

RESEARCH ARTICLE

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## Aerobacterial Vaginosis among Women Attending an Infertility Clinic at a Tertiary Care Hospital in Chennai, India and Susceptibility Pattern of Isolates

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### Abstract

Genital infections and subsequent vaginosis diagnosed through high vaginal swab in women is caused due to fungi or bacteria. The presented study focused on determining the types, numbers and antibacterial susceptibility pattern of aerobic bacteria causing vaginosis in 147 female patients attending infertility centre in Chennai, Tamil Nadu, India. *Candida* spp. caused 17% of infections with 15.7% of vaginosis caused by *E.coli*, *Klebsiella* spp., *Acinetobacter* spp., *Citrobacter* spp. and Gram positive cocci - Methicillin Sensitive *Staphylococcus aureus* (MSSA), Coagulase Negative Staphylococci(CONS), *Staphylococcus aureus* and *Enterococcus* spp. causing 12.9% of vaginitis in the study population. A total of 20 different antibiotics - cell wall inhibitors, protein synthesis inhibitors and nucleic acid synthesis inhibitors; were tested to determine the response of bacterial isolates by Kirby-Bauer disc diffusion method. The study result determined that the most effective drug for treating Gram positive bacterial vaginitis as per CLSI guidelines based on susceptibility pattern as: Linezolid(100%), Gentamycin(91.6%), Amikacin(87.5%), Erythromycin(79.2%), Co-Trimoxazole(72.2%), Ciprofloxacin(65.6%) and least Chloramphenicol(44.3%). High level gentamycin(83.3%) was found to be effective in treating Enterococci. The descending order of susceptibility of Gram negative aerobacteria causing vaginitis as per CLSI guidelines are: Amikacin(87.5%), Gentamycin(82.5%), Cefoperazone sulbactam (76.3%), Ciprofloxacin(68.5%), Ceftazidime(62.5%) and least Amoxyclav(25%). All Gram negative bacteria tested were susceptible to— Imipenem and Meropenem as well as Chloramphenicol. Ceftriaxone (87.5%) and Nitrofurantoin(72.3%) among other antibiotics was effective against Gram negative bacteria while all Enterobacteriaceae members were found to be resistant to tetracycline.

**Keywords:** Vaginitis, Antibiotic Susceptibility pattern, Imipenem, Meropenem, Amikacin, CLSI guidelines, Nitrofurantoin

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## INTRODUCTION

Bacterial vaginosis (BV) occurs due to imbalance in biofilm bacteria of vagina especially inherent *Lactobacillus* spp. and the increased presence of opportunistic bacterial pathogen<sup>1</sup>. Predisposing factors for BV are generally associated with change in pH, increased glycogen levels, hormonal status, age, and demographic factors. Clinical symptom of BV is vaginitis presented as vaginal inflammation with accompanying vaginal discharge with fishy odour, itching, vaginal irritation etc. Vaginitis is one of the most frequently presented clinical picture among women reporting to Gynaecology division in hospitals. Grey-white thin vaginal discharge with fishy odour may be due to microbial causes such as Aerobic Vaginitis (AV), BV, Trichomonal Vaginitis (TV) or Vulvovaginal Candidiasis (VVC)<sup>2</sup>.

Women in the reproductive age group of 19-45 years are most susceptible to bacterial vaginosis. Worldwide prevalence rate of BV ranges from 5% - 70%<sup>3</sup> and in India the rate of prevalence is 30%<sup>4</sup>. Salpingitis occurs in 35% of women with BV and 2.5% of such patients become infertile<sup>5</sup>. Aerobic vaginitis and the treatment of infections in pregnant women is associated with higher incidence of premature rupture of membranes for the patient<sup>6</sup>. High prevalence (70%) of vulvovaginal candidiasis caused by *Candida* spp. leads to routine treatment with anticandidal drugs and recurrent candidiasis is prevalent<sup>7</sup>. The lower one-third of the vagina is home to hundreds of bacteria both aerobic and anaerobic belonging to gram-positive and gram-negative groups<sup>8</sup>. The collection of high vaginal swab and isolation of infective organism should be a mandated procedure as misdiagnosis is common in infertile women<sup>9</sup>. The existing method of treatment with antifungals for vaginosis is a cause for concern. The present study aims to identify and categorize the bacterial agents of vaginosis and analyse their antibacterial susceptibility pattern.

