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KEY WORDS: Antibiotic resistance, multidrug resistance, urinary tract infection

BACTERIOLOGICAL PROFILE ANTIBIOTIC RESISTANCE PATTERN OF BACTERIA ISOLATED FROM URINARY TRACT INFECTIONS IN CENTRAL INDIA

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ABSTRACT

Background: This study aimed to investigate the bacteria associated with urinary tract infection (UTI) and antibiotic susceptibility pattern of the isolates during LAST one year in a tertiary care hospital of central india.
Materials and Methods: Overall 380 patients attending OPD with clinical symptoms of UTI were subjected as samples, and they were cultured and pure isolated bacteria were identified using biochemical tests and subjected to antibiogram assessment using disc diffusion method.
Results: Totally, 57 (15%) from 380 patients had positive UTI result. 51 (89.5% of positive case) patients were resistant to at least one antibiotic. *Escherichia coli, Staphylococcus* spp., and *Pseudomonas* spp. were the most prevalent bacteria which were present in 25(43.8%), 17(29.8%) and 5(8.7%) of the positive samples, respectively.
Conclusion: The results of this study revealed a great concern for emerging UTI-related multidrug-resistant strains of bacteria causing UTI in Iran.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common diseases in human societies which occur in women more than men.[1,2,3] The UTI occurrence depends on several factors provide the presence of bacteria (more than 105/ml) in urine.3 These bacteria cause UTI and if not treated, the infection will spread and cause serious damage to the patient.[2,4,5] UTI treatment with antibiotics is carried out usually before receiving microbiology test results. This therapy, without rational drug prescription occasionally leads to antibiotic resistance and treatment failure as its result.[3,6]

According to the World Health Organization in 2014, antimicrobial resistance is increasingly a global threat for public health and all countries have focused on this problem which is a serious threat to modern medicine.[7]

The most important factor in increasing microbial resistance is improper use of antibiotics.[8,9] which includes incorrect and unreasonable antibiotics prescription. Considering time, the appropriate dose and manner of administration are the most important aspects of rational drug prescription.[9,10] Studies have shown that 30%–60% of the prescribing and use of antibiotics has been improper. Thus it is the need of hour to turn the supervision on the use of antimicrobial agents to check on this worrying trend all over the world.

UTI is a common disease and can be treated easily if antibiotics are used reasonably.[2] Identification of bacteria that cause UTI and analysis of antibiotic susceptibility pattern of them is effective in the treatment. The aim of this study was to investigate the bacteria associated with UTI cases and their antibiotic susceptibility pattern over a period of one year in this tertiary care hospital.

MATERIALS AND METHODS

This cross-sectional study was performed from January 2017 to December 2017 in a tertiary care hospital in india. Overall, 380 patients with clinical symptoms of UTI attending different OPDs were referred to the laboratory .

Urine specimens were collected from each patient. Proper sampling instructions were given to each patient to collect a clean-catch mid-stream urine specimen.[15,16]

Accordingly, about [10-20] ml urine specimen was collected from each patient in a sterile screw-capped, wide-mouth container and labelled with the unique sample number, date and time of collection. Immediately, it was delivered to bacteriology laboratory and processed. Isolation and Identification of bacterial UTI: Using calibrated wire loop (0.001 mL) samples were inoculated in to Cystine Lysine Electrolyte Deficient medium (CLED). After overnight incubation at 37°C for [24–48] hours colonies were counted to check significant growth. Colony counts yielding bacterial growth of 105 CFU/mL of urine were regarded as significant for bacteriuria. Colonies from CLED were subcultured into MacConkey agar and blood agar plates (BAP) (Oxoid) and incubated at 37°C for [24–48] hours. Identification of bacteria was done using colony characteristics, Gram reaction of the bacteria and biochemical tests following standard procedure.[12,13]

