

Bacterial Etiology and their Antibigram in Aerobic Vaginitis Patients at Tertiary Care Hospital, Kota, Rajasthan

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Abstract

Background: Aerobic vaginitis (AV) is a recently identified type of vaginitis that is diagnosed according to microscopic criteria. The clinical characteristics of AV include severely depressed levels of lactobacilli, increased levels of aerobic bacteria and vaginal inflammation. Hence, the present study aims at analyzing the prevalence of AV in females in the reproductive age group (15-45 years) with special reference to pregnancy, pathogens involved and their antibiogram.

Materials and Methods: Over 1-year period, high vaginal swabs were collected from 200 women with clinical suspicion of vaginitis. They were then 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.

Results: The prevalence of AV in this study was 26% (52/200), and the most common age group affected was between 26 and 30 years. Out of the 52 positive samples, 48 samples yielded monobacterial growth and 4 samples polybacterial growth. The most common organism isolated was *Staphylococcus aureus* followed by *Escherichia coli*, *Enterococcus* spp., and β -hemolytic streptococci. Antibiotics such as β -lactams/ β -lactamase inhibitor combinations, vancomycin, linezolid, and moxifloxacin were found to be more effective against all Gram-positive isolates, whereas the Gram-negative isolates were more sensitive toward β -lactams/ β -lactamase inhibitor combination, meropenem, and moxifloxacin.

Conclusions: The prevalence of aerobic bacterial vaginitis patient with gynecological symptoms is investigated thoroughly. Culture with an antimicrobial sensitivity of causative organism must invariably be done to reduce pregnancy complications such as ascending chorioamnionitis, preterm rupture of the membranes, and preterm delivery.

Key words: Aerobic vaginitis, Bacterial vaginosis, Lactobacillus, *Staphylococcus aureus*, *Escherichia coli*

INTRODUCTION

Inflammation is vaginal mucosa, called vaginitis, is a common clinical syndrome accounting for approximately 10 million office visits each year. Women who present with vaginal symptoms often complain of an abnormal

discharge and additional symptoms such as an offensive odor or itching.¹

A complex and intricate balance of microorganisms maintains the normal vaginal flora. It is mainly dominated by members of the genus *Lactobacillus*, which maintains the generally acidic vaginal pH.²

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, chorioamnionitis, premature rupture of membranes, and preterm delivery.³⁻⁶ Ascending infection during gestation

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www.ijss-sn.com

Month of Submission : 04-2016
Month of Peer Review : 05-2016
Month of Acceptance : 05-2016
Month of Publishing : 06-2016

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may also result in maternal complications such as sepsis, septic arthritis, and maternal respiratory distress and even menstrual toxic shock syndrome.⁷

MATERIALS AND METHODS

Source of Data

Vaginal swab specimens were obtained from females in the reproductive age group of 15-45 years with symptomatic vaginal discharge, attending the Gynaecology Department (Outpatients and inpatients) of Jay kay lon maternal and child hospital and New Medical College Hospital, Kota.

Study Design

It was a prospective type of study, conducted on samples collected during a period of 1-year between October 2014 and September 2015.

Inclusion Criteria

High vaginal swab (HVS) from females in the reproductive age group of 15-45 years, with symptomatic vaginal discharge and pregnant females, irrespective of their gestational age, were included in the study.

Exclusion Criteria

Patients with a diagnosis of BV, candidiasis, trichomoniasis, *Neisseria gonorrhoea*, and *Chlamydia trachomatis* cervicitis were excluded from the analysis. Patients treated with oral or parenteral or with local application of antibiotics for at least 1 month before attendance to the hospital were omitted from the study.

Sample Collection Transport and Processing

An unmoistened sterile speculum was inserted before examination of vagina. Three HVSs were collected in sterile saline solution to avoid dryness of samples. The first swab was used for Gram's-staining.⁸ Second swab was used for the preparation of wet mount and KOH mount. The AV score was calculated by microscopic examination under $\times 40$ 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." 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.⁹ gram staining of normal vaginal flora shown in Figure 3.

The third swab was inoculated onto MacConkey's agar, blood agar, and chocolate agar.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 Clinical and Laboratory Standards Institute 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 200 HVSs collected from patients with suspicion of vaginitis were sent from the obstetrics and gynecology department to the laboratory for culture, out of which 52 samples yielded growth under aerobic conditions. Hence, the prevalence of AV in this study was 26%.

