

STUDY OF MULTIDRUG RESISTANCE PATTERN AMONG *ESCHERICHIA COLI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION

SONIYA GOYAL, VIKAS BENIWAL*

Department of Biotechnology, Maharishi Markandeshwar University, Mullana, Ambala - 133 203, Haryana, India.

Email: beniwalvikash@gmail.com

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ABSTRACT

Objective: Urinary tract infections (UTIs) are some of the most common bacterial infections encountered in community and cause of significant morbidity and high medical cost. *Escherichia coli* is the most common pathogen responsible for the majority of UTI infections. Antimicrobial drugs have been routinely prescribed for the empirical treatment of UTIs which has led to a dramatic increase in antibiotic resistance pattern of *E. coli*. The aim of the present study was to analyze the multidrug resistance (MDR) patterns of *E. coli* isolated from UTI patients.

Methods: A total of 80 urine samples collected from the patients suspected of having UTI attending Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala, were cultured using standard microbiological techniques. Antibiotic susceptibility testing of *E. coli* was done using minimum inhibitory concentration (MIC). MIC of tetracycline, doxycycline, azithromycin, erythromycin, ciprofloxacin, levofloxacin, ampicillin, amoxicillin, and amikacin was done by agar dilution method.

Results: Of the total 46 isolates contributing 33 females and 13 males were confirmed as *E. coli*. About 51.34% of the female patients belonged to the age group 21-40 years and 53.84% of the male population belonged to 41-80 years were found to be more susceptible to UTI infection. All isolates confirmed as *E. coli* were found to be MDR. 80% of the isolates exhibited MICs higher than 1000 mg/L against β -lactams. 20% of the *E. coli* isolates exhibited MICs higher than 1000 mg/L against ciprofloxacin, amikacin, and erythromycin. 23% and 95% of *E. coli* isolates exhibited MICs <128 mg/L against doxycycline and levofloxacin, respectively.

Conclusion: The present study revealed the decreased susceptibility of the *E. coli* to all drugs. *E. coli* resistance profile to β -lactams, quinolones, macrolides, tetracyclines, and aminoglycosides were also found to be quite high in this study emphasizing the need to educate public about appropriate use of antibiotics.

Keywords: Antibiotics, *Escherichia coli*, Minimum inhibitory concentration, Multidrug resistance, Urinary tract infection.

INTRODUCTION

Urinary tract infection (UTI) is the most common and life-threatening infection among the Indian population and exist in all age group people [1,2]. UTI remains a significant cause of morbidity in all age groups [3]. It is classified as complicated UTI and uncomplicated UTI. Complicated UTIs are mainly associated with anatomical, metabolic, or functional abnormalities of the urinary tract, and uncomplicated UTIs are mainly due to bacterial infection. UTI may occur in the lower urinary tract termed as cystitis as well as in upper urinary tract termed as pyelonephritis [4]. Women are more prone to UTI infection due to their structural features such as short urethra and some other factors such as pregnancy and sexual activity [5-7]. About 80% UTIs are caused by *Escherichia coli*; a Gram-negative facultative anaerobe rod-shaped pathogen belongs to Enterobacteriaceae family [8]. *E. coli* having P-fimbriae binds specific to galactose residue present on the surface of uroepithelial cells in 99% population termed as uropathogenic *E. coli* (UPEC) is mainly responsible for UTI [9].

UTIs are treated with broad spectrum of antibiotics, i.e. quinolones, tetracyclines, macrolides, and β -lactams [10,11]. The extensive and improper use of these antimicrobial agents causes remarkable increase in the antibiotic resistant pattern among bacterial species especially *E. coli* has become a major problem worldwide [12-15]. The antibiotic treatment is usually started empirically in patients with suspected UTI before urine culture results are available. It is mandatory to know the clinical history of patients and microbial resistant pattern before prescribing antibiotics [16,17]. Genetic mutations and horizontal gene transfer through plasmids are main reasons of developing resistance

among bacterial species [18,19]. Microorganisms considered to be MDR when they develop resistance against three or more than three antibiotics [20,21]. A very recent study from India showed high level (80%) of expression of efflux pumps specific to antimicrobial agents indicates antimicrobial resistance [22]. *E. coli* cultures isolated from urinary samples showed a high level of resistance to β -lactam and fluoroquinolones in recent study from South India [23]. The surveillance data show the highest resistance pattern of antimicrobial agents in *E. coli* that have been used from the long time in human medicine [24]. The past two decades also showed a remarkable increase in emergence and spread of MDR *E. coli* and increasing resistance to newer antimicrobial agents such as fluoroquinolones and some cephalosporins. The MDR *E. coli* has been frequently reported from different parts of the world as an emergence of treatment problem [25,26].

