# **Original Research Article**

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# Coexistence of fluoroquinolone resistance and ESBL production in urinary isolates

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#### **ABSTRACT**

**Background:** Enterobacteriaceae group of organisms are commonly implicated for causing urinary tract infection (UTI). Fluoroquinolones (FQ) are widely used as empirical therapy for UTI. Enterobacteriaceae exhibits high resistance rates to FQs. The plasmid mediated extended spectrum beta lactamase (ESBL) production confers resistance for FQs as well. The aim of the study is to determine the prevalence of fluoroquinolones resistance in urinary isolates and to study the coexistence of FQ resistance and ESBL production in urinary isolates.

**Methods:** Retrospective data of the urine samples received in our microbiology laboratory between December 2018 to May 2019 were reviewed. The organisms were isolated and identified as per standard procedures and the antibiotic susceptibility testing was done following CLSI 2019 guidelines. Ceftazidime (30  $\mu$ g) and ceftazidime-clavulinic acid (30  $\mu$ g/10  $\mu$ g) were used for detection of ESBL production. Levofloxacin (5  $\mu$ g) and ciprofloxacin (5  $\mu$ g) were used for detecting FQ resistance.

**Results:** Out of a total of 1403 urine samples received in our laboratory, 240 (17.1%) sample showed growth of *Enterobacteriaceae*. The different species were *E. coli* (80.4%), *Klebsiella* spp (12.2%), *Citrobacter* spp (4.2%), *Enterobacter* spp (1.6%) and *Proteus mirabilis* (1.6%). Amongst these organisms 121(50.4%) were ESBL producers. Co-existence of FQ resistance among the ESBL producers was observed in 106(87.6%) isolates which is higher as compared to that among the ESBL non producers 71(59.7%).

**Conclusions:** Around 50% of the UTI *Enterobacteriaceae* bacteria are ESBL producers. Fluroquinolone resistance amongst ESBL producing *Enterobacteriaceae* is very high (87.6%).

Keywords: ESBL, Fluroquinolone, Infection, Resistance, Urinary tract infection

# INTRODUCTION

Urinary tract infections (UTIs) being one of the commonest bacterial infections, affecting around 150 million people each year globally. In India as well UTI forms a major cause of economic burden in society and can affect all age groups and sexes. Untreated or partially

treated UTIs can lead to sequelae like recurrence, pyelonephritis and renal damage.<sup>2</sup> The causative agents are now showing increased resistance rates to both commonly prescribed antibiotics as well as to the more potent ones. Fluoroquinolones (FQs) are broad spectrum antibiotics and are amongst the most commonly prescribed group of antibiotics for treating UTI.<sup>3</sup> Due to

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their inappropriate use, high degree of FQ resistance is commonly observed now a days. Extended spectrum beta lactamases (ESBLs) are a group of plasmid mediated, diverse, complex and rapidly involving enzymes which hydrolyze third-generation cephalosporins, penicillins and aztreonam but are inhibited by clavulanic acid. Production of Extended Spectrum Beta Lactamase (ESBL) confers resistance to penicillins, cephalosporins, monobactam and ESBL producers show co- resistance to other commonly used antimicrobials like, cotrimoxazole, and aminoglycosides.<sup>4</sup> Recent studies have shown that co-transfer of the anr determinant on ESBLplasmids mediates producing fluoroquinolone (a commonly prescribed antibiotic), further minimising the treatment horizon.<sup>5,6</sup> So we carried out the present study to find out the prevalence of ESBL production with fluroquinolones resistance in a tertiary care hospital.

#### **METHODS**

The retrospective data of urine samples of patients presented with UTI to our tertiary care hospital between December 2018 to May 2019 period were reviewed. Data of urine samples obtained from all suspected case of UTI during the study period were included in this study.

The urine samples showing polymicrobial contamination (>3 microorganism) were excluded from the study and we analysed the data of all the eligible urine samples. No formal sample size was calculated.

The urine samples were processed and the identification of the organisms from the urine samples were done as per standard microbiological techniques and antibiotic sensitivity testing was done using Kirby Bauer disk diffusion method as recommended by latest CLSI guidelines. Ciprofloxacin (5µg) and Levofloxacin (5µg) were used to assess FQ resistance. Production of ESBL was detected as follows:

- a. Phenotypic screening test for detection of ESBL Production: The isolates were screened for resistance to Ceftazidime (30 $\mu$ g) by Kirby Bauer disk diffusion test. The isolates that displayed resistance to this antimicrobial were considered positive for screening test.
- b. Phenotypic confirmatory test for detection of ESBL Production: The isolates positive for ESBL production on screening test as described above was further confirmed using both ceftazidime (30µg)/ceftazidime-, clavulanic acid (30µg/10µg) disks. A  $\geq$  5-mm increase in the zone diameter for either antimicrobial agent tested in combination with clavulanate vs. the zone diameter of the agent when tested alone was considered as positive as per CLSI guidelines 2019. Statistical analysis was carried out using SPSS software 21. Data was presented as percentages and proportions. The critical value of 'p' indicating the probability of significant difference was taken as <0.05.

