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Antimicrobial susceptibility pattern of human pathogenic bacteria related to *Enterobacteriaceae* family causing urinary tract infection

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ABSTRACT

Gram negative bacteria are most commonly involved in causing Urinary tract infection (UTI), a urinary disease most commonly found in developing countries. The regular monitoring of specific areas gains the knowledge about the prevalence of these in the UTI and their susceptibility pattern is useful for the clinicians to choose correct empirical treatment. Therefore, the aim of this study was to determine the type of Gram negative bacteria related to Enterobacteriaceae involved in UTI and antimicrobial susceptibility pattern of the urinary pathogens. Total 132 urine samples were collected by mid stream clean catch method and tested bacteriologically using standard procedures. Antimicrobial susceptibility testing was performed by using Kirby-Bauer disk diffusion method. Total 48.48% urine samples showed significant bacterial growth. The most common pathogens were Escherichia coli (42.71%), Klebsiella pneumoniae (23.96%), Proteus spp (19.79%) and Enterobacter spp (13.54%). 90.24% E. coli showed resistance to Nalidixic acid, however, Amikacin showed 100% sensitivity to isolated E. coli. Ciprofloxacin and imipenem showed 69.57% resistance in K. pneumonia, however, Levofloxacin showed 100% sensitivity. Nitrofurantoin showed 92.30% resistance in Enterobacter spp and most quinolones and carbenicillins was susceptible to Enterobacter spp. Proteus spp was 100% resistant against Third generation cephalosporin, however, Carbepenems was highly susceptible to isolated Proteus spp. Meropenem (90.63%) was most sensitive among all isolated UTI pathogens and Nalidixic acid showed 67.71% sensitivity among all isolates.

Key words: Antibiogram, *E. coli*, UTI, Drug resistant, Susceptibility monitoring

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INTRODUCTION

Urinary tract infections (UTIs) are the most common extra intestinal infections affecting people of all age groups [1]. Each year about 150 million people are diagnosed with UTI in all over the world [2]. In most cases UTIs are not life threatening and causes reversible damage, however, when a main urinary organ kidneys are involved the risk of irreparable tissue damage and bacteremia increased [3]. Gram negative bacteria play an important role in UTI. It has been estimated that more than 7 million visits to emergency units and 100,000 in hospitals occurs annually in USA [4]. *Escherichia coli* remained the most common causative agent of uncomplicated UTI for many years with 75-90% causes of UTI infection [5, 6, 7]. The other gram negative pathogens causing UTI are *Klebsiella* spp., *Proteus mirabilis* and *Pseudomonas aeruginosa*, however, *Enterococci* and coagulase negative *Staphylococci* are the most frequently encountered gram positive bacteria in UTI [8]. The antibiotic susceptibility patterns of UTI causing pathogens have been varying from time too time and from place to place in both community and hospital settings [9, 10, 11]. Increasing drug resistance in pathogens is now a serious problem to treat diseases like malaria, Tuberculosis, diarrheal diseases, UTI etc., [12]. The main cause of this issue is the improper and uncontrolled use of antibiotics [13] as well as improper prescription, inappropriate dosage and duration of treatment [14]. The genetic

causes of drug resistance n pathogenic microorganisms are horizontal gene transfer via plasmid, transposons and bacteriophages, recombination of foreign DNA in bacteria chromosome and mutations in chromosomal loci [15]. A large number of drug resistant bacteria have been discovered during the past decades as methecillin resistant *Staphylococcus aureus* (MRSA) [16], multi drug resistant *Pseudomonas aeruginosa* [17], *Serratia marcescens* [18], vancomycin resistant *Enterococci* (VRE) [19] and extended spectrum beta lactamase (ESBL) resistant *Enterococci* [20] which is a very serious public health issue mainly in developing countries where high level of poverty, poor hygienic conditions as well as fake and spurious drugs are in the circulation of medical practices [12]. Hence, the changing susceptibility patterns of microorganisms causing UTI leads to conduct antimicrobial susceptibility testing studies of these pathogens in various regions and on regular basis.

MATERIALS AND METHODS

Collection and preparation of test clinical bacterial isolates

Total 132 urine samples were collected by clean catch mid-stream urine collection method in a 4 to 5 ml of sterile, wide mouthed glass bottles with screw cap tops and immediately transported to the laboratory. Guidelines for proper specimen collection were given to all patients on a printed card [21].

