereas none of the CONS were cefoxitin resistant.

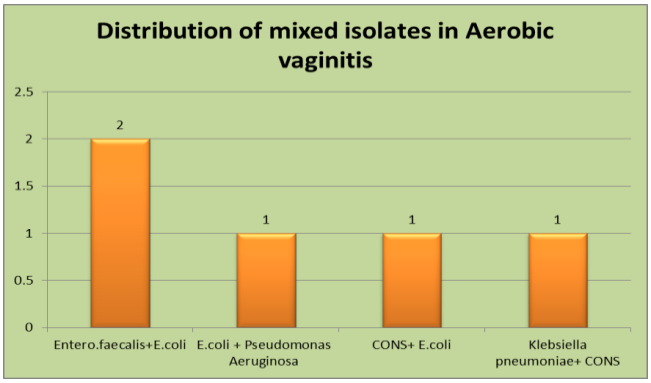

Figure 4: Distribution of mixed isolates in aerobic vaginitis cases.

Table 5: Antibiotic sensitivity pattern of gram positive organisms.

	Enterococcus faecalis(n=10)	*Staph. aureus* (n=6)	*CONS* (n=2)	*β-hemolytic streptococci*(n=3)
P	60	33.3	50	100
Amp	60	NT	NT	100
AC	NT	66.67	100	NT
Cx	NT	66.67	100	NT
E	NT	83.33	100	66.67
Cd	NT	83.33	100	66.67
Gm	90	NT	NT	NT
Sm	90	NT	NT	NT
Lf	80	83.33	100	NT
Dx	NT	83.33	100	NT
Ctx	NT	NT	NT	100
Lz	100	100	100	100
Va	100	100	100	100

P- Penicillin, Amp-Ampicillin, AC- Amoxy-Clav, Cx- Cefoxitin, E- Erythromycin, Cd- Clindamycin, Gm - Gentamicin, Sm- Streptomycin, Lf - Levofloxacin, Dx- Doxycycline, Ctx - Cefotaxime, Lz- Linezolid, Va- Vancomycin, NT – Not tested

Table 6: Antibiotic sensitivity pattern of gram negative organisms.

	E. coli(n=8)	*K. pneumoniae(n=1)*	*P. aeruginosa(n=1)*
Amp	50	0	NT
Pc	NT	NT	0
AC	75	100	NT
PT	NT	NT	100
Ak	87.5	100	0
Gm	87.5	100	100
Tbr	NT	NT	100
Ce	75	100	100
Ctx	75	100	NT
Ctz	NT	NT	0
Cf	87.5	100	100
Cot	75	100	0
Azt	50	100	100
Mr	100	100	100

Amp-Ampicillin, Pc- Piperacillin, AC- Amoxy-Clav, PT - Pipercillin-Tazobactam, Ak – Amikacin, Gm - Gentamicin, Tbr- Tobramycin, Ce- Cefepime, Ctx - Cefotaxime , Ctz – Ceftazidime, Cf - Ciprofloxacin, Cot- Cotrimoxazole, AZT – Aztreonam, Mr - Meropenem. NT – Not tested

The gram positive organisms were maximum sensitive towards β–lactams/ β–lactamase inhibitor combinations, vancomycin and linezolid [Table 5].

The gram negative isolates were least sensitive to ampicillin but showed moderate sensitivity towards third generation cephalosporin, aminoglycosides and fluoroquinolones but were highly sensitive to amoxy-clav and meropenem. The most effective antibiotics against Pseudomonas aeruginosa were gentamicin, tobramycin and meropenem [Table 6].

DISCUSSION

Vaginitis is a common medical problem in women that is associated with substantial discomfort, significant morbidity and hence frequent medical visits. These infections if not treated or ignored could debilitate the patient and could become a source of infection for the neonates especially in case of women belonging to the childbearing age.[13] Therefore this study was designed to assess frequency of various aerobic pathogens in vaginal infections in females in the childbearing age group of 15-45 years.

