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ORIGINAL ARTICLE

Antimicrobial Susceptibility Pattern of Selected Bacterial Pathogens Isolated from High Vaginal Swab

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ABSTRACT

The study determines the prevalence and resistance among pathogenic bacteria against used antibiotics. The isolates were identified biochemically and subjected to antibiotic sensitivity using disk-diffusion method. A total of 210 bacterial isolates were tested, 175 (83.3%) were reported positive whereas 35 (16.6%) were found negative for HVS. The isolates were initially identified on colonies morphology and later via biochemical tests. The gram positive bacteria isolates i,e Staphylococcus aureus was found 48 (23.3%), followed by lactobacillus and least value belonged to Staphylococcus epidermidis i.e, 7 (3.3%). While gram negative bacteria Escherichia coli were 56 (26.6%), followed by Pseudomonas aeruginosa 36 (17.14%) and least value was of Proteus vulgaris. i.e, 7 (3.3%). All the isolates were subjected to antibiotics susceptibility testing. At least six antibiotics were shown to be resistant in bacterial isolates. Antibiotic resistance was found in considerable abundance in the clinical isolates, according to the findings. Antibiotic susceptibility testing and surveillance are essential on a regular basis to avoid treatment failure and the emergence of antibiotic resistance.

INTRODUCTION

The causative agent of many infectious diseases are bacteria which is small single-celled that adopt a diverse environment on the earth [1]. Urinary tract infections (UTIs) in women are a major public health concern, with an estimated 340 million new cases each year, out of 340 million 151 million of them occurring in Asia [2]. Urinary tract infections are a common infection among bacterial diseases, female sex workers are at high risk of contracting and spreading of diseases [3]. UTIs can lead to infertility, ectopic pregnancy, cervical cancer, menstruation irregularities, pregnancy waste, and low birth weight kids [4]. Bacterial vaginosis, among developing countries are a major public-health problem; vaginosis accounts for 40-50 percent of vaginal infections in women [5].

The majority of infections are caused by bacteria from the feces that enter the body through the urethra, particularly in females who have a shorter and wider urethra that allows the microorganisms to easily pass through [6]. Antibiotics are prescribed for the treatment of bacterial infections and their function is to either inhibit the growth of pathogens or to kill them. The bacterial infection threat posed by human have substantially reduced via antibiotics therapy. Antibiotic use has resulted in a significant reduction in fatalities from formerly lethal diseases [7]. Antibiotics' future effectiveness, however, is in doubt due to microorganism resistance. Antibiotic resistance is exacerbated by the fact that bacteria and their resistance genes spread more quickly from one bacterial species to the next [8]. The global distribution of food also aids the spread of resistance [9] poor hygiene in developing countries [10]. The antibiotic resistance has also due to antibiotics availability over-the-counter drugs, and purchase without a physician prescription or patients who do not complete antibiotic therapy, and the use of low standard antibiotics [10]. The vast growing problem in microbial disease treating are drug resistance, methicillin resistant Staphylococcus aureus and Pseudomonas aeruginosa resistant strains of various types have been found [11].

Misuse and over use of antibiotics lead to antibiotic resistance and also produce many complications. Antibiotic resistance not only makes it difficult to provide effective therapy, but it also raises morbidity and death [12]. The rise of resistance against antibiotics in many developing countries is observed potentially a disastrous due to lack of resources and purchase of effective antibiotics which are more expensive [13]. Therefore, this study has been designed to explore major pathogenic bacteria which cause RTIs in female, and also explore current antibiotic resistance in Pakistan which is a serious threat in the next five year that will be posing by health care providers and patients. It is necessary to take note of the routine antimicrobial treatment, awareness among people about antibiotic use and bane over the counter antibiotic availability.

MATERIALS AND METHODS

A total of 210 samples of HVS were collected, from female patients of age between 25-50 years. The sample were brought into laboratory of Khyber Teaching Hospital, Peshawar and stored at 4^0C for further processing. Streaked the swab samples on Nutrient agar plates (Merck Germany) aseptically and incubate at 37°C for bacterial growth. Two different media were used to obtained pure culture. MacConkey Agar was used for isolation of G^{-ve} and Blood Agar was used for of G^{+ve} bacteria. Microbial identification based on colony morphologies and biochemical testing was used to identify all clinical bacterial isolates [14].

Antimicrobial susceptibility: Antimicrobial susceptibility pattern was performed on Mueller Hinton agar (Merck, Germany) utilizing disc diffusion. This approach was used to test the susceptibility of UTI agents according to Clinical and Laboratory Standards Institute (CLSI) [15]. The antibiotic disks consists of Ampicilin (10µg), Kanamicin (30µg), Tetracycline (30µg), Cephalothin (30µg). Tobramicin (10µg), Ciprofleoxacin (5µg), Amikacin (30µg), Ceftriaxone (30µg), Cefotaxime (10µg), Gentamicin (10µg) and Trimethoprim-sulfamethoxazole (25µg).

