Acta Microbiologica et Immunologica Hungarica 64 (1), pp. 63–69 (2017)
DOI: 10.1556/030.63.2016.011
First published online November 16, 2016

DETECTION OF *acrA*, *acrB*, *aac(6′)-Ib-cr*, AND *qepA* GENES AMONG CLINICAL ISOLATES OF *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE*

MOHSEN HEIDARY[1,2], AGHIL BAHRAMIAN[3], ALI HASHEMI[3], MEHDI GOUDARZI[1,3], VAHID FALLAH OMRANI[4], GITA ESLAMI[3] and HOSSEIN GOUDARZI[1,3]*

[1]Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[3]Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

(Received: 1 March 2016; accepted: 25 August 2016)

Background: The distribution of drug resistance among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* has limited the therapeutic options. The aim of this study was to report the prevalence of quinolone resistance genes among *E. coli* and *K. pneumoniae* clinical strains isolated from three educational hospitals of Tehran, Iran. *Materials and methods:* A total of 100 strains of *E. coli* from Labbafinejad and Taleghani Hospitals and 100 strains of *K. pneumoniae* from Mofid Children and Taleghani Hospitals were collected between January 2013 and May 2014. Antimicrobial susceptibility tests were done by disk diffusion method based on Clinical and Laboratory Standards Institute guidelines. Detection of *qepA*, *aac(6′)-Ib-cr*, *acrA*, and *acrB* genes was done by polymerase chain reaction (PCR). *Results:* In this study, fosfomycin and imipenem against *E. coli* and fosfomycin and tigecycline against *K. pneumoniae* had the best effect in antimicrobial susceptibility tests. PCR assay using specific primers demonstrated that the prevalence of *qepA*, *aac(6′)-Ib-cr*, *acrA*, and *acrB* genes among the 100 *E. coli* isolates was 0 (0%), 87 (87%), 92 (92%), and 84 (84%), respectively. The prevalence of *qepA*, *aac(6′)-Ib-cr*, *acrA*, and *acrB* genes among the 100 *K. pneumoniae* isolates was 4 (4%), 85 (85%), 94 (94%), and 87 (87%), respectively. *Conclusion:* The distribution of *qepA*, *aac(6′)-Ib-cr*, *acrA*, and *acrB* resistance determinants in *E. coli* and *K. pneumoniae* is a great concern.

*Corresponding author; E-mail: Hgod100@yahoo.com

Therefore, infection control and prevention of spread of drug-resistant bacteria need careful management of medication and identification of resistant isolates.

Keywords: *Escherichia coli*, *Klebsiella pneumoniae*, quinolone, Iran

Introduction

Escherichia coli and *Klebsiella pneumoniae* are the main gram-negative opportunistic pathogens in nosocomial infections [1, 2]. Virulent strains of *E. coli* mostly can cause urinary tract infections, neonatal meningitis, abdominal cramps, and diarrhea [3–5]. *K. pneumoniae* can cause clinical infections including respiratory tract infection, pneumonia, urinary tract infection, wound infection, and bacteremia [3]. Resistance to fluoroquinolones in clinical isolates of *Enterobacteriaceae* family was first studied in a *K. pneumoniae* strain [6, 7]. Recently, fluoroquinolone resistance has emerged in clinical strains of *E. coli* and *K. pneumoniae* [8, 9]. Mutations in topoisomerase IV and DNA gyrase and overexpression of AcrAB efflux system are the main mechanisms of quinolone resistance in *E. coli* and *K. pneumoniae* isolates [10–12]. Plasmid-mediated quinolone resistance (PMQR) mechanisms have also been reported: Qnr determinants, namely, QnrA, QnrB, QnrC, QnrD, and QnrS; QepA and OqxAB efflux pumps; and AAC(6′)-Ib-cr enzyme that acetylates aminoglycosides and ciprofloxacin [13]. Efflux pumps are transport proteins involved in the extrusion of toxic substrates from intracellular into the extracellular environment. AcrAB and QepA efflux pumps have been reported in clinical isolates of *E. coli* and *K. pneumoniae*. These efflux proteins can modify the permeability of the bacterial membrane by drug extrusion to outside, therefore the antibiotic resistance occurs. AcrAB and QepA multidrug-resistant efflux proteins belong to the major facilitator superfamily group and confers decreased susceptibility to quinolone. The *aac(6′)-Ib-cr* gene encodes the ability of quinolones acetylation such as ciprofloxacin and norfloxacin. Therefore, *aac(6′)-Ib-cr* gene can diminish susceptibility to quinolones in addition to aminoglycosides. The aim of this study was to report the prevalence of quinolone resistance genes, including *qepA*, *aac(6′)-Ib-cr*, *acrA*, and *acrB* genes, among *E. coli* and *K. pneumoniae* clinical strains isolated from three educational hospitals of Tehran, Iran.

