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RESEARCH ARTICLE

ISOLATION AND ANTIMICROBIAL SENSITIVITY PATTERN OF MICROORGANISMS FROM VAGINAL SWAB IN A RURAL TERTIARY CARE HOSPITAL

[1]Shilpa, K., [2]Jiju Janardhanan, [3]Nisha, K.V., [4]Saju Varghese, [5]Benita Mary, L., [6]Vipin Unni, [6]Bernaitis, L. and [7]Preesly, M. S.

[1]Department of Microbiology, East Point College Medical Sciences and Research Centre, Avalahalli, Bangalore, Karnataka
[2]Department of Microbiologgy,Co-operative institute of Health sciences, Thalassery, Kerala – 670105
[3]Department of Microbiology, Century International Institute of Dental Science and Research centre, Kasaragod, Kerala - 671541
[4]Department of Microbiology,Sivagiri sree Narrayana college of Nursing, Varkala, Kerala -695145
[5]Department of Physiology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka
[6]Department of Microbiology, Ratnavel Subramaniam (RVS) Dental College and Hospital, Kumaran Kottam Campus, Coimbatore, Tamilnadu
[7]Department of Biostatistics, Ratnavel Subramaniam (RVS) Siddha Medical College and Hospital, Kumaran Kottam Campus, Coimbatore, Tamilnadu

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**Corresponding author:*

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ABSTRACT

Aim: To study the prevalence of pathogenic organisms and sensitivity patterns of various isolates from vaginal swab.

Method: Samples from October 2016 to October 2017 which had come to the Department of Microbiology at East Point College of Medical Science and Research Centre were included in the study. 120 Vaginal swab samples were collected and direct examined, microscopic Gram stain examination and culture techniques. Isolated microorganisms were identified using microscopical, morphological, biochemical tests. The results showed that positive vaginal cultures were detected in 52 (43.33%) women patients. Further antimicrobial sensitivity analysis were done by Kirby-Bauer disc diffusion method.

Results: These isolates were distributed between Gram- positive bacteria 17 (32.69%), Gram-negative bacteria 32 (61.53%) and fungi 3 (5.76%). The most frequent microorganisms isolated were Escherichia coli 17 (32.69%), followed by Staphylococcus aureus 11 (21.15%), Klebsiella pneumonia 9 (17.30%), coagulase negative Staphylococcus species (CONS) 6 (11.53%), Acinetobacter species 3 (5.76%), Pseudomonas aeruginosa 3 (5.76%), and Candida albicans 3(5.76%). The highest prevalence of vaginal infections were observed in the age group (20-50) years were 49 (94.23%), followed by greater than 51 age group were 3 (5.76%).Antimicrobial sensitivity analysis showed higher percentage of sensitivity to Piperacillin/Tazobactum (94%) in Escherichia coli, followed by Amikacin (81.81%) in Staphylococcus aureus, Meropenem (88.88%) in Klebsiella pneumonia, Meropenem (100%) in Acinetobacter species and Pseudomonas aeruginosa, Gentamicin showed 100% for CONS.

Conclusion: Vaginal infections were more commonly found in 20-50 age group in our study, Gram negative organisms were highly susceptible to Meropenem and Piperacillin/Tazobactum followed by Gram positive organisms to Amikacin and Gentamicin.

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INTRODUCTION

The vagina is a complex system which contain a mixture of microorganisms. This distinctive environment undergoes major changes in all stages of life, from birth to the age of puberty and menopause. Females are more susceptible to urinary tract and vaginal infections due to short urethra and anatomical and physiological proximity to the anal canal. In normal women, estrogen supports vaginal epithelium resulting in glycogen accumulation which in turn helps in maintenance of vaginal

