The characterization and antibiotic resistance profiles of clinical Escherichia coli O25b-B2-ST131 isolates in Kuwait

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

Abstract

Background: Escherichia coli O25b-B2-ST131 are considered virulent extra-intestinal pathogens causing serious clinical complications such as urinary tract infection and bacteraemia. Our main objectives in this study were to characterise the multi-drug resistant (MDR) isolates of this lineage in Kuwait, and to demonstrate whether reduced susceptibility is spread clonally.

Results: A subset of 83 (10%) non-duplicate and non-selective E. coli O25b-B2-ST131 out of 832 MDR E. coli was identified and collected. Minimum inhibitory concentrations of the isolates were determined and pulsed-field gel electrophoresis was used for typing. The majority (95.2%) of the 83 E. coli O25b-B2-ST131 harboured at least one bla gene with blaCTX-M-15 being the most prevalent. blaCTX-M-2 was present in one isolate. Also one isolate harboured blaCTX-M-56, qnrB1 and blaCMY-2 genes and carried IncF1 plasmids of about 97 kb and 160 kb. qnrB and qnrS were found in 8 other blaCTX-M-15 containing isolates. The blaNDM, blaIMP, blaVIM and qnrA were not detected, however, the blaOXA-48 was present in two (2.4%).

Conclusions: The majority of isolates harbouring qnr genes demonstrated relatedness (≥85%) by PFGE. However, the diversity in PFGE profiles for the other MDR isolates reflected the changes in population genetics of E. coli O25b-B2-ST131. We identified for the first time the appearance of blaCTX-M-2 in the Middle East and blaCTX-M-56 outside the Latin American countries. The isolate harbouring blaCTX-M-56 also contained qnrB1 and blaCMY-2 genes and carried IncF1 plasmids. The appearance of a highly virulent E. coli O25b-ST131 that is resistant to penicillins, most cephalosporins, β-lactamase inhibitors as well as fluoroquinolones is a cause for concern.

Keywords: Escherichia coli ST131, Pulsed-field gel electrophoresis, Extended spectrum beta-lactamases, qnrB

Background

Escherichia coli belonging to the phylogenetic group B2, serotype O25b:H4 and Multi-Locus Sequence Type (ST) 131 (E. coli O25b-B2-ST131), producing extended-spectrum β-lactamase (ESBL) is regarded as a major pandemic clone in community and hospitals causing serious clinical infections such as urinary tract infections and bacteraemia [1]. It has been shown that E. coli O25b-B2-ST131 exhibits a high virulence score compared to other lineages [2] and is capable of acquiring antibiotic resistance by different mechanisms [3-6]. The fact that E. coli O25b-B2-ST131 is able to exhibit antibiotic resistance means that the clinical environment within a hospital or community may actively select certain resistant strains [7] making the treatment of these infections increasingly difficult. Analysis by pulsed field gel electrophoresis (PFGE) has identified a high degree of genetic diversity among the E. coli O25b-B2-ST131 isolates; however, some types appear to be more common in certain regions than others [4].

An important cause of resistance in E. coli O25b-B2-ST131 is the production of β-lactamase enzymes. Some of the most prevalent of these are CTX-M-like enzymes as well as other types specifically TEM-1, TEM-24, SHV-12 and the plasmid-mediated AmpC CMY-2 [8-10]. Furthermore, CTX-M-15 producing strains often co-produce...
both OXA-1 as well as variants of an aminoglycoside-modifying enzyme that is responsible for reduced susceptibility both to the aminoglycosides and to some fluoroquinolones expressed by aac(6’)-Ib-cr genes [5,6]. Fluoroquinolone (FQ) resistance in Enterobacteriaceae is usually caused by mutations in the chromosomal genes coding for type II topoisomerases and changes in the expression of efflux pumps and porins. The rise of plasmid-mediated FQ resistance protein Qnr [11] has caused concern in antimicrobial treatment of Enterobacteriaceae whereby carbapenems are considered the best therapeutic option [12]. Nevertheless some Enterobacteriaceae can produce clinically important carbapenemases; the Ambler class B metallo-β-lactamases (NDM, IMP, VIM), the class A enzymes (KPC) and the class D oxacillinase enzymes (OXA-48). Until recently the class C β-lactamases (e.g., TEM, SHV, CTX-M, TEM-1) isolated from pet dogs [20] and CTX-M-15 and OXA-48 carbapenemase is mainly associated with the Tn1999-like transposon inserted into a single 62-kb IncL/M-type plasmid [19]. It has been detected in sporadic cases; E. coli ST1196 (also containing resistance genes: bla_CMV, bla_SHV-12 and bla_TEM-1) and E. coli ST1431 (containing β-lactamase genes: blaCTX-M-1, blaOXA-2 and blaTEM-1) isolated from pet dogs [20] and E. coli (containing blaCTX-M-15 and blaTEM-1 genes) isolated from a Belgian patient with ventilator-associated pneumonia travelling back from Egypt [21].