All identified pure bacterial isolates were subjected to in vitro susceptibility testing using Kirby Bauer disk diffusion method as described in Clinical Laboratory Standard Institution (CLSI) guideline and interpreted accordingly. From a pure culture 3-5 identical colonies of bacteria were taken and transferred to a tube containing 5 mL sterile nutrient broth (Oxoid) and mixed gently until a homogenous suspension was formed and incubated at 37oC until the turbidity of the suspension become adjusted to a McFarland standard 0.5% Baso4. A sterile cotton swab was used and the excess suspension was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the surface of Muller Hinton agar (PH=7.2-7.4) (oxoid) following discs were used: - ceftriaxone, tetracycline, nitrofurantin, gentamycin, ampicillin, ticracilin, picracilin, and trimethoprim-sulphamethaxzole for Gram-negative bacteria, whereas vancomycin, nitrofarantin, erythromycin, ciprofloxacilin, trimethoprime/sulfamethoxazole, chloramphinicol and tetracyclin for pathogenic Gram- positive cocci. The criteria used to select the antimicrobial agents were based on both their availability for the management of UTIs and CLSI guideline.

The plates were then incubated at 37oC for 24 hours. Diameters of the zone of inhibition around the discs were measured to the nearest millimetre using a metal calibre, and the isolates were classified as susceptible and resistant.[12]

The reliability of the study findings were guaranteed by implementing quality control (QC) measures throughout the whole process of the laboratory work. Culture media was tested for sterility and performance. To standardize the inoculum density of bacterial suspension for a susceptibility test, 0.5 McFarland standard was used.13The data entry was checked by double entry. Data were entered using IBM Statistical Package for the Social Sciences (IBM SPSS Statistics; Armonk, NY, USA) version 20 software, analyzed, tabulated and summarized. The results were presented in tables.

RESULTS

In this study, 57 (15%) from 380 patients had positive UTI result. Among them, 51 (89.5% of positive case) patients were resistant to at least one antibiotic. *Escherichia coli*, *Staphylococcus* spp., and *Pseudomonas* spp. were the most prevalent bacteria which were present in 25(43.8%), 17(29.8%) and 5(8.7%) of the positive samples, respectively. The number of isolated bacteria is

presented in [Table 1].

Table 1: bacterial isolates from positive urinary tract infection patients

Bacterial isolates	number	%
Escherichia coli	25	43.8
Staphylococcus aureus	7	12.3
CONS	10	17.5
Pseudomonas aeruginosa	5	8.7
Klebsiella pneumoniae	4	7
Enterobacter spp.	2	3.5
Proteus mirabilis	1	1.7
Acinetobacter baumanii	1	1.7
Enterococcus faecalis	1	1.7
Citrobacter freundii	1	1.7
TOTAL	57	

Table 2: Resistant Gram-positive bacteria and the percentage of their antibiotic resistance

		n(%)											
bacterial isolates	**number**	**amp**	AmoxC	ak	cd	cip	ctr	gen	ipm	me	ox	cot	va
staphylococcus aureus	7	6(85.7)	1(14.3)	1(14.3)	0(0)	2(28.6)	4(57.2)	2(28.6)	1(14.3)	1(14.3)	1(14.3)	2(28.6)	0(0)
CONS	10	8(80)	1(10)	2(20)	0(0)	1(10)	3(30)	1(10)	1(10)	1(10)	1(10)	3(30)	0(0)
Enterococcus faecalis	1	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
TOTAL	18	15(83.3)	2(11.1)	3(16.6)	0(0)	4(22.2)	7(38.9)	3(16.6)	2(11.1)	2(11.1)	2(11.1)	5	0

*n= Resistant Gram-positive bacteria ; %= the percentage of their antibiotic resistance; Ampicillin (Amp), Amoxyclav(Amoxc), Amikacin (Ak), Clindamycin(Cd); Ciprofloxacin(Cip); Ceftriaxone(Ctr), Gentamycin(Gen); Imipenem(Ipm); Methicillin(Me); Oxacillin(Ox); Cotrimoxazole (Cot); Vancomycin(Va)

