

Coexistence of quinolone resistance and extended spectrum beta lactamase production in urinary isolates of *Escherichia coli* - an emerging challenge to antimicrobial prescribing pattern

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Abstract

Background: Urinary tract infections are the second most common infections in community practice and *Escherichia coli* is the most commonly isolated organism in community acquired UTI. Fluoroquinolones are the preferred antimicrobial agents for empirical therapy of UTI. However, it is seen that Fluoroquinolone resistance among urinary isolates and its association with multi drug resistance, especially beta lactam antibiotics is on the rise. Hence, this study was undertaken to evaluate the prevalence of quinolone resistance and its co-existence with Extended Spectrum β -Lactamase (ESBL) production among urinary isolates of *E.coli*.

Objectives: 1. To determine the prevalence of quinolone resistance among urinary isolates of *E.coli*. 2. To evaluate co-existence of quinolone resistance with ESBL production among them.

Materials and Methods: All urine samples received at Microbiology laboratory, McGann hospital, attached to Shimoga Institute of Medical Sciences, Shimoga, was included for study. *E.coli* was isolated by following standard conventional microbiological methods. These isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method and ESBL production was tested according to CLSI guidelines 2014.

Results: In a total of 932 urine samples, 82 *E.coli* were isolated. Out of these 82 *E.coli* isolates, 62 (75.6%) isolates showed quinolone resistance. And out of these 62 quinolone resistant isolates, 54(88.5%) isolates showed ESBL production.

Conclusion: Our study shows significant co-existence of quinolone resistance and ESBL production among the urinary isolates of *E.coli*. The result of this study justifies the need for continuous surveillance, monitoring and revision of the antibiotic use policies to control the dissemination of resistance determinants among the pathogen.

Keywords: UTI, *Escherichia coli*, Fluoroquinolones, ESBL.

Introduction

Urinary tract infections are one of the commonest bacterial infections. *Escherichia coli* are the most frequently isolated bacteria in both community acquired and hospital acquired urinary tract infections. Fluoroquinolone antibiotics which act by inhibition of enzyme DNA gyrase, are the preferred antimicrobial agents for the empirical therapy of UTI.¹ Quinolones have been widely used in the treatment of a variety of infections due to their broader antimicrobial spectra. It is widely accepted that, overuse of antibiotics in hospitals and communities is one of the major contributing factors to antimicrobial resistance. And the problem of quinolone resistance is becoming increasingly serious with the extensive use of these agents in various infections.² It is seen that Fluoroquinolone resistance among the urinary isolates is also associated with multidrug resistance.³

β -lactam antibiotics are the commonly used antibiotics for the treatment of Enterobacteriaceae related infections. However, resistance to these agents have increased world-wide mainly due to the production of β -lactamases, among which, extended spectrum β - lactamases is of great significance.⁴ Mukherjee M et al in their study have reported 37 out of total 40 urinary *E.coli* isolates as multi drug resistant(MDR). Ciprofloxacin was one of the three

drugs considered for labelling it as MDR *E.coli*. And 18 out of these 40 MDR isolates are reported to show ESBL production¹. Raei F et al in a similar study have reported ESBL production among 65.2% of the quinolone resistant *E.coli* isolates.⁵

The reports on high prevalence of multidrug resistance and ESBL production among urinary isolates of *E.coli*, has forced the importance of reassessment of empirical therapy and other antimicrobial usage for the management of UTI. Hence, this study was undertaken with the objective to evaluate co-existence of quinolone resistance, ESBL production and multi drug resistance among the urinary isolates of *E.coli*.

Materials and Methods

1. **Source of Data:** The present study was conducted at Microbiology laboratory, McGann Hospital, attached to Shimoga Institute of Medical Sciences, Shimoga for a period of 6 months from January 2015 to June 2015. All urine samples were included in our study.
2. **Method of collection of data:** All urine samples received in Microbiology laboratory of McGann Hospital, attached to Shimoga Institute of Medical Sciences, Shimoga were processed. Samples were inoculated on to Blood agar and MacConkey agar plates and incubated aerobically overnight at 37°C.

On the basis of colony morphology, Gram staining, motility and biochemical reactions, the organisms were identified as *E.coli*.^{6,7} Antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory Standards Institute (CLSI) guidelines 2014(M100-S24). Commercially available antibiotic sensitivity discs (SD) as per CLSI (Himedia Labs, India) were used for antimicrobial susceptibility testing.⁸

ESBL detection methods⁸:

a. Phenotypic screening test for detection of ESBL

Production: *E.coli* isolates were screened for resistance to Ceftazidime (30µg) and Cefotaxime (30µg) by Kirby Bauer disk diffusion test. The isolates that displayed resistance to one or both of these antibacterials were considered positive for screening test.

b. Phenotypic confirmatory test for detection of ESBL

Production: *E.coli* isolates positive for ESBL production on screening test as described above was further confirmed by using both Ceftazidime (30µg)/Ceftazidime-Clavulanic acid (30µg/10µg) and Cefotaxime (30µg)/Cefotaxime-Clavulanic acid (30µg/10µg) disks as per CLSI guidelines. A ≥ 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 2014.

Results

82 *E.coli* were isolated from a total of 932 urine samples. Out of these 82 *E.coli* positive samples, 53(65%) samples were from female patients and 29(35%) samples were of male patients. The antibiotic susceptibility pattern of *E.coli* isolates was as shown in Table 1 and Fig. 1. Out of 82 *E.coli* isolates, 62(76%) isolates showed resistance to norfloxacin, the quinolone commonly used in empirical therapy of UTI. Lowest sensitivity was shown to Ampicillin(0.01%) and highest to Imipenem(96%).

