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A Study To Determine Prevalence of Quinolone Resistance Genes Among Extended-Spectrum B-Lactamase-Producing Escherichia Coli

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ABSTRACT

Objectives: The aim of this study was to determine the frequency of qnr genes in ESBL-producing and non-ESBL-producing E. coli isolated from outpatient and hospitalized patient clinical specimens

Materials and Methods: Two hundred E. coli strains, isolated from different clinical specimens were used. ESBL-producing E. coli were detected by determining susceptibility to ceftazidime, cefotaxime, and cefpodoxime with the phenotypic confirmatory test (PCT).

Results: Eighty-six (43%) isolates were ciprofloxacin-resistant. The PCT identified 85 (42.5%) of 200 E. coliisolates as ESBL-producing. The blaTEM, blaSHV, qnrA, qnrB, and qnrS gene were found in 65 (76.47%), 23 (27%), 63 (31%), 34 (17%), and 14 (7%) isolates, respectively.

Conclusions: The antibiotics prescription policy should be revised, and infection control measures should be improved.

KEYWORDS : Extended-Spectrum Beta-Lactamas, Quinolone Resistance, *Escherichia coli*

1. Background

Since the detection of plasmid-mediated quinolone resistance (PMQR) in *Klebsiella pneumoniae* strains isolated from clinical specimens from the USA in the late 1990s (2, 3), three PMQR mechanisms have been discovered. One PMQR mechanism has been reported worldwide in various enterobacterial species in the presence of the *qnr* genes (4). Another mechanism is related to the *AAC (6´)-Ib-cr* gene, which is responsible for decreasing susceptibility to quinolones by N-acetylation of a piperazinyl amine substituent of some fluoroquinolones (5). A third PMQR mechanism, which is based on a quinolone efflux pump protein, QepA, has been reported recently (6).

Qnr is a member of the pentapeptide repeat family that protects DNA gyrase and topoisomerase IV from quinolone inhibition (7). Extended-spectrum beta lactamase (ESBL) strains are often plasmid-mediated, and most of these enzymes belong to the *TEM* or *SHV* families (8) that have been reported in many countries (9). In recent studies, a significant relationship was observed between quinolone resistance and the latest cephalosporin antibacterial agents (10-13). The association of *qnr* and ESBL production genes or AmpC-type β lactamase has been observed (14). Furthermore, many studies have demonstrated that plasmids harboring *qnr* may also carry other genes that respond to ESBL-producing strains (15, 16).

2. Objectives

Considering the worldwide spread of the *qnr* gene, especially in hospitalized patients, and the fact that few studies have focused on *qnr* genes in ESBL-producing *Escherichia coli*, the aim of this study was to determine the frequency of three *qnr* gene types in ESBL-producing and non-ESBL-producing *E. coli* clinical isolates at a tertiary Hospital in south india.

3. Materials and Methods

3.1. Bacterial Isolates

The descriptive cross-sectional study included 200 *E. coli* clinical isolates collected from outpatients and hospitalized patients in a tertiary care Hospital from May 2014 to July 2015. We classified the patients as hospitalized if they were admitted to the hospital and stayed for a minimum of 24 hours. We classified the patients as outpatients if they were not hospitalized for more than 24 hours but visited a hospital, clinic, or associated facility for diagnosis or treatment (17). The sample size was calculated based on level of confidence, expected prevalence, and precision (corresponding to effect size) (18).). Prior to specimen collection, criteria such as previous antimicrobial therapy, immune suppression, and presence of bacteremia because of other pathogens before and after colonization by *E. coli* were taken into consideration. Standard biochemical tests were used to detect *E. coli* strains (19).

3.2. Antimicrobial Susceptibility Testing

Susceptibility tests for ciprofloxacin, ceftazidime, cefotaxime, and cefpodoxime were performed for ESBL- and non-ESBL-producing *E. coli* by using the standard disc diffusion test according to the clinical and laboratory standards institute guidelines (20). Isolated *E. coli* were cultured on Muller-Hinton agar. Ciprofloxacin (5 µg), ceftazidime (30 µg), cefotaxime (30 µg), and cefpodoxime (10 µg) disks (Mast, UK) were placed on the inoculated plate and incubated at 35°C for 16 - 20 h. Drug resistance was recognized according to their zone sizes compared with the break point announced by the clinical and laboratory standards institute for *Enterobacteriaceae* (20).

