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Research Article

PREVALENCE OF FLUOROQUINOLONES RESISTANCE AMONG ESBL-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM URINE SAMPLES

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ABSTRACT

Objectives: Resistance among uropathogens has emerged as a growing concern necessitating re-evaluation of the efficacy of recommended empiric antimicrobial regimens. Misuse and overuse of various antibacterial agents in hospitals are a key cause of the emergence of anti-microbial resistance.

The study aimed to identify the prevalence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from urine specimens and to know the prevalence of Fluoroquinolone resistance among ESBL-producing *E. coli* isolates.

Methods: The study was conducted on 500 *E. coli* isolates from urine samples received in the Department of Microbiology, MMIMSR, Mullana, Ambala. The organism isolation and identification were done as per the standard procedures and antibiotic sensitivity testing was done following Clinical Laboratory Standard Institute (CLSI) guidelines. All the strains were screened out for ESBL production as per CLSI guidelines. Ciprofloxacin, Norfloxacin, and Ofloxacin discs were used for the detection of fluoroquinolone resistance.

Results: Out of the total 500 *E. Coli isolates* from urine samples received in our laboratory, 69% were found to be ESBL producers.

Conclusion: We found a high level of quinolone resistance among ESBL-producing *E. coli* strains isolated from patients with urinary tract infections. Because of the widespread use of fluoroquinolones in our country, there is a need for sensitive antibiotic stewardship. Further research is needed to ascertain the gravity of quinolone resistance and to swiftly act against its spread among other nosocomial pathogens.

Keywords: Extended-spectrum beta-lactamase, anti-microbial resistance, Fluoroquinolone resistance

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INTRODUCTION

Urinary tract infections (UTIs) are among the most common infectious diseases in the community as well as in health-care settings [1]. There is a broad range of pathogens that can cause UTI and *Escherichia coli* remains the most common. In the past few years, the number of cases of UTI due to antibiotic-resistant *E. coli strains* has risen posing a significant therapeutic challenge [2]. Misuse and overuse of various antibacterial agents in the hospital context are regarded as key causes of the emergence of antimicrobial resistance [2]. Extended-spectrum beta-lactamases (ESBL) are a group of plasmid-mediated, diverse, complex, and rapidly involving enzymes that hydrolyze penicillin, broad-spectrum cephalosporins, and monobactams, but not cephamycin or carbapenems, and their action is inhibited by clavulanic acid [3]. Production of ESBL confers resistance to penicillins, cephalosporins, and monobactam. ESBL-producing organisms typically show resistance to various antimicrobial classes such as fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole due to large plasmids carrying multiple antibiotic-resistant genes [4,5]. Cotransfer of the quinolone's resistant gene determinant based on ESBL-producing plasmids decreases the treatment horizon even further [6,7]. Hence, this study was conducted to determine the prevalence of fluoroquinolone resistance in ESBL-producing *E. coli* strains isolated from urinary samples.

METHODS

The present study was conducted in the Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala on 500 consecutive, non-duplicate strains of *E. coli* isolated from urine samples from UTI cases. The study was undertaken after the approval from the ethical committee vide letter no. MMIMSR/IEC/2101.

Detailed clinical history and related relevant data were obtained from the patients and were included in the study. Polymicrobial contamination (> 3 microorganisms) of urine samples were excluded from the study.

Specimen processing

Urine samples were collected and processed and a wet film of uncentrifuged urine was observed under the microscope (40×). Evidence of bacteria, pus cells, epithelial cells, tubular casts, RBCs, and crystals seen in wet mount examination were noted. The clinical specimen was then cultured on cystine-lactose-electrolyte-deficient agar. After overnight incubation, colonies were identified by colony characteristics and gram staining. Colony Forming unit in the case of non-catheterized patients that were considered significant was $>10^5$ Colony-forming unit (CFU)/mL and in the case of catheterized patients was $\geq 10^3$ CFU/mL. The final identification and antimicrobial susceptibility testing of the isolates were performed using GN I.D Card and GN-235 in Vitek-2 compact. Ciprofloxacin, norfloxacin, and ofloxacin discs were used for the detection of fluoroquinolone resistance. ESBL confirmation was done by combination disc diffusion test (CDDT)/Kirby Bauer disc diffusion method on Mueller–Hinton agar as recommended by Clinical Laboratory Standard Institute (CLSI) guidelines [8,9]. *E.coli* ATCC 25922 strain was used as the reference strain.