Antibiotic resistant patterns may vary between different geographical areas, and it is indispensable to study about the occurrence of uropathogens in particular area, their distribution among males and females and antimicrobial resistant pattern among uropathogens in that area against different antibiotics, viz., β -lactams, tetracyclines, quinolones, and macrolides [27]. The study in Haryana showed the resistant pattern among uropathogens with special reference to quinolones [28]. However, bacterial antibiotic susceptibility pattern studies are very rare from Haryana which makes patients care largely empirical. This result in the use of multiple antibiotics prescribes by the physicians who increase both cost and morbidity [29]. Due to the excessive usage of antibiotics, it is exigent to study the level of resistance among *E. coli* (most common uropathogen among UTI patients) against different classes of antibiotics in Haryana. Keeping this in mind, the aim

of the present study was to assess the different antibiotics resistant pattern among UPEC isolated from UTI patients from a local hospital in Ambala, Haryana (India).

METHODS

Population under study

The present study was carried out in the Department of Biotechnology, MM University, Mullana, Ambala, on 80 patients who attended Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Ambala, having UTI infection during June 2015 to December 2015. Patient-specific data collected were age and gender. UTI patients were classified into three age groups ≤ 20 years, 21-40 years, and 41-80 years.

Collection and processing of samples

Urine samples were collected from the Microbiology Laboratory of MMIMSR in sterile container. All the media used were obtained from Himedia Laboratories Pvt. Ltd. Samples were inoculated onto cysteine lactose electrolyte deficient media followed by incubation for 24 hrs at 37°C.

Identification and screening of *E. coli* isolates

All isolates were selectively plated onto MacConkey agar and eosin methylene blue (EMB) agar plates followed by incubation for 24 hrs at 37°C for confirmation between lactose and non-lactose fermenting strains. Various biochemical tests including indole test, citrate test, methyl-red test, Voges-Proskauer test, triple sugar iron test, urease test, and Mannitol motility test were done to confirm *E. coli*.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed by agar dilution method on Mueller-Hinton agar to determine the minimum inhibitory concentration (MIC) at varying concentrations of different antibiotics (tetracycline, doxycycline, azithromycin, erythromycin, ciprofloxacin, levofloxacin, ampicillin, amoxicillin, and amikacin) belongs to different classes [30]. The MICs of each isolate were determined as per the interpretive standards defined by Clinical and Laboratory Standards Institute M100-S23 [31]. The antibiotics were obtained from Cipla Ltd, Mumbai.

RESULTS

Urine samples were cultured of all the 80 patients, admitted to Maharishi MMIMSR, Mullana, Ambala, during June 2015 to December 2015. Out of 80 urine samples tested, 46 isolates (33 females/13 males) were suspected to be *E. coli* and used for further study. Pure bacterial colonies were isolated on MacConkey agar and EMB agar plates and confirmed through morphological and biochemical examinations. All the selected isolates were dry round convex colonies capable of fermenting lactose, gave pink-colored colonies on MacConkey agar and dark blue-black colonies with metallic green sheen on EMB agar plates, respectively. The biochemical test of the selected isolates is presented in Table 1.

About 57.5% of the isolates comprising 71.73% females/28.26% were confirmed as *Escherichia coli*. Females in the reproductive age group of 21-40 years constituted 51.15% (17/33) of the total female population susceptible to *E. coli* and male in the reproductive group of 40-80 years constituted 53.84% (7/13) of the total male population sensitive to *E. coli*. Table 2 outlines the age- and gender-wise distribution of *E. coli* isolated from UTI patients. It was observed that female were more susceptible to *E. coli* infection than males, which were more liable to other bacterial infection, i.e., *Klebsiella* and *Enterobacter* species.

The antibiotic sensitivity test was done for different classes of drugs (tetracyclines, macrolides, quinolones, β -lactams, and aminoglycoside) of all *E. coli* isolates isolated from UTI patients. The overall resistance rates for the 46 *E. coli* isolates analyzed are provided in Table 3.

The antibiotic resistance pattern is shown separately for different classes of drugs (Fig. 1). Doxycycline and amikacin were found to be effective against *E. coli* in this study. A high resistance level was observed in *E. coli* against ampicillin (89.13%), amoxicillin (93.47%), and erythromycin (58.69%) exhibited MICs value ≥ 1000 mg/L. It was also observed that *E. coli* isolates showed moderately resistant against tetracycline (93.47%) and ciprofloxacin (45.67%) exhibited MICs value between 128 and 512 mg/L. A low level of resistance was found against levofloxacin (95.65%) and azithromycin (41.30%) exhibited MICs < 128 mg/L in *E. coli* isolates.

DISCUSSION

Antimicrobial resistance is now recognized as an increasing global problem, especially with *E. coli*. [32]. The present study was conducted to find out the distribution of UPEC and to assess the resistant pattern of different antibiotics among *E. coli* isolates isolated from UTI patients in MMIMSR, Mullana, Ambala. *E. coli* is the most common uropathogen has been reported to be responsible for UTI infections all over the world. Out of 80 urine samples, 57.5% (46) samples were reported to be *E. coli* and included in the present study. This finding is similar to the studies done in India by Dash *et al* and Niranjana and Malini. They reported the prevalence of 54.05% and 56.8% *E. coli* isolates in their studies, respectively [33,34].

