Original Article

Frequency of Fluoroquinolone (Ofloxacin) Resistance In Escherichia Coli Urinary Isolates

Shazia Hyder, Samina Baig, Sambreen Zameer, Shaheen Sharafat, Rafique Khanani, Shaheen Akbar Ågha, Muhammad Akbar Ågha

ABSTRACT

Objectives: To determine the frequency of Escherichia coli and fluoroquinolone (Ofloxacin) resistant Escherichia coli isolates from urinary specimen.

Study Design: Observational study.

Place and Duration: Department of Microbiology, Dow Diagnostic Reference and Research Laboratory, from January to December 2013.

Material & Methods: A total of 10753 urine specimen were received and out of them 1778 isolates of Escherichia coli were obtained included in this study. The susceptibility was determined by swabbing the Mueller- Hinton Agar (MHA) plates using Ofloxacin 5 μ g disc. Clinical Laboratory Standards Institute (CLSI) recommendation, =16 mm zone of inhibition being sensitive, =12 mm as resistant and 13-15 mm as intermediate, were followed in interpreting the results.

Results: Out of the 1778 Escherichia coli isolates, 1189 (66.9%) isolates were resistant to Ofloxacin, 570 (32%) were sensitive to Ofloxacin while 19(1.1%) were in intermediate range.

Conclusion: High prevalence of fluoroquinolone (Ofloxacin) resistance among Escherichia coli in urinary isolates was detected which is alarming. There is an urgent need for regular testing and surveillance for fluoroquinolone resistant producing Escherichia coli.

Keywords:: Fluoroquinolone resistant Escherichia coli, Ofloxacin, Urinary tract infections.

INTRODUCTION:

Urinary tract infection (UTI) is among the most common bacterial infection for which the patients seek medical advice. Many Gram positive and Gram negative bacteria are implicating in causing UTI. Escherichia coli are one of the most frequent organism of urinary tract infection¹. Resistance to antimicrobials of

*	Assistant Professor, Microbiology, DOW International	
	Medical College, Karachi.	
**	Post Graduate Candidate, Microbiology, DOW	
	International Medical College, Karachi.	
***	Professor, Microbiology, DOW International	
	Medical College, Karachi.	
****	Professor, Community Medicine, DUHS, Karachi.	
*****	Professor & Director	
	DIEKIBD, DUHS, Karachi.	
Cor	respondence to:	
	Sambreen Zameer	
	tant Professor	
Dow	International Medical College, Karachi.	
	obiology Department International Medical College, Karachi.	

classes has arisen in many bacterial different species and may complicate the management of predominant urinary tract infection². These organisms are mostly resistant to commonly used oral agents³. Fluoroquinolones are being used as the most common antimicrobial in community for the treatment of urinary tract infections. Excessive use of the antimicrobial agents led to considerable increase in the rate of Escherichia coli resistant isolates⁴. Success of antimicrobial therapy depends on correct choice of antibiotics that should be based on known susceptibility of causative organism⁵. Although the UTI is a major problem for community at large, there is lack of the antimicrobial resistance report about causative agents of UTIs and their empiric treatment choice^b. The main aim was to find out the frequency of fluoroquinolone (ofloxacin) resistant Escherichia coli in positive urine culture samples. Through this study we will be able to acquire data that will help our clinicians to decide the better empirical

antimicrobial and this will help in reduction of morbidity and mortality. This study will eventually help in reducing fluoroquinolone-resistant Escherichia coli by selection of other antimicrobial agents.

MATERIAL & METHODS:

The Study was performed in the Department of Microbiology, Dow Diagnostic Research and Reference Laboratory, Karachi, Pakistan, from January to December 2013. Permission from institutional ethical and research committee was taken. Sampling technique was non-probability consecutive sampling. No discrimination was made on age and gender of the patient. Clinical isolates other than Escherichia coli, duplicate samples, repeat specimens of the same patient, Samples less than 25 ml in quantity, Specimen which is delayed for more than one hour in transportation, samples collected and submitted in leaking containers and contaminated specimens were excluded. All urine specimens were processed on Cystine Lactose Electrolyte Deficient (CLED) agar medium (Oxoid Ltd., England). Plates were incubated in ambient air aerobically at 37°C for 18 hours, using standard microbiological techniques. After 18 hours of incubation, colonies of Escherichia coli on CLED agar were observed which were 2-3 mm in diameter lactose fermenting, translucent to opaque and convex. Gram stain of colonies shows gram negative rods. All the lactose fermenting rods were tested for catalase test, oxidase test and further identified by Analytic Profile Index (API 20E) (Figure I).

Antimicrobial susceptibility testing of these isolates was performed on Mueller-Hinton agar (MHA) (Oxoid Ltd., England) using Kirby Bauer's disc diffusion method according to CLSI (Clinical Laboratory Standard Institute). Ofloxacin disc (5µg) was tested. Escherichia coli ATCC 25933 were included as the control strains for susceptibility testing. Resistant and sensitive cases were differentiated by measuring zone diameter around disc as mentioned in CLSI. Statistical Package for Social Sciences (SPSS, version 17 software) was used for data analysis. Descriptive statistics were calculated for both qualitative and quantitative variables. Qualitative variables like Ofloxacin susceptibility, frequencies and percentages were calculated, and presented as tables and bar-chart.