3.3. Phenotypic Confirmatory Test

The combination disc method was used for detecting ESBL strains. Bacteria were cultured on a Muller-Hinton agar plate, and ceftazidime (30 µg) versus ceftazidime/clavulanate (30/10 µg), cefotaxime (30 µg) versus cefotaxime/clavulanate (30/10 µg), and cefpodoxime (10 µg) versus cefpodoxime/clavulanate (30/10 µg) (Mast Diagnostics, UK) were tested. Antibiotic disks were placed in media at a distance of 20 - 30 mm from other disks. After 18 - 24 hours incubation at 37°C, ESBL-producing organisms were detected by observation of a zone diameter increase of at least 5 mm around antibacterial agents in combination with clavulanic acid (20). The reference strain *Klebsiella pneumonia* ATCC 700603 was used as an ESBL-positive control (10).

4. Results

Two hundred *E. coli* isolates were tested from 84 outpatients and 116 hospitalized patients at a tertiary Hospital in south India from May 2014 to July 2015. Overall, 61% of the hospitalized patients were male and 39% were female. The average age of the patients was 55 years ± 0.8 years. The phenotypic confirmatory test identified 85 (42.5%) isolates as ESBL-producing *E. coli*. The greatest proportion [72.9% (62 of 85)] of ESBL-producing isolates was isolated from urine samples, followed by 8.23% (7 of 85) from wound samples, 8.23% (7 of 85) from blood samples, 3.52 (3 of 85) from ascitic fluid samples, and 2.35% (2 of 85) from secretion samples. The remaining 4.7% (4 of 85) of isolates were isolated from CSF, pleural fluid, ear, and nose secretion samples. bla_{TEM} was present in 65 (76.47%) of 85 phenotypic ESBL-producing

isolates, while 23 (27%) ESBL-producing isolates were positive for the bla_{SHV} gene. Some ESBL-producing isolates possessed both the-bla_{TEM} and bla_{SHV} genes.. *qnrB*, and *qnrS* gene frequencies were 63 (31%), 34 (17%), and 14 (7%), respectively. *qnr* (A, B, S) frequencies in hospitalized patients and outpatients are summarized in Figure 3.

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5. Discussion

In this study, 85 (42.5%) of 200 isolates were recognized as ESBL-producing *E. coli*. Furthermore, the prevalence of ESBL-producing *E. coli* was high, especially in hospitalized patients. More than 70% of ESBL-producing organisms were bla_{TEM}- and/or bla_{SHV}-positive. In a study conducted in Mashhad, Iran, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* were 15.62% and 20%, respectively (21). In contrast, our results suggested that there is evidence of a higher prevalence of ESBL-producers. Our molecular findings indicated that bla_{TEM} was more prevalent than bla_{SHV}. This molecular pattern was similar to previous studies by Pakzad et al. and Masjedian Jazi et al. (10). In the present study, the most prevalent gene among all isolates was *qnrA,* followed by *qnrB* and *qnrS*. In a similar study in Iran, *qnrA* and *qnrB* were recognized as the dominant genes. This finding is in agreement with our results (10). To our knowledge, there have not been any previous reports of the *qnrS* gene in ESBL-producing organisms in Iran. *qnr* gene frequency in ESBL-producing *E. coli* varies by country. In the USA, among 313 ceftazidime-resistant *Enterobacteriaceae* isolates, 23% were positive for either *qnrA* or *qnrB,* while *qnrS* was absent (12).

Our findings revealed that some ciprofloxacin-resistant isolates did not have *qnr* genes. Hence, the antibiotics prescription policy should be revised, and infection control measures should be improved.

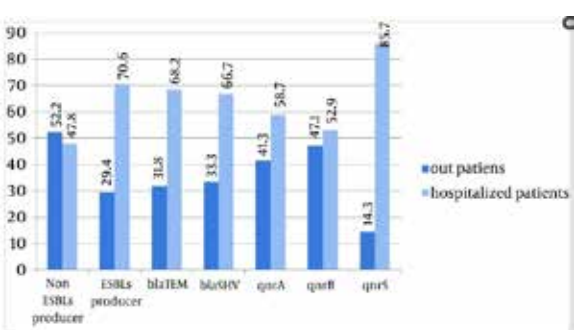
Tables:

Table 1: *qnrA, qnrB,* and *qnrS* Gene Frequencies in Isolated *E. coli*

qnr	ESBL	
	ESBL-Producing *E. coli*	**Non ESBL-Producing *E. coli***
qnrA	29 (34.1)	34(29.6)
Qnr B	15 (17.6)	19(16.5)
Qnrs	9 (10.6)	5(4.3)

Values are presented as No. (%)

