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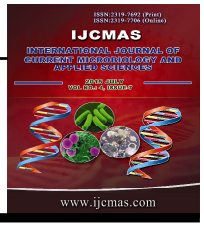

Original Research Article

Profile of Urinary Tract Infection and Quinolone Resistance among *Escherichia coli* and *Klebsiella* species isolates

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Keywords

UTI, Antibacterial resistance, *Escherichia coli*, *Klebsiella spp.*, Quinolone resistance

A B S T R A C T

Urinary tract infections are caused by to specific pattern of microorganisms and their susceptibility to antibiotics depends on the use, overuse or misuse of antibiotics for common conditions in these geographical regions. This study was undertaken to know the microbial profile of UTI and the resistance pattern of *Escherichia coli* and *Klebsiella species* especially to Quinolone group of antibiotics. The study was conducted for a period of six months. Urine samples from suspected UTI patients formed the study subjects. The standard conventional methods were followed in urine culture and sensitivity. The pattern of bacterial isolates and their susceptibility especially to quinolone group of antibiotics were determined among *Escherichia coli* and *Klebsiella species* isolates. 593 of urine samples revealed monomicrobial growth with significant bacteriuria out of 2193 total samples processed. *Escherichia coli* was the most commonly isolated bacteria and constituted 54.5% which was followed by *Klebsiella species* of 21.5%. Resistance to cephalosporins among *E.coli* ranged from 63% to 72 % and 79% to 80% among *Klebsiella spp.* Quinolone resistance ranged from 71% to 91% in *E.coli* and 65% to 94% among *Klebsiella spp.* Extended spectrum beta lactamases (ESBL)production among *E.coli*resistant to Quinolones was 46.3% of *E.coli*and38.5% in *Klebsiella spp.*Resistance to Nitrofurantoin among *E.coli* was 23% and 20% in *Klebsiella spp.* Lowest drug resistance was noted for carbapenems like Imipenem.

Introduction

Urinary tract infection (UTI) is the most common condition causing individuals to seek medical consultation. [1,2] It is considered as one of the major health problem next to respiratory and gastro intestinal infections in human. The primary organisms that cause UTI are bacteria and the diseases ranging from asymptomatic to severe sepsis affecting millions of people every year. [3,4,5]

About 150 million people are estimated to be diagnosed with UTI each year Worldwide, which is costing the global economy in excess of 6 billion US dollars. [6]

Escherichia coli and *Klebsiella species* are the most common members causing UTI. In developing countries most of UTI are treated

on an empirical basis in the absence of any laboratory investigation. [7]

Quinolones especially Fluoroquinolones (FQ) are broad spectrum antimicrobial agents used widely, irrationally and indiscriminately practiced by clinicians and general practitioners for many bacterial infections.[8, 9] Among the FQs, Ciprofloxacin is the frequently used FQ and has shown an excellent activity against pathogens commonly encountered in UTIs.[6] It reaches high concentrations in the prostatic tissue and in the seminal fluid, there for they are considered to be the first therapeutic choice in males with UTI.[10]

The ready availability of broad spectrum antimicrobials particularly quinolones has changed the prescribing habit of physicians treating UTI leading to enhanced resistance in recent years. Resistance to this FQ group of antibiotics is caused by mutations in the chromosomal genes that code for DNA gyrase and/or DNA topoisomerase IV, which are the target enzymes, resulting in alteration in drug accumulation. [8] The widespread use of FQ in veterinary medicine and in the treatment of many human infectious diseases has led to an increasing resistance to these agents.[10]

Worldwide, many studies have reported an increase in ciprofloxacin resistance, by bacteria causing UTIs. The rate of resistance varies from place to place and also among organisms causing UTI. It is in the range of 3.5% to 59.4% over last few years. [6, 11] In India, studies have indicated high rate of resistance by *Proteus species* of 83.4%, followed by 76.3% among *Escherichia coli (E.coli)* and 60.7% by *Klebsiella species*.[12]

Escherichia coli, a commonly isolated uropathogen has become resistant to many antimicrobial agents since last decade. Even other members of *Enterobacteriaceae* which can cause UTI have become less susceptible to widely used antibiotics, this can be considered as public health importance. Hence it is necessary for all institutions to keep continuous surveillance of all uropathogens and their antibiotic susceptibility pattern depending on the locality or regions. [13]

