The emergence of plasmid mediated quinolone resistance qnrA2 in extended spectrum β-lactamase producing *Klebsiella pneumoniae* in the Middle East

Leila Vali*, Ali A. Dashti, Mehrez M. Jadaon and Sherief El-Shazly

**Abstract**

**Background:** *Klebsiella pneumoniae* is one of the most important opportunistic pathogens causing serious complications in patients in hospitals and community. The clinical significance of *K. pneumoniae* is mainly due to its ability to acquire multiple antibiotic resistance genes. In this study we report the findings of a survey of plasmid mediated quinolone resistance in Extended-Spectrum β-lactamase (ESBL)-producing *K. pneumoniae* in Kuwait.

**Methods:** Clinical samples were collected from the microbiology laboratories of three major hospitals. Isolates were confirmed as ESBL-producers by disc diffusion method and PCR for the presence of bla genes. Antimicrobial susceptibility testing and genetic analysis were performed to detect the presence of a number of genes conferring resistance to β-lactam and fluoroquinolone antimicrobial agents including *bla*SHV, *bla*TEM, *aac* (6′)-Ib-cr, qnrA, qnrB and qnrS. Pulsed-field gel electrophoresis (PFGE) was used for typing the isolates.

**Results:** In total 173 ESBL-producing *K. pneumoniae* were detected. qnr gene was identified in 27 (15.6%) isolates and *aac* (6′)-Ib-cr gene in 26 (96%). One (3.7%) contained qnrA2, 21 harbored qnrB1 (78%) and 5 (18.5%) contained qnrS. Twenty one (78%) isolates contained all three *bla* genes. PFGE showed diverse profiles.

**Conclusion:** We identified for the first time the emergence of the mobile fluoroquinolone resistance qnrA2 in a clinical isolate in the middle east and also showed the dissemination of *aac* (6′)-Ib-cr, qnrB, and qnrS genes among ESBL-producing *K. pneumoniae* in Kuwait. The abundance of plasmid mediated resistance to fluoroquinolones among ESBL-producing *K. pneumoniae* is alarming as it facilitates therapy failure. Preventing the spread of these isolates is crucial if we are to sustain the effectiveness of the limited choices we have left in antimicrobial therapy.

**Keywords:** *Klebsiella pneumoniae*, Fluoroquinolones, qnr, Pulsed-field gel electrophoresis, Extended spectrum β-lactamase

**Background**

Members of the Enterobacteriaceae family in particular the multi-drug resistant *Klebsiella pneumoniae* are amongst the opportunistic pathogens. *K. pneumoniae* causes a wide range of infections from urinary and respiratory tract infections to bacteraemia, particularly in elderly or debilitated patients. Antibiotic resistance is an important factor that hinders therapy and delays improvement in patients’ health. In *K. pneumoniae* resistance to antimicrobial agents is caused by different mechanisms however the production of extended spectrum β-lactamases (ESBLs) such as CTX-M and other enzymes including TEM-1, TEM-24, SHV-12 and the plasmid-mediated AmpC (CMY-2) are considered highly important [1–3]. CTX-M-15 producing strains can often contain variants of an aminoglycoside-modifying enzyme expressed by *aac*(6′)-Ib-cr genes. Aminoglycoside-modifying enzymes cause reduced susceptibility to aminoglycosides and to some fluoroquinolones [4]. Fluoroquinolone resistance in *K. pneumoniae* can arise by mutations in the chromosomal type II topoisomerases and changes in the expression of efflux pumps and porins. Furthermore fluoroquinolone resistance can also be
facilitated by plasmid-mediated resistance determinants \textit{qnr} that express the pentapeptide repeat proteins. These proteins protect the quinolone targets (DNA gyrase or topoisomerase IV enzymes) from the inhibitory activity of fluoroquinolone antibiotics [5, 6]. In view of the genetic heterogeneity variants of \textit{qnrA}, \textit{B} and \textit{S}-like genes share 94 % to 99 %, 85 % to 99 % and 90 % nucleotide identity respectively [7].