The study group included women in the reproductive age group, i.e., between 15 and 45 years. The maximum number of AV cases fell in the age group of 26-30 years (37.5%), followed by 31-35 years (31.57%) depicted in Table 1.

The prevalence of AV cases was higher among non-pregnant (33.57%) compared to pregnant cases (8.33%) depicted in Table 2.

Table 1: Age wise distribution of AV cases

Age group (years)	Number of samples studied (n=200)	Number of culture positive samples (n=52) (%)
15-20	12	1 (8.33)
21-25	37	10 (27.02)
26-30	64	24 (37.5)
31-35	38	12 (31.57)
36-40	17	3 (17.64)
41-45	32	2 (6.25)

AV: Aerobic vaginitis

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

Category	Total number of samples collected (n=200)	Number of culture positive samples (n=52) (%)
Pregnant	60	5 (8.33)
Non-pregnant	140	47 (33.57)

AV: Aerobic vaginitis

Table 3: Grading of AV

Grading of AV	Number of positive samples (n=52) (%)
Mild AV	42 (80.76)
Moderate AV	8 (15.38)
Severe AV	2 (3.84)

AV: Aerobic vaginitis

In this study, 80.76% of women had mild AV, 15.38% with moderate AV, and only 3.84% of women with severe AV were detected (Table 3).

In the present study of 52 culture positive samples, 92.31% yielded single organism on culture and 7.69% samples yielded dual organisms (multiple) (Table 4).

The most common etiological agent of AV in this study was *Staphylococcus aureus* (41.07%), followed by *Escherichia coli* (21.43%), *Enterococcus* spp. (12.5%), and β -hemolytic streptococci (8.93%) (Figure 1).

There were 4 samples of AV with multibacterial growth, each with two bacteria. 50% samples had a combination of *S. aureus* + *E. coli*, 25% samples had a combination of CONS + *Enterobacter cloaca*, and another 25% was with *Enterococcus* spp. + *E. coli*, shown in Figure 2.

The Gram-positive organisms were maximum sensitive toward β -lactams/ β -lactamase inhibitor combinations, vancomycin, and linezolid (Table 5).

The Gram-negative isolates were least sensitive to ampicillin but showed moderate sensitivity toward third generation cephalosporin, aminoglycosides, and levofloxacin but were highly sensitive to amoxy-clav and meropenem and moxifloxacin. The most effective antibiotics against *Pseudomonas aeruginosa* were amoxicillin-clavulanic acid, gentamicin, tobramycin, meropenem, and moxifloxacin (Table 6).

Table 4: Growth pattern of the culture positive samples (n=52)

Growth pattern	Number of culture positive samples (%)
Single organism	48 (92.31)
Multiple organism	4 (7.69)
Total	52 (100)

DISCUSSION

The prevalence of AV in the present study was 26% which correlates with that of Fan *et al.*, in China,¹³ who reported the prevalence rate of 23.74% and Sangeetha *et al.*¹⁴ in Bangalore, India, also reported a culture positivity of 20.8%. Even higher prevalence of AV was observed by Ling C (80%), in 2009, and by Razzak *et al.* (95.45%) in 2011,^{15,21} whereas Donders *et al.*, in 2002 (Belgium¹⁷) reported a lower prevalence rate of AV, i.e., 7.9% and Donders *et al.*, in 2009¹⁸ reported a prevalence of 8.3% among pregnant women.

