HIGH VAGINAL SWABS;
FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY OF GRAM NEGATIVE RODS ISOLATED FROM HIGH VAGINAL SWABS

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ABSTRACT... Normal vaginal flora contains a wide range of microorganisms. Hydrogen peroxide produced by Lactobacillus strains plays a vital role in maintaining the microenvironment of the vagina and in the inhibition of overgrowth of potentially pathogenic bacteria. Bacterial vaginosis BV is the main reason of vaginal discharge. Many gram positive and gram negative rods i.e. E.coli, Klebsiella, Proteus, Acinetobacter and Pseudomonas spp. are major contributors in bacterial vaginosis. **Aim:** The present study was conducted to elucidate the frequency of various gram-negative rods in high vaginal swabs and sensitivity pattern of bacteria to antibiotics that are currently used. **Study Design:** Cross sectional study. **Setting:** Department of Obstetrics and Gynecology of Benazir Bhutto Hospital Rawalpindi, a tertiary health care center for the people of Rawalpindi. **Period:** January 2015 to May 2016. **Material and Methods:** A total of 220 High vaginal swabs (HVS) were collected both from indoor and outdoor patients presenting with symptoms of vaginal discharge aged between 20 to 65 years. Swabs were inoculated on blood, Chocolate and MacConkey's agar. After overnight incubation plates were examined for growth, colonial morphology, final confirmation was done on the basis of biochemical testing and API 20-E system (BioMerieux, France) up to species level. Antibiotic sensitivity testing was done by (modified Kirby-Bauer's) disc diffusion method using amikacin, ampicillin, amoxicillin clavulanic acid, imipenem, ceftazidime, tigecycline, ciprofloxacin, sulzone and cefixime. After overnight incubation plates were examined to read the susceptibility zone. **Results:** Out of 220 HVS samples, 100 samples showed bacterial growth and confirmed as Gram negative bacilli. Age wise distribution of infection showed highest rates b/w age 20-30 was 36% followed by 31-40 (23%), 41-50 (25%) and 11% above 50 years of age. Bacteria isolated from HVS were E.coli (53%), Klebsiella (22%), Pseudomonas (12%), citrobacter (6%), Proteus (5%) and Acinetobacter (2%) respectively. Highly sensitive antibiotics against bacteria were imipenem (96%), sulzone (90%) and Ciprofloxacin (88%), whereas least affective antibiotics against gram negative rods were penicillins (ampicillin, amoxicillin-clavulanic acid), amikacin due to indiscriminate use of antibiotics. **Conclusion:** High prevalence of gynecological infections demands that the patients who have vaginosis must be investigated regularly and carefully through culture and identification of causative bacteria. Emergence of antibiotic resistance must be controlled in order to avoid improper use, frequent abuse, insufficient dosages, trouble-free availability of antibiotics and treatment schedule must be designed subsequent to proper laboratory investigations.

Key words: HVS, Bacterial vaginosis, Imipenem, E.coli, Sulzone.

INTRODUCTION

The vagina is a complex system which contain a mixture of microorganisms.[1] This distinctive environment undergoes major changes in all stages of life, from birth to the age of puberty and menopause.[2] Females are more susceptible to urinary tract and vaginal infections due to short urethra and anatomical and physiological proximity to the anal canal.[3] In normal women, estrogen supports vaginal epithelium resulting in glycogen accumulation which in turn helps in maintenance of vaginal pH.[4]

Naturally vaginal flora contains a wide range of microorganisms. The vagina of a healthy premenopausal woman is occupied by Lactobacillus spp.[5] Lactobacilli are Gram positive rods that produce secretions in vitro having

antimicrobial properties, with acidolin, lactacidin, lactacin B and hydrogen peroxide. Lactobacillus strains producing H2O2 play a vital role in maintaining the microenvironment of the vagina and in the inhibition of overgrowth of organisms that are potentially pathogenic.[6,7] Hydrogen peroxide producing lactobacilli are cidal to (HIV-1), probably due to the effect of H2O2 with halides and myeloperoxidase in vaginal fluid thus maintains acidic vaginal PH and protection against a diversity of pathogens.[8]

In symptomatic women the most common vaginal infections are bacterial vaginosis (40-45%), vaginal candidiasis (20-25%) and trichomoniasis (15-20%), while 7-72% of women with vaginal infections stay undiagnosed. Vaginitis is the leading problems in gynecology and the main reason that led to females to seek out physician advice nearly 10 million office visits per annum.