## MATERIALS AND METHODS

The study was conducted for a period of one year from April 2018 to March 2019. Patients reporting to infertility clinic in ACS Medical College and Hospitals, Chennai, Tamil Nadu, India were included in the study. The study was approved by the institutional Ethics committee

(Approval number: 001/04/2017/IEC/SU dated: 27/04/2017). Women patients in the reproductive age group 19-45 years were included in the study<sup>10</sup>. Patients having genital malignancy, antibiotic utilization by any route in the month prior to sample collection, having TV, *Gardnerella vaginalis* or *Neisseria gonorrhoea* and those above or below the reproductive age were excluded from the study<sup>11,12</sup>. A detailed questionnaire on age, medical history, infertility status and relevant parameters was requested and informed consent for inclusion in the study obtained. As per study design a total of 153 samples were collected and 6 were excluded as they did not match inclusion criteria.

High vaginal swab (HVS) in triplicate from each patient was collected as per standard protocol by trained nurses. In brief: Sterile speculum was inserted into vagina and from the vaginal walls samples were aseptically collected and placed in sterile saline, transported to microbiology laboratory for investigations<sup>13</sup>. First HVS swab was used for bacterial cytology by Gram staining and second HVS sample used for wet mount analysis. Third HVS sample was used for isolation of bacteria using MacConkey agar and Blood agar<sup>14</sup>. The culture plates were incubated at 37°C overnight for cultivation of aerobacteria<sup>15</sup>. The suspected growth of aerobacteria on culture plates were subjected to various biochemical tests as per standard guidelines in Quality assured microbiology laboratory at Department of Microbiology, ACS Medical College and Hospitals<sup>16,17</sup>.

Kirby Bauer disk diffusion method for antibiotic susceptibility testing was performed as per CLSI guidelines<sup>18</sup>. The Muller-Hinton agar (MHA) plates were swabbed with 0.5/ 1.0 McFarland standard broth culture of bacteria. Standard commercially available antibiotic discs (Hi-Media, India) were placed on the surface of MHA plate as per the pattern wherein 24 mm distance maintained between adjacent discs and 20 mm distance between edge of plate and disc. The plates were incubated at 35°C ± 2°C overnight and results recorded<sup>19</sup>. Antibiotics included in susceptibility testing of bacterial isolates were as follows: Amikacin (AK; 30 mcg/disc), Amoxycylav (AMC; 30 mcg/disc), Ampicillin (AMP; 10 mcg/disc), Cefoperazone sulbactam (CFS; 50/50 mcg/disc), Cefotaxime (CTX; 30 mcg/disc), Ceftazidime

(CAZ; 30 mcg/disc), Ceftriaxone (CTR; 30 mcg/disc), Chloramphenicol (C; 30 mcg/disc), Ciprofloxacin (CIP; 5 mcg/disc), Co-Trimoxazole (COT; 25 mcg/disc), Erythromycin (E; 15 mcg/disc), Gentamycin (G; 10 mcg/disc), High Level Gentamycin (HLG; 120 mcg/disc), Imipenem (IPM; 10 mcg/disc), Linezolid (LZ; 10 mcg/disc), Meropenem (MRP; 10 mcg/disc), Nitrofurantoin (NIT; 300 mcg/disc), Norfloxacin (NX; 10 mcg/disc), Penicillin (P; 10 mcg/disc), and Tetracycline (T; 10 mcg/disc). Data analysis was done using IBM SPSS Statistics for Windows, version XX (IBM Corp., Armonk, N.Y., USA) and results presented.

## RESULTS

The presented study documented a cross sectional study for the presence of microbial agents in 45.6% of patients from a total of 147 female patients attending infertility clinic – screened for vaginal infections and the results calculated using excel calculator. *Candida* species was present in 17% of patients. 28.6% of infertility patients had bacteria as etiological agents in vaginitis (Table 1). Gram positive cocci(12.7%) and Gram negative bacteria (15.7%) made-up the 28.6% of isolates. Methicillin Sensitive *Staphylococcus aureus*(MSSA; 15.8%), Coagulase Negative *Staphylococcus*(CONS; 42.1%), *Staphylococcus aureus* (10.5%) and *Enterococcus* species(31.6%) were the gram positive cocci isolated from high vaginal swab(HVS) samples. The four Gram negative Enterobacteriaceae causative agents of vaginitis in the study were *E. coli*(43.5%), *Klebsiella* species(21.7%), *Acinetobacter* species(17.4%) and *Citrobacter* species(17.4%). Maximum infections