In screening test for ESBL detection, 70(85%) isolates were resistant to ceftazidime and 67(82%) isolates were resistant to cefotaxime as shown in Table 2. Hence, a total of 71 isolates were subjected to ESBL confirmatory test, out of which 61(74%) isolates were positive for ESBL production in combined disk diffusion method.

Coexistence of quinolone resistance and ESBL production was seen in 54(65%) isolates (Table 3) and multi-drug resistance was seen as an important observation among the isolates resistant to quinolone and ESBL producers (Fig. 2).

Table 1: Antibiotic susceptibility pattern of *E.coli* isolates

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	1(0.01%)	81(99.99%)
Gentamicin	58(71%)	24(29%)
Amikacin	65(79%)	17(21%)
Ampicillin-sulbactam	15(18%)	67(82%)
Cefotaxime	15(18%)	67(82%)
Imipenem	80(96%)	2(4%)
Ceftazidime	12(15%)	70(85%)
Norfloxacin	20(24%)	62(76%)

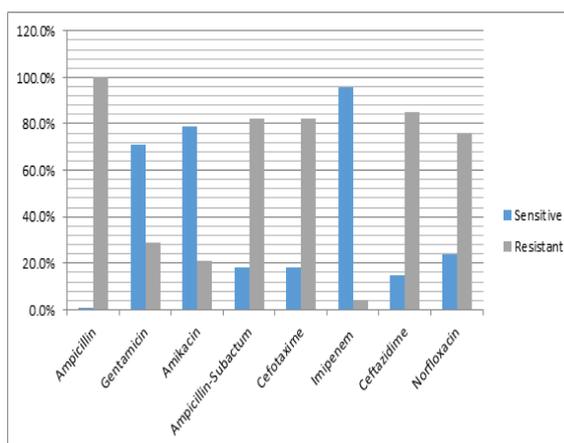


Fig. 1: Bar chart showing antibiotic susceptibility pattern of *E.coli* isolates

Table 2: Screening test for ESBL production among *E.coli* isolates

	Cefotaxime resistant	Cefotaxime sensitive
Ceftazidime resistant	66	04
Ceftazidime sensitive	01	11

Table 3: Coexistence of quinolone resistance and ESBL production

	ESBL +ve	ESBL -ve
Norfloxacin sensitive	07	13
Norfloxacin resistant	54	08

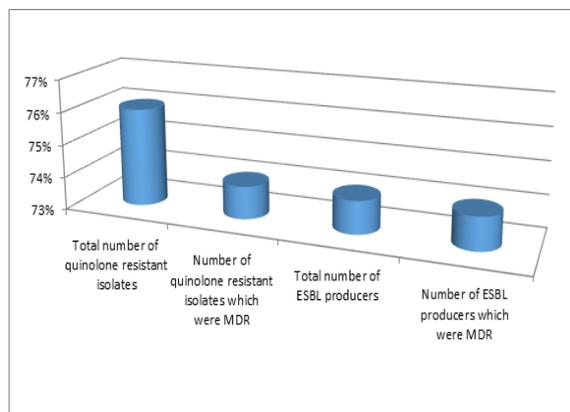


Fig. 2: Multi drug resistance among quinolone resistant and ESBL producing isolates

Discussion

Fluoroquinolones are potent antimicrobial agents for the treatment of a wide variety of community-acquired and nosocomial infections. The mechanisms of quinolone resistance involves decreased cellular accumulation of the drug and chromosomal mutations affecting the quinolone resistance determining regions of DNA gyrase (GyrA and GyrB) and topoisomerase IV (ParC and ParE).⁹

Extended spectrum β -lactamases, hydrolyse the oxymino beta lactams like ceftazidime, cefotaxime, ceftriaxone and monobactams but not carbapenems. The genes encoding ESBL production may be plasmid mediated or chromosomal. Production of ESBL has emerged as a major mechanism of resistance amongst the uropathogens against β -lactam antibiotics and has become a major problem in clinical practice in last few years.^{9,10}

The discovery of plasmid borne quinolone resistance genes *qnr* in the late 1990s has added a new dimension to quinolone resistance. Recent studies have demonstrated the cotransfer of *qnr genes*, encoding reduced susceptibility to the quinolones, with ESBLs on the same plasmid. Therefore, use of quinolones may consequently select bacteria that are not only quinolone resistant but also beta lactam resistant or vice versa.¹⁰

In the present study, out of the 82 *E.coli* isolates, 62(76%) isolates showed resistance to quinolone antibiotic and 61(74%) isolates showed ESBL production. Coexistence of quinolone resistance and ESBL production was seen in 54(65%) isolates. Quinolone resistant isolates were also multi-drug resistant. Imipenem was the most sensitive antibiotic followed by amikacin. The present study results are in agreement with the reports of Paterson et al¹¹ and Tolun et al.¹²

In the present study, Quinolone resistance is associated with multi drug resistance and production of extended spectrum beta lactamases. Indiscriminate use of quinolones as empirical therapy for UTIs will facilitate emergence of resistance to quinolones and

also emergence of multi-drug resistant strains. Coexistence of quinolone resistance and ESBL production is a serious public health problem. Hence, it requires repeated surveillance, formulation of antibiotic policy, prudent prescription of antibiotics and recycling of antibiotics.

Conclusion

Since most of the UTIs are treated empirically, the selection of the antimicrobial agent should be determined not only on the basis of the most likely pathogen, but also on the basis of its susceptibility pattern. Random use of quinolones in UTI should be discouraged because of increased resistance to it, its coexistence with ESBL production and multi drug resistance.

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