Test for confirmation of ESBL production [9]

Ceftazidime (30 µg) alone as well in combination with clavulanic acid (30/10 µg) and Cefotaxime (30 µg) alone as well in combination with clavulanic acid (30/10 µg) were used for the test. ≥5 mm increase in

the zone diameter for ceftazidime or cefotaxime in combination with clavulanic acid (30/10 µg) than that for ceftazidime/cefotaxime was taken positively for ESBL production (Fig. 1).

RESULTS AND DISCUSSION

About 3/4th (370/500) strains of *E. coli* were isolated from indoor patients while 1/4th (130/500) was from outdoor patients. Among the patients, 289 (58%) were female and 211 (42%) were males. The antibiotics sensitivity pattern of *E. coli* isolated from urine samples demonstrated high resistance to ampicillin (96%), third-generation cephalosporins such as cefixime (95%), ceftriaxone (92%), and fluoroquinolones (ciprofloxacin 87%, norfloxacin 86%, and ofloxacin 87%). Lower resistance was seen towards amoxicillin-clavulanic acid (35%), amikacin (14%), nitrofurantoin (13%), imipenem (8%), and meropenem (6%). (345/500) 69% of strains were found to be ESBL producers using the CLSI-recommended phenotypic confirmatory CDDT.

The highest distribution of ESBL-producing strains was from the department of urology (24.23%), followed by medicine (21%), ICU (13.5%), obstetrics and gynecology (12%), and emergency (11%).

ESBL-producing *E. coli* is the significant cause of increased morbidity in patients with UTI. Antimicrobial resistance patterns of *E. coli* continue to pose a great threat to public health worldwide and lead to serious health problems such as prolonged hospitalization and treatment failure. An attempt was made to study the prevalence of fluoroquinolones resistance in ESBL-producing *E. coli* in urine samples in 500 successive, non-duplicated clinical specimens received in the Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research Mullana, Ambala. The majority of the 500 clinical isolates of *E. coli* in this study, that is, 76% were from indoor patients, whereas just (26%) came from outdoor patients. In a study of

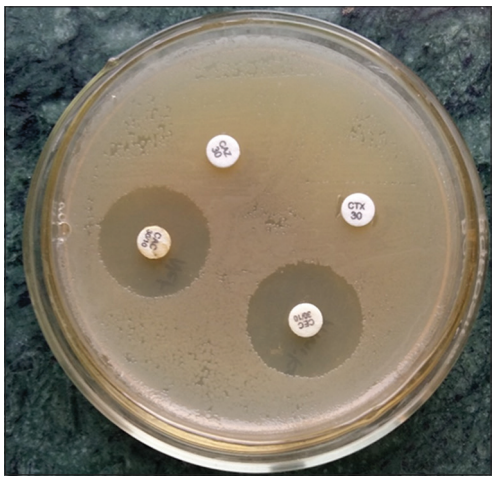

Fig. 1: Depict positive ESBL production

Table 1: Antibiotics susceptibility pattern of fluoroquinolones sensitivity in ESBL Producing *E. coli* isolates versus non-ESBL producing *E. coli*

Antibiotics	Susceptibility (%) among ESBL-producing strains n=345	Susceptibility (%) among non-ESBL producing strains n=155
Ciprofloxacin	34 (10.1%)	22.5%
Ofloxacin	34 (10.1%)	22.5%
Norfloxacin	34 (10.1%)	25.8%

E. coli: *Escherichia coli,* ESBL: Extended-spectrum beta-lactamase

200 urine isolates of extended-spectrum-lactamase-producing *E. coli*, Khodare *et al.*, [10] discovered that (53%) of isolates were obtained from In-patient department (IPD) and (47%) of isolates were obtained from outpatient department (OPD). FarajzadehSheikh *et al.*, [11] on the other hand, conducted a study on ESBL-producing *E. coli* strains isolated from UTI and found that the majority of *E. coli* isolates were obtained from outdoor patients (59.21%) and in-door patients (40.72%) due to hospital-acquired infection.