In addition \textit{K. pneumoniae} has been reported to produce clinically important carbapenemases; such as Ambler class B metallo-\(\beta\)-lactamases (NDM), the class A enzymes (KPC) and the class D oxacillinase enzymes (OXA-48) [8, 9]. Following the rise in the use of cephalosporins in hospitals; the incidence of infections caused by ESBL-producing \textit{K. pneumoniae} has soared [2, 10]. Resistance to 3rd generation cephalosporins is predominantly associated with the presence of \textit{bla}_{\text{CTX-M}} genes located on plasmids [11]. Therefore plasmid mediated resistance to fluoroquinolones among ESBL producing \textit{K. pneumoniae} is alarming as it facilitates therapy failure. Moreover; the exchange of the genetic information among bacterial species threatens the efficacy of fluoroquinolones and increases the dependency on carbapenem antibiotics for treatment. The purpose of the present study was to determine the prevalence of plasmid-mediated fluoroquinolone resistance in ESBL-producing \textit{K. pneumoniae} in hospitalized patients in Kuwait.

Material and methods

Bacterial isolates

In-patient clinical samples were collected from the microbiology laboratories of three major governmental hospitals that serve the six governorates of Kuwait, namely Al-Ahmadi hospital, Al-Amiri hospital and Adan hospital. All three hospitals are tertiary health care providers with bed capacities of 300, 500 and 700, respectively. The average number of specimens processed every day in each hospital varies from 500 to 700 which includes samples from out-patient and in-patient specialists units. A table was created based on the patients’ records containing information such as age, gender, hospital, and type of specimen. Specimens were processed by clinical staff members of the diagnostic microbiology laboratories using standard protocols. The species identification was carried out by the API (bioMérieux, Marcy l’Etoile, France) and the VITEK 2 systems (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO, USA). The results were analyzed according to the Clinical and Laboratory Standards Institute (CLSI) (2012) guidelines [12]. The isolates were stored in 10 % skim milk at -70 °C.

Susceptibility testing

Antibiotic susceptibility tests were performed by the Kirby-Bauer method disc diffusion test following the CLSI (2012) criteria and recommendations [12]. The antibiotics tested were ampicillin, ampicillin/sulbactam, amoxicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefazidine, cefepime, ceftriaxone, cefazolin and cefuroxime. The Minimum Inhibitory Concentration (MIC) was determined by agar dilution method and E-test when available for the following antibiotics: trimethoprim gentamicin, cefotaxime, imipenem, meropenem, ciprofloxacin, levofloxacin and tetracycline. Isolates that showed resistance to at least three classes of antibiotics were considered as MDR. Isolates that were detected as resistant to cefoxitin were further investigated for the presence of an \textit{ampC} \(\beta\)-lactamase by using multiplex PCR [13, 14].

Double-disc synergy method

ESBLs were detected as previously described using the disc approximation and double-disc synergy methods and confirmed with cefotaxime and ceftazidime E-test ESBL strips (AB Biodisk, Biomerieux-diagonstics, Durham, NC, USA) [2]. For the disc approximation test, clavulanate diffusion from an amoxicillin–clavulanate (AMC30) disc was used to test for synergy with cefotaxime, ceftazidime, cefuroxime, cefepime and cefixime (Oxoid) as described previously [15]. For the double-disc synergy test, a ceftazidime disc (30 µg) was placed 30 mm away from a disc containing amoxicillin–clavulanate (60/10 µg). ESBL production was considered positive when an enhanced zone of inhibition between the \(\beta\)-lactam and \(\beta\)-lactamase inhibitor-containing discs. For the E-test, ESBL strips containing ceftazidime and ceftazidime–clavulanate and strips containing cefotaxime and cefotaxime–clavulanate were used to determine the MIC ratio according to the manufacturer’s instructions (AB Biodisk, Biomerieux-diagnostics, Durham, NC, USA). Cultures were incubated aerobically at 37 °C for 18–24 h. CTX-M-15 \(\beta\)-lactamase enzyme displays a catalytic activity toward ceftazidime.