Table 1: Biochemical reaction of the isolates

Biochemical test	Result
Gram-staining	-
Indole test	+
Methyl-red	+
Voges-Proskauer	-
Citrate	-
Urease	-
TSI	A/AG
Motility	Motile

+: Positive, -: Negative, TSI: Triple sugar iron

Table 2: Age- and gender-wise distribution of *E. coli* isolated from UTI patients

???	Female (%)			Male (%)		
	≤ 20 years	21-40 years	41-80 years	≤ 20 years	21-40 years	41-80 years
<i>E. coli</i>	9.09	51.15	39.39	38.46	7.69	53.84

E. coli: *Escherichia coli*. UTI: Urinary tract infection

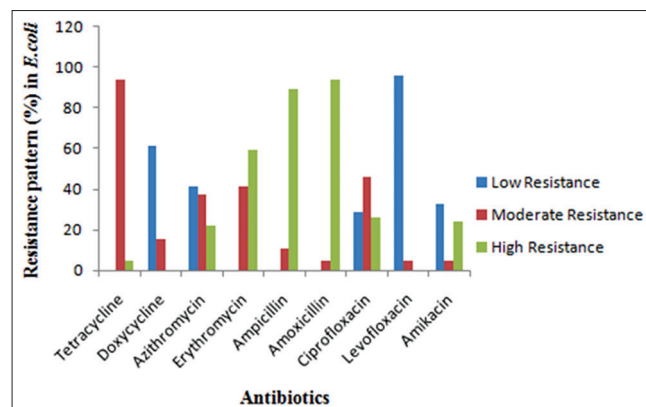


Fig. 1: Resistance pattern of different antibiotics against *Escherichia coli*

Table 3: Antibiotic sensitivity and resistance pattern of isolated *E. coli* (n=46) from UTI patients

Antibiotic class	Antibiotic	Sensitivity (%)	Low resistance (%)	Moderate resistance (%)	High resistance (%)
Tetracycline	Tetracycline	2.17 (1)	0 (0)	93.47 (43)	4.34 (2)
	Doxycycline	23.91 (11)	60.86 (28)	15.21 (7)	0 (0)
Macrolides	Azithromycin	0 (0)	41.30 (19)	36.95 (17)	21.73 (10)
	Erythromycin	0 (0)	0 (0)	41.30 (19)	58.69 (27)
Quinolones	Ciprofloxacin	0 (0)	28.26 (13)	45.65 (21)	26.08 (12)
	Levofloxacin	0 (0)	95.65 (44)	4.34 (2)	0 (0)
β -lactams	Ampicillin	0 (0)	0 (0)	10.86 (5)	89.13 (41)
	Amoxicillin	2.17 (1)	0 (0)	4.34 (2)	93.47 (43)
Amino glycosides	Amikacin	39.13 (18)	32.60 (15)	4.34 (2)	23.91 (11)

E. coli: *Escherichia coli*. UTI: Urinary tract infection

Distribution pattern based on the basis of age and gender has significantly proved that females in the reproductive age group of 21-40 years and both males and females in the reproductive age group of 41-80 years are more susceptible to UTI infections. Young women are more prone to UTI infection due to their structural features and some other factors such as pregnancy and sexual activity, whereas in older women kidney failure and diabetics are important factors responsible for UTI infections. In males, UTI infections are mainly due to prostate infections, urinary stone, and use of catheters in the age group of 41-80 years [35].

In this study, we concluded that the majority of *E. coli* isolates showed the high resistance (≥ 1000 mg/L) to ampicillin (89.13%), amoxicillin (93.47%), and erythromycin (58.69%). 93.47% and 45.65% *E. coli* isolates were moderately resistant (<1000 mg/L) against tetracycline and ciprofloxacin, respectively. Some *E. coli* isolates were sensitive against doxycycline (23.91%) and amikacin (39.13%). To conclude, this study showed that 100% UPEC isolates were found to be MDR which is consistent with other study done by Dash *et al.* This is quite high when compared to other study done by Niranjana *et al.* and Hasan *et al.* The prevalence of MDR *E. coli* was about 75.6% and 52.9%, respectively, in those studies. The increased occurrence of UTI due to MDR *E. coli* could be due to increased prevalence of MDR strains in the community. This could be due to self-prescription policy, intake of comparatively cheaper antibiotics as well as inadequate doses.

CONCLUSION

UTI infections due to MDR *E. coli* were found to be quite high in the present study. *E. coli* resistance profile to β -lactams, quinolones, macrolides, tetracyclines, and aminoglycosides were also found to be quite high in this study emphasizing the need to educate public about appropriate use of antibiotics and for continuous surveillance of antimicrobial resistant trends worldwide of particularly MDR *E. coli* strains causing UTI.

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