Methods

Bacterial isolates

A total of 100 strains of *E. coli* from Labbafinejad and Taleghani Hospitals and 100 strains of *K. pneumoniae* from Mofid Children and Taleghani Hospitals were

Table I. *Primer sequence* and product size

Primer	Sequence (5′-3′)	Gene	Product size (bp)
QepA-F	CTGCAGGTACTGCGTCATG	*qepA*	403
QepA-R	CGTGTTGCTGGAGTTCTTC		
AcrA-F	TCTGATCGACGGTGACATCC	*acrA*	157
AcrA-R	TCGAGCAATGATTTCCTGCG		
AcrB-F	CAATACGGAAGAGTTTGGCA	*acrB*	64
AcrB-R	CAGACGAACCTGGGAACC		
Aac(6′)-Ib-F	TTGCGATGCTCTATGAGTGGCTA	*aac(6′)-Ib*	611
Aac(6′)-Ib-R	CTCGAATGCCTGGCGTGTTT		

collected between January 2013 and May 2014. The following conventional biochemical tests were done: motility, indole, methyl red, Voges–Proskauer, ornithine decarboxylase and lysine decarboxylase, Simmons citrate test, and triple sugar iron.

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed by the Kirby-Bauer disk diffusion method (Mast Group Ltd., Merseyside, UK) according to Clinical and Laboratory Standards Institute guidelines [14]. The antimicrobial agents tested were cefpodoxime (30 μg), ciprofloxacin (30 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ampicillin (10 μg), tigecycline (15 μg), cefotaxime (30 μg), ceftazidime (30 μg), levofloxacin (5 μg), and fosfomycin (50 μg). *E. coli* ATCC25922 was used as quality control strain.

PCR detection and DNA sequencing

The DNA was extracted by GeNet Bio company (Korea, Cat. no. K-3000) and used as a template for polymerase chain reaction (PCR). The quinolone

Table II. Temperature and time of PCR assay

	Temperature (°C)				Time			
Step	*qepA*	*acrA*	*acrB*	*aac(6′)-Ib*	*qepA*	*acrA*	*acrB*	*aac(6′)-Ib*
Initial denaturation	94	94	94	94	5 min	5 min	5 min	5 min
Denaturation	94	94	94	94	45 s	45 s	45 s	45 s
Annealing	51	57	52	55	45 s	45 s	45 s	45 s
Extension	72	72	72	72	45 s	45 s	45 s	45 s
Final extension	72	72	72	72	5 min	5 min	5 min	5 min
Cycle	36	36	36	36				

resistance encoding genes, including *qepA*, *aac(6′)-Ib-cr*, *acrA*, and *acrB* genes were amplified for all *E. coli* and *K. pneumoniae* strains by means of PCR using the primer sets and thermal cycling conditions described in Tables I and II. PCR products were analyzed by electrophoresis in a 1%–1.5% agarose gel. One of the PCR products was purified and direct sequencing was done.

Statistical analysis

Our study was a descriptive study. Analysis of results was done by MINITAB16 software. *P* value and confidence intervals were <0.05 and 95%, respectively.

Results

In all the 100 strains of *E. coli* recovered, 70 strains were isolated from Labbafinejad Hospital (70%) and 30 from Taleghani Hospital (30%). A total of 33 strains were isolated from male patients (33%) and 67 from female patients (67%). In all the 100 strains of *K. pneumoniae* recovered, 50 strains were isolated from Mofid Children Hospital (50%) and 50 from Taleghani Hospital (50%). A total of 57 strains were isolated from male patients (57%) and 43 from female patients (43%). In this study, fosfomycin and imipenem against *E. coli* and fosfomycin and tigecycline against *K. pneumoniae* had the best effect in antimicrobial susceptibility tests. Antibiotic susceptibility testing results for clinical isolates of *E. coli*

Table III. Antibiotic susceptibility testing results

	Resistant no. (%)		Sensitive no. (%)		Intermediate no. (%)	
Antibiotic	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*
Gentamicin	43 (43%)	80 (80%)	55 (55%)	10 (10%)	2 (2%)	10 (10%)
Amikacin	34 (34%)	17 (17%)	65 (65%)	83 (3%)	2 (2%)	0 (0%)
Imipenem	24 (24%)	3 (3%)	66 (66%)	91 (91%)	10 (10%)	6 (6%)
Cefotaxime	66 (66%)	84 (84%)	33 (33%)	13 (13%)	2 (2%)	3 (3%)
Levofloxacin	70 (70%)	68 (68%)	20 (20%)	20 (20%)	10 (10%)	12 (12%)
Fosfomycin	10 (10%)	5 (5%)	85 (85%)	90 (90%)	5 (5%)	5 (5%)
Ampicillin	62 (62%)	100 (100%)	17 (17%)	0 (0%)	21 (21%)	0 (0%)
Ciprofloxacin	83 (83%)	86 (86%)	14 (14%)	10 (10%)	3 (3%)	4 (4%)
Cefpodoxime	72 (72%)	88 (88%)	26 (26%)	12 (12%)	2 (2%)	0 (0%)
Tigecycline	15 (15%)	–	30 (30%)	–	55 (55%)	–
Ceftazidime	62 (62%)	80 (80%)	33 (33%)	15 (15%)	5 (5%)	5 (5%)