Table 3: Resistant Gram-negative bacteria and the percentage of their antibiotic resistance

		n(%)											
Bacterial isolates	Number	Amp	Amoxc	Ak	Caz	Cip	Ctr	Nit	Gen	Ipm	Na	Cot	Cl
Escherichia coli	25	22(88)	15(60)	5(20)	9(36)	19(76)	10(40)	8(32)	6(24)	1(4)	19(76)	12(48)	0(0)
Pseudomonas aeruginosa	5	5(100)	4(80)	1(20)	3(60)	3(60)	3(60)	2(40)	1(20)	1(20)	5(100)	5(100)	0(0)
Klebsiella pneumoniae	4	3(75)	2(50)	1(25)	2(50)	3(75)	2(50)	2(50)	1(25)	0(0)	4(100)	2(50)	0(0)
Enterobacter spp.	2	2(100)	1(50)	0(0)	1(50)	2(100)	1(50)	0(0)	1(50)	0(0)	2(100)	2(100)	0(0)
Proteus mirabilis	1	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)
Acinetobacter baumanii	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)
Citrobacter freundii	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
TOTAL	39	34 (87.1)	24 (61.5)	8 (20.5)	17 (43.5)	29 (74.3)	18 (46.1)	12 (30.7)	9 (23)	2 (5.1)	32 (82)	23 (58.9)	0

*n= Resistant Gram-negative bacteria ; %= the percentage of their antibiotic resistance; Ampicillin (Amp), Amoxyclav(Amoxc), Amikacin (Ak), Ceftazidime(Caz); Ciprofloxacin(Cip); Ceftriaxone(Ctr), Nitrofurantoin (Nit), Gentamycin(Gen); Imipenem(Ipm); Nalidixic Acid (Na); Cotrimoxazole (Cot); Colistin (Cl)

According to the results presented in [Table 2] and [Table 3], among the isolated bacteria, the most resistant Gram-positive bacteria were Staphylococcus spp. and the most resistant Gram-negative bacteria were *E. coli* . Gram-positive and Gram-negative bacteria showed the highest antibiotic resistance to ampicillin, respectively, and also demonstrated the most sensitivity to imipenem and amikacin.

DISCUSSION

The improper use of the antimicrobials for the treatment of the infections has adverse effects on public health organization of a country both in economic impact and increasing of the drug resistance among causative bacteria. Hence, it is essential to continuously evaluate the antimicrobial resistance condition in a society which was the first purpose of the present study, particularly in the case of UTIs. At first glance, results of the study demonstrate relatively high occurrence of the positive urine culture among samples collected from patients with UTI clinical signs, in comparison with other studies.[17,18] It may be due to the climate and nature of the central india, which has humid and relatively hot weather.[19]

As bacterial resistance increased in recent decades,[6,20] the isolates of the present study recovered from UTIs showed high resistance. Hence, 89.5% of them demonstrated resistance to at least one antibiotic. E. coli and Staphylococcus, similar to other studies were the most prevalent Gram-negative and Gram-positive bacteria, respectively.[17] The most resistance of the bacterial isolates was against ampicillin, as more than 80% of the Staphylococcus strains were resistant. However vancomycin resistance was not seen at all in this study and it could be because of only outdoor patient registration. Among Gram-negative bacteria, multidrug-resistant (MDR) strains have been reported as important and increasing strains which can spread the resistance among different populations of bacteria. E. coli and Pseudomonas spp. are the most significant Gram-negative MDRs, particularly in UTI patients.[17,21] None of the isolates were pan drug resistant (PDR).

Results of antibiogram test for 57 bacterial isolates recovered from UTI revealed that amikacin, nitrofurantoin, vancomycin, colistin and imipenem were the most effective antimicrobials against the strains. Some Gram-negative bacteria were resistant to antibiotics, which are widely used for treating hospital-acquired infections with MDR Gram-negative bacteria such as Pseudomonas and Acinetobacter. Carbapenems are resistant to the -lactamase enzymes produced by numerous MDR Gram-negative bacteria, so, playing a significant role in the treatment of infections not cured with other antibiotics.[22] Hence,judicious use of these antibiotics is required as probable increase of the imipenem-resistant strains can be an emerging concern for health control systems of a

country. It seems that administrators should have a special precision and care in the use of these drugs for treatment of the UTI and/or other infections.

To survey the antimicrobial resistance pattern among patients with specific infection within a few years, it is essential to evaluate and compare antibiotic resistance condition in each of the years. and first step to obtain a proper management and good control policy for decreasing the development of antibiotic resistance among microorganisms, particularly the pathogens is the evaluation and practical assessment of the antibiotic resistance patterns among definite populations of the patients of a country.

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Conflicts of interest
There are no conflicts of interest.

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