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# PREVALENCE OF ANTIMICROBIAL RESISTANCE AND INTEGRONS IN *ESCHERICHIA COLI* FROM PUNJAB, PAKISTAN

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## ABSTRACT

Antimicrobial resistance was studied in *Escherichia coli* strains isolated from urine samples of 457 patients suffering from urinary tract infection. High prevalence of class 1 integrons (43.56%), sulfamethoxazole resistance genes *sul1* (45.54%) and *sul2* (51.48%) along with occurrence of quinolone resistance genes was detected in multi drug resistance isolates.

**Key words:** *Escherichia coli*; integrons; antimicrobial resistance; urinary tract infection.

Urinary tract infections (UTIs) are the most common bacterial infections and *Escherichia coli* is the most prevalent cause of UTIs (12, 15). Empirical treatment of UTIs in the developed countries is based on the susceptibility data originated from the local surveillance schemes (1, 7, 8). However, countries like Pakistan do not have any surveillance system to provide reference guidelines for empirical therapy. Thus errors are expected in most of the estimates for empirical therapy for UTIs in such conditions.

Integron system is a dynamic force in the evolution of multidrug resistance (MDR) and it helps bacteria to acquire novel combinations of resistance genes (6). Integrons are horizontally transferable genetic elements which play an important role in dissemination and accumulation of resistance genes in bacteria (15). A strong association between the presence of integrons and antimicrobial resistance has been established by recent studies on Uropathogenic *E. coli* (UPEC) in various regions of Asia, Europe and USA (1, 6, 13, 15, 17). However, the prevalence of integrons and related gene cassettes in *E. coli* strains isolated from UTI patients has not been previously reported from Pakistan.

The aim of this study was to determine the prevalence of antimicrobial resistance, integrons and related genetic elements among *E. coli* isolates from UTI patients from Pakistan. The study was conducted for duration of 1 year (June 2006- June 2007).

The prior approval was obtained from departmental ethical committee. Medical history of the participants was obtained with the help of a questionnaire after obtaining a signed informed consent from all the individuals who participated in the study. Clinically diagnosed UTI cases (n = 457), hospitalized in 11 different hospitals located mainly in 3 different cities of Punjab, were included in this study. Diagnoses was based on clinical history, physical examination, and urine tests including urine microscopy within first 1 hour of urine sample collection and subsequent two consecutive

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urine cultures. The median age of 280 (61.27%) female and 177 (38.73%) male patients was 40 and 45 years respectively. Exclusion criteria was as follows: (A) age < 15 years (B) hospital-acquired infection; (C) hospitalization for more than 48 hours before taking urine samples; (D) patients with permanent indwelling catheters; (E) patients with history of previous use of urinary catheter. However, cases with first time short term use of catheters after admission to the hospital were included and urine samples were taken from such cases within 6 hours of first catheterization.

All urine samples were collected before starting empirical antimicrobial therapy. Urine samples were collected from non-catheterized patients by midstream clean-catch method. Urine samples were collected directly from the catheter tubings into the sterile syringes in case of catheterized patients. All urine samples were processed on MacConkey agar medium (Oxoid, UK) with a standard wire loop and incubated at 37°C overnight. Significant growth was evaluated as $\geq 10^5$ colony forming units (CFU)/ml for midstream urine, and $\geq 10^{2-3}$ CFU/ml for urine samples from catheterized patients (11). Urine samples not complying with the criteria of significant growth were excluded. Identification of *E. coli* was performed by standard biochemical methods. Bacterial isolates other than *E. coli* were excluded. Antimicrobial susceptibility of *E. coli* isolates was determined using standard disk diffusion techniques and strains were classified as susceptible (S), intermediate (I) and resistant (R) according to recommendations of CLSI (Clinical Laboratory Standards Institute) (5). Susceptibility to trimethoprim, sulfamethoxazole-trimethoprim, ampicillin, cefotaxime, ceftazidime, nitrofurantoin, nalidixic acid and ciprofloxacin was determined.