Detection of \textit{bla} genes and other resistance determinants

The presence of resistant genes (listed below) were investigated by PCR assays as previously reported [16]. PCR was conducted in a GeneAmp 9700 (Perkin-Elmer, Illinois, USA) system using the conditions specified for each primer; corresponding to the source references. The resistant genes investigated were \textit{bla}_{\text{TEM-1}}, \textit{bla}_{\text{SHV-1}}, \textit{bla}_{\text{CTX-M-like}}, \textit{bla}_{\text{NDM}}, \textit{bla}_{\text{OXA-1}}, \textit{qnrA}, \textit{qnrB}, \textit{qnrS}, \textit{aac(6’)-Ib} \text{II-cr}, \textit{gyrA}, \textit{parC}, \textit{gyrB}, \textit{parE}, \textit{intI1}, \textit{intI2}, \textit{bla}_{\text{KPC}}, \textit{bla}_{\text{TIM}}, \textit{bla}_{\text{IMP}}, \textit{bla}_{\text{OXA-48}}, and \textit{ampC}. The detection of \textit{bla}_{\text{PER}}, \textit{bla}_{\text{GES}} and \textit{bla}_{\text{VEB}} was performed by PCR according to Opazo et al. [17].

Isolates resistant to ciprofloxacin and for which the ceftaxidime or cefotaxime MICs were >8 mg/L were screened for ESBLs and \textit{qnr} genes. Double disc and combination disc tests were used for ESBLs confirmation.

Amplified PCR products were purified with Qiagen purification kit (Qiagen, Limburg Netherlands) according to the
manufacturer’s instructions and both strands were sequenced by automated ABI3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The BLAST program of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov) was used to search and compare databases for similar nucleotide sequences.

### Pulsed-Field Gel Electrophoresis (PFGE)

PFGE was performed by using the restriction enzyme *Xba*I [18], with a run time of 20 h and switch times of 5 to 50 and 5 to 35 s at 14 °C and 6 V/cm (CHEF-DR II System; Bio-Rad, Hercules, CA, USA). The PFGE profiles were analyzed by using BioNumerics software version 7.1 (Applied Maths, Sint-Martens-Latem, Belgium). Statistics was added at the end of the methods section.

### Statistical analysis

Out of the total number of isolates included hereby, the percentage of the different ESBL-producing bacteria were calculated in each of the three hospitals participating in the study.

### Table 1 The total number of ESBL producing Enterobacteriaceae isolates containing *qnr* genes during this study

| Hospital | Enterobacteriaceae (ESBL producers) | *Escherichia coli* | Enterobacter cloacae | *Proteus mirabilis* | *Klebsiella oxytoca* | *Klebsiella pneumoniae* | *qnr* positive *K. pneumoniae* |
|----------|------------------------------------|-------------------|---------------------|-------------------|-------------------|-----------------------|-------------------------------|
| Amiri    | 480                                | 383 (80 %)        | 1 (0.2 %)           | 18 (3.8 %)        | 6 (1 %)           | 72 (15 %)             | 5 (6.9 %)                     |
| KOC      | 137                                | 83 (61 %)         | 6 (4 %)             | 7 (5 %)           | 4 (3 %)           | 37 (27 %)             | 10 (2.7 %)                    |
| Adan     | 215                                | 140 (65 %)        | 4 (1.8 %)           | 3 (1.4 %)         | 4 (1.8 %)         | 64 (30 %)             | 12 (18.3 %)                   |
| Total    | 832                                | 606 (73 %)        | 11 (1.3 %)          | 28 (3 %)          | 14 (1.7 %)        | 173 (21 %)            | 27 (15.6 %)                   |