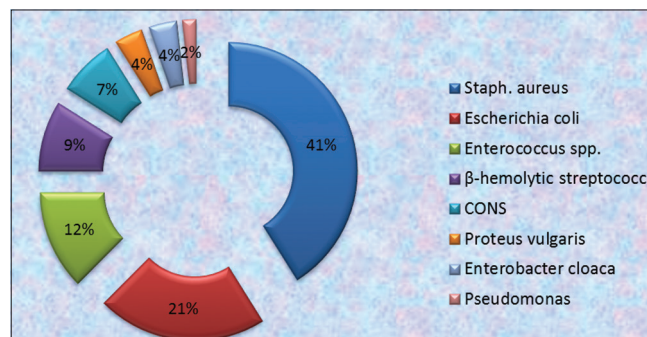


Figure 1: Distribution of organisms isolated from aerobic vaginitis cases

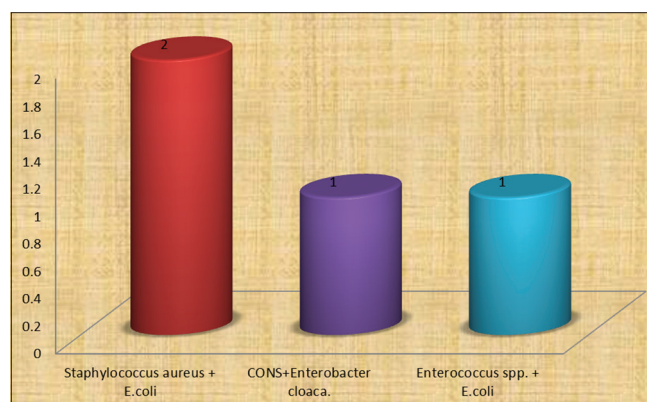


Figure 2: Distribution of mixed isolates in aerobic vaginitis cases

Table 5: Percentage of sensitivity of gram positive isolates to various antibiotics

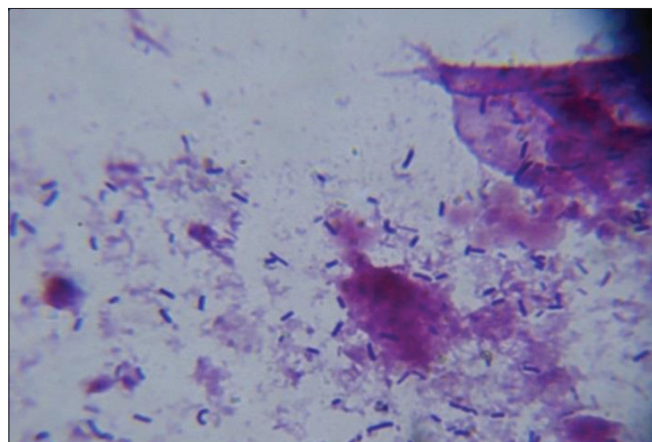
Antimicrobial agent	<i>Staphylococcus aureus</i> (n=23) (%)	<i>Enterococcus</i> spp. (n=7) (%)	β -hemolytic streptococci (n=5) (%)	CONS (n=4) (%)
Penicillin	9 (39.1)	3 (42.8)	5 (100)	2 (50)
Ampicillin	NT	4 (57.1)	5 (100)	NT
Amoxicillin-clavulanic acid	17 (73.9)	NT	5 (100)	3 (75)
Cefoxitin	17 (73.9)	NT	NT	4 (100)
Clindamycin	21 (91.3)	NT	3 (60)	4 (100)
Vancomycin	23 (100)	7 (100)	NT	4 (100)
Linezolid	23 (100)	7 (100)	NT	4 (100)
Doxycycline	20 (86.9)	3 (42.8)	3 (60)	4 (100)
Levofloxacin	21 (91.3)	4 (57.1)	3 (60)	4 (100)
Moxifloxacin	21 (91.3)	5 (71.4)	4 (80)	4 (100)
Gentamicin	17 (73.9)	6 (85.71)	3 (60)	3 (75)

NT: Not tested

Table 6: Percentage of sensitivity of gram negative isolates to various antibiotics

Antimicrobial agent	<i>Escherichia coli</i> (n=12) (%)	<i>Proteus vulgaris</i> (n=2) (%)	<i>Enterobacter cloaca</i> (n=2) (%)	<i>Pseudomonas aeruginosa</i> (n=1) (%)
Ampicillin	4 (33.3)	NT	1 (50)	NT
Amoxicillin-clavulanic acid	9 (75)	NT	2 (100)	1 (100)
Gentamicin	9 (75)	1 (50)	2 (100)	1 (100)
Amikacin	8 (66.6)	2 (100)	2 (100)	0
Levofloxacin	9 (75)	2 (100)	1 (50)	0
Moxifloxacin	10 (83.3)	2 (100)	2 (100)	1 (100)
Cotrimoxazole	8 (66.6)	NT	1 (50)	NT
Ceftriaxone	9 (75)	1 (50)	1 (50)	NT
Cefotaxime	9 (75)	1 (50)	1 (50)	NT
Ceftazidime	NT	2 (100)	NT	0
Aztreonam	6 (50)	1 (50)	1 (50)	0
meropenem	12 (100)	2 (100)	2 (100)	1 (100)
Piperacillin	NT	1 (50)	NT	0
Piperacillin-tazobactam	NT	2 (100)	NT	1 (100)
Tobramycin	NT	NT	NT	1 (100)