DNA was extracted from *E. coli* isolates by Phenol-Chloroform extraction method (9). All *E. coli* isolates were screened for the presence of class 1, 2 and 3 integrons with the help of PCR-RFLP method (17). Integrase PCR products were digested using *Hinf1* according to the manufacturer's instructions (Fermentas). Integrons were classified on the bases of the sizes of restricted fragments after treatment of *hep* amplified PCR products with *Hinf*1 (491bp fragment for class 1 integrons, 300 bp and 191 bp fragments for class 2 integrons, while 119 bp and 372 bp fragments for class 3 integrons) (17). Sulfamethoxazole resistance determinants *sul1* and *sul2* were identified by PCR amplification using gene specific primers as described previously (1). Ciprofloxacin and / or nalidixic acid resistant strains were screened for the plasmid associated quinolone resistance genes. Plasmids (extracted by using Miniprep (Qiagen) plasmid extraction kit) were used as templates to amplify internal fragments of the *qnr*A (580 bp), *qnr*B (264 bp) and *qnr*S (428 bp) (3).

A total of 101 non-repetitive *E. coli* isolates (representing 7-10 isolates from each hospital) were isolated from UTI patients. The results indicate a high incidence of antimicrobial resistance along with related genetic elements among *E. coli* isolates from UTI patients. In order of descending prevalence, 89 (88.12%) of 101 *E. coli* isolates were resistant to sulfamethoxazole-trimethoprim, 87 (86.14%) were resistant to trimethoprim, 85 (84.16%) were resistant to ceftazidime, 85 (84.16%) were resistant to nalidixic acid, 79 (78.22%) were resistant to ampicillin, 79 (78.22%) were resistant to nitrofurantoin, 77 (76.24%) were resistant to cefotaxime, and 35 (34.65%) were resistant to ciprofloxacin. Majority of isolates were resistant to ≥3 antimicrobials tested. Our observed antimicrobial resistances were either similar or higher than the resistance levels previously reported in various other regions. Sulfamethoxazole-trimethoprim resistance (88.12%) in this study was similar to previously reported levels (up to 90 %) among UPEC isolates (12). The prevalence of trimethoprim resistance in our study (86.14%) is clearly higher than resistance levels reported in the previous studies (25% to 68%) in Asia, Africa and South America (10). Our observed trends in sulfamethoxazole resistant allele distributions show that *sul2* is the most common resistance gene (*su*l2 > *sul*1) in sulfamethoxazole-trimethoprim resistant isolates. The

prevalence of *sul*1 & *sul*2 was 45.54% (n = 46) and 51.48% (n = 52) respectively among 101 *E. coli* isolates.

A high level of antimicrobial resistance (84.16%) against nalidixic acid was observed during current study compared to a resistance level of 26.5% reported by Siddiqui (14). However, resistance was low against ciprofloxacin (34.65%) in our study as compared to 95% resistance to fluoroquinolones in enterobacteriaceae strains reported by Fluit *et al.* in Europe (7). However, Goldstein (8) reported even lower level of fluoroquinolones resistance in *E. coli* (<10% resistance) in France as compared to our study. Our observed prevalence of plasmid associated quinolone resistance genes, *qnr*B, *qnr*A and *qnr*S, was 4 (4.70%), 3 (3.53%) and 3 (3.53%) respectively out of 85 nalidixic acid resistant strains. Similarly, 2 (5.71%), 1 (2.86%) and 2 (5.71%) of 35 ciprofloxacin resistant strains were positive for *qnr*B, *qnr*A and *qnr*S respectively. This study constitutes the first report of quinolone resistance determinants among Uropathogenic *E. coli* isolates from Pakistan and the results are in consistent with recent studies from Europe and Asia (4, 16).