To date reports from the Middle East has been focused on the sporadic and selective E. coli O25b-B2-ST131 cases [22] and a comprehensive study on the epidemiology of this lineage was lacking. Therefore we aimed to address this issue by systematically characterising the multi-drug resistant (MDR) isolates of E. coli O25b-B2-ST131 recovered from patients in order to use these findings as a source for future reference studies and surveillances.

Methods

Bacterial isolates
A survey of Extended Spectrum β-lactamase (ESBL)-producing Enterobacteriaceae was undertaken from January 2010 to December 2012. A subset of 832 MDR E. coli strains was collected from the microbiology laboratories of three major hospitals that serve the three governorates of Kuwait. All the three hospitals are tertiary health care providers with bed capacities of 300 for Ahmadi, 500 for Amiri and 600 for Yiaco-Adam. The average number of specimens processed each day varies from 500 to 700 which includes samples from outpatient and in-patient specialists units. 832 original isolates represent a subset of the isolates submitted to the clinical diagnostic laboratories of these centres.

Each patient was included only once in this study. A database was created based on the patient’s records that contained information; such as age, sex, hospital, location of care on each site, type of specimen and date of sampling. Specimens were processed by clinical staff members of the diagnostic laboratories using standard protocols. Cultures were performed on blood agar, MacConkey, Cystine lactose electrolyte deficient agar (CLED) and incubated aerobically and anaerobically as required. All isolates were identified at the species level based on colony morphology, biochemical analysis and by using Vitek2 (Vitek AMS; BioMérieux Vitek Systems Inc., Hazelwood, MO, USA). The isolates were stored in 10% skim milk and at -70°C.

To confirm the phylogenic grouping of E. coli O25b-B2-ST131, PCR amplification of the pabB, trpA, chuA, yjaA genes [23] and DNA fragment of TSPE4.C2 were carried out as described before [24]. The products were sequenced from both directions and analysed.

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was determined by automated broth microdilution method (Vitek2) (Vitek AMS; BioMérieux Vitek Systems Inc., Durham, NC, USA) and the results were analysed according to the Clinical and Laboratory Standards Institute, CLSI (2012) guidelines [25]. The antibiotics tested in this study were: Amikacin, amoxicillin/clavulanic acid, amp/sulbactam, ampicillin, cefazolin, cefepime, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cefoxitin, cefpodoxime, cephalothin, ceftriazone, ciprofloxacin, gentamicin, imipenem, meropenem, levofloxacin, nitrofurantoin, norfloxacin, tetracycline, tobramycin, trimethoprim/sulfamethoxazole, piperacillin/tazobactam, piperillin and tigecycline.

ESBL production was confirmed by vitek2 analyzer and disk diffusion. Minimum inhibitory concentration (MICs) of quinolones, fluoro-quinolones and β-lactams including carbapenems were determined using the E-test method (CLSI 2012) [25]. 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 ampC β-lactamase by using multiplex PCR [8,26].

Double-disc synergy method
ESBLs were detected as previously described [27] using the disc approximation and double-disc synergy methods and confirmed with cefotaxime and ceftazidime E-test ESBL strips (AB Biodisk, Biomerieux-diagnostics, Durham, NC, USA). For the disc approximation test, clavulanate
diffusion from an amoxicillin–clavulanate (AMC30) disc was used to test for synergy with ceftaxime, cef-tazidime, cefuroxime, cefepime and cefixime (Oxoid) as described previously [28]. 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 was visible between the β-lactam and β-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 β-lactamase enzyme displays a catalytic activity toward ceftazidime.

Modified Hodge test
The test inoculum (0.5 McFarland turbidity) was spread onto Mueller-Hinton agar plates and disks containing 30 μg ceftazidime (with and without 10 μg clavulanic acid) and 10 μg imipenem (with and without 750 μg EDTA) were placed on the surface of the media. The plates were incubated at 37°C overnight. P. aeruginosa NCTC 10662, E. coli NCTC 10418, and S. aureus NCTC 6571 were used as controls on every plate.

