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Prevalence of Aerobic Vaginitis its Microbial spectrum and Antibiogram at a Tertiary care Hospital, Patna.

Dr. Neelima Singh, Junior Resident, Department of Microbiology, IGIMS, Patna
Dr. Dipali Prasad, Associate Professor, Department of Obstetrics & Gynarcology, IGIMS
Dr. Namrata Kumari, Additional Professor, Department of Microbiology, IGIMS, Patna
Dr. Anima Xess, Professor, Department of Microbiology, IGIMS, Patna

Corresponding Author: Dr. Neelima Singh, Junior Resident, Department of Microbiology, IGIMS**,** Patna

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Abstract

Aerobic Vaginitis (AV) is recently identified type of vaginitis that is diagnosed according to microscopic criteria. The clinical characteristics of AV include severely depressed levels of Lactobacillus and vaginal inflammation. So, the present study aims at analyzing the prevalence of AV in females in the reproductive age group (15-45 years), pathogens involved and their antibiogram.

Materials and Methods: Over 1 year period, high vaginal swabs were collected from 220 women with clinical suspicion of vaginitis. They were subjected to Gram-staining, wet mount and culture. The AV score was determined, and the organisms were then identified and antibiotic sensitivity test of isolates was performed as per Clinical and Laboratory Standards Institute guidelines.

Result: The prevalence of AV in this study was 35. The most common organism isolated was Enterococcus species followed by E.coli and S. aureus.

Conclusion : The aerobic vaginitis patient with gynaecological symptoms should be investigated thoroughly. Aerobic vaginitis (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.

Keywords: Aerobic Vaginitis, Enterococcus species, Lactobacillus, Staphylococcus aureus, Escherichia coli.

Introduction

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.[1] Vaginal discharge is common problem among women of reproductive age group which remains unattended and undiagnosed due to ignorance and unawareness. So proper isolation and identification of causative organisms is the main implication of this study.

Between 7% and 70% of women who have vaginal discharge complaints will have no definitive diagnosis.[2] Classifications of Candida vaginitis, Trichomonas vaginitis, Bacterial Vaginosis are insufficient to explain all clinical symptoms, therapy failures and the surprising outcomes of such studies. Such forms of abnormal vaginal flora have been termed as “intermediate flora” and this type of abnormality as “Aerobic Vaginitis.”[3]

Aerobic Vaginitis is defined as disruption of the Lactobacillary flora, accompanied by signs of inflammations and the presence of rather scarce, predominantly aerobic microflora, composed of enteric

commensals or pathogens.[4] Aerobic Vaginitis corresponds to a type of disturbed microflora, in which the lactobacilli are replaced by aerobic facultative pathogens like Escherichia coli, Staphylococcus aureus, Group B Streptococcus, Klebsiella pnumoniae and Enterococcus species. Disruption of the vaginal ecosystem during aerobic vaginitis cause an increase in pH to >6, a decrease in lactate concentrations and an increase in leucocytes and pro-inflammatory cytokines concentrations in the vaginal discharge. Common presenting feature are yellow discharge and dyspareunia with red inflammation of vagina.[3]

Because of the poor recognition of AV, this condition is often misdiagnosed as Bacterial Vaginosis (BV), which may lead to treatment failures and severe complications, such as pelvic inflammatory disease, infertility, miscarriage, chorioamniotis, premature rupture of membranes and preterm delivery.[5-8]

Materials and Methods

It was a cross-sectional hospital based study, conducted on vaginal swab specimens obtained from females in the reproductive age group of 15-45 years with symptomatic vaginal discharge attending Gynaecology OPD, Indira Gandhi Institute of Medical Sciences, Patna. The samples were collected during a period of 1 year between December 2016 to December 2017. Patients in pregnancy, puerperium and laboratory confirmed STD were excluded from the study. Institutional ethical committee clearance was taken. The samples were collected only after obtaining written informed consent from the patients. They were explained about the purpose of the study and the procedures involved

Sample collection Transport and Processing

Three High Vaginal Swabs(HVS) were collected in sterile Normal Saline to avoid dryness of samples. The first swab was used for Gram's staining. Second swab was used for the preparation of wet mount and KOH mount.