Figure 1: The distribution of Non-ESBL- and ESBL-Producers and bla_{TEM}, bla_{SHV}, *qnrA, qnrB,* and *qnrS* Frequencies in *E. coli* Strains Isolated From Outpatients and Hospitalized Patients



References

1. Takahashi H, Hayakawa I, Akimoto T. [The history of the development and changes of quinolone antibacterial agents]. Yakushigaku Zasshi. 2003;38(2):161–79.
2. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis. 2006;6(10):629–40.
3. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet.1998;351(9105):797–9.
4. Stavoe AKH. Plasmid-mediated quinolone and fluoroquinolone resistance. MMG445 Basic Biotech J.2006;2(1):154–9.
5. Robicsek A, Strahilevitz J, Jacoby GA, Macielag M, Abbanat D, Park CH, et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med.2006;12(1):83–8.
6. Ma J, Zeng Z, Chen Z, Xu X, Wang X, Deng Y, et al. High prevalence of plasmid-mediated quinolone resistance determinants qnr, aac(6′)-Ib-cr, and qepA among ceftiofur-resistant Enterobacteriaceae isolates from companion and food-producing animals. Antimicrob Agents Chemother. 2009;53(2):519–24.
7. Tran JH, Jacoby GA. Mechanism of plasmid-mediated quinolone resistance. Proc Natl Acad Sci U S A.2002;99(8):5638–42. doi: 10.1073/pnas.082092899.
8. Behrooozi A, Rahbar M, Yousefi JV. Frequency of extended spectrum beta-lactamase (ESBLs) producing Escherichia coli and klebseilla pneumonia isolated from urine in an Iranian 1000-bed tertiary care hospital. Afr J Microbiol Res. 2010;4(9):881–4.
9. Rupp ME, Fey PD. Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment. Drugs. 2003;63(4):353–65.
10. Pakzad I, Ghafourian S, Taherikalani M, Sadeghifard N, Abtahi H, Rahbar M, et al. qnr Prevalence in Extended Spectrum Beta-lactamases (ESBLs) and None-ESBLs Producing Escherichia coli Isolated from Urinary Tract Infections in Central of Iran. Iran J Basic Med Sci. 2011;14(5):458–64.
11. Cerquetti M, Garcia-Fernandez A, Giufre M, Fortini D, Accogli M, Graziani C, et al. First report of plasmid-mediated quinolone resistance determinant qnrS1 in an Escherichia coli strain of animal origin in Italy. Antimicrob Agents Chemother. 2009;53(7):3112–4.
12. Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC. qnr prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. Antimicrob Agents Chemother. 2006;50(8):2872–4.
13. Kim MH, Lee HJ, Park KS, Suh JT. Molecular characteristics of extended spectrum beta-lactamases in Escherichia coli and Klebsiella pneumoniae and the prevalence of qnr in Extended spectrum beta-lactamase isolates in a tertiary care hospital in Korea. Yonsei Med J. 2010;51(5):768–74.
14. Pai H, Seo MR, Choi TY. Association of QnrB determinants and production of extended-spectrum beta-lactamases or plasmid-mediated AmpC beta-lactamases in clinical isolates of Klebsiella pneumoniae.Antimicrob Agents Chemother. 2007;51(1):366–8.
15. Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae.J Antimicrob Chemother. 2005;56(3):463–9.
16. Poirel L, Cattoir V, Soares A, Soussy CJ, Nordmann P. Novel Ambler class A beta-lactamase LAP-1 and its association with the plasmid-mediated quinolone resistance determinant QnrS1. Antimicrob Agents Chemother. 2007;51(2):631–7
17. Centers for Medicare, Medicaid Services . Are You a Hospital Inpatient Or Outpatient?: If You Have Medicare--ask! United States: Centers for Medicare & Medicaid Services, Department of Health and Human Services; 2009. pp. 1–8.
18. Hajian-Tilaki K. Sample size estimation in epidemiologic studies. Caspian J Intern Med.2011;2(4):289–98.
19. Mahon CR, Lehman DC, Manuselis Jr G, editors. Textbook of Diagnostic Microbiology. Elsevier Health Sciences; 2014.
20. Wikler MA. Performance standards for antimicrobial susceptibility testing: Sixteenth informational supplement. Clinical and Laboratory Standards Institute; 2012.
21. Zaniani FR, Meshkat Z, Naderi Nasab M, Khaje-Karamadini M, Ghazvini K, Rezaee A, et al. The Prevalence of TEM and SHV Genes among Extended-Spectrum Beta-Lactamases Producing Escherichia Coli and Klebsiella Pneumoniae. Iran J Basic Med Sci. 2012;15(1):654–60.