Patients suffering from vaginitis complain of symptoms including increased vaginal secretions, odor in the vaginal discharge and itching or irritation in the vagina. Vaginal secretions characterized by consistency (curd like, thin, thick, watery), color (clear, white, grey, green or yellow), amount (more or less), odor (fishy, foul smelling, pungent) dyspareunia and bleeding.[9]

Bacterial vaginosis (BV) is a poly microbial disease characterized by less hydrogen peroxide producing lactobacilli and greater concentration of Gram negative bacilli. It is main reason of vaginitis leading to vaginal discharge. In reproductive age it is least understood and widespread disease. Worldwide frequency of BV is 3.6-40% reported in different population. Prevalence of BV varied based on the surveyed population, ranging from 4% in developed countries to 61% in the third world countries, with 14% is the mean prevalence in developed and developing region. Today bacterial vaginosis is the most common vaginal disease in the United States. In USA BV is 26-37% prevalent whereas 4-37% in general population of European countries[10]. The common reason of vaginal discharge is colonization of vagina with different iatrogenic, endogenous microorganisms transmitted sexually. Lower one third of vagina harbors hundreds of gram-positive and gram-negative species.[11]

Most common organisms that cause vaginal discharge are Trichomonas vaginalis Neisseria gonorrhea, Chlamydia trachomatis and (group B Streptococcus) Streptococcus agalactiae whereas other sources lead to transmission of gram negative rods i.e. E.coli, Klebsiella, Proteus, Acinetobacter and Pseudomonas spp.[12]

Vaginal discharge is a common problem but often neglected. These infections can easily be detected by simple tests, such as wet mount and Gram stain is the cost effective and "Gold standard" method used for the diagnosis of BV, requires less time and is used on large scale. Amsel's criteria and Nugent scoring system are most commonly used in hospitals for the detection of BV. High vaginal swab for culture and sensitivity testing in addition confirm organism that causes infection and suggestion of antibiotics against infection.[13] According to the Centre for Disease control and prevention (CDC) guidelines, treatment based on sensitivity pattern, partner information, health promotion and follow up was included in the management.[14]

Previously, no extensive studies conducted relating to BV and vaginal flora in Asian communities. This study was aimed at to elucidate the frequency of various gram negative rods in high vaginal swabs and sensitivity pattern of bacteria to antibiotics that are currently used. By studying antibiotics sensitivity pattern in the patients, we may modify the recommendations of therapy protocol.

MATERIALS AND METHODS

This is a cross sectional study conducted at Department of Obstetrics and Gynecology of Benazir Bhutto Hospital Rawalpindi, a tertiary health care center for the people of Rawalpindi from January 2015 to May 2016. A total of 220 high vaginal swabs were collected both from indoor and outdoor patients aged between 20 to 65 years presented with symptoms of vaginal discharge, malodor, dysuria, dyspareunia, itching and fever. Patients who have vaginal

instrumentation recently and history of usage of past two weeks antibiotics were excluded from the study. Sterile swabs were labeled with a unique identifier to make sure secrecy and freedom from bias. After collection of sample, all the swabs were inoculated on blood, Chocolate and MacConkey's agar plates. Plates were incubated aerobically for 18-24 hours. Preliminary identification was done on the basis of colonial characteristics (shape, translucency, color, margins and surface elevation), Gram stain and biochemical reactions (catalase test, cytochrome oxidase test). Mucoid, lactose fermenting colonies of Gram negative rods were further analyzed by the API 20-E system (BioMerieux, France) for the identification up to specie level.

Antibiotic sensitivity testing was done using (modified Kirby-Bauer's) disc diffusion method. Antimicrobials tested for sensitivity were amikacin, ampicillin, amoxicillin clavulanic acid, imipenem, ceftazidime, tigecycline, ciprofloxacin, sulzone and cefixime. 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. The frequency and antimicrobial sensitivity patterns of microbes were presented in percentages. Statistical analysis was done by Microsoft Excel program. However, the analysis was completed with respect to age only, because the details of social factors were not available.

RESULTS

In this study, majority of the females were in the age group between 20-65 years. Out of 220 high vaginal swab cultures, 100 samples were positive for bacterial growth and confirmed as Gram negative bacilli were E.coli, Klebsiella, Citrobacter, Proteus, Pseudomonas and Acinetobacter. Age wise distribution and frequency of infections caused by Gram negative rods is given in Table-I. Distribution of bacteria isolated from high vaginal swab was E.coli (53%), Klebsiella (22%), Pseudomonas (12%), citrobacter (6%), Proteus (5%) and Acinetobacter (2%) respectively as shown in Figure-1. The detailed percentages of antimicrobial sensitivity of the isolated gram negative bacilli against various antibiotics are shown in Table-II.