Sample processing

A calibrated sterile platinum wire loop for the semi-quantitative method was used for the plating and it has a 4.0 mm diameter designed to deliver 0.01 ml. A loopful of the well mixed urine sample was inoculated into triplicate plates of Mac-Conkey agar. All plates were then incubated at 37°C aerobically for 24 h. The plates were then examined macroscopically and microscopically for bacterial growth. The bacterial colonies were counted and multiplied by 100 to give an estimate of the number of bacteria present per milliliter of urine. A significant bacterial count was taken and a sample was considered positive for UTI if as any count equal to or in excess of 10^5 cfu/ml [22, 23]. The mean of three replicated experiments was considered.

Bacterial isolation and identification procedures

Each well mixed urine sample (5 µl) was inoculated on Mac-Conkey agar. The inoculum on the plate was streaked out for discrete colonies with a wire loop following standard procedures [24, 25]. The culture plates were incubated at 35°C - 37°C for 24 h and observed for growth through formation of colonies. The bacterial isolates were collected on nutrient agar slants and sub cultured periodically. All the bacteria were identified using morphological, microscopy and biochemical tests following standard procedures described by Cowan and Steel and Cheesborough [26, 24].

Antimicrobial susceptibility testing

Antimicrobial sensitivity testing of all isolates was performed on diagnostic sensitivity test plates by the Kirby Bauer disk diffusion method [27] following the definition of the Clinical and Laboratory Standards Institute [28]. Bacterial inoculums were prepared by suspending the freshly-grown bacteria in 25 ml sterile nutrient broth and inoculums were adjusted to 0.5 McFarland. A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates. Filter paper disks containing designated amounts of the antimicrobial drugs obtained from commercial supply firms (Himedia Labs, Mumbai, India) were used. The antimicrobial agents tested were Imepenem (10µg), Meropenem (10µg), Ciprofloxacin (5µg), Tobramycin (10µg), Moxifloxacin (5µg), Ofloxacin (5µg), Sparfloxacin (5µg), Levofloxacin (5µg), Ceftazidime (30µg), Amikacin (30µg), Nitrofurantoin (300µg), Netillin (30µg), Nalidixic acid (30µg), Cephotaxime (30µg), Co-Trimoxazole (25µg), Gentamicin (10µg), Ceftrixone (5µg), Gatifloxacin (30µg).

Statistical analysis

The student t-test for paired samples was used to compare resistance versus sensitivity against all isolates with Statistical Package for Social Sciences (SPSS) software for Windows, version 20. Susceptibility was calculated as percentages with 95% confidence intervals and a p-value of <0.05 was considered to be statistically significant.

RESULTS

Out of total 132 urine samples only 64 (48.48%) showed a significant bacterial growth ($\geq 10^5$ cfu/ml) and considered positive for UTI.

Total 96 Gram negative bacteria were isolated from 64 positive samples of urine. Among all 96 isolates, *E. coli* showed the high prevalence 41 (42.71%) in total followed by *Klebsiella pneumoniae* 23 (23.96%); *Proteus spp.* 19 (19.79%) and *Enterobacter spp.* 13 (13.54%) (Table 1).

Table 1. Percentage of isolated Gram negative pathogens causing UTI.

Gram negative isolates	No. in total	Percentage
E. coli	41	42.71
Klebsiella pneumoneae	23	23.96
Enterobacter spp.	13	13.54
Proteus spp.	19	19.79
Total	**96**	**100**

Table 2. Overall number and percentage (%) of susceptibility to the antimicrobial agents among 96 UTI isolates.

