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Research Article

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

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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 Niranjan 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
E. coli	9.09	51.15	39.39	38.46	7.69	53.84

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

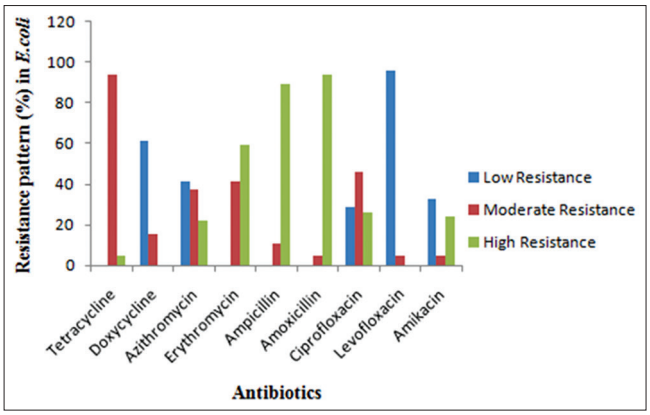

Fig. 1: Resistance pattern of different antibiotics against *Escherichia coli*

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 Niranjan *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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REFERENCES

1. Stamm WE, Hooton TM. Management of urinary tract infections in adults. N Engl J Med 1993;329(18):1328-34.
2. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). Clin Infect Dis 1999;29(4):745-58.
3. Kolawale AS, Kolawale OM, Kandaki-Olukemi YT, Babatunde SK, Kolawale CF. Prevalence of urinary tract infections among patients attending Dalhatu Araf specialist hospital, Lafia, Nasarawa state, Nigeria. Int J Med Sci 2009;1(5):163-7.
4. Stamm WE, Norrby SR. Urinary tract infections: Disease panorama and challenges. J Infect Dis 2001;183 Suppl 1:S1-4.
5. Hotchandani R, Aggarwal KK. Urinary tract infections in women. Indian J Clin Pract 2012;23(4):187-92.
6. Patel S, Taviad PP, Sinha M, Javadekar TB, Chaudhari VP. Urinary tract infections (UTI) among patients at GG hospital and medical college, Jamnagar. Natl J Community Med 2012;3(1):138-41.
7. Chaudhuri SR, Thakur AR, Nandy P, Samanta S. Urinary tract infection - A survey of local population. Am J Infect Dis 2008;4(2):117-23.
8. Paape MJ, Schultze WD, Desjardins C, Miller RH. Plasma corticosteroid, circulating leukocyte and milk somatic cell responses to *Escherichia coli* endotoxin-induced mastitis. Proc Soc Exp Biol Med 1974;145(2):553-9.
9. Todar K. Pathogenic *E. coli* online Textbook of Bacteriology. Wisconsin-Madison: University of Wisconsin-Madison, Department of Biotechnology; 2007.
10. Taur Y, Smith MA. Adherence to the infectious diseases society of America guidelines in the treatment of uncomplicated urinary tract infection. Clin Infect Dis 2007;44(6):769-74.
11. Zervos MJ, Hershberger E, Nicolau DP, Ritchie DJ, Blackner LK, Coyle EA, *et al*. Relationship between fluoroquinolone use and changes in susceptibility to fluoroquinolones of selected pathogens in 10 United States teaching hospitals, 1991-2000. Clin Infect Dis 2003;37(12):1643-8.
12. World Health Organization. Antimicrobial Resistance. Available from: http://www.who.int/mediacentre/factsheets/fs194/en/. [Last updated on 2014 Apr; Last cited on 2015 Apr].
13. Goldstein FW. Antibiotic susceptibility of bacterial strains isolated from patients with community - Acquired urinary tract infections in France. Multicentre study group. Eur J Clin Microbial Infect Dis 2000;19(2):112-7.
14. Mooljuntee S, Chansiripomchai P, Chansiripornchai N. Prevalence of the cellular and molecular antimicrobial resistance against *E. coli* isolated from Thai broilers. Thai J Vet Med 2010;40(3):311-5.
15. Nickle JC. Urinary tract infections and resistant bacteria, in highlights of a symposium at the combined meeting of the 25th international congress of chemotherapy (ICC) and the 17th European congress of clinical microbiology and infectious diseases (ECCMID), held on March 31 - April 3, 2007, Munich, Germany. Rev Urol 2007;9(2):78-80.
16. Ashkenazi S, Even-Tov S, Samra Z, Dinari G. Uropathogens of various childhood populations and their antibiotic susceptibility. Pediatr Infect Dis J 1991;10(10):742-6.
17. McKellar QA, Sanchez Bruni SF, Jones DG. Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine. J Vet Pharmacol Ther 2004;27(6):503-14.
18. Levy SB, Marshall B. Antibacterial resistance worldwide: Causes, challenges and responses. Nat Med 2004;10 12 Suppl: S122-9.
19. Maxwell A. DNA gyrase as a drug target. Trends Microbiol 1997;5(3):102-9.
20. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18(3):268-81.
21. Santo E, Salvador MM, Marin JM. Multidrug-resistant urinary tract isolates of *Escherichia coli* from Ribeirão Preto, São Paulo, Brazil. Braz J Infect Dis 2007;11(6):575-8.
22. Sarkar SK, Bhattacharyya A, Mandal SM. YnfA, a SMR family efflux pump is abundant in *Escherichia coli* isolates from urinary infection. Indian J Med Microbiol 2015;33(1):139-42.
23. Somashekara SC, Deepalaxmi S, Jagannath N, Ramesh B, Laveesh MR, Govindadas D. Retrospective analysis of antibiotic resistance pattern to