Nitrofurantoin is one of the most appropriate antibacterial agents for empirical therapy of UTIs because it is highly concentrated in the urine and it is administered orally. However, the resistance level in our isolates (78.22%) was significantly high against this antimicrobial as well. Our observed resistance level against cefotaxime was 76.24% whereas 84.16% of isolates were resistant to ceftazidime which is very high level of resistance as compared to up to 5% resistance levels reported in some developed countries for cefotaxime and ceftazidime (8). Susceptibility to penicillin is also decreasing globally (8). We observed a high level of resistance (78.22%) against ampicillin. Saffer *et al.* (13) reported a similarly high level of resistance (82-100%) against ampicillin & similar trend (100% ampicillin resistance) was described by Nicolle (12). *E. coli* isolates sensitive to penicillins and / or cephalosporins were not screened in the current study for detection of ESBL (Extended Spectrum Beta Lactamases) production. This is a limitation of the current study as *E. coli* isolates producing ESBL are classified as resistant to these antimicrobials even if the *in vitro* tests indicate susceptibility (2, 3). We will further investigate the prevalence of ESBL in these isolates in future study.

The presence of integrons in *E. coli* isolates is a serious risk factor for spreading antimicrobial resistance. The prevalence of integrons ranging from 22% to 59% has been reported in clinical *E. coli* isolates in several previous studies on UPEC in Europe and Asia (6, 15). We also observed a similar trend of prevalence of class 1 integrons (44 (43.56%) of 101 isolates) in our study. Arrangement of various genotypes' combinations revealed that the independent occurrence of *int1* among the studied samples was relatively rare as evident by a small number, i.e., 4 (3.96%) of 101 isolates (Table 1). However relatively higher number possessed *sul2* without *int1* and *sul*1 (23 (22.77%) of 101 isolates) as compared to occurrence of *sul*1 without *int*1 and *sul*2 (4 (3.96%) of 101 isolates). Moreover, the occurrence of class 1 integrons was noted in almost all those isolates which were positive for *sul*1. These results are in accordance with the previous studies (15). The occurrence of a significant number of *sul*2 alleles was independent of the presence of integrons. Perhaps these *sul*2 alleles are carried on transposons and / or plasmids which may also transfer them horizontally (1). On the other hand no indication of presence of class 3 integrons was noted among the studied isolates, while class 2 integrons were detected in 3 (2.97%) isolates (Table 1) as compared to 5 to 15 % prevalence of class 2 integrons reported from other regions (15).

The results indicate that antimicrobial resistance level in *E. coli* isolates from UTI patients in Pakistan is relatively higher for most of the antimicrobials tested compared to the developed countries. The data also suggested that urine cultures and susceptibility tests can not be neglected in order to avoid worrisome trend of development of resistance to most commonly used antimicrobials for treatment of UTIs. The prevailing trend of co-occurrence of class 1 integrons and

antimicrobial resistance genes in our study is an additional threat for spread of the antimicrobial resistance traits which may further complicate future strategies for empirical therapy of UTIs.

**Table 1.** Resistance gene combinations (of *sul*1, *sul*2, *int*1, *int*2, *int*3) found in 101 *E. coli* isolates cultured from UTI patients' urine samples.

| Gene combinations | Frequency (%) |
|---|---|
| *int*1 alone | 4 (3.96%) |
| *int*1 + *sul*1 | 13 (12.87%) |
| *int*1 + *sul*2 | 0 (0%) |
| *int*1 + *sul*1+ *sul*2 | 27 (26.73%) |
| *int2* alone | 3 (2.97%) |
| *int2* + *sul*1 | 0 (0%) |
| *int2* + *sul*2 | 0 (0%) |
| *int2* + *sul*1+ *sul*2 | 0 (0%) |
| *int*3 alone | 0 (0%) |
| *sul*1 alone | 4 (3.96%) |
| *sul*2 alone | 23 (22.77%) |
| *sul*1 + *sul*2 | 2 (1.98%) |

