# Etiology of vaginal infections and antimicrobial resistance pattern of aerobic bacterial isolates in women of reproductive age group attending a tertiary care hospital

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# ABSTRACT

**Background:** The vaginal flora is a complicated environment containing dozens of microbiological species in variable quantities and relative proportions. Many women with vaginitis may remain undiagnosed and such forms of abnormal vaginal neither considered as normal nor can be called bacterial vaginosis and are termed as "intermediate flora" and its management probably differs from that of bacterial vaginosis. It is of crucial importance in pregnant females at risk of preterm delivery. **Materials and Methods:** Six hundred and ten high vaginal swabs both from indoor and outdoor patients were collected and cultured, and their susceptibility to various antimicrobials was determined by standard methods. **Result:** Significant growth was obtained in 357 (58.5%) samples. Among the positive samples, 314 were positive for aerobic bacterial isolates, and 43 showed the growth of *Candida* species. The common aerobic isolates were *Escherichia coli* (24.92%), *Klebsiella pneumoniae* (23.50%), *Staphylococcus aureus* (16.52%), *Enterococcus* species (8.40%), and coagulase-negative *Staphylococcus* (6.44%). About two-third of the positive samples (71.0%) were from outdoor patients, while one-third (29%) were from indoor patients. The highest frequency of infection was observed at 25-30 years followed by 31-35 years of age group. The prevalence of aerobic vaginitis cases was higher among nonpregnant compared to pregnant cases. The Gram-negative isolates showed maximum resistance to ampicillin, followed by aminoglycosides, and ciprofloxacin. The Gram-negative isolates showed maximum resistance to ciprofloxacin followed by ceftriaxone, ceftazidime, gentamicin, and amikacin. **Conclusion:** The high prevalence of gynecological infections demands that the patients with gynecological symptoms be investigated thoroughly. As the culture provides, the identification of causative microorganisms, it must invariably be done.

Key words: Aerobic vaginitis, antimicrobial resistance, vaginal infections

## **INTRODUCTION**

The vaginal microflora is a complex environment composed of varying microbiological species in variable quantities and relative proportions.<sup>[1]</sup>

Vaginitis is the inflammation and infection of vagina commonly encountered in clinical medicine. Whether infectious or not, vaginitis poses one of the most common problems in gynecology and is one of the main reasons, leading the females to seek advice from a physician approximately 10 million office visits annually.<sup>[2-4]</sup> Some pathological conditions causing vaginitis are well-defined such as bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis, yet, 7-72% of women with vaginitis may remain undiagnosed, and its management probably differs from that of bacterial vaginosis.<sup>[5]</sup> The problem is that some forms of abnormal vaginal microflora 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. This type of undefined abnormal flora may be of crucial importance in pregnant women at risk of preterm delivery.<sup>[5-7]</sup> 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.<sup>[8]</sup>

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 resistance pattern.

### MATERIALS AND METHODS

A retrospective study was carried out in a tertiary care center during a period of 1 year from January 2016 to December 2016. Vaginal swabs were taken from females in their reproductive age groups (15-45 years) with symptomatic vaginal discharge attending the gynecology department. A total of six hundred and ten high vaginal swabs were received in the microbiology

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laboratory for processing. All the samples were processed by standard methodology.<sup>[9]</sup> The aerobically incubated organisms were identified with the help of colonial morphology, gram staining, and biochemical analysis. Isolated organisms were subjected to sensitivity testing by Kirby-Bauer disc diffusion method, using Clinical Laboratory Standards criteria 2017, to interpret diameter of inhibition zone.<sup>[10]</sup>

# RESULTS

Of all the cultured samples (610), significant growth was obtained in 357 (58.5%) samples. Among the positive samples, 314 were positive for aerobic bacterial isolates, and 43 showed the growth of *Candida* species. About two-third of the positive samples (71.0%) were from outdoor patients, while one-third (29%) were from indoor patients. The highest frequency of infection was observed at 25-30 years followed by 31-35 years of age group [Table 1]. The prevalence of AV cases was higher among nonpregnant compared to pregnant cases [Figure 1].

There was greater predominance of Gram-negative isolates than Gram-positive isolates. The common aerobic isolates were *E. coli* (24.92%), *Klebsiella pneumoniae* (23.50%), *S. aureus* (16.52%), *Enterococcus* species (8.40%), and coagulase-negative *staphylococcus* (6.44%) [Table 2].

The Gram-positive organisms showed maximum resistance to ampicillin, followed by aminoglycosides (amikacin, gentamicin), and ciprofloxacin. Among the *E. faecalis*, only 3 (10.0%) showed high-level resistance to gentamicin (120  $\mu$ g), and 2 (6.6%) isolates were resistant to vancomycin (VAR). Among the *S. aureus*, 3 (5.08%) isolates were resistant to methicillin. All Gram-positive isolates were found to be sensitive to linezolid [Table 3a].