RESULTS:

During the study period, total 10,753 clinical specimens were received from outpatient in the Microbiology Department. Out of these a total 5025 (46.73%) of which had cultures positive for different micro-organisms. Out of 5025, 1778 were Escherichia coli and 3247 were other microorganisms. Consequently, the prevalence of Escherichia coli was 35.38% (1778) out of 5025 specimens. Mean age of the patients in our study was 44.64 years (SD 23.05) and targated age groups are 3 and 6. Table I. Predominantly the isolates were from female patients that were 1395/1778 (78.45 %); while male patients were 383/1778 (21.55%). In all of these isolates the overall ofloxacin resistant Escherichia coli were 1189 out of 1778 (66.9%), whereas rest of 570 (32%) were ofloxacin sensitive Figure II.

DISCUSSION:

Escherichia coli are an important urinary tract pathogen, with a rising prevalence of community-acquired infection. Escherichia coli are a pathogen primarily associated with hospitalization^{7.8}. It causes health care associated urinary tract infections outbreaks in special clinical settings such as an ICU, as well as community acquired infections with increasing incidence^{8,10}.



Figure-I. A representative photograph of biochemical reactions of Escherichia coli with API 20E strip

Journal of Peoples University of Medical & Health Sciences 2014;4(2):63-7.

Age grouping	Ofloxacin			Total
	S	Ι	R	-
1(1-10 year)	46	0	114	160
2 (11-20 year)	34	0	75	109
3 (21-30 year)	92	6	204	302
4 (31-40 year)	56	3	134	193
5 (41-50 year)	89	3	150	242
6 (51-60 year)	103	5	213	321
7 (61-70 year)	86	1	167	254
8 (71-80 year)	41	1	101	143
9 (81-90year)	21	0	30	51
10 (91-100 year)	02	0	1	3
Total	570 (32%)	19 (1.1%)	1189 (66.9%)	1778 (100%)

Shazia Hyder, Samina Baig, Sambreen Zameer, Shaheen Sharafat, Rafique Khanani, Shaheen Akbar Agha, Muhammad Akbar Agha



Table 1: Ofloxacin Susceptibility Pattern in Different Age Groups

Figure-II. Ofloxacin Susceptibility Pattern in Escherichia Coli

Journal of Peoples University of Medical & Health Sciences 2014;4(2):63-7.

The frequency of isolation of Escherichia coli from all urine specimens was 35.38% (1778 of 5025) indicating its importance as a most predominant urinary tract pathogen. Nasim et al in a study conducted in a tertiary care hospital of Karachi found Escherichia coli as a most frequent isolate (53%) in the total positive specimen^{11,12}. Mean age of the patients in our study was 44.97 years (SD 24.99). In another study mean age was 39 years¹³. In our study a higher incidence of urinary tract infection occurrence was observed in females than males (1359 females and 383 males) but the difference was not statistically significant and is almost similar with those reported elsewhere. One study demonstrated the gender distribution of urinary tract infection of Escherichia coli in females 65.0% in males 35.0 %¹⁴. Similar findings were reported by Khan et al, in Aligarh¹⁵. Higher prevalence of UTI among females was due to the factors that predispose women to UTI more than men.

Isolation of ofloxacin resistant Escherichia coli causing urinary tract infection in our study were 1189 (66.9%) out of 1778 was comparable to earlier reports from some of the countries such as China but much less than in reports from Canada and Netherland¹⁶. In our study predominantly the ofloxacin resistant Escherichia coli isolates were from female patients that are 933/1189 (78.46%), while isolates from male patients were 256/1189 Resistance to quinolones was (21.53%).comparable to the study by Khan and Ahmed $(50\% \& 46\%)^{17}$, which is much higher than reported by Farooqi et al (25% in 1997)¹⁸. While a study from Ghaza strip, the overall distribution of ofloxacin resistant Escherichia coli was 75% in females and 25% males¹⁹. In our study the mean age of patients with ofloxacin resistant Escherichia coli were 48.32. In vitro ofloxacin sensitivity demonstrates a high prevalence (80%) of resistance among the E.coli strains, a drug considered to be of choice in urinary tract infection. The high prevalence of resistance to commonly used antibiotic has caused considerable alarm. The factors favouring development of antibiotic resistance include previous use of an antibiotic by the individual or widespread use of antibiotics in the community. The latter may lead to shift in the species resistance pattern of organisms prevalent in a community²⁰. Antibiotic misuse is very common in developing countries, mainly because of failure to restrict the use of antibiotics in hospitals and to control their sale and use in community²¹. Continued blind empiric therapy with failure to send a urine culture may be the key reason for the emergence of resistance to ofloxacin.

CONCLUSION:

Escherichia coli are emerging as ofloxacin resistant pathogens in community where the selective pressure of antibiotic is high, showing a need for rational use of antimicrobials. Early recognition of infection and appropriate antibiotic therapy are necessary to prevent increasing drug resistance.