pH. Naturally vaginal flora contains a wide range of microorganisms. The vagina of a healthy premenopausal woman is occupied by Lactobacillus species (Shamas Pervaiz, 2017). Lactobacilli maintain the normal vaginal pH (3.8- 4.2) by producing lactic acid, stabilizing the vaginal ecosystem and hydrogen peroxide, suppressing the growth of gram- negative and Gram- positive facultative and obligate anaerobes. The predominant bacterial pathogen associated with vaginal infection were aerobic isolates Escherichia coli, coagulase negative Staphylococcus (CONS), Staphylococcus aureus, Klebsiella pneumonia. Organisms may also be introduced into the genital tract by instrumentation, presence of a foreign body, or irritation can cause infection. Infections transmitted in this way are often caused by the same organisms that cause skin and wound infections. The frequent cause of vaginal discharge is an infection or colonization of different microorganisms (Shilan, 2015). Infections of the genitourinary tracts or reproductive tract infections are a major problem of women's sexual health. They are commonly seen in women of reproductive age and usually present with vaginal discharge[3]. Bacterial vaginosis is the invasion of the vagina with anaerobic bacteria organisms. It occurs when there is alteration of the vaginal ecology with gross depletion of the normal bacteria flora lactobacilli with overgrowth of anaerobic polymicrobial organisms (Okiki Pius, 2015). It is the commonest form of vaginal infections in women of reproductive age (Deborah Money, 2005). It constitutes almost 40% of cases in women attending sexually transmitted disease clinic (YasmeenHouso, 2011). In pregnant women, it constitutes almost 30% of all cases (Sadiya Shaikh, 2018). The common organisms implicated in bacterial vaginosis include Gardnerella vaginalis, Mycoplasma hominis, and anaerobic bacteria such as Peptostretococci, Prevotella spp, and Mobiluncus spp (Sujata Singh, 2016). Vaginal discharge is a common presentation in general practice, potentially indicating the presence of STIs. What little is known about GPs' investigation and management of vaginal discharge suggests that GPs commonly rely on the high vaginal swab (HVS)—an investigation rarely used by specialists. Despite this there is little information on what GPs expect from HVS, or whether they are satisfied with the reports they receive (Julius Schachter, 2003). Processing HVS samples accounts for a considerable proportion of the workload of most microbiology laboratories. Although Public Health Laboratory Service (PHLS) laboratories have a standard operating procedure (SOP) for HVS samples there are no universally accepted guidelines on how to process HVS samples, and this appears to be reflected by variability in processing and reporting between laboratories. In addition, it is not clear whether GPs are receiving the information they want from HVS reports (Go, 2006). Because many investigators in the field felt that vaginal swab specimens would be useful in screening for chlamydial infections, a multicenter study was designed to evaluate vaginal swabs as specimens (Hacer, 2012). The aim of the study was to isolate pathogenic organisms and sensitivity patterns of various bacterias from vaginal swab.

MATERIALS AND METHODS

Samples collection: High vaginal swabswere collected from two hundred and fifty (120) women patients with vaginal symptoms who attended the gynecology clinics and obstetrics outpatient and inpatient department of East Point College Medical Sciences and Research Centre, Avalahalli, Bengaluru, a rural tertiary care hospital, during the period from October 2016 to October 2017 . The age of these patients ranged between (20- 55) years. High vaginal swabs were taken from women patients suffering with abnormal vaginal discharge, itching, burning and lower abdominal pain. The samples were taken from each women patient (by doctors) using sterile swabs stick and speculum. Vaginal swab for each patient were transported to the laboratory by inoculating the swab into a sterile tube containing 3 ml of normal saline. The samples were examined by staining with Gram stain. for culture and sensitivity in the Microbiology laboratory, all the swabs were inoculated on blood, Chocolate and MacConkey's agar plates. Plates were incubated aerobically for 18-24 hours.

Isolation of microorganisms: For isolation of microorganisms, the specimen of vaginal swab was directly inoculated on culture media: Blood agar, MacConkey agar, and Sabouraud dextrose agar plates were incubated aerobically at 37˚C for 24-48 hours, and Chocolate agar plates were incubated microaerophilically at 37˚C for 24-48 hours.

Identification of microorganisms: Pure colonies of isolated microorganisms were identified using morphological, biochemical test, Candida speciation was done by Germ tube test.

Antibiotic sensitivity testing: It was done for Gram positive and Gram negative isolates using Kirby-Bauer's disc diffusion method. Antimicrobials tested for sensitivity were amikacin, cefepime, colistin, amoxicillin clavulanic acid, meropenem, imipenem, Piperacillin tazobactum, cotrimoxazole, ciprofloxacin, and gentamicin. After overnight incubation plates were examined to read the susceptibility zone. Data obtained were presented as distribution of microorganisms with respect to age, number and percentage of patients from which the microorganisms were isolated.

RESULTS

Total percentage of organisms isolated from vaginal swabs (Table 1), (Figure 1). A total of 120 samples had received for culture and sensitivity out of which 52 (43.33%) showed positive for culture.Out of which, Escherichia coli 17 (32.69%), followed by Staphylococcus aureus 11 (21.15%), Klebsiella pneumonia 9 (17.30%),coagulase negative Staphylococcus species (CONS) 6 (11.53%), Acinetobacter species 3 (5.76%), Pseudomonas aeruginosa 3 (5.76%), and Candida albicans 3(5.76%).

Table 1. Percentage of isolates obtained

Organisms	Percentage of isolates
Klebsiella pneumoniae	17.3
Escherichia coli	32.69
Pseudomonas aeruginosa	5.76
Acinetobacter species	5.76
Candida albicans	5.76
CONS	11.53
Staphylococcus aureus	21.15

Agewise distribution of isolates (Table 2), (Figure 2). The highest prevalence of vaginal infections were observed in the age group (20-50) years were 49 (94.23%), followed by greater than 51 age group were 3 (5.76%). Escherichia coli (16) was found to be more prevalent in 20-50 age group, followed by Staphylococcus aureus (11), Klebsiella pneumonia (8), CONS(6), Acinetobacter species, Candida albicans, Pseudomonas aeruginosa were found to be least prevalent.