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## REFERENCES

1. Blahna, M.T.; Zalewski, C.A.; Reuer, J.; Kahlmeter, G.; Foxman, B.; Marrs, C.F. (2006). The role of horizontal gene transfer in the spread of trimethoprim–sulfamethoxazole resistance among uropathogenic *Escherichia coli* in Europe and Canada. *J. Antim. Chemother.,* 57, 666-671.
2. Caio, F.de.O.; Adenilde S.; Valéria M.L.; Alexandre R.; Jorge A.H.; Sydney H.A. (2010). Prevalence of extended-spectrum beta-lactamases-producing microorganisms in nosocomial patients and molecular characterization of the shv type isolates. *Braz. J. Microbiol.,* 41, 278-282.
3. 3. Cattoir, V.; Poirel, L.; Rotimi, V.; Soussy, C.J.; Nordmann, P. (2007). Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J. Antimicrob. Chemother.,* 60 (2), 394-397.
4. Cavaco, L.M.; Aarestrup, F.M. (2009). Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new transmissible resistance mechanisms *qnr*A, *qnr*B, *qnr*S, and aac(6')Ib-cr, in *Escherichia coli* and *Salmonella enterica* and determinations of wild-type distributions. *J. Clin. Microbiol.,* 47 (9), 2751-2758.
5. Clinical and Laboratory Standards Institute (CLSI): (2006). Performance Standards for Antimicrobial Susceptibility Testing; 16th Informational Supplement M100-S16, Clinical and Laboratory Standards Institute, Wayne.
6. Farshad, S.; Japoni, A.; Hosseini, M. (2008). Low distribution of integrons among multidrug resistant *E. coli* strains isolated from children with community-acquired urinary tract infections in Shiraz. *Iran. Pol. J. Microbiol.,* 57 (3), 193-198.
7. Fluit, A.C.; Jones, M.E.; Schmitz, F.J.; Acar, J.; Gupta, R.; Verhoef, J. (2000). Antimicrobial resistance among urinary tract infection (UTI) isolates in Europe: results from the SENTRY Antimicrobial Surveillance Program 1997. *Antonie Van Leeuwenhoek.,* 77 (2), 147-152.
8. Goldstein, F.W. (2000). Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections in France. Multicentre Study Group. *Eur. J. Clin. Microbiol. Infect. Dis.,* 9 (2), 112-117.
9. Hai-Rong, C.; Ning, J. (2006). Extremely rapid extraction of DNA from bacteria and yeasts. *Biotechnol. Lett.,* 28 (1), 55-59.
10. Lee, J.C.; Oh, J.Y.; Cho, J.W.; Park, J.C.; Kim, J.M.; Seol, S.Y.; Cho, D.T. (2001). The prevalence of trimethoprim-resistance-conferring dihydrofolate reductase genes in urinary isolates of *Escherichia coli* in Korea. *J. Antimicrob. Chemother.,* 47 (5), 599-604.
11. Maki, D.G.; Tambyah, P.A. (2001). Engineering out the risk of infection with urinary catheters. *Emerg. Infect. Dis.,* 7 (2), 342–347.
12. Nicolle, L.E. (2003). Urinary tract infection: traditional pharmacologic therapies. *Dis. Mon.,* 49 (2), 111-128.
13. Saffar, M.J.; Enayti, A.A.; Abdolla, I.A.; Razai, M.S.; Saffar, H. (2008). Antibacterial susceptibility of uropathogens in 3 hospitals, Sari, Islamic Republic of Iran, 2002-2003. *East. Mediterr. Health. J.,* s14 (3), 556-563.

14. Siddiqui, AA. (2008). Prevalence of quinolone-resistant urinary tract infections in Comanche County Memorial Hospital. *J. Okla. State Med. Assoc.,* 101 (9), 210-212.
15. Solberg, O.D.; Ajiboye, R.M.; Riley, L.W. (2006). Origin of class 1 and 2 integrons and gene cassettes in a population-based sample of uropathogenic *Escherichia coli. J. Clin. Microbiol.,* 44 (4), 1347-1351.
16. Usein, C.R.; Palade, A.M.; Tatu-Chițoiu, D.; Ciontea, S.; Ceciu, S.; Nica, M.; Damian, M. (2009). Identification of plasmid-mediated quinolone resistance *qnr*-like genes in Romanian clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Roum. Arch. Microbiol. Immunol.,* 68 (1), 55-57.
17. White, P.A.; McIver, C.J.; Rawlinson, W.D. (2001). Integrons and gene cassettes in the Enterobacteriaceae. *Antimicrob. Agents. Chemother.,* 45, 2658-2661.

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