and *K. pneumoniae* are shown in Table III. The prevalence of *qepA*, *aac(6′)-Ib-cr*, *acrA*, and *acrB* genes among the 100 *E. coli* isolates was 0 (0%), 87 (87%), 92 (92%), and 84 (84%), respectively. The prevalence of *qepA*, *aac(6′)-Ib-cr*, *acrA*, and *acrB* genes among the 100 *K. pneumoniae* isolates was 4 (4%), 85 (85%), 94 (94%), and 87 (87%), respectively.

Discussion

First, PMQR genes were identified in *Enterobacteriaceae* family. Then, *qnr* determinants, including *qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*, have been identified. Furthermore, AAC(6′)-Ib-cr that acetylates aminoglycosides and ciprofloxacin and *qepA*, which encodes an efflux pump, have been identified [15]. In this study, the prevalence of *qepA* gene among *K. pneumoniae* isolates was reported 4 (4%), but this gene was not seen among *E. coli* isolates. These results are consistent with the study performed by Chen et al. [16] in China, where 4.9% of *qepA* gene was positive. Despite this low prevalence rate, this plasmid-encoding gene, can be moved between hospitals and health-care centers increase the resistance rate. The AAC(6′)-Ib-cr causes decreased susceptibility to quinolones in addition to aminoglycosides. In this study, we showed that 87 (87%) clinical isolates of *E. coli* and 85 (85%) clinical isolates of *K. pneumoniae* carried *aac(6′)-Ib-cr* gene. This high prevalence rate of resistance is a serious threat to use both the quinolone and aminoglycoside drugs for treatment programs in the future. It was demonstrated in this survey that ciprofloxacin and levofloxacin had the most drug resistance against *E. coli* and *K. pneumoniae* isolates. The antibiotic susceptibility testing performed by Ma et al. [17] confirms our results. Recent studies reported several mechanisms for antimicrobial resistance ability among clinical isolates of *E. coli* and *K. pneumoniae*. AcrAB efflux pump is one of the main chromosomal mechanisms of resistance to quinolones in *Enterobacteriaceae* family [18–21]. This efflux protein is one of the important mechanisms in multidrug-resistant *E. coli* and *K. pneumoniae* isolates. This study showed high prevalence rate of *acrA* and *acrB* genes among *E. coli* isolates, 92% and 84%, respectively, and in *K. pneumoniae* isolates, 94% and 87%, respectively. Since the neighboring countries of Iran have a similar distribution of quinolone resistance genes studied in this survey, the geographical location can play a key role in spreading of these genes. Recent studies showed increase in the quinolone-resistant clinical isolates of *E. coli* and *K. pneumoniae* among hospitalized patients in Iran [22, 23]. Much more investigations need to simplify the actual incidence rate of quinolones resistance encoding genes.

Acknowledgments

The authors would like to thank the Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Funding Sources

This work was supported by a research grant from Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences (Grant No. 1863).

Conflict of Interest

None.

References

1. Tsai, Y.-K., Fung, C.-P., Lin, J.-C., Chen, J.-H., Chang, F.-Y., Chen, T.-L., Kristopher Siu, L.: *Klebsiella pneumoniae* outer membrane porins OmpK35 and OmpK36 play roles in both antimicrobial resistance and virulence. Antimicrob Agents Chemother **55**, 1485–1493 (2011).
2. Hakemi-Vala, M., Makhmor, M., Kobarfar, F., Kamalinejad, M., Heidary, M., Khoshnood, S.: Investigation of Antimicrobial Effect of *Tribulus terrestris L.* against Some Gram Positive and Negative Bacteria and *Candida spp*. Novelty Biomed **2**, 85–90 (2014).
3. Livermore, D.M.: Current epidemiology and growing resistance of gram-negative pathogens. Korean J Intern Med **27**, 128–142 (2012).
4. Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., Finlay, B. B.: Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev **26**, 822–880 (2013).
5. Brumbaugh, A. R., Mobley, H. L.: Preventing urinary tract infection: Progress toward an effective *Escherichia coli* vaccine. Expert Rev Vaccines **11**, 663–676 (2012).
6. Roy, S., Viswanathan, R., Singh, A. K., Das, P., Basu, S.: Sepsis in neonates due to imipenem-resistant *Klebsiella pneumoniae* producing NDM-1 in India. J Antimicrob Chemother **66**, 1411–1413 (2011).
7. Robicsek, A., Jacoby, G. A., Hooper, D. C.: The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis **10**, 629–640 (2006).
8. Yang, J., Ye, L., Wang, W., Luo, Y., Zhang, Y., Han, L.: Diverse prevalence of 16S rRNA methylase genes armA and rmtB amongst clinical multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates. Int J Antimicrob Agents **38**, 348–351 (2011).
9. Zhou, X., Gao, J., Huang, Y., Fu, S., Chen, H.: Antibiotic resistance pattern of *Klebsiella pneumoniae* and *Enterobacter sakazakii* isolates from powdered infant formula. Afr J Microbiol Res **5**, 3073–3077 (2011).