### Table 2 The list of *K. pneumoniae* isolates

| Hospital | Isolate number | Specimen  | Gender | Age | Species |
|----------|----------------|-----------|--------|-----|---------|
| Adan     | ADA-17         | Urine     | M      | NA  | K. pneumoniae |
|          | ADA-28         | Urine     | M      | NA  | K. pneumoniae |
|          | ADA-30         | Urine     | M      | NA  | K. pneumoniae |
|          | ADA-39         | Bile      | F      | 33  | K. pneumoniae |
|          | ADA-45         | Urine     | M      | 76  | K. pneumoniae |
|          | ADA-49         | Bile      | F      | 33  | K. pneumoniae |
|          | ADA-69         | Urine     | F      | 78  | K. pneumoniae |
|          | ADA-89         | Urine     | M      | 84  | K. pneumoniae |
|          | ADA-111        | Urine     | M      | 77  | K. pneumoniae |
|          | ADA-140        | Urine     | F      | 77  | K. pneumoniae |
|          | ADA-214        | Urine     | M      | 79  | K. pneumoniae |
|          | ADA-215        | Urine     | F      | 8   | K. pneumoniae |
| Ahmadi   | KOC-2          | Urine     | M      | 82  | K. pneumoniae |
|          | KOC-7          | Urine     | F      | 47  | K. pneumoniae |
|          | KOC-12         | Catheter Tip | F   | 14  | K. pneumoniae |
|          | KOC-32         | Urine     | F      | 36  | K. pneumoniae |
|          | KOC-37         | Urine     | F      | 36  | K. pneumoniae |
|          | KOC-63         | Urine     | F      | 90  | K. pneumoniae |
|          | KOC-64         | Urine     | F      | 58  | K. pneumoniae |
|          | KOC-66         | Catheter Tip | F   | 72  | K. pneumoniae |
|          | KOC-74         | Urine     | F      | 60  | K. pneumoniae |
|          | KOC-105        | Diabetic Foot | M   | 59  | K. pneumoniae |
| Al-Amiri | Y-2            | Diabetic Foot | M   | 52  | K. pneumoniae |
|          | Y-4            | Urine     | M      | 78  | K. pneumoniae |
|          | Y-12           | Bedsore Swab | F   | 65  | K. pneumoniae |
|          | Y-15           | Penile Swab | M   | 81  | K. pneumoniae |

NA Not available
DNA profiles were analysed by the unweighted pair method with arithmetic average (UPGMA) using BioNumerics v.7.1. The development of the algorithms necessary for the comparison of fingerprinting profiles of isolates was based on the Dice correlation coefficient. The hierarchic Cluster analysis and phylogenetic trees were subsequently analysed with an optimization of 1.0 % and a tolerance of 1 %. Isolates were considered to belong to the same PFGE clone if their Dice similarity index was ≥ 85 %.

**Results**

**Bacterial isolates**

All three governmental hospitals participated during our study period from 2010 to 2012; however there were inconsistencies in the level of strain contribution from each hospital. Therefore under-representation of ESBL-mediated *K. pneumoniae* might exist. A total of 832 ESBL-producing *Enterobacteriaceae* isolates were obtained Table 1. They comprised of 606 *Escherichia coli* (73 %), 11 *Enterobacter cloacae* (1.3 %), 28 *Proteus mirabilis* (3 %), 14 *Klebsiella oxytoca* (1.7 %) and 173 *Klebsiella pneumoniae* (21 %).

The antibiotic sensitivity testing for *K. pneumoniae* isolates was performed followed by double-disc synergy tests. The results were confirmed with PCR and sequencing the product.

From the total of 173 (21 %) ESBL-producing *K. pneumoniae* *qnr* genes were identified in 27 (15.6 %) isolates. The distribution of the isolates is presented in Table 2.

**Sensitivity to antimicrobial agents**

All the 27 *qnr* positive isolates were resistant to ampicillin (MIC > 16), ciprofloxacin (MIC > 4), cefotaxime (MIC > 4), ceftazidime (MIC > 4), ceftriaxone (MIC > 4), and gentamicin (MIC > 16); but sensitive to imipenem (MIC > 1) and meropenem (MIC > 1).

Sequencing the PCR products showed the distribution of the resistance determinants (Table 3). *blaCTX-M-15* was detected in 25 (93 %), *blaCTXM-2* in 2 (7 %), *blaTEM* in 21 (78 %), *blaSHV* and *aac(6')-Ib-cr* gene was found in 26 (96 %) isolates. Twenty one (78 %) isolates contained all three *bla* genes. From the 27 *K. pneumoniae* isolates only 1 (3.7 %) contained *qnrA2*, 21 harbored *qnrB1* (78 %) and 5 (18.5 %) contained *qnrS*. Class I integrons (*intI1*) was detected in 25 (93 %) isolates but class II integrons (*intI2*) was not found in any of the isolates tested. *blaKPC*, *blaVIM*, *blaPER*, *blaGES*, *blaEEB*, *blaIMIB*, *blaOXA1* and *blaOXA48* and *ampC* were not detected in this study. No mutations were found in *gyrA* and *parC*.