The AV score was calculated by microscopic examination under 40X magnification, according to a modified Donder's score.[9] An AV score of <3 was taken as "no signs of AV", 3-4 as "light AV", 5-6 as "moderate AV", and any score >6 as "severe AV". Aeobic Vaginitis was diagnosed if smears were deficient in lactobacilli, positive for cocci or coarse bacilli, positive for parabasal epithelial cells, and positive for vaginal leucocytes.

The third swab was inoculated in MacConkey's Agar and Blood Agar. The aerobically incubated bacterial growth was identified by standard biochemical reactions.[10] The antibiotic sensitivity of aerobic bacterial isolates was performed by standardised Kirby-Bauer disc diffusion technique as per the Clinical and Laboratory Institute guidelines.[11] The antimicrobial discs were obtained from Hi Media Laboratories Private Limited, Mumbai.

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

Result

A total of 660 HVSs collected from 220 patients with suspicion of vaginitis were sent from the Gynaecology department to the laboratory for culture, out of which 77 cases yielded growth under aerobic conditions. Hence the prevalence of AV in this study was 35%.

The study group included women in the reproductive age group of 18-45 years. The maximum number of AV cases fell in the age group of 26 to 30 years followed by 31 to 35 years.

Table 1. Age wise distribution of AV cases.

Age(yrs)	No. of patients n=77	% of patients
15-20	2	2.59
21-25	9	11.68
26-30	33	42.85
31-35	15	19.48
36-40	10	12.98
41-45	8	10.38

In this study, 62 cases (80.51%) had mild AV, 12(15.58%) had moderate AV and 3(3.89%) had severe AV.

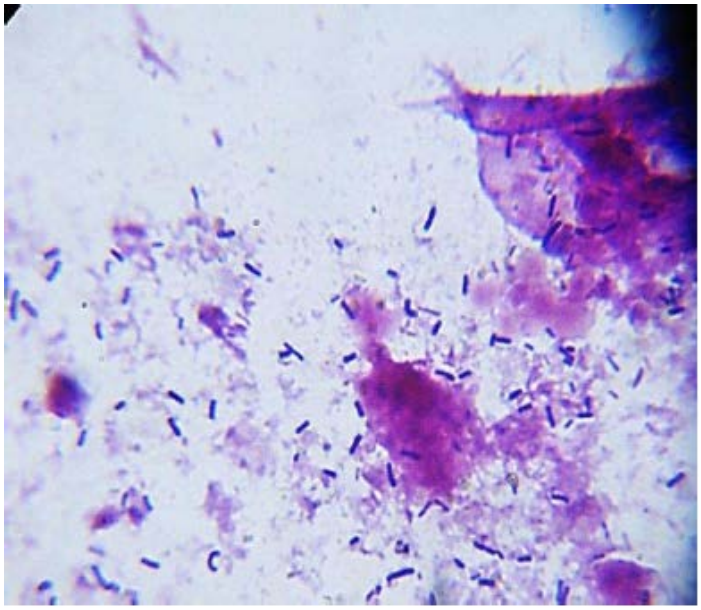

Figure 1.Gram's staining: Normal Vaginal flora

Table 2. Grading of AV

Grading of AV	No. Of patients(n=77)	Percentage%
Mild	62	80.51
Moderate	12	15.58
Severe	3	3.89

Out of 77 culture positive cases, 73(94.8%) yielded single organism on culture and only 4(5.19%) showed mixed growth.

