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Research Article

vaginitis

Candida and Aerobic Bacterial Isolates with Antibiotic Susceptibility pattern from vaginitis among Reproductive age group women in a Tertiary Care Center, a Retrospective Analysis

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Introduction: Vaginitis, a common clinical condition among women of reproductive age, results in considerable morbidity. This study aimed to analyze the frequency of Candida and aerobic bacterial isolates from the vagina with antibiotic susceptibility testing. **Methods:** A retrospective study was done in a tertiary care center, South India, Kolar. Data was collected from one year. Two high vaginal swabs collected were subjected to Gram staining, culture. The isolate was identified phenotypically, and an antibiotic sensitivity test was performed. **Result:** Prevalence of aerobic and Candidal vaginitis is 19.43% in the age group of women between 16 to 53years. The majority of the subjects, 50.8%, belongs to the 21-25yrs age group, and the highest positivity was seen among women in the age group 16 to 20 years. Of the 97 isolates, bacterial isolates included 60(61.85%), 37(38.15%) had Candida species. The most common etiological agent in aerobic vaginitis was Escherichia coli (E Coli), followed by Staphylococcus aureus. 35(94.60%) were Candida albicans, 1(2.70%) each was Candida krusei and Candida paropsilosis Gram-negative organisms showed more sensitivity to Carbapenems, least sensitivity was seen among Ampicillin, third-generation Cephalosporin Piperacillin and Ciprofloxacin. **Conclusion:** Gynecological conditions like vaginitis and vaginosis causes severe morbidity and mortality especially in pregnancy which needs to be addressed appropriately and adequately hence identification of the etiologic agents becomes the necessity to provide adequate and appropriate treatment

Keywords: Aerobic vaginitis, Candida, Escherichia coli, Antibiotic susceptibility testing

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Introduction

The healthy flora of the vagina, made up of diverse microorganisms in different quantities and ratios, is predominated by *Lactobacilli* responsible for acidic PH, remaining in harmony with the host [1]. Vaginitis (inflammation of the vagina) or vaginosis is a common clinical condition among women of reproductive age group where other diverse pathogenic agents replace normal vaginal flora [2]. The term Aerobic Vaginitis (AV) and Bacterial Vaginitis (BV), though there is a thin silver line to differentiate these two terms where there is a dysbiotic outgrowth of typical commensal flora, though they may coexist, they are different entities having different treatment options and clinical risk [3, 4].

Etiology among AV differs from BV, including aerobic and facultative anaerobic bacteria. Anaerobic bacteria are mainly associated with BV [3]. Other agents like *Candida*, *Trichomonas vaginitis and* Herpes *simplex* implicates different complications [2]. Commensals become opportunistic pathogens if the environment favors their overgrowth [5]. 3/4th of the women is most likely to experience Vulvovaginal Candidiasis (VVC) in their life time which often leads to recurrences. Treating appropriately and adequately is the need of the hour [6]. Though *Candida albicans* has been reported to be the commonest isolate in most of the studies, there is some proportion of non-albicans *Candida* that is responsible for treatment failure with first-line antifungal agents [7].

These conditions, if not diagnosed, can lead to various complications like pelvic inflammatory disease, infertility, ectopic pregnancy, reproductive dysfunction. Vaginal infections in pregnancy lead to serious consequences like miscarriage, chorioamnionitis, premature rupture of membranes, and preterm delivery. Ascending infection during pregnancy may also result in maternal complications such as sepsis, septic arthritis, maternal respiratory distress and even menstrual toxic shock syndrome [8]. Though the etiology differs, there are no pathognomic clinical features to differentiate any entities like bacterial vaginitis, aerobic vaginitis, vaginal candidiasis, which can occur either as a single or mixed infection. The syndromic approach in the diagnosis and treatment may often be unsuccessful, Resulting in complications. Therefore, microbiological evaluation has considerable value in providing appropriate treatment and reducing complications. This study aimed to analyze the frequency of Candida and aerobic bacterial isolates from the vagina with their antibiotic susceptibility testing.