were observed in 26-30 years age group of patients with age group less than 20 years having the least infection at 2.4% (Table 2). Six HVS samples from patients showed mixed infections: out of the six mixed infections four were of two different bacteria with three mixed infections involving two Enterobacteriaceae members and one involving *E.coli* and *Enterococcus* sp. One of the remaining two mixed infections was of *E.coli* and *Candida* with the remaining mixed infection involved three microbes - *E.coli*, *Acinetobacter* sp. and *Candida*. Gentamycin at 91.7% susceptibility was the antibiotic of choice against all types of Staphylococci with Chloramphenicol being the least effective of

**Table 1.** Microbial etiology in HVS samples of infertility patients

Result/ Isolated Microorganism	Frequency (n)	Percentage
Negative	80	54.4
Fungi	25	17
<i>Candida</i> spp	25	100
Gram Positive Bacteria	19	12.9
Methicillin Sensitive <i>Staphylococcus aureus</i>	3	15.8
Coagulase Negative <i>Staphylococcus aureus</i>	8	42.1
<i>Staphylococcus aureus</i>	2	10.5
<i>Enterococcus</i> spp.	6	31.6
Gram Negative Bacteria	23	15.7
<i>E.coli</i>	10	43.5
<i>Klebsiella</i> spp.	5	21.7
<i>Acinetobacter</i> spp.	4	17.4
<i>Citrobacter</i> spp.	4	17.4
Total	147	100

**Table 2.** Demography of aerobacterial isolates from causing vaginitis in infertility patients

Age group - years	MSSA n(%)	CONS n(%)	S.a n(%)	Entero. spp. (%)	<i>E.coli</i> (%)	Kleb. spp. (%)	<i>Acineto.</i> spp (%)	<i>Citro.</i> spp. (%)	Total (%)
>20	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(2.4)
21-25	1(7.7)	4(30.7)	1(7.7)	3(23.1)	1(7.7)	1(7.7)	2(15.4)	0(0)	13(30.9)
26-30	2(13.3)	2(13.3)	1(6.7)	3(20)	4(26.7)	1(6.7)	0(0)	2(13.3)	15(35.7)
31-35	0(0)	1(33.3)	0(0)	0(0)	2(66.7)	0(0)	0(0)	0(0)	3(7.2)
36-40	0(0)	0(0)	0(0)	0(0)	2(50)	0(0)	2(50)	0(0)	4(9.5)
<41	0(0)	0(0)	0(0)	0(0)	1(16.7)	3(50)	0(0)	2(33.3)	6(14.3)
Total	3(7.1)	8(19)	2(4.8)	6(14.3)	10(23.8)	5(11.9)	4(9.5)	4(9.5)	42(100)

MSSA - Methicillin Sensitive *Staphylococcus aureus*; CONS - Coagulase Negative *Staphylococcus aureus*; S.a- *Staphylococcus aureus*; Entero. spp.- *Enterococcus* species; Kleb. spp.- *Klebsiella* species; Acineto.spp.- *Acinetobacter* species; Citro. spp.- *Citrobacter* species; n(%) - frequency (percentage)

**Table 3.** Antibacterial Susceptibility pattern of Gram Positive cocci associated with vaginitis in infertility patients including CLSI guidelines

Organism	n	AMP n(%)	AK n(%)	COT n(%)	C n(%)	CIP n(%)	E n(%)	G n(%)	HLG n(%)	LZ n(%)	P n(%)
MSSA	3	NT	3(100)*	2(66.6)	1(33.3)	1(33.3)	3(100)	3(100)	NT	2(100)*	NT
CONS	8	NT	8(100)*	4(50)	4(50)	5(62.5)	3(37.5)	6(75)	NT	7(100)*	NT
<i>Staph. aureus</i>	2	NT	2(100)*	2(100)	1(50)	2(100)	2(100)	2(100)	NT	NT	NT
<i>Enterococcus</i>	6	5(83.3)	3(50)	NT	NT	4(66.6)	2(50)*	NT	5(83.3)	6(100)	4(66.6)