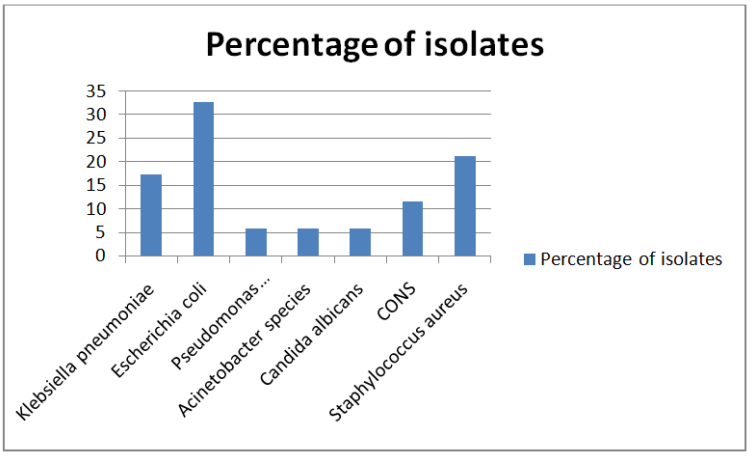

Figure 1. Percentage of isolates obtained

Table 2. Age wise distribution of isolates

Organisms	Age group	
	20-50	>51
Klebsiella pneumoniae	8	1
Escherichia coli	16	1
Pseudomonas aeruginosa	2	1
Acinetobacter species	3	0
Candida albicans	3	0
CONS	6	0
Staphylococcus aureus	11	0

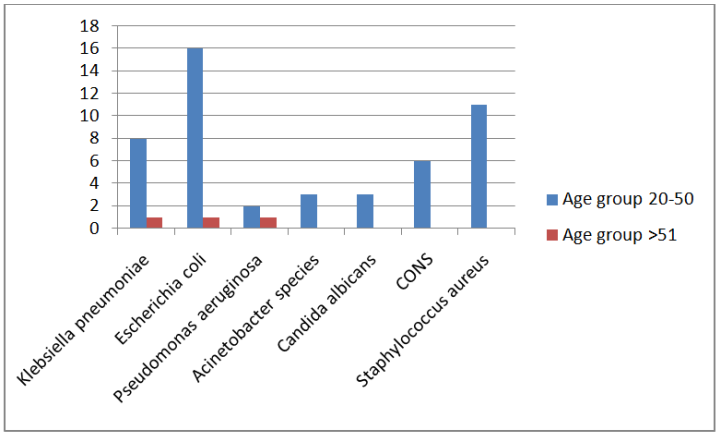

Figure 2. Age wise distribution of isolates

Table 3. Percentage sensitivity of isolates

Antibiotics						
	Escherichia coli	Staphylococcus aureus	Klebsiella pneumoniae	Acinetobacter species	Pseudomonas aeruginosa	CONS
Amikacin	58.8	81.81	33.33	100	-	50
Cefepime	64.7	45.45	11.11		-	-
Colistin	58.8	-	11.11	100	100	83.33
Meropenem	70.5	-	88.88	-	100	-
Amoxycillin/Clavulanicacid	70.5	81.81	-	100	-	50
Imipenem	70.5	-	88.88	100	100	-
Piperacillin/Tazobactum	94.1	-	44.44	100	100	-
Cotrimoxazole	52.9	-	44.44	-	100	-
Ciprofloxacin	11.7	81.81	11.11	-	-	-
Gentamicin	52.9	72.72	44.44	-	-	100

Among greater than 51 age group Klebsiella pneumonia, Escherichia coli and Pseudomonas aeruginosa were found to be prevalent. Percentage of isolates (Table 3), (Figure 3). Antimicrobial sensitivity analysis showed higher percentage of sensitivity to Piperacillin/ Tazobactum (94%) in Escherichia coli, followed by Amikacin (81.81%) in Staphylococcus aureus, Meropenem (88.88%) in Klebsiella pneumonia, Meropenem (100%) in Acinetobacter species and Pseudomonas aeruginosa, Gentamicin showed 100 percentage of sensitivity for CONS. Escherichia coli showed 58.8 percentage of sensitivity to Amikacin, 64.7 percentage of sensitivity to Cefepime, 58.8 percentage of sensitivity to Colistin, 70.5 percentage of sensitivity to Amikacin Meropenem, Amoxycillin/ Clavulanicacid, Imipenem respectively, 94.1