RERERENCE:

- Nielubowicz GR, Mobley HL. Hostpathogen interactions in urinary tract infection. Nature reviews Urology. 2010;7(8):430-41.
- Lindback H, Lindback J, Sylvan S, Melhus A. Low frequency of antibiotic resistance among urine isolates of Escherichia coli in the community, despite a major hospital outbreak with Klebsiella pneumoniae producing CTX-M-15 in Uppsala County. Scandinavian journal of infectious diseases. 2010;42(4):243-8.
- 3. Locatelli C, Caronte I, Scaccabarozzi L, Migliavacca R, Pagani L, Moroni P. Extended-spectrum beta-lactamase production in E. coli strains isolated from clinical bovine mastitis. Veterinary research communications. 2009;33 Suppl 1:141-4.
- Kamenski G, Wagner G, Zehetmayer S, Fink W, Spiegel W, Hoffmann K. Antibacterial resistances in uncomplicated urinary tract infections in women: ECO.SENS II data from primary health care in Austria. BMC infectious diseases. 2012;12:222.
- 5. Sabir N, Khan E, Sheikh L, Hassan R. Impact of antibiotic usage on resistance in

Journal of Peoples University of Medical & Health Sciences 2014;4(2):63-7.

Shazia Hyder, Samina Baig, Sambreen Zameer, Shaheen Sharafat, Rafique Khanani, Shaheen Akbar Agha, Muhammad Akbar Agha

microorganism; urinary tract infections with E.coli as case point. J pak Med Assoc 2004;54(9):472-5.

- Al-Hasan MN, Lahr BD, Eckel-Passow JE, Baddour LM. Antimicrobial resistance trends of Escherichia coli bloodstream isolates: a population-based study, 1998-2007. J Antimicrob Chemother. 2009;64(1):169-74.
- Anatoliotaki M, Galanakis E, Schinaki A, Stefanaki S, Mavrokosta M, Tsilimigaki A. Antimicrobial resistance of urinary tract pathogens in children in Crete, Greece. Scand J Infect Dis. 2007;39(8):671-5.
- Smith SP, Manges AR, Riley LW. Temporal changes in the prevalence of communityacquired antimicrobial-resistant urinary tract infection affected by Escherichia coli clonal group composition. Clin Infect Dis. 2008;46(5):689-95.
- 9. Roveta S, Marchese A, Debbia EA. Antibiotic susceptibility tests directly on urine samples using Uro-Quick, a rapid automated system. J Chemother. 2006;18(1):12-9.
- Rock W, Colodner R, Chazan B, Elias M, Raz R. Ten years surveillance of antimicrobial susceptibility of community-acquired Escherichia coli and other uropathogens in northern Israel (1995-2005). Isr Med Assoc J. 2007;9(11):803-5.
- 11. Pitout JD, Gregson DB, Campbell L, Laupl and KB. Molecular characteristics of extended-spectrum-beta-lactamaseproducing Escherichia coli isolates causing bacteremia in the Calgary Health Region from 2000 to 2007: emergence of clone ST131 as a cause of community-acquired infections. Antimicrob Agents Chemother. 2009;53(7):2846-51.
- 12. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011;52(5):e103-20.

- Zhanel GG, Hisanaga TL, Laing NM, DeCorby MR, Nichol KA, Weshnoweski B, et al. Antibiotic resistance in Escherichia coli outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). International journal of antimicrobial agents. 2006;27(6):468-75.
- Linhares I, Raposo T, Rodrigues A, Almeida A. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000-2009). BMC infectious diseases. 2013;13:19.
- 15. Stuck AK, Tauber MG, Schabel M, Lehmann T, Suter H, Muhlemann K. Determinants of quinolone versus trimethoprimsulfamethoxazole use for outpatient urinary tract infection. Antimicrobial agents and chemotherapy. 2012;56(3):1359-63
- Khan SW, Ahmed A. Uropathogens and their susceptibility pattem:aretrospective analysis. J.Pak.Med.Assoc., 2001;51:98-100.
- 17. Farooqi BJ, Shareeq F, Rizvi QK, et at. Changing pattern of antimicrobial susceptibility of organisms causing community acquired urinary tract infections. J.Pak.Med.Assoc; 2000;50:369-73
- Farhat Ullah, Malik SA, Ahmed J. Antibiotic susceptibility pattern and ESBL prevalence in nosocomial Escherichia coli from urinary tract infections in Pakistan. Afr J Biotechnol. 2009;8:3921–6.
- 19. Ramzan M, Bakhsh S, Salam A, Khan G M, Mustafa G. Risk factors in urinary tract infection. Gomal J Med Sci. 2004; 2: 1-4.
- Khattak A M, Khattak S, Khan H, Ashiq B, Mohammad D, Rafiq M. Prevalence of asymptomatic bacteriuria in pregnant women. Pak J Med Sci, 2006; 22: 162-165.
- Zareef S, Zafar H, Izhar K, Dodhy Ehsan M, Hayat A. The culture and sensitivity pattern of uropathogens detected at Benazir Bhutto hospital. Ann. Pak. Inst. Med. Sci. 2009; 5: 121-125.