n- Number of isolates; n(%) - Number of susceptible isolates (percentage susceptible); AMP- Ampicillin 10 µg/disc; AK- Amikacin 30 µg/disc; CO- Co-Trimoxazole 25 µg/disc; C- Chloramphenicol 30µg/disc; CIP- Ciprofloxacin 5µg/disc; E- Erythromycin 15 µg/disc; G- Gentamycin 10 µg/disc; HLG- High level Gentamycin 120 µg/disc; LZ- Linezolid 10 µg/disc; Penicillin 10µg/disc; T- Tetracycline 10 µg/disc; NT- Not Tested; MSSA - Methicillin Sensitive *Staphylococcus aureus*; CONS- Coagulase Negative *Staphylococcus aureus*; *Staph. aureus*- *Staphylococcus aureus*; *Enterococcus*- *Enterococcus* species; \*- NOT INCLUDED IN CLSI GUIDELINES

**Table 4.** Antibacterial Susceptibility pattern of Enterobacteriaceae members isolated from infertility patients as per CLSI guidelines

Organism	n	AMP n(%)	AK n(%)	AMC n(%)	CFS n(%)	CAZ n(%)	CIP n(%)	G n(%)
<i>E.coli</i>	10	4(40)	10(100)	0(0)	8(80)	9(90)	5(50)	8(80)
<i>Klebsiella</i> species	5	0(0)	5(100)	0(0)	5(100)	3(60)	5(100)	4(100)
<i>Acinetobacter</i> species	4	2(50)	4(100)	2(100)	3(75)	3(75)	1(25)	3(75)
<i>Citrobacter</i> species	4	1(25)	2(50)	0(0)	2(50)	1(25)	4(100)	3(75)

n- Number of isolates; n(%) - Number of susceptible isolates (percentage susceptible); AMP- Ampicillin 10 µg/disc; AK- Amikacin 30 µg/disc; AMC- Amoxycylav 30 µg/disc; CFS- Cefoperazone sulbactam 50/50 µg/disc; CAZ- Ceftazidime 30 µg/disc; CIP- Ciprofloxacin 5 µg/disc; G- Gentamycin 10 µg/disc.

**Table 5.** Antibacterial Susceptibility pattern for miscellaneous antibiotics against Enterobacteriaceae members isolated from infertility patients

Organism	C n(%)	CTX n(%)	CTR n(%)	IPM n(%)	MRP n(%)	NIT n(%)	NX n(%)	T n(%)
<i>E.coli</i>	1(100)	4(40)	3(75)	2(100)	2(100)	4(80)	6(66.6)	0(0)
<i>Klebsiella</i> species	NT	3(60)	NT	NT	NT	3(100)	4(100)	0(0)
<i>Acinetobacter</i> species	1(100)	0(0)	NT	1(100)	1(100)	2(50)	3(75)	0(0)
<i>Citrobacter</i> species	1(100)	1(25)	1(100)	1(100)	2(100)	3(75)	1(50)	0(0)

n(%) - Number of susceptible isolates (percentage susceptible); C- Chloramphenicol 30 µg/disc; CTX- Cefotaxime 30 µg/disc; CTR- Ceftriaxone 30 µg/disc; IPM- Imipenem 10 µg/disc; MRP- Meropenem 10 µg/disc; NIT- Nitrofurantoin 300 µg/disc; NX- Norfloxacin 10 µg/disc; T- Tetracycline 10 µg/disc.

all the CLSI guideline antibiotic for Staphylococci. Amikacin and Linezolid among the non-CLSI panel of antibiotic tested in our study was found to be the most effective drug against Staphylococci with all strains tested being 100% susceptible to these two antibiotics. Enterococci isolated from infertility patients were found to be 100% susceptible to Linezolid followed by 83.3% susceptibility to High

level gentamycin. *Enterococcus* sp. were least susceptible to Ciprofloxacin and Penicillin (Table 3).