Hence the present study has been undertaken to know the pattern of uropathogens causing UTI and their antibacterial susceptibility pattern with special interest in the prevalence of Quinolone resistance among *Escherichia coli and Klebsiella species*

Materials and Methods

This study was conducted in Department of Microbiology at KIMS Hospital Hubli between Jan 2014 to June 2014.After thorough instructions to the patients, clean-catch midstream urine was collected into a sterile universal container. The specimens were labelled and transported to the laboratory for immediate processing. A loop-full (0.001 ml) of well mixed un-centrifuged urine was streaked onto the surface of blood agar and MacConkey's agar. The plates were incubated aerobically at 37^0 C for 18-24 hours and counts were expressed in colony forming units (CFU) per milliliter (mL). A count of $\geq 10^5$ CFU/mL was considered significant bacteriuria. Organisms identified were based on standard conventional bacteriological techniques.[14,15]

All the isolates were subjected for antimicrobial susceptibility testing and this was performed as per Kirby-Bauer disc diffusion method as per CLSI guidelines 2013.[16] Antimicrobial discs used were Amoxycillin-clavulanic acid (20/10 µg), Co-

Trimoxazole (1.25/23.75µg), Gentamicin (10µg), Amikacin (30µg),Nalidixic acid (30µg), Ciprofloxacin (5µg), Norfloxacin (10 µg), Nitrofurantoin (300 µg), Cephotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Ceftazidime +clavulanic acid (30µg/10µg),Cefaperazone (30µg), Cefaperazone +sulbactum (30µg/10µg),Imipenem (10µg).

The isolates were also tested for extended spectrum beta lactamase production by phenotypic confirmation test by using ceftazidime & ceftazidime+clavulanic acid disc at 15 mm distance. Quality controls were regularly checked by employing standard strains of *E.coli* ATCC 25922, interpretive criteria for susceptibility or resistance was followed as per CLSI guidelines.

The plates for the susceptibility tests were prepared in the laboratory. All the antibiotic discs and dehydrated media were obtained from Hi-media laboratories Pvt. Limited, Mumbai, India.

Results and Discussion

A total of 2193 consecutive urine samples were included in the study during the study period. Of these 1360 (62%) were sterile, 593 (27%) showed significant growth of $>10^5$ CFU/ml and 57 (2.6%) showed insignificant growth and 183 (8.4%) were found contaminated with poly microbial growth of more than three types of organisms.

Of these 593 mono microbial culture positive urine samples, *E.coli* (323, 54.5%) was the most common bacteria isolated. Second most common organism isolated in the study was *Klebsiella spp* accounting for 21.5% (127), followed by *Proteus spp* 5.5% (33) and *Pseudomonas spp* 4.5% (27).Other less commonly isolated organisms are *Staphylococcus aureus* 4%, *Enterobacterspp* and *Acinetobacterspp* 2.5% each. Candida species was isolated in 12 samples constituting nearing 2%. *Co-agulase negative Staphylococcus, Enterococcus spp, Providencia species* were isolated less commonly in 17 samples.

The two major isolates *E.coli* and *Klebsiellaspp* showed varying resistance to various antibiotics.*E. coli* exhibited highest resistance to cephalosporins. Resistance to Amoxycillin-clavulanic acid was 74%, 76% of isolates were also resistant to Co-Trimoxazole.

Similar nature of resistance was also noticed among Klebsiella isolates for the same antibiotics. Resistance to cephalosporins like Ceftriaxone, ceftazidime and cefaperazone was 72%, 68% and 63% respectively among *E.coli* isolates. Among *Klebsiella species* resistance noticed was 79% to 80% for the same cephalosporins. However 94% of *Klebsiella spp* and 92% of *E. coli* were sensitive to ceftazidime+clavulanic acid.

Among the aminoglycosides, resistance to Gentamicin was more among both isolates and was 41% and 46% in *E. coli* and *Klebsiella spp*. Amikacin was more useful to these isolates as only 37% of *Klebsiella spp* and 23% of *E. coli* were resistant.

Among the quinolones tested, resistance to Nalidixic acid, Ciprofloxacin and Norfloxacin was 91%, 79% and 71% respectively among *E. coli* isolates. Among *Klebsiella spp* the resistance rates were 94%, 68% and 65% respectively against them.