In our study, female patients were found to have the highest percentage of *E. coli* strains (58%), compared to the male patients (42%). In contrast to author Abduzaimovic *et al.*, [12] prevalence of UTI found that (89.87%) of *E. coli* were isolated from female patients whereas (10.13%) from male patients. According to another study conducted by Moue *et al.*, [13] on the occurrence of UTIs in both OPD and IPD, females were more prone to have UTI (79.5%) than males (20.0%). UTIs are probably more common in women because their urethras are shorter than men's, allowing bacteria to migrate upward more easily.

In the present study, the antibiotic susceptibility pattern of the *E. coli* isolates was found to be variable. The maximum sensitivity was found for imipenem (94%), meropenem (92%), and nitrofurantoin (87%). In contrast with author Jia *et al.*, [14] study on community-acquired UTIs, reported that the maximum susceptibility to *E. Coli* was reported for ertapenem (98.9%), nitrofurantoin (96%), and Fosfomycin (95.4%). Another study done by Cebeci and Keskin [15] on the prevalence of ESBL among *E. Coli* isolates and their susceptibility mentioned that the maximum susceptibility rate to *E. Coli* was to ertapenem (93.7%), nitrofurantoin (91.6%), Fosfomycin (89.5%), respectively. While amoxicillin clavulanic acid was found effective in 61% of strains. Of the other antibiotics used, maximum resistance was detected against ampicillin (96%) followed by the third-generation cephalosporins, cefixime (95%), and fluoroquinolones like ciprofloxacin (87%). In similarity with author Noor *et al.* [16], the study reported a resistance rate against ampicillin, ciprofloxacin, and cefotaxime (83.4%). Another study by Madani *et al*., [17] reported that the *E. coli* strain showed resistance to ampicillin (91.4%) and cefixime (61%), ciprofloxacin (66.7%), respectively.

In the present study, ESBL production was observed in (69%) of *E. Coli* strains while (31%) were non-ESBL producers using the recommended double disc diffusion test Furthermore, the prevalence of ESBL-producing *E. Coli* was high, especially in hospitalized patients. Accordance to a study conducted by Nimri and Azaizeh [18] showed that ESBL-producing *E. coli* were (80.7%). Another study was done by author Park *et al.*, [19] on the classification and occurrence of *E. coli* and *Klebsiella pneumonia* isolates producing ESBL which reported the prevalence variability in different years 17.7% (2003) and 84% (2009) of ESBL-positive *E. coli* strains, respectively. A study conducted by Pakzad *et al*., [20] reported a lower prevalence (28%) of ESBLs. A similar study was done by author Tayebi *et al*., [21] which reported that the prevalence of ESBL differs depending on species and environmental regions. Another study by Singh and Singh [22] reported a minimum (27%) ESBL producers in isolates of *E. coli.* The prevalence of ESBL production in gram-negative bacilli varies widely among different geographical regions and in different clinical settings. Plasmids responsible for ESBL production tend to be large and frequently encode for resistance to other classes of antimicrobials also, thus limiting the choice of antimicrobials available for the treatment of infections. The most common coresistance found in ESBL-producing organisms are aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfamethoxazole-trimethoprim.

A high proportion of our isolates (91.3%) showed resistance to fluoroquinolones. Our results showed that resistance to tested fluoroquinolones in ESBL isolates was higher than in non-ESBL-producing isolates. Another study done by Hassan and Jamal [23] demonstrated resistance to ciprofloxacin to 85%.

In our study, non-ESBL producing *E. coli* isolates showed resistance to ciprofloxacin (77.5%), ofloxacin (77.5%) and norfloxacin (74.2%), respectively, which is by the study done by the author Ahmed *et al*., [24] which showed resistance to ciprofloxacin (55%). In another study, done by Jamil *et al.*, [25] 46% resistance to ciprofloxacin was found.

CONCLUSION

In our study, we found a high level of quinolone resistance (more than 91.3%) among ESBL-producing *E. coli* strains isolated from patients with UTI. Because of the widespread use of fluoroquinolone in our country, its resistance is increasing, and the coexistence of ESBL and fluoroquinolone resistance can aggravate the problem of UTI treatment further exhausting the treatment options. A judicious and culture-sensitivity-based approach might help in overcoming this problem. Rational use of antimicrobial policy as well as stopping the unnecessary prescription and non-prescription sales in retail pharmacies can be performed as strategies to prevent the increase of quinolone resistance. There is a need for sensitive antibiotic stewardship. Further research is needed to ascertain the gravity of quinolone resistance and to quickly act against its spread among other nosocomial pathogens.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Nil.

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