Material and methods

This study was conducted in the Department of Microbiology and Obstetrics and Gynecology at Sri Devaraj Urs Medical College, South India, Kolar. It is a cross-sectional observational, retrospective study. Data for one year (January 2019 to February 2020) was collected from the Laboratory information system in the Central diagnostic laboratory services, Microbiology section and was entered in an Excel sheet and analyzed.

Inclusion criteria: Symptomatic women with complaints of vaginal discharge in the reproductive age group who attended Obstetrics and Gynecologic outpatient and inpatient department were enrolled in the study.

Two high vaginal swabs were collected from each woman. One swab was used for Gram staining, and the other swab was used for inoculation into culture media. 5% sheep Blood agar, Chocolate agar and Mac Conkey agar were used for inoculation incubated at 37oC for 48hrs at atmospheric air. Chocolate agar was incubated in a CO_2 incubator. The colonies were identified phenotypically by morphological characteristics, Gram staining reactions and standard biochemical tests [9]. The isolate was considered significant if the Gram staining picture showed a considerable number of pus cells, Para basal epithelial cells, altered microbial flora with scanty or no lactobacilli associated with predominant heavy to moderate growth of one or two organisms and the absence or suppression of normal flora Candida has grown on the culture media were identified by Gram staining and speciated by inoculation onto Hichrom agar which was recognized by the colour of the colony.

Antimicrobial susceptibility test: Antibiotic susceptibility testing of all the bacterial isolates was done on Mueller Hinton agar (MHA) plate by modified Kirby Bauer's disc diffusion technique [10]. Following antibiotic disc (procured from Hi-Media Mumbai) was used according to CLSI (Clinical and

Laboratory Standards Institute, 2018) guidelines [11].

Amoxycillin/ Clavulanic acid (20/10mcg), Gentamicin (10mcg), Ciprofloxacin (5mcg), Tetracycline (30mcg), Doxycycline (30mcg), Linezolid (30mcg), Erythromycin (15mcg), and Clindamycin (2mcg), Cotrimoxazole(1.25/23.75mcg), Chloramphenicol(30mcg) were put for Gram positive organisms and Gentamicin (10mcg), Amikacin (30mcg), Tobramycin (10mcg), Imipenem (10mcg), Meropenem (10mcg), Ertapenem (10mcg) Piperacillin/Tazobactam (100/10mcg), Cefepime (30mcg), Ampicillin (10mcg), Amoxycillin Clavulanic acid (20/10mcg), Ceftriaxone (30mcg), Cefotaxime (30mcg), Piperacillin (100mcg), Cotrimoxazole (1.25/23.75mcg), Chloramphenicol (30mcg), Ciprofloxacin (5mcg), Levofloxacin (5mcg), Doxycycline (30mcg), Tetracycline (30mcg) were put for Gram negative organisms. For non-fermenters following antibiotics were used, Amikacin(30mcg), Gentamicin (10mcg), Tobramycin (10mcg), Piperacillin (100mcg), Piperacillin/Tazobactam (100/10mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), Cefotaxime (30mcg), Ciprofloxacin (5mcg), Levofloxacin (5mcg), Imipenem (10mcg), Meropenem (10mcg).

The inhibition zone diameter was measured and interpreted according to the standards recommended by CLSI 2018.

Statistical analysis: Data were entered into a Microsoft Excel datasheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions.

Graphical representation of data: MS Excel and MS word were used to obtain various types of graphs.

P-value (Probability that the result is true) of <0.05 was considered statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

Results

The total number of samples received in the microbiology laboratory included 463.

Out of 463 subjects, growth was positive in 90(19.43%), which say the prevalence of vaginitis is 19.43%. AV included 11.44 % (53/463), and Candidal vulvovaginitis had 7.99 % (37/463).

The age group of the women was between 16 to 53years. The majority of the subjects, 50.8%, belongs to the 21-25yrs age group, and the isolation rate was more in the 16 to 20 years age group (Table 1).

Table 1: Distribution of subjects studied and positive cases according to age group.