percentage of sensitivity to Piperacillin/ Tazobactum, 52.9 percentage of sensitivity to Cotrimoxazole, 11.7 percentage of sensitivity to Ciprofloxacin, 52.9 percentage of sensitivity to Gentamicin. Staphylococcus aureus showed 81.81 percentage of sensitivity to Amikacin, 45.45 percentage of sensitivity to Cefepime, 81.81 percentage of sensitivity to Amoxycillin/Clavulanicacid, 81.81percentage of sensitivity to Ciprofloxacin, 72.72 percentage of sensitivity to Gentamicin. Klebsiella pneumoniae showed 33.33 percentage of sensitivity to Amikacin, 11.11 percentage of sensitivity to Cefepime, 11.11 percentage of sensitivity to Colistin, 88.88 percentage of sensitivity to Meropenem and Imipenem,44.44 percentage of sensitivity to Piperacillin/Tazobactum. Acinetobacter species showed 100 percentage of sensitivity to Amikacin, Colistin, Amoxycillin/Clavulanicacid, Imipenem, Piperacillin/ Tazobactum. Pseudomonas aeruginosa also showed 100 percentage of sensitivity to Colistin, Meropenem, Imipenem, Piperacillin/ Tazobactum. CONS showed 50 percentage of sensitivity to Amikacin, 83.33 percentage of sensitivity to Colistin, 50 percentage of sensitivity to Amoxycillin/ Clavulanicacid.

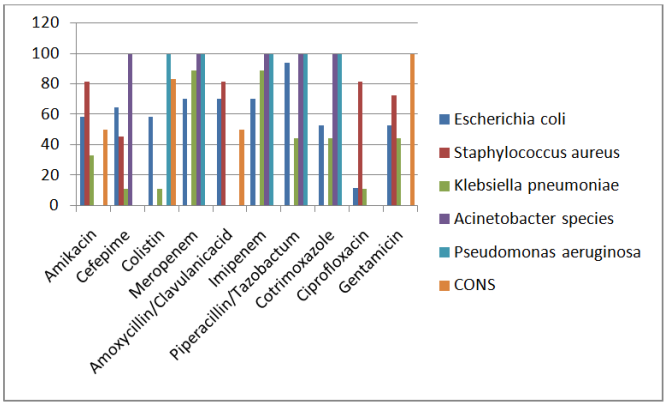

Figure 3. Percentage sensitivity of isolates

DISCUSSION

Vaginal infections are a great threat for women's health related to common gynecological problem.Our study demonstrates the prevalence of vaginal pathogens in symptomatic women (Spinillo, 1997). Vaginal infections are increasing due to vagina colonization by pathogenic bacteria other than the protective bacteria. The maximum frequency (36%) of infection was noted at the age of 20 to 30 years with a reduction in the frequency as the age advanced (Adeyba, 2003). In present study, the overall prevalence of vaginal infections (43.3 %) was coherent with reports in Vietnam (49.5 %) (http://womanhealthgate.com/focus-candidatrichomonas bacteria-atrophic-vaginitis/) and Thandalam (44 %) (Chalechale, 2010). However, it was higher compared to reports in India (14.7 %) (Lamichhane, 2014), Iran (27.6 %) (Hng, 2009). This variation might be methodology difference in isolation and identification of etiologies of vaginal infections. Moreover, environmental factors and difference on the actual study participants might also explain the above discrepancy. In this study, vaginal candidiasis infection rate is very less. Buta study conducted in Vietnam (Eshete, 2013), Bangladesh (Saleh, 2014) and (Trabert, 2008), where candidiasis was the most prevalent vaginal infection. However, it differs from a study done in India (Filho, 2010) where trichomoniasis was the most prevalent.

In this study of microorganisms associated with vaginal infections, both bacterial and fungal isolates have been implicated. Staphylococcus aureus belongs to those pathogenic bacteria not commonly present in the vagina but however, have been implicated in vaginitis. Infection of the vagina by intestinal flora is quite common due to the close proximity of the anus to the vagina. Furthermore, it was also reported that whenever Lactobacillus species is displaced by an overgrowth of pathogens like Escherichia coli, S. aureus. The culture method was used in this study because it is the "gold standard" against which the performance of other diagnostic methods is compared. Although, bacterial vaginal infections are one of the major causes of frequent antibiotic use in women of reproductive age, the level of antibiotic resistance in vaginal isolate was not studied before in the place. Thus, this study presents the antibiogram of the most predominant vaginal isolates.

Conclusion

Vaginal infections were more commonly found in 20-50 age group in our study, Gram negative organisms were highly susceptible to Meropenem and Piperacillin/Tazobactum followed by Gram positive organisms to Amikacin and Gentamicin.

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