Age (years)	Frequency (%)	Bacteria
20-30	75(36%)	E.coli, Acinetobacter
31-40	42(23%)	E.coli, Klebsiella, Citrobacter
41-50	56(25%)	E.coli, Proteus, Klebsiella, Citrobacter
›50	17(11%)	E.coli, Proteus, Klebsiella, Pseudomonas

Table-I. Percentage distribution of Gram negative rods isolated from High vaginal swab with respect to age and infection rate.
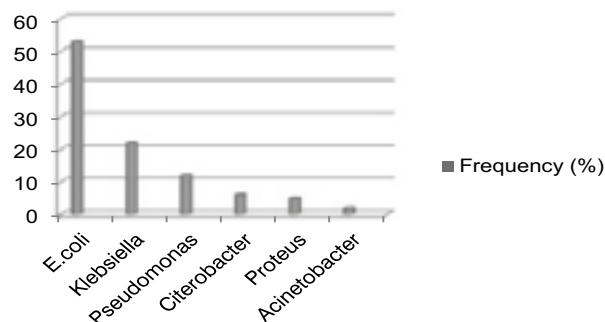
Figure-1. Percentage distribution of bacteria recovered from vaginal discharge at Department of Obstetrics and Gynecology of Benazir Bhutto Hospital Rawalpindi.

Bacteria	AK	AMP	AMC	IPM	CAZ	CIP	SCF	TGC	CFM
E.coli	76%	16%	7%	95%	52%	98%	95%	45%	70%
Klebsiella	49%	62%	62%	85%	82%	68%	91%	41%	71%
Pseudomonas	32%	30%	4%	72%	50%	51%	82%	78%	55%
Citrobacter	12%	26%	55%	88%	27%	63%	23%	66%	45%
Proteus	67%	30%	4%	92%	62%	82%	73%	58%	48%
Acinetobacter	46%	65%	45%	71%	55%	88%	74%	38%	52%

Table-II. Antibiotic sensitivity pattern of Gram negative rods isolated from high vaginal swab.
* AK-Amikacin, AMP-Ampicillin, IMP-Imipenem, CFM-Cefixime, TGC-Tigecycline, AMC-Amoxicillin-clavulanic acid, CAZ-Ceftazidime, SCF- Sulzone, CIP-Ciprofloxacin

DISCUSSION

Vaginal flora contain a range of microorganisms normally, Lactobacillus spp. play fundamental role in maintaining acidic vaginal PH and prevent the overgrowth of potentially harmful and opportunistic bacteria.[15] Vaginal infections are a great threat for women's health related to common gynecological problem.[16] Our study demonstrates the prevalence of latent vaginal pathogens in symptomatic women.

Vaginal infections are increasing due to vagina colonization by pathogenic bacteria other than the protective bacteria[17]. 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. Similar findings with respect to age were seen in a Kenyan study[18]. The results of our study are similar to the study conducted by Lakshmi et al., they reported the prevalence of vaginal infections in india.[19]

In our study E.coli (53%) was the most frequent gram negative rods followed by Klebsiella (22%), Pseudomonas (12%), Citrobacter (6%) Proteus (5%) and Acinetobacter (2%) respectively, as seen in a study conducted by Dutta et al. in Dhaka.[20] McDonald et al. also found E. coli to be the important bacteria associated with bacterial vaginosis and mid–trimester of pregnancy losses.[21]

The most useful antibiotics against gram negative rods in our study were imipenem (96%), sulzone (90%) and Ciprofloxacin (88%), antibiotics like imipenem are extremely effective but expensive. Tariq et al., reported similar findings.[22] Whereas the antimicrobials with least affectivity against gram negative rods were Penicillins (ampicillin, amoxicillin-clavulanic acid), amikacin due to indiscriminate use of antibiotics.

CONCLUSION

High prevalence of gynecological infections demands that the patients who suffer from the symptoms of gynecology must be investigated carefully. The elevated prevalence of vaginal gram negative bacteria in the recent study stress that high vaginal swab culture provides the identification of causative bacteria, it must be regularly done. There is a need to be aware of antibiotic resistance in order to avoid improper use, frequent abuse, insufficient dosages, trouble-free availability of antibiotics and treatment schedule must be designed subsequent to proper laboratory investigations.

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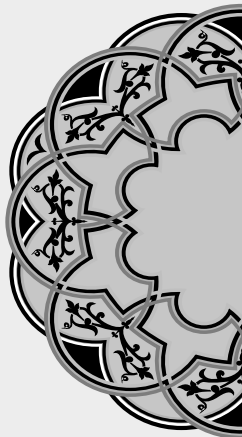
"Never be a prisoner of your past.
It was just a lesson, not a life sentence."

Unknown

AUTHORSHIP AND CONTRIBUTION DECLARATION

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