Age group	Number of cases studied	Number of positive cases
16-20yrs	97(21%)	27(27.8%)
21-25yrs	235(50.8%)	41(17.4%)
26-30yrs	102(22%)	17(16.66%)
31-35yrs	18(3.9%)	1(5.5%)
36-40yrs	8(1.7%)	3(37.5%)
>40yrs	3(0.6%)	1(33.3%)
Total	463	90(19.43%)

Ninety-seven isolates were obtained from the 90 women with positive growth, of which bacterial isolates included 60(61.85%) of which Gram-negative were 43(71.66%), Gram-positive were 17(28.34%), and 37(38.15%) included Candida species (Figure 1 &2).

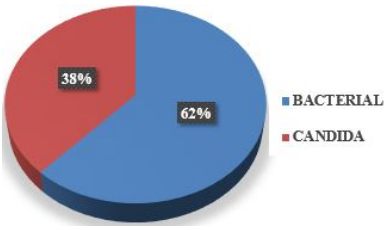

Figure 1: Percentage of aerobic bacterial and Candida isolates.

Total 97isolates among these bacterial isolates were 60 (61.85%), and Candida were 37 (38.15%).

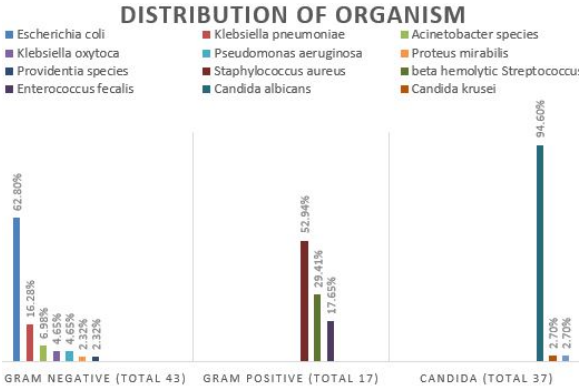

Figure 2: Total isolates.

The most common etiological agent in aerobic vaginitis was *Escherichia coli* (E Coli), followed by *Staphylococcus aureus* (Table 2).

Table 2: **Distribution of organisms isolated from the study subjects.**

Organism	Frequency(n)	Percentage (%)
Candida albicans	35	38.89
Escherichia coli	27	30
Staphylococcus aureus	9	10
Klebsiella pneumoniae	7	7.77
β haemolytic Streptococcus	5	5.55
Enterococcus species	3	3.33
Acinetobacter species	3	3.33
Pseudomonas aeruginosa	2	2.22
Klebsiella oxytoca	2	2.22
Proteus mirabilis	1	1.11
Providentia species	1	1.11
Candida krusei	1	1.11
Candida Paropsilosis	1	1.11

Out of 90 subject's single organism was found in 83(92.33%) subjects, and more than one organism was present in 7(7.77%) subjects (Figure 3& 4).

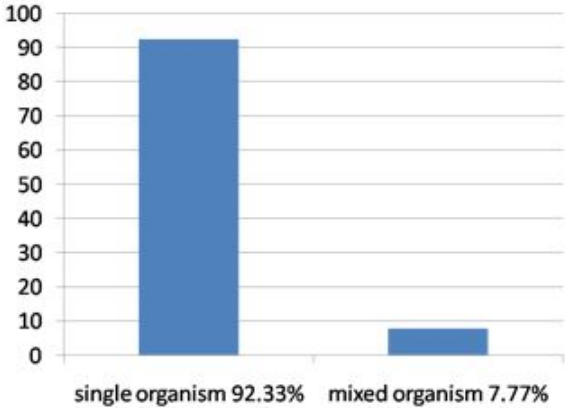

Figure 3: Percentage of single and mixed organisms.

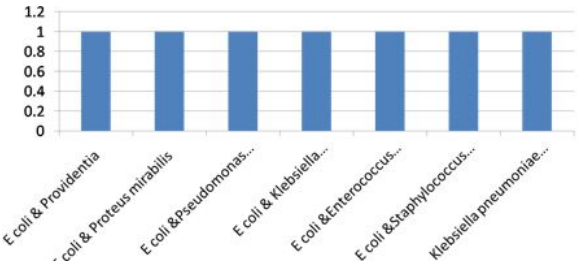

Figure 4: Distribution of mixed organism.