# **RESULTS**

A total of 1403 urine samples were received during the study period of which total, 305 (21.74%) sample showed growth of various organisms (Figure 1).

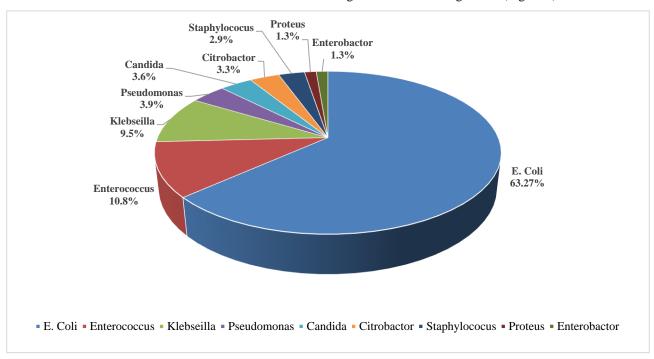


Figure 1: Distribution of different organisms isolated (n=305).

Male:Female ratio was found to be 1:1.1. Out of the total 305, 240 (79%) of the isolates belonged to *Enterobacteriaceae* family. Rest of the isolates were 12 (5%) *Pseudomonas* spp., gram-positive organisms [33 (78.6%) *Enterococcus* spp and 9 (21.4%) *Staphylococcus* spp.] and *Candida* spp 11 (3.6%). The different organisms belonging to *Enterobacteriaceae* family were

Escherichia coli 193 (80.4%), Klebsiella spp 29 (12.1%), Citrobacter spp 10 (4.2%), Enterobacter spp 4 (1.6%) Proteus mirabilis 4 (1.6%). The resistance pattern in gram positive cocci (GPC) for both ciprofloxacin and levofloxacin was similar in the study. Out of the total 42 GPC, 26.1% isolates were sensitive and 73.8% isolates were resistant to both the fluoroquinolones (Table 1).

Table 1: Percentage resistance of Gram-positive organisms to fluoroquinolones (n=42).

CDC (** 42)	Ciprofloxacin		Levofloxacin	
GPC (n= 42)	S	R	S	R
Staphylococcus aureus (n=9)	1 (11.1%)	8 (88.9%)	1 (11.1%)	8 (88.9%)
Enterococcus spp (n=33)	10 (30.3%)	23 (69.7%)	10 (30.3%)	23 (69.7%)
Total	11 (26.1%)	31 (73.8%)	11 (26.1%)	31 (73.8%)

Table 2: Percentage resistance of Gram-negative organisms to fluoroquinolones (n=252).

Organism		E. coli (n=193)	Klebsiella spp (n=29)	Citrobacter spp (n=10)	Enterobacter Spp (n=4)	Proteus spp (n=4)	Pseudomonas spp (n=12)
Ciprofloxacin	S	30 (15.5%)	10 (34.5%)	6 (60%)	0 (0%)	0 (0%)	6 (50%)
	R	163 (84.5%)	19 (65.5%)	4 (40%)	4 (100%)	4 (100%)	6 (50%)
Levofloxacin	S	38 (19.7%)	16 (55.2%)	7 (70%)	0 (0%)	2 (50%)	5 (41.7%)
	R	155 (80.3%)	13 (44.8%)	3 (30%)	4 (100%)	2 (50%)	7 (58.3%)

For Gram negative isolates, higher rates of resistance for ciprofloxacin was observed as compared to levofloxacin (79.4% ciprofloxacin Vs. 73.0% levofloxacin). Out of the total Gram-negative organisms isolated 80% (201/252) of the isolates showed resistant to either of these fluoroquinolones.

Out of the total 240 *Enterobacteriaceae* members subjected to ESBL detection, 121 (50.4%) isolates were ESBL producers (Table 3).

Table 3: Distribution of ESBL positive and ESBL negative organisms of Enterobacteriaceae family in terms of Fluroquinolones resistance (n=240).