**PFGE**

PFGE showed diverse profiles among the isolates tested (Fig. 1). Only two isolates (KOC 7 and KOC 64) from urinary tract infection had identical PFGE patterns. The majority of isolates with similar *qnr* genes; displayed similar antimicrobial resistance phenotypes but their PFGE profiles were not identical. In total three isolates (ADA-45, ADA-49 and KOC-37) were not typable by PFGE.

Table 3 The profiles of *qnr* positive *K. pneumoniae* isolates and other resistance genes studied

| Profiles of the antibiotic resistance genes | No. of isolates |
|--------------------------------------------|-----------------|
| *qnrB1, blaCTX-M-15*                       | 2               |
| *qnrB1, blaCTX-M-2, blaSHV, aac(6')-Ib-cr* | 1               |
| *qnrB1, blaCTX-M-15, blaSHV, aac(6')-Ib-cr* | 3               |
| *qnrA2, blaCTX-M-15, blaTEM-1, blaSHV, aac(6')-Ib-cr* | 1               |
| *qnrA2, blaCTX-M-15, blaTEM-1, blaSHV, aac(6')-Ib-cr* | 4               |
| *qnrB1, blaCTX-M-15, blaSHV, aac(6')-Ib-cr* | 15              |
| **Total**                                   | **27**          |
Discussion
In the present study we detected a similar rate of ESBL-producing *K. pneumoniae* (21%) as previously reported (28%) [19]. However, we found 15.6% (27/173) of ESBL-producing *K. pneumoniae* contained a plasmid-mediated *qnr* gene. Previous research undertaken in Kuwait in 2007 did not identify either *qnrA* or *qnrS* in ESBL-producing Enterobacteriaceae and reported a low prevalence of 4.7% (3 out of 64 isolates) of *qnrB* in *E. coli* [7]. Here we identified *qnr* A, B and S in ESBL-producing *K. pneumoniae*. The incidence rate of 15.6% *qnr* in Kuwait compared to other countries in Asia such as Iran (30.4%) [20], Thailand (34.6%) [21], Malaysia (37%) [22], India (67%) [23] and Saudi Arabia (44%) [24] is considerably lower.

The key findings in our study are the emergence of *qnrA2* in the Middle East for the first time and also the spread of *qnrS* among the clinical ESBL-producing *K. pneumoniae* isolates in Kuwait. *qnrA* is the first plasmid-mediated quinolone resistance identified however, in *K. pneumoniae* it is not as widespread as *qnrB* [25]. *qnrA* confers resistance to quinolones such as nalidixic acid and increases minimum inhibitory concentration (MIC) values of fluoroquinolones up to 20-folds [26]. It has also been shown that when both *qnrA* and *aac(6’)-Ib-cr* are present in the same cell, the level of ciprofloxacin resistance is increased fourfold more than that conferred by *qnrA* alone [5]. Therefore the detection of *qnrA2* gene in an ESBL producing *K. pneumoniae* from the catheter tip of a 14 year old female patient was alarming and a challenge for empirical antibiotic therapy such as fluoroquinolones in clinical settings. The rise of plasmid-mediated fluoroquinolone resistance is concerning for antimicrobial treatment of *K. pneumoniae* whereby the only option left will be carbapenems.

In this study *blaCTX-M-15* was the predominant β-lactamase and in 93% of the cases (25 out of 27 isolates) it was associated with the presence of *aac(6’)-Ib-cr*. We also identified *qnrB1* and *qnrS* in isolates harbouring *aac(6’)-Ib* *Ib-cr* and *blaCTX-M*. These isolates were resistant to penicillins, most cephalosporins, β-lactamase inhibitors as well as fluoroquinolones posing a risk to combination β-lactam/β-lactamase inhibitor therapy. The correlation between ESBL and ciprofloxacin resistance is an indication of the important contribution of the plasmid-mediated resistance to fluoroquinolone resistance in this region.