Identification of resistance genes
The presence of resistant genes listed below was investigated by PCR assays. PCR was conducted in a GeneAmp 9700 (Perkin-Elmer, Waltham Massachusetts, USA) system using the conditions specified for each primer; corresponding to the source references. blaTEM-1& blashV5, blaCTX-M-like [9], blaNDM [13], blaOXA-1 [3]. qnrA and qnrS [29], qnrB [30], aac(6’)-Ib ib-cr [31], gyrA & parC [32], gyrB & parE [33]; int11 [34] & int12 [35], blavIMβ, blaIMβ, blaOXA-48 [19], ampC [8].

Amplified PCR products were purified with Qiagen purification kit (Qiagen Valencia, CA, USA) according to the manufacturer’s instructions and both strands were sequenced by automated ABI3100 DNA sequencer (Applied Biosystems, Carlsbad, CA, USA) system. 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 acid sequences.

Pulsed-field gel electrophoresis
Pulsed-Field Gel Electrophoresis (PFGE) analysis was based on techniques described elsewhere [37]. After PFGE, the gels were stained with ethidium bromide and scanned. The analysis of the gels was performed using BioNumerics software version 7.1 (Applied Maths, Ghent, Belgium). This software facilitates the development of the algorithms necessary for the comparison of profiles of isolates based on the Dice coefficient and the hierarchic unweighted pair arithmetic average algorithm. Cluster analysis and phylogenetic trees were subsequently analysed with an optimization of 1.0% and a tolerance of 0.7%. Isolates were considered to belong to the same PFGE clone if their Dice similarity index was ≥85%.

Plasmid analysis
Plasmids were extracted (Promega, Fitchburg, WI, USA) and characterized by PCR as described previously [38]. Plasmids from clinical isolates were detected using PFGE. A single block was incubated at 55°C for 1 hour with 1 unit of S1 nuclease (New England Biolabs, Ipswich, MA, USA) in Zinc Buffer (200 μl of 50 mM NaCl, 30 mM sodium acetate and 5 mM ZnSO4). Electrophoresis was performed at 6 V, 5-50s for 20 h [39].

Resistance transfer assays
Mating experiments were performed with E. coli J62-2 (RifR) as the recipient strain. Cultures of the donor (KOC-10 harbouring blacTX-M-56, qnrB1 and blacMY-2 genes) and the recipient strain were grown in Luria-

Table 1: The distribution of 832 isolates by hospital, year, and sample from which the isolates were originally recovered

| Hospital     | Total | Year   | Urine | Blood | Wound | Gastric Fluid | Catheter tip | Ascitic Fluid | Eye | Pleural Fluid | Sputum | Tissue | Pus |
|--------------|-------|--------|-------|-------|-------|---------------|--------------|--------------|-----|---------------|--------|--------|-----|
| Amiri (ADA)  | 240   | 2010   |       |       |       | 2             | 1            | 1            | 1   | 1             |        |        |     |
|              |       | 2011   | 49    | 1     | 2     |               |              |              |     |               |        |        |     |
|              |       | 2012   | 177   | 5     | 1     |               |              |              |     |               |        |        |     |
| Ahmadi (KOC) | 236   | 2010   | 87    | 7     | 13    | 2             | 8            | 7            | 3   |               |        |        |     |
|              |       | 2011   | 48    | 1     |       |               |              |              |     |               |        |        |     |
|              |       | 2012   | 51    | 2     | 1     |               |              |              |     |               |        |        |     |
| Yiaco-Adan (Y)| 356  | 2010   |       |       |       |               |              |              |     |               |        |        |     |
|              |       | 2011   |       |       |       |               |              |              |     |               |        |        |     |
|              |       | 2012   | 305   | 13    | 24    | 1             | 2            | 1            | 2   |               |        |        | 8   |

Dashti et al. BMC Microbiology 2014, 14:214
http://www.biomedcentral.com/1471-2180/14/214
Table 2 The distribution of 83 isolates by hospital, year, and sample from which the isolates were originally recovered

| Hospital         | Total no. | Year | Urine | Blood | Wound | Pus | Catheter tip | Ascetic Fluid | Eye | Pleural Fluid | Sputum |
|------------------|-----------|------|-------|-------|-------|----|--------------|---------------|-----|---------------|--------|
| Amiri (ADA)      | 9         | 2010 |       |       |       |    |              |                |     |               |        |
|                  |           | 2011 |       |       |       |    |              |                |     |               |        |
|                  |           | 2012 |       |       |       |    |              |                |     |               |        |
| Ahamdi (KOC)     | 57        | 2010 | 38    | 5     | 2     | 2  | 2            | 1              |     |               |        |
|                  |           | 2011 | 3     |       |       |    |              |                |     |               |        |
|                  |           | 2012 | 3     |       |       |    |              |                |     |               |        |
| Yiaco-Adan (Y)   | 17        | 2010 |       |       |       |    |              |                |     |               |        |
|                  |           | 2011 |       |       |       |    |              |                |     |               |        |
|                  |           | 2012 | 13    | 2     |       |    |              |                |     |               |        |