The most common etiological agent of AV in this study was *Enterococcus* species 20/77 (25.97%)

The Gram positive organisms were maximum sensitive towards β-lactams/β-lactamase inhibitor combinations, Vancomycin, and Linezolid.(Table no.4)

The Gram negative isolates were least sensitive to ampicillin but showed moderate sensitivity toward third generation Cephalosporin, Aminoglycoside and Levofloxacin but were highly sensitive to Amoxy-clav and Meropenem.(Table no.5)

Figure 2.Distribution of Aerobic Vaginitis pathogens

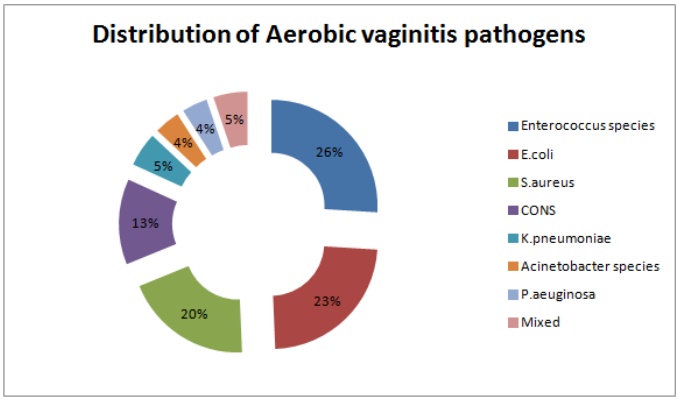

Table 3. Growth pattern of the culture positive samples.

Organisms isolated	No. Of patients	Percentage%
Enterococcus species	20	25.97
E.coli	18	23.37
Staphylococcus aureus	15	19.48
CONS	10	12.98
K. pneumoniae	4	5.19
Acinetobacter species	3	3.89
P.aeruginosa	3	3.89
Mixed Growth	4	5.19
Total	77	100

Table 4. Percentage of sensitivity of Gram positive isolates to various antibiotics

Antimicrobial agent	Enterococcus species n=20(%)	Staphylococcus aureus n=15(%)	CONS n=10(%)
Penicillin	15(75)	4(26.67)	2(20)
Ampicillin	14(70)	NT	NT
Amoxycillin-clavulanic acid	NT	11(73.4)	6(60)
Cefoxitin	NT	11(73.4)	9(90)
Clindamycin	NT	13(86.7)	10(100)
Vancomycin	20(100)	15(100)	10(100)
Linezolid	20(100)	15(100)	10(100)
Levofloxacin	16(80)	13(86.7)	10(100)
Gentamycin	14(70)	12(80)	8(80)

NT=Not Tested

Table 5. . Percentage of sensitivity of Gram negative isolates to various antibiotics.

Antimicrobial agent	E.coli n=18(%)	K.pneumoniae n=4(%)	P.aeruginosa n=3(%)	Acineto bacter species n=3(%)
Ampicillin	6(33.3)	1(25)	NT	0
Amoxycillin-clavulanic acid	13(72.2)	3(75)	3(100)	2(66.7)
Gentamycin	13(72.2)	3(75)	3(100)	1(33.3)
Amikacin	10(55.5)	2(50)	2(66.7)	2(66.7)
Levofloxacin	14(77.7)	3(75)	1(33.3)	3(100)
Cotrimoxazole	11(61.1)	4(100)	NT	2(66.7)
Cefotaxime	13(72.2)	3(75)	NT	1(33.3)
Ceftazidime	NT	NT	1(33.3)	NT
Aztreonam	8(50)	3(75)	2(66.7)	2(66.7)
Meropenem	18(100)	4(100)	3(100)	1(33.3)
Piperacillin	NT	NT	1(33.3)	NT
Piperacillin-tazobactam	NT	NT	3(100)	NT
Tobramycin	NT	NT	1(33.3)	1(33.3)

NT=Not tested

Discussion

The prevalence of Aerobic vaginitis (AV) in this study was 35% which correlates with that of Mumtaz *et al* (Pakisthan) who reported a prevalence rate of 38.01% (731/1923).[12] Ayengar V *et al* (India) also reported a culture positivity of 57%.[13] 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]

Fan and colleagues in 2013 observed a prevalence rate of 23.74% 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.