Antimicrobial class	Antimicrobial agents	Number & percentage of isolates					
		R		I		S	
		N	%	N	%	N	%
Quin.	Cf	47	48.96	0	0	49	51.04
	Mo	35	36.46	5	5.21	56	58.33
	Of	25	26.04	5	5.21	66	68.75
	Sc	32	33.33	3	3.13	61	63.54
	Le	10	10.42	5	5.21	81	84.37
	Na	65	67.71	3	3.12	28	29.17
	Gf	19	19.79	8	8.33	69	71.88
Amn.	Tb	29	30.21	4	4.17	63	65.62
	Ak	26	27.08	0	0	70	72.92
	Ge	27	28.12	5	5.21	64	66.67
Cep[3]	Ca	53	55.21	9	9.37	34	35.42
	Ce	40	41.67	2	2.08	54	56.25
	Ci	49	51.04	4	4.17	43	44.79
Carb.	Im	18	18.75	1	1.04	77	80.21
	Mr	9	9.37	0	0	87	90.63
Others	Nf	45	46.87	6	6.25	45	46.88
	Nt	21	21.87	5	5.21	70	72.92
	Co	46	47.92	2	2.08	48	50.0

Statistical interference: significant at p<0.05

Quin.= Quinolones; Amn.= Aminoglycosides; Cep[3]= III generation cephalosporin; Carb.= Carbenicillin; Cf= Ciprofloxacin; Mo= Moxifloxacin; Of= Ofloxacin; Sc= Sparfloxacin; Le= Levofloxacin; Na= Nalidixic acid; Gf= Gatifloxacin; Tb= Tobramycin; Ak= Amikacin; Ge= Gentamycin; Ca= Ceftazidime; Ce= Cefotaxime; Ci= Ceftrixone; Im= Imipenem; Mr= Meropenem; Nf= Nitrofurantoin; Nt= Netellin; Co= Co-trimaxazole; R= Resistant; I= Intermediate; S= Sensitive; N= Number

Table 3. Determination of the relationship between sensitive and resistant pathogens using paired Samples t-Test

	Paired Samples Test							
	Paired Differences							
				95% Confidence interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1 Sensitive-resistant	26.05556	31.32462	7.38328	10.47819	41.63292	3.529	17	0.003

The calculated P-value was lower than 0.05 in paired t tests performed on sensitive vs. resistant pathogens indicating that the mean differences between the paired observations was significant. The P-value for the sensitive vs. resistant variables was found p=0.003 at 95% level of confidence (Table 3).

The percentages of resistance of all 96 isolates to the antimicrobial agents were: 67.71% to Nalidixic acid followed by 55.21% to Ceftazidime and 51.04% to Ceftriaxone. The percentages of pathogens resistance varied between 67.71% and 9.37% to the antimicrobial agents, while in susceptible of the pathogens varied between 29.17% and 90.63%. The most effective drug was Meropenem (90.63%), followed by Levofloxacin (84.37%) and Netellin (72.92%) among all 96 UTI isolates (Table 2).

According to table 4 Nalidixic acid was found to be most resistant drug in 90.24% cases of *E. coli* followed by Ciprofloxacin (65.85% cases) and Co-trimaxazole (63.41% cases). However, Amikacin showed the highest sensitive drug in 100% isolates of *E. coli* followed by Imipenem, Meropenem which both showed sensitivity in 92.68% cases and Tobramycin in 82.93% cases. The resistance and sensitivity range of tested antimicrobial agents against *E. coli* was 0%-90.24% and 4.88%-100% respectively.

Table 4. Antimicrobial susceptibility profiling of isolated Gram negative UTI pathogens.