Resistance to Nitrofurantoin was less compared to any other antibiotics used in this study, and observed 23% among *E. coli* isolates and 20 % in *Klebsiella spp*.

Lowest drug resistance was noticed for Carbapenems like imipenem, where the resistance was just 3% among *E. coli* and 9% in *Klbsiella spp*.

ESBL production rate among *Escherichia coli* resistant to all three quinolones was 46.3%, whereas 38.5% in *Klebsiella spp*. The same in sensitive isolates was 11.6% & 25% in *Escherichia coli* and *Klebsiella species* respectively as tested by phenotypic confirmatory test.

Most of these ESBL producers were multidrug resistant with a high level of resistance to more than three groups of antibiotics.

Worldwide many studies have proved that the members of *Enterobacriaceae* family as the predominant uropathogens. Studies have indicated that this group of uropathogenic bacteria constitutes between 60% and 79% in different setups.[13,5, 9, 17] In the present study these group also accounted for 86.5%.

Among monomicrobial growths with significant bacteriuria, the most common isolate identified was *E.coli* constituting 54.5% in the present study. In most of the situations in the world still even today, *E.coli* is observed to be the most common uropathogen, rate of isolation was varying from 24.5% to 88% and this also varied over time due to various reasons in these places. [1,4,7,9,12,13,14]

Next to *E.coli*, *Klebsiella spp* constituted 21.5%, which ranks as second uropathogen in *Enterobacteriaceae* group causing UTI. The similar rates of *Klebseilla spp* isolation in urine sample of patients from many developing countries was noticed in other studies.[4,7,13, 16] The reasons for existence of this bacterial species especially in developing countries as second common uropathogen is not clear, as following *E.coli* many other species have been noted as second most common isolates in some of developed nations.

Most of polymicrobial growth which constituted 8.4% of total samples in this study cultures, the cultures were in different combinations, they mainly consisted of *Candida, Bacillus subtilis, Diphtheroids, Klebsiella spp* and *E.coli* . Most of the polymicrobial cultures were in females than males.

It is usual to find uropathogenic *E.coli* which are resistant to many commonly used antibiotics. However, the degree of resistance varies in different set ups depending upon underlying conditioning influences, diseases and irrational use of antimicrobials in the locality .The extent to which the development of drug resistance also depends on frequency of use of same antimicrobial drug for similar conditions over varying durations.[13,16]

In the present study *E.coli* and *Klebsiella spp* exhibited a almost a similar degree of resistance to antibiotics such as Augmentin 74%& 81%, Co-trimaxazole 76% and 78%. Resistance to cephasoporin groups of antibiotics was high in *Klebsiella spp* ranging from 78% to 80% than *E.coli* where it was 63% to 72%. Among aminoglycosides resistance to Gentamicin was 41% to 46% and to Amikacin, 23% to 37%.

Resistance by *E.coli* to Fluoroquinolones(FQ) group antibiotics like ciprofloxacin and norfloxacin was 79% and 71% respectively. However resistance by *Klebsiella spp* was slightly lower of 68% and 61% respectively in the present study. *E.coli* developing resistance to FQ is

reported to be on the raise, from 3% to 26.4% among UTI patients in developed countries like Spain and Japan.[18]Increased and easy availability and over use of different antibiotics of FQ group as shown an increase in resistance among *E.coli* isolates in many regions of world like Nigeria, Iran, Bangladesh and Pakistan which was varying from 21.6% to 84%.[1,4,7, 13,14, 16]. Studies across India also reported various degree of FQ resistance up to 68.5% which is almost comparable to the present study.[12]

An established fact is that, there is direct relation between frequency of antibiotics used or misused and pattern of antibiotic resistant bacterial strains in human beings. There is also overuse of FQ in veterinary farms. The resistance to antimicrobial agents can be readily transferred among bacteria by transmissible elements/plasmids. [1,4] There are many reasons for this alarming situation, including rigorous marketing of antibiotics, inappropriate prescription of antibiotics without appropriate sensitivity testing, easy availability in the pharmacy and poor infection control strategies.

E.coli and *Klebsiella spp* have shown susceptibility to Nitrofurantoin of 77% and 80% respectively. Resistance to Nitrofurantoin across worldwide was also less, as reported in few studies.[3,4,78,13,14]. For treatment of UTI, even today Nitrofurantoin can be a good choice, as observed in the present study.