NT: Not tested

**Figure 3: Gram's-staining (×1000 magnification): Normal vaginal flora**

In the present study, the highest prevalence of vaginal infections was noted among young sexually active females at the age group of 26-30 years (37.5%) followed by 31-35 years (31.57%) and 21-25 years (27.02%). This was in concordance with studies done by Sangeetha *et al.*, Mumtaz *et al.*, and Khan and Khan.^{2,14,16} The frequency of culture positivity seems to decline progressively with increasing age.

The prevalence of AV among pregnant women in this study was 8.33%. It correlates with the study of Zodzika *et al.*, in 2010,¹⁷ which accounts for a prevalence rate of 10.79%. Donders *et al.*, 2009¹⁸ studied 759 pregnant women among which 8.3% had coccoid AV flora. The low incidence of AV among pregnant women in present study may be due to the fact that pregnancy is a period, in which the vaginal microbiota conditioned by high estrogen levels has a good supply of glycogen and a high percentage of lactobacillary flora which significantly reduces the multiplication of pathogenic organisms, more due to production of defense factors by lactobacilli.¹⁹

The maximum number of cases in this study was diagnosed with mild AV (80.76%). Moderate AV was reported in 15.38% of cases and severe AV in only 3.84% of cases which is in accordance with studies done by Zodzika *et al.* and Sobel *et al.*^{17,20}

About 92.31% of cases yielded single bacterial growth, whereas 7.69% yielded multiple organisms (two bacterial species in culture) which are nearby to study of Sangeetha *et al.* (India)¹⁴ which detected 88.77% single bacterial isolate and 19.23% multibacterial isolates. The present study is, in contrast, to study done by Razzak *et al.*, 2011 (Iraq),²¹ who observed 50 (47.62%) out of 105 cases as a polymicrobial organism.

In the present study, *S. aureus* (41.07%) was the most prevalent organism isolated from AV cases followed by *E. coli* (21.43%) and *Enterococcus* spp. (12.5%). In a study by Mumtaz *et al.* (Pakistan),¹⁶ *S. aureus* (46.07%) was the most prevalent isolated pathogen. Tansarli *et al.*⁴ and Zarbo *et al.*¹⁹ also reported a high prevalence of *S. aureus* which is 41.7% and 27.9%, respectively.

About 8.93% of the vaginal isolates in the present study were β -hemolytic streptococci. Similar rates have been observed by Mumtaz *et al.*¹⁶

The Gram-positive organisms in this study showed more resistance to penicillin and ampicillin. 39.1% of the *S. aureus* isolates were resistant to penicillin. Most of the β -hemolytic streptococci were sensitive to penicillin and is in tallying with Mumtaz *et al.*¹⁶ In most 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 results²² as clearly seen from the present study.

The most effective chemotherapeutic agents against Enterobacteriaceae were amoxy-clav, aminoglycosides moxifloxacin and meropenem which is in correlation with study done by Tariq *et al.*²³

Most of the *Pseudomonas* species were found resistant to piperacillin and ceftazidime, whereas 100% of the isolates were sensitive to piperacillin-tazobactam, gentamicin, and meropenem. Similar antibiogram pattern was observed Mumtaz *et al.*¹⁶

CONCLUSIONS

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. This study is also helpful in the prevention of neonatal morbidity and mortality using appropriate antibiotics along with their rational use to avoid emergence of drug resistance.

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How to cite this article: Nahar D, Soni G, Chand AE, Mourya S. Bacterial Etiology and their Antibigram in Aerobic Vaginitis Patients at Tertiary Care Hospital, Kota, Rajasthan. Int J Sci Stud 2016;4(3):103-107.

Source of Support: Nil, **Conflict of Interest:** None declared.