Distribution	ESBL positive	ESBL negative	Total
Fluoroquinolone sensitive	15 (12.4%)	48 (40.3%)	63 (26.3%)
Fluoroquinolone resistant	106 (87.6%)	71 (59.7%)	177 (73.7%)
Total	121 (50.4%)	119 (49.6%)	240

The different ESBL producers were 105 (86.8%) *E. coli*, 12 (9.9%) *Klebsiella* spp, 2 (1.7%) *Citrobacter* spp, 1 (0.8%) *Enterobacter* spp and 1 (0.8%) *Proteus mirabilis*. We observed that 87.6% of ESBL positive organisms

were FQ resistant and 73.7% of ESBL negative organisms were FQ resistant. Thus, co-existence of ESBL production and FQ resistance was observed in 106 (87.6%) isolates. Individually, coexistence of ESBL production and FQ resistance was observed in of 100% of ESBL positive *Citrobacter* spp, *Enterobacter* spp and *Proteus mirabilis*. It was 75% and 88.6% for *Klebsiella* spp and *E. coli* respectively (Figure 2).

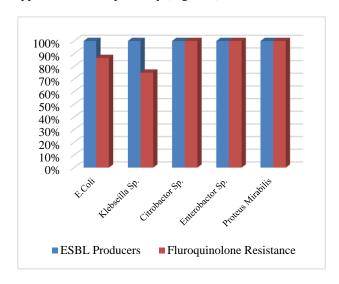


Figure 2: Coexistence of ESBL production and FQS resistance in gram negative isolates (n=106).

# **DISCUSSION**

Gram negative organisms are commoner than gram positive organism as a cause for UTI.8 Since most of the UTI are treated with empirical treatment regimes, and antibiotic resistance rates are on the rise, it is important for the clinicians to know the latest antibiotic resistance trends. It is now an accepted fact that antimicrobial resistance is a major public health problem. Fluroquinolones are widely used in empirical treatment of infections. High resistance fluroquinolones is an emerging concern now a days and amongst all the modes of FQ resistance, presence of transferable PMQR gene is of prime concern.9 The FQ resistance in our study is 80%. This could be attributed to empirical use of FQs by the clinicians or inappropriate treatment due to patient noncompliance. Ciprofloxacin resistance rates were higher than levofloxacin amongst the enterobacteriaceae in our study. The resistance to ciprofloxacin is observed in 100% of Enterobacter spp and *Proteus* spp and 50% in Pseudomonas spp. Sensitivity rates in Gram positive organisms were almost equal. Fluroquinolone resistance varies from country to country. Resistance to FQs in various countries are as follows: Latin America 38.7%, India 75%, Canada 22%, USA 24%, Turkey 49%. The average resistance rates in Asian countries is 33.2%.<sup>10</sup>

In our study 121 (50.4%) isolates were ESBL positive which comprised of 86.8% *E coli*, 9.9% *Klebsiella* spp, 1.7% *Citrobacter* spp, 0.8% *Enterobacter* spp and 0.8% *Proteus* spp. Kim, et al. in a Korean study concluded 17.7% E coli and 26.5% K. pneumonia to be ESBL producers. The discovery of plasmid mediated FQ resistance gene, (*qnr* gene) has embarked a new line of thought of antimicrobial resistance in clinical isolates. Qnr protein leads to reduced susceptibility to FQ. Recent studies have demonstrated that *qnr* gene is co-transferred with ESBL resistance gene on the same plasmid. Thus, rampant use of FQ coincidently selects the ESBL resistant isolates and ESBL positive isolates show more FQ resistance.<sup>11</sup>

Coexistence of FQ resistant and ESBL positive isolate in our study is found to be 87.6% (n=106) which is comparatively higher than that is reported in other studies done in India or abroad. Arundhati, et al. reported FQ-ESBL coexistence in *E coli* to be 65% whereas only 5.8% E. coli and 40.5% K. pneumoniae showed co-existence in a Korean study. 11,12 Studies done in other countries by Lautenbach (USA), Shahcheraghi in (Iran) and Tumbarello (Italy) showed 60%, 48% and 32% of ESBL producing isolates of K. pneumoniae to be resistant to ciprofloxacin, respectively. 12-14 Another Indian report from Chennai also showed that 61% of ESBL producing K. pneumoniae were resistant to ciprofloxacin and 52% to levofloxacin. 15 Higher FQ resistance in our study can probably be attributed to over the counter availability and inappropriate antibiotic in our region.

There are certain limitations in our study. Being a retrospective study, some of the data regarding course of disease, outcome could not be included. We believe a prospective study will certainly be better in this regard.

# **CONCLUSION**

Because of widespread use of FQ in our country, its resistance is increasing and coexistence of ESBL and FQ resistance can aggravate the problem of UTI treatment further by exhaustion of the treatment options. A judicious and culture sensitivity-based approach might help in overcoming this problem.

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