The increasing resistance to third-generation cephalosporins was associated with the presence of ESBLs in the present study, as 46.3% of *Escherichia coli* resistant to FQ were ESBL producers. Among *Klebsiella spp* the rate of ESBL production was 38.5%. However, among FQ sensitive isolates ESBL production was 11.6% and 25% respectively in *Escherichia coli* and *Klebsiella species* as tested by phenotypic confirmatory test.

Most of these ESBL producers were multidrug resistant's (MDR). In seventies MDR (i.e. resistance to $\geq$ 3 groups of antibiotics) was practically non-existent and the cause of which was restricted to mutation of chromosomal genes. During the last two decades bacterial resistance scenario was worsened by plasmids, which carry resistance genes to a large number of antibiotic and these are rapidly transferred among different bacteria. [9, 12, 14]

Imipenem was found to act against *E coli*and *Klebsiella spp* in 97% and 91% respectively. A similar finding was also noticed in other studies. [2,4,19] But the alarming issue is 9% of *Klebsiella spp* and 3% of *E.coli* are Carbapenem (Imipenem) resistant, which further may worsen the resistance pattern and limit the treatment options.

Analysis of the plasmid DNA of *E.coli* shows multiple plasmids ranging from 1.0 to >140 MDa, with diverse pattern.Analysis of plasmid profile may be useful tool to document the appearance of plasmid with important phenotypic characteristics. Most importantly the drug resistance character is most often encoded on plasmids, which can easily be transferred among isolates. [4]

As the resistance to FQ group of antibiotics is caused by mutations in the chromosomal genes that code for DNA gyrase and/or DNA topoisomerase IV, which are the target enzymes, resulting in alteration in drug accumulation, the analysis of plasmid profile will give accurate picture on genotypic mechanisms in the present isolates.[8]

The frequent use of antimicrobial agents, their dosage and period of administration vary greatly from country to country, and

variable within the country and even among treating physicians. Even the widespread use of FQs and many other antibiotics in veterinary medicine is added to the existing resistance problem. This has led to large differences in the emergence of resistant strains. Since UTI has large socio economic impact and may contribute to the emergence of bacterial drug resistance; the periodic surveillance of antibiotic susceptibility in a systematic manner will help in clinical management.

Table.1 Profile of Uropathogens isolated in mono microbial cultures (N=593)

Sl NO	Organism isolated	Total numbers	Percentage
1.	*Escherichia coli*	323	54.5
2.	*Klebsiella spp*	127	21.5
3.	*Proteus spp*	33	5.5
4.	*Pseudomonas spp*	27	4.5
5.	*Enterobacter spp*	15	2.5
6.	*Citrobacter spp*	9	1.5
7.	*Acinetobacter spp*	15	2.5
8. .	*Staphylococcus aureus*	23	4.0
9.	*Candida spp*	12	2.0
10.	*Others*	9	1.5

Table.2 Antimicrobial resistance pattern of Escherichia coli and Klebsiella spp.

Antibiotic	*Escherichia coli* (N=323)		*Klebsiella* spp (N=127)	
	Resistant (%)	Sensitive (%)	Resistant(%)	Sensitive (%)
Amoxycillin-Clavulanic acid	239 (74%)	84 (26%)	103(81%)	24 (19%)
Co-Trimoxazole	245(76%)	78 (24%)	102 (80%)	25 (22%)
Ceftriaxone	233 (72%)	90 (18%)	102 (80%)	25 (22%)
Ceftazidime	220 (68%)	103 (32%)	101 (79%)	26 (21%)
Cefaperazone	203 (63%)	120 (27%)	102 (80%)	25 (22%)
Ceftazidime - Clavulanic acid	25 (8%)	298 (92%)	8 (6%)	119 (94%)
Gentamicin	133(41%)	190(59%)	58(46%)	69(54%)
Amikacin	75(23%)	248(77%)	47(37%)	80(63%)
Nalidixic acid	294(91%)	29 (9%)	119 (94%)	8 (6%)
Ciprofloxacin	255(79%)	68(21%)	86 (68%)	41 (22%)
Norfloxacin	229(71%)	94(19%)	83 (65%)	40(35%)
Nitrofurantoin	75(23%)	248(77%)	25(20%)	102 (80%)
Imipenem	10 (3%)	313 (97%)	15 (9%)	112 (91%)

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