Enterobacteriaceae group of organisms showed higher sensitivity to Carbapenems, Chloramphenicol, Amikacin, and combination drugs Like Ceftazidime & Clavulanic acid, Piperacillin & Tazobactam.

Moderate sensitivity was seen among Gentamicin, Tobramycin, Doxycycline, Cotrimoxazole and Levofloxacin. Least sensitivity was seen among Ampicillin, Ampicillin & Clavulanic acid, third-generation Cephalosporin like Ceftriaxone & Cefotaxime, Ceftazidime, Piperacillin and Ciprofloxacin (Table 3).

Table 3: Antibiotic sensitivity of Enterobacteriaceae group of organisms.

Antibiotic	E coli (n=27) %	K. pneumoniae (n=7) %	K. oxytoca (n=2) %	Providentia (n=1) %	Proteus mirabilis(n=1) %	Overall sensitivity %
PI	(3)11.1	(2)28.5	00	00	00	7.92
PIT	(21)77.7	(4)57.1	(2)100	00	(1)100	66.96
AMP	(3)11.1	(1)14.2	00	00	00	5.06
AMC	(3)11.1	(2)28.5	00	00	00	7.92
CTR	(3)11.1	(2)28.5	00	00	00	7.92
CTX	(4)14.8	(2)28.5	00	00	00	8.66
CAZ	(4)14.8	(3)42.8	00	00	00	11.52
CAC	(19)70.3	(4)57.1	(2)100	00	(1)100	65.48
COT	(15)62.9	(5)71.4	00	(1)100	00	46.86
AK	(22)81.4	(4)57.1	(2)100	(1)100	00	67.7
GEN	(17)62.9	(6)85.7	(1)50	(1)100	00	59.72
TOB	(15)55.5	(6)85.7	(1)50	(1)100	00	58.24
CIP	(11)40.7	(3)42.8	00	(1)100	00	36.7
LE	(12)44.4	(4)57.1	00	(1)100	00	40.3
C	(23)85.1	(4)57.1	(2)100	(1)100	00	68.44
DO	(11)40.7	(3)42.8	00	(1)100	(1)100	56.7
IPM	(15)55.5	(4)57.1	(1)50	(1)100	NT	65.65
MRP	(27)100	(4)57.1	(2)100	(1)100	(1)100	91.42
ETP	(22)81.4	(4)57.1	(2)100	(1)100	00	67.7

PI- Piperacillin, PIT- Piperacillin/Tazobactam, AMP- Ampicillin, AMC- Amoxycillin/ Clavulanic acid, CTR- Ceftriaxone, CTX- Cefotaxime, CAZ- Ceftazidime, CAC- Ceftazidime/ Clavulanic acid/ COT- Cotrimoxazole, AK- Amikacin, GEN- Gentamicin, TOB- Tobramycin, CIP- Ciprofloxacin, LE- Levofloxacin, C- Chloramphenicol, DO- Doxycycline, IPM- Imipenem, MRP- Meropenem, ETP- Ertapenem, NT- Not Tested.

Non-fermenters like Acinetobacter species and Pseudomonas aeruginosa showed 100% sensitivity to Carbapenems, Aminoglycosides, Fluoroquinolones and Cotrimoxazole. Moderate sensitivity was seen among Ceftriaxone, Cefotaxime, Ceftazidime, Piperacillin, and Piperacillin/ Tazobactam (Table 4).

Table 4 Antibiotic sensitivity of non-fermenters.