	Antimicrobial agents	Isolated UTI pathogens											
		E. coli (N=41)			*K. pneumoniae* (N=23)			*Enterobacter spp* (N=13)			*Proteus spp* (N=19)		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Quin.	Cf	27 (65.85)	0 (0)	14 (34.15)	16 (69.57)	0 (0)	7 (30.43)	0 (0)	0 (0)	13 (100)	4 (21.05)	0 (0)	15 (78.95)
	Mo	21 (51.22)	5 (12.19)	15 (36.59)	14 (60.87)	0 (0)	9 (39.13)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)	19 (100)
	Of	12 (29.27)	4 (9.76)	25 (60.97)	0 (0)	1 (4.35)	22 (95.65)	0 (0)	0 (0)	13 (100)	13 (68.42)	0 (0)	6 (31.58)
	Sc	16 (39.02)	3 (7.32)	22 (53.66)	6 (26.08)	0 (0)	17 (73.92)	0 (0)	0 (0)	13 (100)	10 (52.63)	0 (0)	9 (47.37)
	Le	10 (24.39)	5 (12.20)	26 (63.41)	0 (0)	0 (0)	23 (100)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)	19 (100)
	Na	37 (90.24)	2 (4.88)	2 (4.88)	14 (60.87)	1 (4.35)	8 (34.78)	5 (38.46)	0 (0)	8 (61.54)	9 (47.37)	0 (0)	10 (52.63)
	Gf	13 (31.71)	8 (19.51)	20 (48.78)	6 (26.08)	0 (0)	17 (73.92)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)	19 (100)
Amn.	Tb	3 (7.32)	4 (9.75)	34 (82.93)	15 (65.22)	0 (0)	8 (34.78)	6 (46.15)	0 (0)	7 (53.85)	5 (26.32)	0 (0)	14 (73.68)
	Ak	0 (0)	0 (0)	41 (100)	7 (30.43)	0 (0)	16 (69.57)	5 (38.46)	0 (0)	8 (61.54)	14 (73.68)	0 (0)	5 (26.32)
	Ge	4 (9.76)	5 (12.20)	32 (78.04)	6 (26.08)	0 (0)	17 (73.92)	7 (53.85)	0 (0)	6 (46.15)	10 (52.63)	0 (0)	9 (47.37)
Cep[3]	Ca	14 (34.15)	7 (17.07)	20 (48.78)	16 (69.57)	2 (8.69)	5 (21.74)	4 (30.77)	0 (0)	9 (69.23)	19 (100)	0 (0)	0 (0)
	Ce	19 (46.34)	0 (0)	22 (53.66)	8 (34.78)	1 (4.35)	14 (60.87)	6 (46.15)	1 (7.70)	6 (46.15)	7 (36.84)	0 (0)	12 (63.16)
	Ci	17 (41.46)	3 (7.32)	21 (51.22)	6 (26.08)	1 (4.35)	16 (69.57)	7 (53.85)	0 (0)	6 (46.15)	19 (100)	0 (0)	0 (0)
Carb.	Im	2 (4.88)	1 (2.44)	38 (92.68)	16 (69.57)	0 (0)	7 (30.43)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)	19 (100)
	Mr	3 (3.72)	0 (0)	38 (92.68)	6 (26.08)	0 (0)	17 (73.92)	0 (0)	0 (0)	13 (100)	0 (0)	0 (0)	19 (100)
Others	Nf	8 (19.51)	6 (14.64)	27 (65.85)	14 (60.87)	0 (0)	9 (39.13)	12 (92.30)	0 (0)	1 (7.70)	11 (57.89)	0 (0)	8 (42.11)
	Nt	2 (4.88)	5 (12.19)	34 (82.93)	6 (26.08)	0 (0)	17 (73.92)	3 (23.08)	0 (0)	10 (76.92)	10 (52.63)	0 (0)	9 (47.37)
	Co	26 (63.41)	1 (2.44)	14 (34.15)	8 (34.78)	1 (4.35)	14 (60.87)	5 (38.46)	0 (0)	8 (61.54)	7 (36.84)	0 (0)	12 (63.16)

Quin.= Quinolones; Amn.= Aminoglycosides; Cep[3]= III generation cephalosporin; Carb.= Carbenicillin; Cf= Ciprofloxacin; Mo= Moxifloxacin; Of= Ofloxacin; Sc= Sparfloxacin; Le= Levofloxacin; Na= Nalidixic acid; Gf= Gatifloxacin; Tb= Tobramycin; Ak= Amikacin; Ge= Gentamycin; Ca= Ceftazidime; Ce= Cefotaxime; Ci= Ceftrixone; Im= Imipenem; Mr= Meropenem; Nf= Nitrofurantoin; Nt= Netellin; Co= Co-trimaxazole; R= Resistant; I= Intermediate; S= Sensitive; N= Number

Both Imipenem and Ciprofloxacin showed highest resistance (69.57%) in *K. pneumonia* followed by Tobramycin (65.22%), Nalidixic acid (82.93%) and Nitrofurantoin (82.93%). The resistance and sensitivity range of tested antimicrobial agents against *K. pneumonia* was 0%-69.57% and 21.74%-100% respectively (Table 4).