Urine samples comprised 67% (18/27) of the specimens followed by wound swabs 15% (4/27). We did not find any correlation between antimicrobial resistance characteristics or specimen type and PFGE profiles. In fact overall the PFGE profiles of the tested isolates were diverse implying that the spread of *qnr* genes is not concentrated to only a specific clonal lineage. A detailed longitudinal study is required to identify the clonal identity of ESBL-producing *qnr*-positive *K. pneumonia* isolates.

Conclusion
This manuscript reports the genotypic variation among strains of *qnr* positive *K. pneumoniae* in hospitalized patients in Kuwait. The presence of *qnr* and *aac(6’)-Ib-cr* genes especially when they co-exist in ESBL-producing isolates contribute to the increase in fluoroquinolone resistance.

In conclusion the clinical importance of our findings is that the circulating MDR plasmids that contain *qnr, aac(6’)-Ib-cr* and *bla* genes are concerning in hospitals as they increase the chances of *K. pneumoniae* adaptability to environmental pressures such as antibiotics. If guidelines are not in place regarding the strict use of antibiotics we will face antimicrobial therapy failure in hospitalized patients. Continuous monitoring of plasmid mediated fluoroquinolone ESBL-producing *Enterobacteriaceae* in clinical settings is crucial.

Abbreviations
ESBL: Extended-Spectrum β-lactamase; *K. pneumoniae*: Klebsiella pneumoniae; *qnr*: Plasmid-mediated quinolone resistance gene; *bla*: β-lactamase gene; PFGE: Pulsed-field gel electrophoresis; MIC: Minimum Inhibitory Concentration; MDR: Multi-drug Resistant; PCR: Polymerase Chain Reaction; AMC: Aminocyclin/Clavulanic acid.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
LV, AAD, MMJ and SE all participated equally in the design of the study, processing the samples, laboratory experiments and analysing the data. LV drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgement
The authors would like to thank Miss Shorooq Barak Al-Inizi for her technical support. The authors would also like to acknowledge the Research Unit for Genomics, Proteomics and Cellomics Studies (OMCS) of the Health Sciences Centre, Kuwait University (Project No. SRL01/13) for assisting in DNA sequencing.

Funding
This work was supported by Kuwait University Research Administration Grant number NW02/10 and the Kuwait Foundation for Advancement of Science (KFAS), Grant no. 2011130204.

Received: 19 April 2015 Accepted: 17 June 2015
Published online: 28 June 2015

References
1. Karaki E, Ellington MJ, Pike R, Warren RE, Livermore DM, Woodford N. Molecular characterization of plasmids encoding CTX-M-15 β-lactamases from Escherichia coli strains in the United States. J Antimicrob Chemother. 2006;58(6):965–8.
2. Dasthi AA, Jadaon MM, Gomaa HH, Noronha B, Udo EE. Transmission of a *Klebsiella pneumoniae* clone harbouring genes for CTX-M-15-like and SHV-112 enzymes in a neonatal intensive care unit of a Kuwait hospital. J Med Microbiol. 2010;59:987–92.
3. Munier GK, Johnson CL, Snyder JW, Moland ES, Hanson ND, Thomson KS. Positive extended-spectrum-beta-lactamase (ESBL) screening results may be due to AmpC beta-lactamas more often than to ESBLs. J Clin Microbiol. 2010;48:673–4. http://www.ncbi.nlm.nih.gov/pubmed/19955269.
4. Park CH, Rovicsek A, Jacoby GA, Sahm D, Hooper DC. Prevalence in the United States of *aac(6’)-Ib-cr* encoding a ciprofloxacin-modifying enzyme: Antimicrob Agent Chemother. 2006;50:3953–5.
5. Mammeri H, Van De Loo M, Poirel L, Martinez-Martinez L, Nordmann P. Emergence of plasmid-mediated quinolone resistance in Escherichia coli in Europe. Antimicrob Agents Chemother. 2005;49:71–6.

6. Vetting MW, Hegde SS, Fajardo JE, Fiser A, Roderick SL, Talik HE, et al. Pentapeptide repeat proteins. Biogeosciences. 2006;3:1–10.

7. Cattoir V, Poirel L, Rotimi V, Soussi CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. J Antimicrob Chemother. 2007;60:934–7.