Table 2 shows the distribution of the 83 isolates of E. coli O25b-ST131 by hospital, year, and sample from which the isolates were originally recovered. The distribution of these 83 isolates did not contain any of the β-lactamase enzymes while the majority (95.2%) harboured at least one β-lactamase resistance gene. Two isolates harboured blaCTX-M-15 and blacTX-M-56, blaNDM, blalMD, and blacIM genes were not found. ISEcp1 was detected upstream region of 25 (33%) of the blaCTX-M-15 positive isolates. blaCMY-2 was only detected in four isolates (4.8%). IS elements were detected in 2 blaCMY-2 positive isolates, 1 contained class 1 integrons and 1 class II integrons.

Class 1 integrons were identified in 30 (36.1%) isolates and only 5 (6%) contained class II integrons. None of the isolates contained both classes of integrons.

Quinolone resistance determinants

All but two isolates were resistant or had intermediate resistance to ciprofloxacin (MIC > 2 mg/l). Two sensitive isolates did not contain aac(6’)-Ib B1-ccr (isolates Y-116 and Y-159). We did not detect qnrA gene in any of the isolates tested. Three isolates harboured qnrB1 and 4 harboured qnrS1. qnrB1 and qnrS1 coexisted in only 2 isolates (Table 4).

Fifty six (67.5%) isolates carried aac(6’)-Ib B1-ccr. Among the aac(6’)-Ib B1-ccr negative strains (27/83) 32.3%, 1 isolate carried qnrB1 and blacTX-M-56 (KOC-10) and 1 isolate carried qnrS1 (ADA-234). The remaining 78% (7/9) qnrB positive isolates also contained aac(6’)-Ib B1.

Table 4 The profile of quinolone resistant E. coli O25b-B2-ST131 isolates

| Profiles of the antibiotic resistance genes | No. of isolates |
|--------------------------------------------|----------------|
| blaCTX-M-56, blagHv-2, qnrB1               | 1              |
| blaCTX-M-15, aac(6’)-Ib-ccr, blatem-1, qnrB1 | 1              |
| blaCTX-M-15, aac(6’)-Ib-ccr, blagOXA-1, blatem-1, qnrB1, ISecp1 | 1 |
| blaCTX-M-15, aac(6’)-Ib-ccr, blagOXA-1, blatem-1, qnrS1, ISecp1 | 1 |
| blaCTX-M-15, aac(6’)-Ib-ccr, blagOXA-1, qnrB1, qnrS1 | 2 |
| blaCTX-M-15, aac(6’)-Ib-ccr, blagOXA-1, qnrS1, ISecp1 | 2 |
| blaCTX-M-15, qnrS1, blagOXA-1, ISecp1 | 1 |
| Total | 9 |

Berani (LB) broth (10⁹ cfu/ml) and mixed in the ratio of 1:4 and incubated for 5 hours at 37°C. Transconjugates (0.1 ml) were selected on LB agar plates containing rifampicin (150 mg/L) and cefotaxime (2 mg/L). The transconjugates were tested for antibiotic resistance followed by PCR of the resistance determinants.

Result

Bacterial isolates and the detection of O25b-ST131

All three hospitals participated during our study period; however, there were inconsistencies in the level of strain contribution for each year. Therefore, under-representation of E. coli multi-drug resistant isolates might exist. We tested a subset of 832 E. coli MDR (Table 1). Of which 83 (10%) were identified as the O25b-sequence type (ST) 131 clone of B2 phylogenic group. The principal source of isolation (81%) was urine; mainly from patients older than 60 years of age, these comprised 49% of all the urine specimens. The distribution of these 83 isolates and the source of isolation are presented in Table 2. (Also see Additional file 1).

PCR amplification and sequencing

Table 3 shows the distribution of the bla genes among the 83 isolates of E. coli O25b-ST131. Four (4.8%) did not contain any of the β-lactamase enzymes while the majority (95.2%) harboured at least one β-lactamase resistance gene. Two isolates harboured blactX-M-15 and blactX-M-56, blanan, blalMD, and blamic genes were not found. ISecp1 was detected upstream region of 25 (33%) of the blactX-M-15 positive isolates. blacMY-2 was only detected in four isolates (4.8%). IS elements were detected in 2 blacMY-2 positive isolates, 1 contained class 1 integrons and 1 class II integrons.