The Gram-negative isolates showed maximum resistance to ciprofloxacin followed by ceftriaxone, ceftazidime, gentamicin, and amikacin. Maximum sensitivity was shown to imipenem, sulbactam-ceftazidime, and piperacillin-tazobactam [Table 3b].

# 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.<sup>[11]</sup>

Vaginal flora of adult females contains Lactobacilli which helps in maintaining the vaginal pH and thereby prevent the overgrowth of potential pathogens, thus reducing the frequency of infections. However, antibiotics like broad-spectrum can kill or suppress helpful bacteria in the genital tract, allowing resistant organisms to grow unchecked.<sup>[12]</sup> AV is a syndrome due to the alteration of the vaginal biota with different bacterial, clinical, and immunological characteristics with respect to the classic form of vaginitis. From a clinical point of view, the infection is characterized by the presence of yellowish, bad-smelling secretions, redness, itching, and congestion of the vaginal mucosa and different levels of dyspareunia, toxic leucocytes, and parabasal epitheliocytes, negative KOH test, and raised vaginal

Table 1: Age wise distribution of AV cases				
Age group	Number of cases	Number of positive		
	studied ( <i>n</i> =610)	cases ( <i>n</i> =357) (%)		
15-20	30	6 (20.00)		
21-25	61	31 (52.45)		
26-30	279	182 (65.27)		
31-35	143	86 (60.10)		
36-40	65	35 (53.84)		
41-45	32	16 (50.00)		

AV: Aerobic vaginitis

Table 2	: Distribution	of	organisms	isolated	from
AV case	s				

Aerobic isolates	<b>n</b> (%)
E. coli	89 (24.92)
K. pneumoniae	84 (23.50)
S. aureus	59 (16.52)
Enterococcus species	30 (8.40)
Coagulase-negative Staphylococcus	23 (6.44)
Acinetobacter species	16 (4.48)
Pseudomonas species	9 (2.52)
Citrobacter species	3 (0.84)
Proteus species	1 (0.20)
Candida species	43 (12.04)

E.coli: Escherichia coli, S. aureus: Staphylococcus aureus, K. pneumoniae: Klebsiella pneumoniae, AV: Aerobic vaginitis



Figure 1: Distribution of aerobic vaginitis among pregnant and nonpregnant women

pH. AV is one of those diseases that is prevalently transmitted by sexual contact and if it is not diagnosed and treated early, during pregnancy can place the health of both the mother and the fetus at risk as it is associated with preterm birth, pPROM, and chorioamnionitis. It is therefore very important diagnosing it early and treating with a correct pharmacological therapy.<sup>[13]</sup> Therefore, this study was designed to assess the frequency of various aerobic pathogens in vaginal infections in females in the childbearing age group.

The prevalence of AV in our study is 58.5% which is found to be higher than that of a study done by Fan *et al.*, who reported the prevalence rate of  $23.74\%^{[14]}$  and Sangeetha *et al.*, who reported the prevalence of  $20.8\%^{[9]}$  Even higher prevalence of AV was observed by Cheng *et al.* and Razzak *et al.*, i.e. 80 % and 95.45%, respectively.<sup>[15,16]</sup>

The highest frequency of infection (65.27%) was observed at 25-30 years followed by 31-35 years of age group with a frequency of 60.01%. Mumtaz *et al.* in 2008 reported the

Table 3a: Antimicrobial resistance pattern in Gram-positive isolates				
Antimicrobial	<i>S. aureus</i> ( <i>n</i> =59)	E. faecalis (n=30)	Coagulase-negative	
			Staphylococcus (n=23)	
Ampicillin (10 mcg) (%)	71.9	60.0	NT	
Azithromycin (15 mcg) (%)	47.3	NT	7.8	
Amikacin (30 mcg) (%)	40.3	46.6	0	
Gentamicin (10 mcg) (%)	61.4	10.0	0	
Ciprofloxacin (5 mcg) (%)	57.8	50.0	0	
Amoxicillin-clavulanate (20 mcg/10 mcg) (%)	10.5	6.6	0	
VAR (30 mcg) (%)	0	3.3	0	
Linezolid (30 mcg) (%)	0	0	0	