Antibiotic	Acineto bacter (n=3) %	Pseudo monas aeruginosa (n=1) %	Overall sensitivity %
PI	(1)33.3	(1)100	66.65
PIT	(1)33.3	(1)100	66.65
CTR	(2)66.6	NT	66.6
CTX	(2)66.6	NT	66.6
CAZ	(1)33.3	(1)100	66.65
COT	(3)100	NT	100
AK	(3)100	(1)100	100
GEN	(3)100	(1)100	100
TOB	(3)100	(1)100	100
CIP	NT	(1)100	100
LE	NT	(1)100	100
IPM	(3)100	(1)100	100
MRP	(3)100	(1)100	100

PI- Piperacillin, PIT- Piperacillin/ Tazobactam, CTR- Ceftriaxone. CTX- Cefotaxime, CAZ- Ceftazidime, COT- Cotrimoxazole, AK- Amikacin, GEN- Gentamicin, TOB- Tobramycin, CIP- Ciprofloxacin, LE- Levofloxacin, IPM- Imipenem, MRP- Meropenem, NY- Not Tested.

Table 5: Antibiotic sensitivity of Gram-positive organisms.

Antibiotic	Staphylococcus aureus(n=9) %	β haemolytic Streptococcus (n=5) %	Enterococcus faecalis (n=3) %	Overall sensitivity %
AMC	(2)22.2	NT	NT	22.2
P	00	(5)100	NT	50
E	(1)11.1	(1)20	00	10.36
CD	(4)44.4	(3)60	NT	52.2
TE	(7)77.7	(2)40	(1)33.3	50.33
DO	(9)100	NT	(1)33.3	66.65
COT	(7)77.7	NT	NT	77.7
C	(7)77.7	(4)80	(3)100	85.9
GEN	(6)66.6	NT	NT	66.6
CIP	(1)11.1	NT	NT	11.1
LE	NT	00	(1)33.3	16.65
LZ	(9)100	(5)100	(3)100	100
VA	NT	(5)100	(3)100	100
HLG	NT	NT	(2)66.6	66.6
AMP	NT	NT	(2)66.6	66.6

AMC- Amoxycillin/ Clavulanic acid, P- Penicillin, E- Erythromycin, CD- Clindamycin, TE- Tetracycline, DO- Doxycycline, COT- Cotrimoxazole, C- Chloramphenicol, GEN- Gentamicin, CIR- Ciprofloxacin, LE- Levofloxacin, LZ- Linezolid, VA- Vancomycin, HLG- High-level Gentamicin, AMP- Ampicillin, NT- Not Tested.

Discussion

The etiology of vaginitis is primarily due to microbial causes, which can be detected in the laboratory by standard techniques [3]. Misdiagnosis of AV as any other cause of vaginitis leads to incorrect treatment, leading to complications like desquamative inflammatory vaginitis, which can be deleterious in pregnancy [4]. Vulvovaginal candidiasis being the second most common infection of the vulvovaginal area, requires specific treatment with antifungal agents, [5]. the prevalence of which in our study was 7.99% Prevalence of aerobic vaginitis in different studies when compared to this study (11.43%) was higher. It was reported as 26%, 21.25 and 20.8% in the study conducted by Nahar et al. [12] Krishnaswamy et al. [13]. (2019) and Sangeetha et al. [14]. (2015) respectively. Still higher rate of 58.5% was reported by Pal K et al. [15]. (2017). The study by Wang ZL et al. (2016) showed an almost similar frequency of AV as that of our study as 15.40% [16].

Overall vaginal infection in our study due to AV & VVC was 19.43%. The other causes of vaginitis due to *Trichomonas* BV were not included, as their diagnostic methods mainly rely on microscopy. A retrospective study in Solapur by Shaikh et al. showed the overall prevalence of vaginitis as 37%, and AV and VVC was 66.48% and 21.62%, respectively [17]. In another study AV & VVC reported was 51% & 17% respectively [3]. Looking into this prevalence of vaginitis mandates the isolation and identification of the organism followed by susceptibility testing. Our study had the frequency of infection higher in the age group of 16 to 20 years (27.8%) followed by 21 to 25 years (17.4%). Our institute having access to the rural population, early marriage, and early sexual activity may be the reason for this population group having the highest frequency.