urinary pathogens in a tertiary care hospital in South India. J Basic Clin Pharm 2014;5(4):105-8.

24. US Food and Drug Administration. National Antimicrobial Resistance Moanitoring System - Enteric Bacteria (NARMS): 2008 Executive Report. Rockville (MD); 2010. Available from: http://www.fda.gov/AnimalVeterinary/SafetyHealth/Antimicrobial Resistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm. [Last cited on 2012 Feb 13].
25. Blaettler L, Mertz D, Frei R, Elzi L, Widmer AF, Battegay M, *et al.* Secular trend and risk factors for antimicrobial resistance in *Escherichia coli* isolates in Switzerland 1997-2007. Infection 2009;37(6):534-9.
26. Hasan AS, Nair D, Kaur J, Baweja G, Deb M, Aggarwal P. Resistance patterns of urinary isolates in a tertiary Indian hospital. J Ayub Med Coll Abbottabad 2007;19(1):39-41.
27. Scheld WM. Maintaining fluoroquinolone class efficacy: Review of influencing factors. Emerg Infect Dis 2003;9(1):1-9.
28. Varughese L, Beniwal V. High quinolone resistant pattern among enteric pathogens isolated from patients with urinary tract infection. Indian J Biotechnol 2015;14:167-71.
29. Williams A, Mathai AS, Phillips AS. Antibiotic prescription patterns at admission into a tertiary level intensive care unit in Northern India. J Pharm Bioallied Sci 2011;3(4):531-6.
30. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother 2001;48 Suppl 1:5-16.
31. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty third Informational Supplement CLSI Document, M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
32. Slama TG. Gram-negative antibiotic resistance: There is a price to pay. Crit Care 2008;12 Suppl 4:S4.
33. Dash SK, Chakraborty SP, Mandal D, Roy S. Isolation and characterization of multidrug resistant uropathogenic *Escherichia coli* from urine sample of urinary tract infected patients. Int J Life Sci Pharm Res 2012;2:25-39.
34. Niranjan V, Malini A. Antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among inpatients. Indian J Med Res 2014;139(6):945-8.
35. Gordon KA, Jones RN. Susceptibility patterns of orally administered antimicrobials among urinary tract infection pathogens from hospitalized patients in North America: Comparison report to Europe and Latin America. Results from the SENTRY antimicrobial surveillance program (2000). Diagn Microbiol Infect Dis 2003;45(4):295-301.