AEROBIC VAGINAL PATHOGENS AND THEIR SENSITIVITY PATTERN

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Background: The vaginal flora is a complicated environment, containing dozens of microbiological species in variable quantities and relative proportions. The frequent cause of vaginal discharge is an infection or colonization with different microorganisms. Some pathologic conditions causing vaginitis are well defined yet, 7–72% of women with vaginitis may remain undiagnosed and such forms of abnormal vaginal flora neither considered as normal, nor can be called bacterial vaginosis have been termed as 'intermediate flora' and its management probably differ from that of bacterial vaginosis. It is of crucial importance in pregnant females at risk of preterm delivery. The present study has been conducted especially to elucidate this type of aerobic vaginal isolates and their culture and sensitivity towards currently used antibiotics. This study was conducted at the Microbiology Department of Fauji Foundation Hospital, Rawalpindi over a period of two years (April 2004–March 2006). **Methods:** One thousand, nine hundred and twenty three high vaginal swabs, both from indoor and outdoor patients were collected, cultured and their susceptibility to various antibiotics was determined. **Results:** Significant growth was obtained in 731 samples. The highest frequency of infection (39.5%) was observed at 31–40 years followed by 41–50 years (35.8%). About 76% were from outdoor and 24% were from indoor patients. *Staphylococcus aureus* was the most prevalent vaginal pathogen at 11–60 yrs & with highest prevalence at 31–40 years followed by 41–50 years. It was a predominant pathogen in both indoor (35%) as well as outdoor (41.6%) patients, followed by enteric gram-negative bacilli and other gram-positive cocci. There were very few antibiotics among the conventionally available aminoglycosides, third generation cephalosporins, penicillin, quinolones, sulfonamides and tetracyclines possessing good sensitivity (>80%) against any one the common aerobic vaginal pathogens. The effective chemotherapeutics agents belong to the groups of carbapenems and β–lactams β–lactamase inhibitor combinations. **Conclusions:** The high prevalence of gynaecological infections demands that the patients with gynaecological symptoms be investigated thoroughly. Culture must invariably be done. Currently the antibiotics showing good sensitivity are very expensive. So there is a need for an effective antimicrobial policy. Effective drug should be reserved for the treatment of serious life threatening situations only.

Key Words: Staphylococcus aureus, Gram–negative bacilli, Escherichia coli, Klebsiella pneumoniae, carbapenems, β–lactams, β–lactamase inhibitor combinations, piperacillin/tazobactam, cefoperazone/sulbactam

INTRODUCTION

The composition of aerobic human vaginal microflora has been extensively studied.[1-3] The vaginal flora is a complicated environment, containing dozens of microbiological species in variable quantities and relative proportions. A complex and intricate balance of microorganisms maintains the normal vaginal flora. It is mainly dominated by members of the genus *Lactobacillus*, which maintains the generally acidic vaginal *p*H.[4]

The frequent cause of vaginal discharge is an infection or colonization with different microorganisms.[5] Vaginitis, whether infectious or not, poses one of the most common problems in gynaecology, and is one of the main reasons leading the females to seek advice from a physician approximately 10 million office visits annually.[6-9] However, diagnosis and treatment can be elusive, if based on clinical symptoms and the characterization of vaginal discharge alone, leading to a lack of relief from the symptoms.[10,11]

Although some pathologic conditions causing vaginitis are well defined like bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis yet, 7–72% of women with vaginitis may remain undiagnosed and such forms of abnormal vaginal flora neither considered as normal, nor can be called bacterial vaginosis have been termed as 'intermediate flora' and its management probably differ from that of bacterial vaginosis.[12] It is of crucial importance in pregnant females at risk of preterm delivery.[13] The present study has been conducted especially to elucidate this type of aerobic vaginal isolates and their culture and sensitivity towards currently used antibiotics.

MATERIAL AND METHODS

One thousands, nine hundred and twenty three high vaginal swabs, both from indoor and outdoor patients, were collected on the sterile swabs and inoculated on blood agar, MacConkey's agar and Chocolate agar. The aerobically incubated organisms

were identified with the help of colonial morphology, gram staining and biochemical analysis.[14] Isolated organisms were subjected to sensitivity testing by Kirby-Bauer disc diffusion method,[15] using National Committee for Clinical Laboratory Standards criteria, to interpret diameter of inhibition zone.[16]

RESULTS

Of all the cultured samples, significant growth was obtained in 731 samples. About two-third of the positive samples (76%) were from outdoor, while one-third (24%) were from indoor patients. The highest frequency of infection 289 out of 731(39.5%) was observed at 31–40 years followed by 41–50 years of age group 262 out of 731 (35.8%). (Figure-1)

Out of 731 isolates Staphylococcus aureus was the most prevalent organism 337 (46%), followed by Escherichia coli 100 (13.7%), Klebsiella pneumoniae 77 (10.5%), Enterococci 66 (9.0%), Beta haemolytic streptococci 64 (8.8%), Pseudomonas zaeruginosa 53 (7.3%) and Candida spp 10 (1.0%). Staphylococcus aureus was the most prevalent vaginal pathogen at 11–60 yrs, with highest prevalence at 31–40 years followed by 41–50 years. Staphylococcus aureus was followed by enteric gram-negative bacilli and other gram- positive cocci. While at the early age (0-10 yrs) Klebsiella pneumoniae and Pseudomonas aeruginosa were the predominant organisms. (Table-1). Staphylococcus aureus was a predominant pathogen in both indoor 60 out of (171–35%) as well as outdoor 229 out of 550 (41.6%) patients, followed by enteric gram-negative bacilli and other gram- positive cocci. (Table-2) The detailed results of the percentage sensitivity of the common isolates against the various antibiotics are shown in Table-3.