The prevalence of Aerobic vaginitis (AV) in this study was 20.8% which correlates with that of Fan and colleagues (2013) who reported prevalence rate of 23.74%.[1] Even higher prevalence of aerobic vaginitis was observed by Ling C (80%) in 2009 and by Razzak et al (95.45%) in 2011.[14,15] whereas Donders in 2002(Belgium) reported a lower prevalence rate of AV i.e.7.9% and in 2009 reported a prevalence of 8.3 % among pregnant women.[7, 16]

In this study, the highest prevalence of vaginal infections was noted among young sexually active females, at the age group of 26-30 years (30%), followed by 31-35 years (26.08%) and 21-25 years (20.83%). This was in concordance with studies done by Khan and khan and Mumtaz et al.[13,17] The frequency of culture positivity seems to decline progressively with increasing age.

The prevalence of AV among Pregnant women in this study was 7.14%. Donders et al in 2009 studied 759 pregnant women among which 8.3% had coccoid AV flora.[16] The low incidence of AV among pregnant women in this study may be due to the fact that pregnancy is a period in which the vaginal micro biota, conditioned by high oestrogen levels has a good supply of glycogen and a high percentage of lacto bacillary flora which significantly reduces the multiplication of pathogenic organisms, more due to production of defence factors by lactobacilli.[18]

Maximum number of cases in this study was diagnosed with light AV (88.46%). Moderate AV was reported in 7.69% of cases and severe AV in only 3.85% of cases which is in accordance with studies done by other researchers.[7,19]

80.77% of cases yielded monomicrobial growth whereas 19.23% yielded polymicrobial growth (two bacterial species in culture) which is in contrast to study done by Razzak et al, 2011 (Iraq) who observed 50 out of 105 cases (47.62%) as polymicrobial infections.[15]

In this study, Enterococcus faecalis (32.26%) was the most prevalent organism isolated from AV cases followed by Escherichia coli (25.8%) and Staphylococcus aureus (22.6%). In a study by Khan and khan in Islamabad (2004), Enterococcus faecalis (31%) was the most frequently isolated aerobic vaginal pathogen.[13] Other researchers have also reported similar organisms in their study.[14,20,21]

9.68% of the vaginal isolates in the present study were β-hemolytic streptococci. Similar rates have been observed by Mumtaz et al.[17] The isolation of K pneumoniae and Pseudomonas aeruginosa in AV cases was also reported by other researchers.[13,15,17]

The gram positive organisms in this study showed more resistance to penicillin and ampicillin. Nearly 40 % of the Enterococcus isolates were resistant to penicillin and ampicillin whereas only 10% of them showed resistance to aminoglycosides which is in contrast to other studies.[21] Most of the β-hemolytic streptococci were sensitive to penicillin and is in tallying with Mumtaz et al.[17] In most cases of Staphylococcus aureus, resistance to penicillin is attributable to ß-lactamase production. Therefore, penicillin in combination with one of the ß lactamase inhibitors gives much better results,[22] as clearly seen from this study.

The most effective chemotherapeutic agents against Enterobacteriacea were amoxy-clav, aminoglycosides and meropenem which is in correlation with study done by Tariq et al.[21] Most of the Pseudomonas species were found resistant to piperacillin and ceftazidime whereas100% of the isolates were sensitive to Piperacillin-tazobactum, aminoglycosides and meropenem. Similar antibiogram pattern was observed by other researchers.[17]

CONCLUSION

The study concluded that the types of antibiotics used to treat vaginitis must be very selective in order not to kill the beneficial bacteria (Lactobacilli) that help in preservation of vaginal health and ecosystem, being one of the probiotic bacteria, while effectively should aid in eradicating the Gram-negative enterics such as *E. coli*, and Gram-positive cocci like *S. aureus*, and *E.faecalis*.

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Ethical approval: Approved by institutional ethics committee

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