8. Poirel L, Hombrouck-Aket C, Frencaux C, Bernabeu S, Nordmann P. Global spread of New Delhi metallo-β-lactamase 1. Lancet Infect Dis. 2010;10:832.

9. Poirel L, Carbonnelle E, Bernabeu S, Gutmann L, Rotimi V, Nordmann P. Importation of OXA-48-producing Klebsiella pneumoniae from Kuwait. J Antimicrob Chemother. 2012;67:2051–2.

10. Jacoby TS, Kuchen RS, Dos Santos RP, Magedanz L, Guzzato P, Moreira LB. Impact of hospital-wide infection rate, invasive procedures use and antimicrobial consumption on bacterial resistance inside an intensive care unit. J Hosp Infect. 2010;75:23–7.

11. Peirano G, Pitout JDD. Molecular epidemiology of Escherichia coli producing CTX-M beta-lactamas: the worldwide emergence of clone ST131 O25:H4. Int J Antimicrob Agents. 2010;35:316–21.

12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. Document M100-S21. Wayne, PA: CLSI; 2012.

13. Pérès-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002;40:2153–62.

14. Pitout JD, Gregson DB, Church DL, Laupland KB. Population-based laboratory surveillance for AmpC beta-lactamase-producing Escherichia coli, Calgary. Emerg Infect Dis. 2007;13:443–8.

15. Sonnevend A, Al Dhaheri K, Mag T, Herpay M, Kolodziejek J, Nowotny N, et al. CTX-M-15-producing multidrug-resistant enteraggregative Escherichia coli in the United Arab Emirates. Clin Microbiol Infect. 2006;12:582–5.

16. Dashti AA, Vali L, El-Shazly S, Jadaon MM. The characterization and antibiotic resistance profiles of clinical Escherichia coli O25b-B2-31 isolates in Kuwait. BMC Microbiol. 2014;14:214.

17. Opazo A, Vali L, Al Obaid K, Dashti AA, Amyes SG. Novel genetic structure harbouring blaadh in ceftazidime-resistant Acinetobacter baumannii isolated from Kuwait. Int J Antimicrob Agents. 2014;43:383–4.

18. Ribot FM, Far NA, Gautorn R, Carmeron DN, Hunter SB, Swaminathan B, et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157, Salmonella and Shigella for pulsenet. Foodborne Path Dis. 2006;3:59–67.

19. Al Benwan K, Al Sweih N, Rotimi VO. Etiology and antibiotic susceptibility patterns of community- and hospital-acquired urinary tract infections in a general hospital in Kuwait. Med Princ Pract. 2010;19:440–6.

20. Seyedpour SM, Eftekhar F. Quinolone susceptibility and detection of qnr genes in clinical isolates of nosocomial Escherichia coli. J Microbiol. 2014. doi:10.5812/jjm.11136.

21. Pasom W, Charanowong A, Luitanond A, Wilaluckana C, Kenprom S, Puang-Ngern P. Plasmid-mediated quinolone resistance genes, qnrS, qnrB, and qnrA, in urinary isolates of Escherichia coli and Klebsiella pneumoniae at a teaching hospital, Thailand. Jpn J Infect Dis. 2013;66:428–32.

22. Al-Marzoq F, Mohd Yusof MY, Tay ST. Molecular analysis of ciprofloxacin resistance mechanisms in Malaysian ESBL-producing Klebsiella pneumoniae isolates and development of mismatch amplification mutation assays (MAMA) for rapid detection of qnrA and parC mutations. Biomed Res Int. 2014. doi:10.1155/2014/601630.

23. Tripathi A, Dutta SK, Majumdar M, Dhara L, Banerjee D, Roy K. High prevalence and significant association of ESBL and QNR genes in pathogenic Klebsiella pneumoniae isolates of patients from Kolkata, India. Indian J Microbiol. 2012;52:557–64.

24. Shibli AM, Al-Agamy MH, Khubnani H, Senok AC, Taufik AF, Livermore DM. High prevalence of acquired quinolone resistance genes among Enterobacteriaceae from Saudi Arabia with CTX-M-15 β-lactamase. Diagn Microbiol Infect Dis. 2012;73:350–3.

25. Martínnez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferrable plasmid. Lancet. 1998;351:797–9.

26. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis. 2006;6:629–40.