S. aureus: Staphylococcus aureus, E. faecalis: Enterococcus faecalis, VAR: Vancomycin

Table 3b: Antimicrobial resistance patterns of Gram-negative isolates						
Antimicrobial	E. coli	K. pneumoniae	Pseudomonas	Acinetobacter	Proteus	Citrobacter
	( <i>n</i> =89)	( <i>n</i> =84)	species (n=9)	species (n=16)	species	species
					( <i>n</i> =1)	( <i>n</i> =3)
Ciprofloxacin (5 mcg) (%)	64.0	61.9	44.4	75.0	100	66.6
Amikacin (30 mcg) (%)	32.5	44.0	22.2	62.0	100	100
Gentamicin (10 mcg) (%)	47.1	51.1	33.3	68.5	100	100
Ceftriaxone (30 mcg) (%)	56.1	65.4	44.4	50.0	100	33-3
Ceftazidime (30 mcg) (%)	43.9	45.2	33.1	25.0	100	33-3
Sulbactam-Ceftazidime (30/10 mcg)	3.3	7.1	11.1	12.5	0	0
Piperacillin-Tazobactam (100 mcg/10 mcg) (%)	4.4	4.7	11.1	0	0	0
Imipenem (10 mcg) (%)	1.12	1.19	0	0	0	0

E.coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae

highest incidence of vaginal infections among young, sexually active females, at the two age groups, i.e., 31-40 years (39.5%), followed by 41-50 years (35.8%).<sup>[17]</sup> Another study by Khan and Khan (2004) showed that these infections are common at the age groups of 15-30 years followed by 31-40 years.<sup>[11]</sup> Although adult women have lactobacillus in their vaginal flora which serves as a check for other pathogenic organisms, other activities of these women like sexual activities and indiscriminate the use of antibiotics can predispose them to vaginal infections.

*E. coli* (24.92%) and *Klebsiella pneumoniae* (23.50%) were the most common aerobes isolated from the samples followed by *S. aureus*. In a study by Mumtaz *et al.*,<sup>[17]</sup> *S. aureus* (46.07%) was the most prevalent isolated pathogen. Tansarli *et al.* and Zarbo *et al.* also reported a high prevalence of *S. aureus* 41.7% and 27.9%, respectively.<sup>[13,18]</sup> In another study by Tariq *et al.*, the common isolates were *Enterococcus* spp. (14.7%), *E. coli* (10.2%), and beta-hemolytic *Streptococcus* spp. (7.3%).<sup>[19]</sup> Donders also stated the causes of AV as *E. coli*, *Enterococci, Staphylococcus* spp., and*E. coli* are cited as one of the most common causes of this vaginitis.<sup>[20]</sup>

In our study, the prevalence of AV in pregnant females was slightly lower (29.30%) than nonpregnant females (72.80%). The prevalence in pregnant in a study by Sangeetha *et al.*<sup>[8]</sup> was 7.14%. Donders *et al.*<sup>[20]</sup> in 2009 studied 759 pregnant women among which 8.3% had coccoid AV flora. The low incidence of AV among pregnant women in our 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 defence factors by Lactobacilli.<sup>[13]</sup>

In this study, common Gram-negative isolates E. coli and K. pneumoniae showed more resistance to ceftriaxone, ceftazidime, amikacin, and gentamicin and showed high sensitivity to beta-lactam/beta-lactam inhibitor combination and carbapenems (Table 3b). Study by Shamim et al. showed that the most effective chemotherapeutic agents against Gramnegative rods (E. coli and K. pneumoniae) were imipenem (96.0%, 100%), piperacillin/tazobactam (92.1%, 95.8%), whereas the antimicrobials with least affectivity against both of them were those belonging to the groups of penicillins, sulfonamides, and tetracyclines.<sup>[17]</sup> In the present study, the Gram-positive isolates showed maximum resistance to ampicillin, aminoglycosides, and ciprofloxacin and high sensitivity to linezolid, VAR, and amoxicillin-clavulanate [Table 3a]. A similar study by Nahar et al., the Gram-positive organisms showed more resistance to penicillin and ampicillin. About 39.1% of the S. aureus isolates were resistant to penicillin.[21]

AV is a frequent infection of the lower genital system, and it does not differentiate from other vaginal infections by its clinical characteristics. In high percentage, it is associated with other infections. It is very important to pay attention to the presence of AV as mixed infection or special entity when diagnosing vaginitis, especially in pregnancy. Therapeutic treatment of AV differentiates from other types of vaginitis and wrong diagnose can lead to wrong treatment and complications.<sup>[22]</sup> Our study concludes that aerobic vaginitis is a common female genital tract infection and most prevalent sexually active females whose vaginal ecosystem demonstrates an abnormal status of vaginal flora. The high prevalence of gynecological infections demands that the patients with gynecological symptoms are investigated thoroughly, and culture must invariably be done. The antibiotics showing good sensitivity must be used to eradicate the infection but with care to not destroy the normal vaginal flora. Hence, there is a need for an effective antimicrobial policy.

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