25 to 30 years followed by 31 to 35 years had the highest frequency of infection in the study by Pal et al. [15]. Sangeetha KT et al. [14] and Krishnaswamy L et al. [13]. Mumtaz et al. noted the highest frequency of AV among 31 to 40 years (39.5%) followed by 41 to 50 years (35.8%) [18]. Vulvovaginal candidiasis was reported to be 13.1% and 5.3%, highest in the age group of 30 to 39years and 18 to 25years, respectively [19].

There is a vast difference in the *Candida* species isolated from the vagina worldwide, which depends on various factors such as geographical area, presence of risk factors like diabetes, use of antibiotics, type of population studied. However, *Candida albicans* (94.60%) is the commonest species of VVC in our study, and there was an increase in non-albicans Candida (58%) isolated. The main species were *Candida glabrata* (71%) and *Candida krusei* (29%) [20].

Gram-positive isolates were lesser compared to Gram-negative isolates. *Escherichia coli* was the commonest organism, followed by *Staphylococcus aureus,* similar to the study by Pal et al., Jahic et al., Mulu et al. In contrast to this, Gram-positive were 83.8%. Gram-negatives were 16.3%, and the predominant species was *Streptococcus agalactiae* which was detected by amplicon sequencing 5 targeting the 16S rRNA V4 region and also by culture [21]. 9.68% and 1.2% β hemolytic *Streptococcus* was detected respectively by Sangeetha et al. [14], and Mulu et al., which was 5.5% in our study Staphylococcus aureus were most common by Mumtaz et al. [18]. Enterococcus fecalis was the commonest organism as per Sangeetha KT et al. Most of the microorganisms isolated in AV are the bacteria that colonize the intestinal tract. Escherichia coli being the highest community in the intestinal tract, is most likely to have gained entry into the vulvovaginal region, causing infection due to proximity of anal orifice with the vaginal orifice and also an imbalance in the normal microbiota of the vagina along with other contributing factors like poor hygiene [5, 22].

7.7% of individuals had AV caused by more than one organism, and one incidence of AV combined with VVC was noted in our study. 16% of the combination of AV with VVC was reported by Wang et al. by using the rapid enzyme kit detection method and microscopy. Polymicrobial growth was also noted to the extent of 19.23% by Sangeetha et al. Enterobacteriaceae group of organisms showed maximum sensitivity to Meropenem (91.42%), almost similar to Krishnamurthy et al., where the sensitivity was 100%. Most minor sensitivities were observed with Ampicillin, Ceftriaxone, Fluoroquinolones similar to study by Sangeetha et al., Mumtaz et al. and Pal K et al. One *Pseudomonas aeruginosa* isolated was sensitive to all antibiotics, sensitivity to Piperacillin/ Tazobactam and Carbapenems was highest for *Pseudomonas*

By Sangeetha et al. and Krishnamurthy et al. With this scenario Carbapenems to be considered especially while treating pregnant women with aerobic vaginitis to prevent the risk of transmission of infection to neonates Isolation of Gram positive's though lesser than Gram-negatives, drug resistance has significant impact such as infertility, toxic shock syndrome, neonatal septicemia. Clindamycin sensitivity 52.2% noted elevates the use of most common antibiotic in the form of pessary Aerobic vaginitis is a newly recognized entity that has a various impact both in pregnant and nonpregnant women which has to be addressed appropriately and adequately to combat the adverse outcome in pregnancy and to prevent infertility, discomfort like dyspareunia [23, 24].

Conclusion

It is necessary to deal with the pathogens of vaginitis after microbiological evaluation. This will differentiate the causes of vaginitis like Vulvovaginal Candidiasis and aerobic vaginitis, included in this study. Error in treatment can be avoided like unnecessary administration of broad-spectrum antibiotics, which carries the risk of depletion of a beneficial organism like *Lactobacilli, the* emergence of drug resistance and hence to focus on the appropriate treatment

What does this study add to existing knowledge?

Aerobic vaginitis being a common condition, most of the time, no efforts will be made to identify the causative agent. In our study, to our surprise, we found a high incidence of resistance and the organisms being resistant to most of the commonly used antibiotics.

Permission from the Institutional Research Board (IRB): Yes.

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