10. Heidary, M., Hashemi, A., Goudarzi, H., Khoshnood, S., Roshani, M., Azimi, H., Goudarzi, M.: The antibacterial activity of Iranian plants extracts against metallo beta-lactamase producing *Pseudomonas aeruginosa* strains. J Paramed Sci **7**, 13–19 (2016).
11. Heidary, M., Goudarzi, H., Hashemi, A., Eslami, G., Goudarzi, M., Chirani, A. S., Amraei, S.: The prevalence of genes that encode quinolone resistance in *Klebsiella pneumoniae* strains isolated from hospitalized patients during 2013–2014. Arch Pediatr Infect Dis (2016).
12. Maramba-Lazarte, C. C.: Etiology of neonatal sepsis in five urban hospitals in the Philippines. PIDSP J **12**, 75–85 (2011).
13. Rodríguez-Martínez, J. M., Cano, M. E., Velasco, C., Martínez-Martínez, L., Pascual, A.: Plasmid-mediated quinolone resistance: An update. J Infect Chemother **17**, 149–182 (2011).
14. Clinical and Laboratory Standards Institue (CLSI): Performance Standard for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. CLSI document M100_s17. CLSI, Wayne, PA, 2013.
15. Kumar, V., Sun, P., Vamathevan, J., Li, Y., Ingraham, K., Palmer, L., Huang, J., Brown, J. R.: Comparative genomics of *Klebsiella pneumoniae* strains with different antibiotic resistance profiles. Antimicrob Agents Chemother **55**, 4267–4276 (2011).
16. Chen, X., Zhang, W., Pan, W., Yin, J., Pan, Z., Gao, S., Jiao, X.: Prevalence of *qnr*, *aac(6′)-Ib-cr*, *qepA*, and *oqxAB* in *Escherichia coli* Isolates from Humans, Animals, and Environment. Antimicrob Agents Chemother **56**, 3423–3427 (2012).
17. Ma, J., Zeng, Z., Chen, Z., Xu, X., Wang, X., Deng, Y., Lü, D., Huang, L., Zhang, Y., Liu, J., Wang, M.: 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 **53**, 519–524 (2009).
18. Hasdemir, U. O., Chevalier, J., Nordmann, P., Pagès, J. M.: Detection and prevalence of active drug efflux mechanism in various multidrug-resistant *Klebsiella pneumoniae* strains from Turkey. J Clin Microbiol **42**, 2701–2706 (2004).
19. Du, D., Wang, Z., James, N. R., Voss, J. E., Klimont, E., Ohene-Agyei, T., Venter, H., Chiu, W., Luisi, B. F.: Structure of the AcrAB-TolC multidrug efflux pump. Nature **509**, 512–515 (2014).
20. Opperman, T. J., Kwasny, S. M., Kim, H. S., Nguyen, S. T., Houseweart, C., D'Souza, S., Walker, G. C., Peet, N. P., Nikaido, H., Bowlin, T. L.: Characterization of a novel pyranopyridine inhibitor of the AcrAB efflux pump of *Escherichia coli*. Antimicrob Agents Chemother **2**, 722–733 (2014).
21. Buffet-Bataillon, S., Tattevin, P., Maillard, J. Y., Bonnaure-Mallet, M., Jolivet-Gougeon, A.: Efflux pump induction by quaternary ammonium compounds and fluoroquinolone resistance in bacteria. Future Microbiol **11**, 81–92 (2016).
22. Seyedpour, S. M., Eftekhar, F.: Quinolone susceptibility and detection of *qnr* and *aac*(6')-*Ib-cr* genes in community isolates of *Klebsiella pneumoniae*. Jundishapur J Microbiol **7**, 1–4 (2014).
23. Lari, A. R., Azimi, L., Rahbar, M., Fallah, F., Alaghehbandan, R.: Phenotypic detection of *Klebsiella pneumoniae* carbapenemase among burns patients: First report from Iran. Burns **39**, 174–176 (2013).