Figure-1: Frequency of infections at different age groups in 731 female Patients

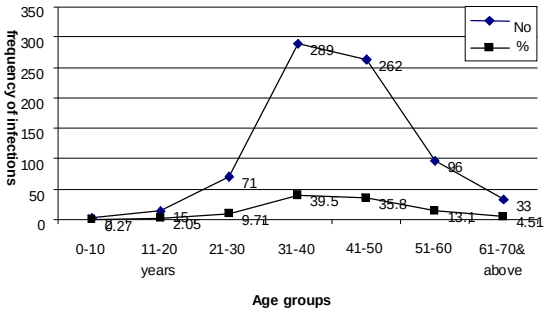

Table-1: Organisms Prevalent at Different Age Groups in Vaginal Isolates

Organisms	0-10	11-20	21-30	31-40	41-50	51-60	61 & above	Total
Staphylococcus aureus	0	12	35	145	104	32	9	337
Escherichia coli	0	0	13	10	40	27	10	100
Klebsiella pneumoniae	1	0	7	25	27	15	2	77
Enterococci	0	0	6	22	28	8	2	66
Beta- hemolytic Streptococci	0	1	2	18	31	5	7	64
Pseudomonas aeruginosa	1	2	5	17	18	8	2	53
Acinetobacter	0	0	2	4	4	0	0	10
Proteus species	0	0	0	5	4	0	1	10
Candida species	0	0	1	2	3	1	0	7
Providencia spp	0	0	0	3	2	0	0	5
Enterobacter spp	0	0	0	1	0	0	0	1
Morganella	0	0	0	0	1	0	0	1
Total	2	15	71	289	262	96	33	731

Table-2: Prevalent Organisms in 721 Indoor/Outdoor Patients

	Indoor		Outdoor	
Organisms	No	%	No	%
Staphylococcus aureus	60	35	229	41.6
Escherichia coli	40	23.3	104	18.9
Klebsiella pneumoniae	22	12.8	55	10
Pseudomonas aeruginosa	22	12.8	34	6.18
Beta- hemolytic Streptococci	10	5.84	45	8.18
Enterococci spp	8	4.67	59	10.7
Proteus species	5	2.92	3	0.54
Acenitobactor	2	1.16	8	1.45
Candida species	1	0.58	6	1.09
Providencia spp	1	0.58	4	0.72
Citrobactor spp	0	-	1	0.18
Morganella spp	0	-	1	0.18
Enterobacter spp	0	-	1	0.18

Table-3: Percentage of Sensitivity of Aerobic Vaginal Isolates to various Antibiotics

Antibiotics	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Beta hemolytic Streptococci*	*Enterococci*
Penicillin group						
Penicillin					90.5	44.4
Ampicillin	14.8	18.2		26.3	96.0	63.8
Amoxicillin	25.0	9.52		32.8	81.3	
Amoxicillin/clavulanic acid	46.8	60.29	30	67.1		85.7
Piperacillin/tazobactam	92.1	95.8	94.4	89.1	100.0	96
Cefoperazone/Sulbactam	100					
Cephalosporins:						
Cephradine				36.8	25.0	
Cefotaxime	73.9	79.1	37.7	70.4	90.0	50.9
Ceftazidime	70.4	71.4	26.2		90.0	
Monobactams:						
Aztreonam	52.3	72	66.7	19.13		70
Carbapenems:						
Imipenem	96.0	100	90.7	98.64	100.0	91.0
Meropenem			91.7			
Quinolones:						
Norfloxacin	52.7	72.7	53.3	67.4	66.7	
Ciprofloxacin	69.0	80.2	78.7	65.51	85.4	59.5
Aminoglycosides:						
Gentamicin	65.4	62.8	36.7	67.6	50.0	43.5
Amikacin	81.3		84.6	76.9		
Miscellaneous:						
Co-trimoxazole	21.5	61.8		23.6	17.5	37.2
Doxycycline	25	57.1		49.3	58.1	42.1
Minocyclin		25				
Lincomycin	42.9			46.9	66.7	
Fosfomycin	81.8	88.5	54.1	71.6	70.6	49.0
Methicillin				69.3		0
Vancomycin				93.6		95.0