RESULTS

In the current study, 174(83.3%) patients out of 210 (100%) were observed to be positive for HVS in clinical samples and 36 (16.6%) samples were found negative. Patients aged 25 to 50 years old had a higher rate of Urinary tract infections. The susceptibility of antibiotics against gram positive and gram negative bacterial isolates differed significantly.

Identification of isolates: The isolates were identified on color basis on various media, the gram positive bacteria Staphylococcus epidermidis was non lactose fermenter, no growth on Macconkey

Agar and give white colonies on Blood Agar. Staphylococcus aureus was gram +ve bacteria non lactose fermenter, no growth on MacConkey Agar, show grey, or golden, small, dry colonies on Blood Agar. Lactobacillus are gram positive, non-lactose fermenter, no growth on the MacConkey Agar and give circular white colonies on blood agar. While the gram negative bacteria such as, Escherichia coli was lactose fermentor, pink, dry, small colonies on MacConkey Agar and white centric round, small colonies on Blood Agar. Proteus vulgaris was non-lactose fermenter, small colourless colonies on MacConkey agar and swarming colonies on blood agar. Pseudomonas aerouginosa was non lactose fermenter, white creamy mucoid colonies on Macconkey agar and dark green color on blood agar.

Gram positive and gram negative bacteria isolated from HVS: The bacterial species were identified by gram staining and later via biochemical tests such as catalase, coagulase, oxidase, citrate test and lactose fermentation. The overall prevalence value of the gram +ve bacteria isolated from the HVS samples in total was 175(83.3%), Staphylococcus aureus were found 48 (23.3%), followed by Lactobacillus 21 (10%) and least value 7 (3.3%) of Staphylococcus epidermidis were found positive in the clinical samples in the present study (table 3). The gram negative were also initially identified by gram staining and later by biochemical tests such as Indole, Methyl Red, Vogues proscure test, citrate utilization test and triple sugar iron test, and lactose fermentation test. E. coli were found 56 (26.6%), followed by Pseudomonas aeruginosa 35 (16.6%) and least value 7 (3.3%) of Proteus vulgaris were found positive in the clinical samples in the present study.

Antibiotic sensitivity of isolates: Antibiotic susceptibility testing was performed on the positive clinical bacterial isolates. Susceptibility testing revealed that all of the bacteria isolated from HVS had the highest level of resistance. The antibiotics zone of inhibition was measured in mm. Antibiotics with a zone of inhibition >20 mm were classified as sensitive, whereas <15 mm were classified as resistant, and 15-20 mm were classified as intermediate. Among these antibiotics Kenamicin, Ciprofloxacin, Tobramicin and Gentamicin were sensitive to all gram positive isolates (S. aureus, S. epidermidis, and Lactobacilli) and gram negative (E. coli). The reference organisms were also observed which show susceptibility (E. coli ATTC 8739) to AMC, CTX, CIP, TE and CFP, antibiotics. S. aureus on the other hand, was the most susceptible to antibiotics. CIP exerted the largest inhibitory zone against the reference pathogenic bacteria. The result also shows that E. coli were resistant to most of the antibiotics due to gut normal flora.

The other bacterial isolates such as Pseudomonas aeroginosa and Proteus vulgaris were subjected to antibiotic tests and results were presented in the tables. Although few in number, but all tested isolates of Proteus and Pseudomonas were resistant to AM, CFT, TE, CRO and CTX. (Table 2). When comparing Proteus isolates to Pseudomonas spp., Proteus isolates were more sensitive to CIP. However, several proteus isolates had a higher susceptibility zone than Pseudomonas and E. coli isolates, as well as the E. coli ATTC 8739 reference bacterial strains.

Table 1: Frequency of Gram positive and gram negative bacteria isolates from HVS

Isolates	Positive	Percentage %
Staphylococcus aureus	48	23.3
Lactobacillus	21	10
Staphylococcus epidermidis	7	3.3
E. coli	56	26.6
Pseudomonas aeruginosa	36	17.14
Proteus vulgaris	7	3.3
Total	175	83.3