Antibiotic susceptibility pattern against the four Enterobacteriaceae – *E.coli*, *Klebsiella* sp., *Acinetobacter* sp. *Citrobacter* sp.; isolated from HVS (Table 4) as per CLSI guidelines indicated Amikacin as the drug of choice in treating such infections as 21 out of 23 strains were determined

to be susceptible. Ampicillin at 28.8% susceptibility followed by Amoxyclav at 25% were determined to be least effective against Enterobacterial pathogens. All the Enterobacteriaceae tested were found to be susceptible to Imipenem, Meropenem and Chloramphenicol. The descending order of effective antibacterials (not included in CLSI guidelines) against enterobacteria as determined in the study was Ceftriaxone, Nitrofurantoin, Nalidixic acid and cefotaxime (Table 5).

## DISCUSSION

*Candida* sp. as the major isolate in HVS samples is in agreement with previous studies done by Abdul-Aziz et al. (2019). This high prevalence is the primary reason for the empirical treatment of vaginitis using antifungals. The high prevalence at 62.7% of bacterial infections in all culture positive among in vaginal swabs of women with infertility mandates the regular isolation of pathogens in women with vaginitis. Prolonged bacterial and fungal infections may cause tubal disorders leading to infertility as documented in studies by Sukatendel et al in 2019. The presence of Chlamydial infections which can be observed in stained tissue sections or serodiagnosed as done in studies done by the authors implies the role of microorganisms in development of infertility in females. The age group 21 to 30 years had the most number of cases with active infectious agents in the vagina implicating the correlation between active sexual behaviour and infections<sup>20</sup>.

Antibiotic susceptibility of all Gram positive bacterial isolates in the study validates the retainment of Gentamycin with 91.7% susceptibility (Table 3) by all strains of Staphylococci which was similar to results from studies by Poutrel et al. (2018) and High-Level Gentamycin at 83.3% susceptibility by Enterococci<sup>23</sup>. Linezolid (100%) is the drug of choice to treat Enterococci which is similar to studies by Mendes et al (2018) where they reported more than 95% susceptibility by Enterococci. Amikacin and Linezolid with 100% efficacy against Staphylococci in the group of antibacterial not recommended in CLSI for treatment is the novel finding in this study that validates such studies and warrants the setting up of National treatment protocols with probably region-specific treatment regimens and also supported by report of Sharma et al (2018).

Antibiotics like Co-Trimoxazole and Ciprofloxacin that inhibit nucleic acid biosynthesis had an intermediate type of response. The present study documents the higher efficacy in treatment of Gram positive bacteria with protein synthesis inhibiting antibiotics like – Linezolid, Amikacin, Gentamycin or Erythromycin and lower response to cell wall inhibiting antibiotic – Ampicillin which is in agreement with the studies by Bush and Bradford (2019) that documents the production of beta-lactamases.

The present study identified the protein synthesis inhibiting antibiotics – Amikacin and Gentamycin as the being most active against gram negative bacteria isolated from the study population based (Table 4). Third generation cephalosporins – Ceftazidime and Cefoperazone/Sulbactam were most effective against Gram negative isolates with other cell wall inhibitors having activity in less than 29% of cases as documented by Krishnasamy et al (2019). Combination of third generation with  $\beta$ -lactamase inhibitors especially sulbactam increased the efficiency of the drug<sup>28</sup>.

Treatment of Enterobacteriaceae with chloramphenicol is most warranted as all strains tested were found susceptible while all strains are resistant to tetracycline<sup>15</sup>. All strains tested in the study were susceptible to Imipenem and Meropenem the recommended second line antibiotic (Table 5). Treatment with nitrofurantoin may be done on a case by case basis as it was found to be efficient against target bacteria in 76.3% cases<sup>30</sup>. Statistical analysis for significance gives data that drugs with high susceptibility rates were significant. This analysis does not take into consideration that antibiotic susceptibility varies between strains and susceptibility testing of each isolate is crucial to completely resolving infections.

## CONCLUSION

*Candida* is the most prevalent organism in vaginitis. Gram positive cocci and gram negative rods were almost equivalent in causing bacterial vaginosis. Overall, Amikacin was found to be the most effective antibiotic. Protein synthesis inhibiting group of antibiotics were found to have greater impact in treatments based on the susceptibility pattern of isolates in the study. Imipenem and Meropenem were found to be

effective and their use should be restricted to special cases.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHORS' CONTRIBUTION

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

#### FUNDING

None.

#### DATA AVAILABILITY

All datasets generated or analysed during this study are included in the manuscript.

#### ETHICS STATEMENT

The study has been approved by the Institutional Research committee and Institutional Human Ethical committee.

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