Nitrofurantoin was the most resistant drug in 92.30% cases of *Enterobacter spp* followed by Ceftriaxone, Gentamycin both in 53.85 % cases and Cefotaxime, Tobramycin both in 46.15% cases. However, Ciprofloxacin, Moxifloxacin, Ofloxacin, Sparfloxacin, Levofloxacin, Gatifloxacin, Imipenem, Meropenem were most sensitive against *Enterobacter spp* in 100% cases followed by Netillin (76.92%) and Amikacin, Co-trimaxazole both in 61.54% cases. The resistance and sensitivity range of tested antimicrobial agents against *Enterobacter spp* was 0%-92.30%% and 7.70%-100% respectively (Table 4).

All 19 isolates (100%) of *Proteus* spp were resistant to Ceftazidime and Ceftriaxone followed by Amikacin (73.68%) and Ofloxacin (68.42%). However, all 19 isolates (100%) were sensitive to Moxifloxacin, Levofloxacin, Gatifloxacin, Imipenem and Meropenem followed by Ciprofloxacin (78.95%) and Tobramycin (73.68%). The resistance and sensitivity range of tested antimicrobial agents against *Proteus* spp was 0%-100% (Table 4).

DISCUSSION

Bacterial urinary tract infection is one of the serious issues which needed an urgent medical attention in community [29]. The most effective management of UTI patients is the identification of pathogens and selection of effective antimicrobial agent against them [30]. The effective and traditional method for the diagnosis of UTI is plate count method in which $>10^5$ bacteria/mL of urine indicates bacteriuria [31, 32]. In this study, the isolation rate of bacteria from urine was 48.48 % which is supported by other reports [29, 33, 34, 35, 36] but not correlated with other [37]. The most predictable and primary etiological bacteria involved in UTI in both out and inpatients is *E. coli* [38, 39, 40, 10], however, the enteropathogenic variety of *E. coli* was also suggested the most common cause of neonatal diarrhea [41]. In this study, *E. coli* was by far the most common bacteria isolated from urine samples and this finding is in agreement with others finding too [42, 33, 36, 43, 44, 37, 45, 46]. In other study done in Ethiopia on UTI investigation from diabetic patients also showed that *E. coli* (31.7%) was the most prevalent bacterial isolate from asymptomatic and symptomatic diabetic patients [47]. In contrary to others study findings where the second reported isolates was *Staphylococcus species* [33, 48, 43, 49, 44, 37], however, in this study it was *K. pneumoniae* which is in agreement with the findings of other studies [50, 51, 46]. Increasing resistance against antimicrobial agents is a worldwide problem [52]. This study revealed that there is a higher prevalence rate of resistance against commonly prescribed antibiotics in India. A considerable reduction is also found in the activity of nitrofurantoin among the commonly used drugs in treatment of UTI. These findings are supported by other studies done in Kuwait [53] and also in the U.S., southern Europe, Israel, and Bangladesh with up to 50% of *E. coli* strains being resistant to antibiotics used [54]. The most useful antibiotics in this study were Meropenem (Carbepenem) and Levofloxacin (Quinolones) in 90.63% and 84.37% overall cases respectively. These drugs are relatively expensive when compared to most antibiotics frequently used. This probably had restricted their procurement and indiscriminate use, therefore making the organisms susceptible to it. These findings differed from other reports where quinolones are the most effective antimicrobial agent against UTI causing bacteria [55, 56, 57, 58].

The findings have no doubt there is an urgent need for constant monitoring of susceptibility of pathogens in different populations to commonly used anti-microbial agents. The data of this study may be used to determine trends in antimicrobial susceptibilities, to formulate local antibiotic policies and overall to assist clinicians in the rational choice of antibiotic therapy to prevent misuse, or overuse, of antibiotics.

CONCLUSION

This study concluded that *E. coli* was the predominant pathogen in urinary tract infection. Isolated UTI pathogens showed highest resistant against Nalidixic acid and Meropenem was the highest sensitive. Finally, empirical antibiotic selection in treatment of UTI should be based on the knowledge of local prevalence of causative organisms and their antimicrobial sensitivities rather than on universal guidelines so as to reduce the incidence of resistance. Indiscriminate prescription and use of antibiotics should be discouraged by continuous public enlightenment on rational antibiotic use as well as adoption of strict national antibiotic policy to regulate the prescription, sale and use of antibiotics.

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