Table 2: Antibiogram assay of isolated pathogens from HVS

Isolates	AM	KA	CF	CIP	TE	AN	SXT	TOB	CRO	CTX	GN
S aureus	0.0	95.2	0.0	96.6	18.4	0.0	12.5	95.6	60.2	20.6	94.2
S epidermidis	0.0	96.4	5.5	95.5	18.5	4.0	12.8	95.4	65.5	19.3	92.5
Lactobacilli	10.4	90.2	10.1	95.2	20.5	16.5	16.4	90.2	70.2	21.5	85.5
E coli	5.8	72.1	20.5	80.0	23.6	85.2	45.6	60.2	75.5	70.4	85.5
Pseudomonas	11.2	26.5	12.5	70.5	12	85.5	4.5	48.6	25.5	75.5	56.5
Proteus spp	18.5	75.4	20.0	98.0	0.0	95.4	40.2	94.2	65.8	64.8	95.0

AM, Ampicilin; KA, Kanamicin; CF, Cephalothin; CIP, Ciprofloxacin, TE, Tetracycline; AN, Amikacin; SXT, Trimethoprim-sulfamethoxazole; TOB, Tobramicin; CRO, Ceftriaxone; CTX, Cefotaxime; GM, Gentamicin.

DISCUSSION

Urinary tract infections are common in female worldwide especially in a female of reproductive age. In the united states UTIs are the most frequent bacterial infections in outpatient door, accounting for seven million clinical visits and 100,000 hospitalizations per year [16, 17]. Before the age of 24, 1 out of 3 women will require antimicrobial treatment for a UTI, and 40-50% percent of women will have a UTI over their lifetime [17]. However, the overuse and misuse of antibiotics is leading to the emergence of resistance to lifesaving drugs. Hospital antibiograms are commonly used for detecting empiric antimicrobial treatment, monitoring trends and antimicrobial resistance. The findings of this study clearly demonstrated that the HVS is a popular tool for detecting bacterial infection in the female population of Lahore, as well as the use of various antibiotics to treat this infection. The current study bacterial isolates were also isolated and identified previously by many investigators in their findings. Strains of test bacteria were isolated from samples of high vaginal swab, manifesting symptoms of genito-urinary tract infections [18]. The gender distribution of patients in our study is similar to that of other studies, revealing a statistically significant female prevalence of UTIs (68 percent of the positive cultures) while the current study results 83.3 percent, the increase is due to years differences of study. The pathogens identified in this study are similarity with other studies conducted in different region or internationally [19], however different results have been reported. The difference in environmental condition and hygiene level of every country results similarities and differences in pathogenesis. Urinary tract infections are commonly caused by E. coli, Proteus sp., and Pseudomonas sp [20]. The current study investigated higher prevalence of E. coli (26.6%) infectivity due to poor hygienic condition of female. In accordance with our study, vaginal colonization by E. coli, Proteus spp., Staphylococci, and P. aeruginosa has also been reported [21]. All isolates of Pseudomonas in this study revealed highest resistant to AMP, TE and SXT and there was also a lot of variation in antibiotic susceptibility between isolates. Furthermore, the current investigation found that P. aeruginosa isolates were more susceptible to CIP and AN than previously thought, possibly indicating a shift in antibiotic resistance trends. However, Pseudomonas was susceptible to CTX, GN, AK, and CIP. Other researchers have observed similar findings involving E. coli and Pseudomonas antibiotic resistance patterns [22, 23]. All the Gram negative organisms were susceptible to KA, CIP, AK, CRO, CTX and GN while E. coli shows highest susceptibility to CIP. A previous study also reported high susceptibility of gram-negative bacteria to AK in 2007 [24]. Alshara[25] in 2011 also reported effectiveness of AK, CTX and CIP against E. coli and other gram negatives but the current finding showed that SXT,TOB, CRO,CTX and GN against the E. coli and Pseudomonas spp. was less effective except against Proteus spp. The emergence of CIP (fluoroquinolone) resistance in many

bacteria that cause health-care-associated and community-acquired illnesses has raised questions regarding the antibiotic's long-term effectiveness [26]. The current study not only reveals high variability in antibiotic susceptibility among pathogenic bacteria, but it also indicated changing antibiotic resistance patterns. More study is also required to establish current criteria for empirical therapeutic regimens, which aid in establishment of current antibiotics resistance control measures [27]. The rise of antibiotic-resistant pathogenic bacteria necessitates a continuous examination of antimicrobial sensitivity profiles among clinically important microorganisms in our hospitals.

CONCLUSION

The harmful bacteria studied showed a low incidence of sensitivity to routinely used antibiotics. Antibiotic resistance was found at the greatest levels. CIP resistance was found slightly among the tested isolates. This study also suggests that if successful infectious disease treatment is to be ensured, ongoing tracking of changes in resistance patterns is critical. Physician should recommend antibiotics based on locally availability and have a proven susceptibility patterns for cost-effective treatment of bacterial illnesses, avoiding the use of more expensive second, third and fourth generation medicines. The study also emphasizes the importance of keeping eyes on changes in antibiotic profiles in order to spot new resistance traits and prevent treatment failure.

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