The prevalence of AV varies considerably from place to place which could be attributable to the changes in the environmental conditions and the socio-cultural habits of the people in the developing countries. In addition poverty, malnutrition, high population density, the unavailability of potable water, low health status, lack of personal hygiene and irrational use of antibiotics like broad-spectrum penicillins or tetracyclines which can kill or suppress helpful bacteria (*Lactobacilli*) in the genital tract, thus allowing the resistant organisms to grow unchecked.[16]

Maximum number of cases in this study was diagnosed with mild AV 62/77 (80.5%). Moderate AV was reported in 12/77(15.6%) of cases and severe AV in only 3/77(3.9%) of cases which is in accordance with studies done by other researchers.[17,18]

In this study, *Enterococcus* species 20/77 (25.9%) was the most prevalent organism isolated from AV cases followed by *Escherichia coli* 18/77(23.4%), *Staphylococcus aureus* 15/77(19.5%) and *Coagulase negative staphylococci* 10/77(12.9%)(Table no.5.8). In a study by Khan and khan in Islamabad (2004), *Enterococcus faecalis* (31%) was the most frequently isolated pathogen in Aerobic vaginitis.[19]

Tariq *et al* in 2006 also reported *Enterococcus* spp. (14.7%) and *E.coli* (10.2%) as the commonest bacterial vaginal pathogens.[20] In 2004, Tempera and Furneri studied a sample of 30 women with a clinical and microbiological diagnosis of aerobic vaginitis. *E. coli* was the most frequently isolated pathogen (n = 29), followed by *E. faecalis* (n = 15).[21]

Ling and colleagues and Zarbo *et al* have also isolated *Staphylococci aureas, Enterococcus species* and *E. coli* in AV cases.[22,14]

Fan and team in 2013, observed that out of the 72 single AV cases, such bacteria as *Enterococcus species, E. coli*, and *CONS* were most frequently isolated.[2] The isolation of *K. pneumoniae* in AV cases was also reported by other researchers.[23] In a study by Chowdareddy in Bangalore (2013), the pathogens derived from the genital tract of the women with PROM were predominantly *Staphylococcus aureus* and *Klebsiella pneumoniae*.[24]

Pseudomonas aeruginosa and *Acinetobacter species* were isolated in 3/77(3.89%) each. These organisms were also reported as vaginal pathogens by other studies.[12,15] These bacteria had been isolated especially from women suffering from offensive odour, and from vaginal discharge, besides, from non-pregnant women using intrauterine device. It is potentially opportunistic bacteria within the vagina. Such microorganism may become an increasing prevalent upon minor alterations of the vaginal environment. Other investigators have also isolated these bacteria from cases of vaginitis.[25]

The pathogenic bacteria of AV in the present study were primarily Gram-positive (GPC) aerobic bacteria 45/77 (58.44%) whereas the prevalence of Gram-negative organisms (GNB) was 28/77(36.36%). This preponderance of GPC was also observed in other studies.[12,14]

The Gram positive organisms in this study showed more resistance to penicillin and ampicillin. Out of 20 isolates of *Eterococci*, 15(75%) were sensitive to penicillin. *Enterococci* are intrinsically resistant to cephalosporins, so not tested. Among 15 isolates of *S.aureus* only 4(26.7%) were sensitive to penicillin. In most of the cases of *S.aureus*, resistance to penicillin is attributable to β-lactamase production. Therefore, penicillin in combination with one of the β-lactamase inhibitors gives much better results[26] as clearly seen from the present study.

The most effective chemotherapeutic agents against Enterobacteriaceae were Amoxy-clav, Aminoglycosides and Meropenem which is in correlation with study done by Tariq *et al*.[20]

Pseudomonas species were found resistant to piperacillin and ceftazidime, whereas all of the isolates were sensitive to piperacillin-tazobactam and meropenem. Similar antibiogram pattern was observed by Mumtaz *et al*.

Conclusion

Aerobic vaginitis (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 and chorioamnionitis. AV is one of those diseases that are more common among young sexually active females as was the case in this study where the most vulnerable age group was between 26-30 years. In our study the most common vaginal pathogen causing AV was *Enterococcus species* followed by *Escherichia coli* and *Staphylococcus aureus.* Proper isolation and identification of causative organisms of AV shoud be done. 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.

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