DISCUSSION

The highest incidence of vaginal infections in this study was noted among young, sexually active females, at the two age groups, i.e., 31–40 years (39.5%), followed by 41–50 years (35.8%), a study by Khan & Khan (2004) showed that these infections are common at the age groups of 15–30 years followed by 31–40 years.[4] Although the vaginal flora of adult females contain lactobacilli responsible maintaining the vaginal *p*H & preventing the overgrowth of potential pathogens, thereby reducing the frequency of infections at this age group. But antibiotics like broad-spectrum penicillins or tetracyclines can kill or suppress helpful bacteria in the genital tract, allowing resistant organisms to grow unchecked.[17]

Staphylococcus aureus was the most prevalent vaginal pathogen at the ages ranging from 11–60 yrs and its highest prevalence was noted at 31–40 years followed by 41–50 years. It was a predominant pathogen in both indoor (35%) as well as outdoor (41.6%) patients. Escherichia coli was 2nd most common organism at all ages & even in the later ages (61–70 & above) of life. According to Khan & Khan (2004), Enterococci was most prevalent at 15–46 years followed by Streptococcus pyogenes and E. coli.[4] The different pattern of infection in the present study may be owing to the prevalent conditions like health education, sanitation and medical coverage available in each country.

Staphylococcus aureus is one of the most persistent pathogens of humans and has remained one of the most common cause of infection, incidence of which has been steadily increasing.[18–20] The vaginal mucosa of 5 (23%) of the females, is colonized by this organism, in whom it predisposes them to toxic shock syndrome.[21–23] Group B Streptococci, is also a common vaginal colonizer, and it continues to be the most important bacterial cause of bacterial sepsis and meningitis in newborns.[23] The presence of members of faecal flora in the vagina was attributed to unhygienic bowel practices in the past.[24] However recent studies shows that the intestinal and urogenital microflora play a central role in maintaining both the health and well being of the humans and replacing the good bacteria in adequate amounts confers the health benefits in the host These colonizers of the female introitus predisposes the women to recurrent UTI.[24,25] According to Gilbert *et al* (2002) vaginal microorganisms associated with aerobic vaginitis were found to be mainly group B Streptococci, S aureus and E. coli which were three to five times more frequent in aerobic vaginitis than in the normal flora.[12] McDonald *et al* (1994) found E. coli and group B. Streptococci to be the important pathogens associated

with mid–trimester pregnancy losses, alongside the classic bacterial vaginosis organisms.[13]

Aerobic vaginitis typically does not respond to antibacterial vaginosis medication so has to be treated by antibiotics according to the culture and sensitivity. An optimal treatment scheme for aerobic vaginitis, which includes antibacterial agents and simultaneously normalizing the vaginal ecosystem, has not been established until today.[12]

The most effective chemotherapeutic agents observed against Staphylococcus aureus in this study were imipenem (98.64%), vancomycin (93.6%), piperacillin/tazobactam (89.13%), lesser activity has been noted against penicillins, tetracycline (49.3%), sulphonamides (23.6%), first generation cephalosporins (36.8%) and monobactams (19.13 %).

For a long time, penicillins have been a main stay for the management of a variety of Staphylococcal infections. But the organism has gradually acquired resistance towards them, evident from this study where only 26.3%, 32.8% of the Staphylococcus aureus were sensitive towards ampicillin and amoxicillin only. In most cases of Staphylococcus aureus, resistance to penicillin is attributable to ß-lactamase production. Therefore, penicillin in combination with one of the ß-lactamase inhibitors gives much better results,[26,27] clearly seen from this study (piperacillin/tazobactam (89.1% and coamoxiclav 67.1%).

Most of the β-haemolytic Streptococci have been found susceptible towards penicillin (90.5%), ampicillin (96.0%), amoxicillin (81.3%). While most of the enterococci were found resistant towards penicillin (44.4%), co-trimoxazole (37.2%) and doxycycline (42.1%) and aminoglycosides (43.5%). But are fairly sensitive towards ampicillin (63.8%), coamoxiclav (85.7%) and piperacillin/tazobactam (96%) and vancomycin (95.0 %). Enterococci are the important human pathogens which have gained resistance to the commonly used antibiotics against gram positive bacteria.[27] but can be best treated with penicillin or vancomycin.[28] A study by Tariq *et al* (2006) showed that Enterococcus, Staphylococcus and Streptococcus were mostly sensitive to penicillin and amoxicillin.[25]

The most effective chemotherapeutic agents against gram–negative rods (Escherichia coli versus Klebsiella pneumoniae) in this study 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 (ampicillin14.8%, 18.2%), tetracycline (25%, 57.1%) and sulphonamides (21.5%, 61.8%). This in accordance with the study by Tariq *et al* (2006) most of the E. coli and Klebsiella were sensitive to piperacillin-tazobactum, imipenem.[25]

CONCLUSIONS

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. There were very few antibiotic among the conventionally available aminoglycosides, third generation cephalosporins and penicillin, quinolones which possess good sensitivity (>80%) against any one of the common organisms. Currently the antibiotics showing good sensitivity, i.e., carbapenems and β–lactams β–lactamase inhibitor combinations are very expensive and out of reach of the poor patients. There is need for an antibiotic policy for its rational use.

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