Class 1 integrons were identified in 30 (36.1%) isolates and only 5 (6%) contained class II integrons. None of the isolates contained both classes of integrons.

Table 3 Molecular characterization of bla genes among E. coli O25b-B2-ST131 in Kuwait

| Profiles of the antibiotic resistance genes | No. of isolates (%) |
|--------------------------------------------|-------------------|
| blatem-1                                   | (24.4%)           |
| blashv-12                                  | (1.2%)            |
| blatX-M-2                                  | (1.2%)            |
| blatX-M-15                                 | (38.6%)           |
| blatem-1, blashv-12                        | (1.2%)            |
| blatX-M-15, blashv-12                      | (10.8%)           |
| blatX-M-15, blankatem-1                    | (25.3%)           |
| blatX-M-15, blankatem-1, blashv-12          | (14.5%)           |
Ib-cr (Figure 1). None of the qnr positive isolates carried \textit{bla}\textsubscript{SHV}.

No mutations were detected in the quinolone-resistance-determining regions of gyrA. However, there was a new mutation in isolate D-140 topoisomerase subunit IV at position 520 G to C that altered 174 Val (GTC) to Leu (CTC) possibly not leading to any additional chromosome encoded fluoroquinolone resistance. We also observed mutations in isolate Y-190 in topoisomerase subunit IV; the amino acid 560 A → V and at position 840 V → A.

**PFGE**

PFGE showed diverse genetic profiles (Figure 2). The isolates that harboured \textit{qnr} genes; although resemble similar phenotypes; some displayed unrelated PFGE profiles suggesting that they were not epidemic cases (Figure 1). The genotyping results of the 5 isolates that contained class II integrons suggested that only two of these isolates have identical PF patterns and harboured similar antibiotic resistant profiles whereas the other three isolates were not closely related and contained different resistance genes including one isolate which contained the AmpC gene \textit{bla}\textsubscript{CMY-2}. All 5 harboured \textit{bla}\textsubscript{CTX-M-15} (Figure 3).

**Antimicrobial susceptibility**

We identified 3 (3.6%) of the \textit{E. coli} O131 isolates did not contain β-lactam resistance genes which reflect the infection caused by cephalosporin-susceptible clones (KOC-3, KOC-47 and Y-136). These isolates were collected from two different hospitals, all from urine specimens and were not related by PFGE to each other but were closely related to other isolates that contained \textit{bla}\textsubscript{CTX-M-15} (Figure 2).

**Plasmid analysis**

IncFII plasmid that also contains β-lactamase gene \textit{bla}\textsubscript{OXA-1} that encodes for OXA-1 and the aminoglycoside/fluoroquinolone acetyl transferase \textit{aac(6')-Ib-cr} was present in 58 (70%) of isolates of which 33 (40%) contained both genes. The isolate (KOC10) harbouring \textit{bla}\textsubscript{CTX-M-56} gene also contained \textit{qnrB1} and \textit{bla}\textsubscript{CMY-2} genes and carried IncF1 plasmids of about 97 kb and 160 kb (Figure 4). Number of transconjugates in 1 ml for KOC10 was on average 40 to 6 × 10^2 which comprised of 4 × 10^8 to 6 × 10^7 transconjugants per donor cell. PCR revealed that only one of the transconjugates contained \textit{qnrB1} and \textit{bla}\textsubscript{CMY-2} genes and one contained \textit{qnrB1} and \textit{bla}\textsubscript{CTX-M-56}.

**Discussion**

This manuscript reports the trend of \textit{E. coli} O25b-ST131 isolated non-selectively in hospitals. During our two year study 10% of MDR \textit{E. coli} isolated belonged to the \textit{E. coli} O25b-ST131 clonal group indicating that the Middle East has joined the countries affected by this virulent pathogen posing a major public health concern. MDR \textit{E. coli} O25b-ST131 isolates were isolated from different age groups of patients (3-94 years old; with the average age of 54.4 years old).

The majority of isolates (38.6%) harboured only \textit{bla}\textsubscript{CTX-M-15} and 10.8% also contained \textit{bla}\textsubscript{TEM} and or \textit{bla}SHV. Among ESBL producers; we detected the presence of \textit{bla}\textsubscript{CTX-M-56} for the first time in the Middle East and outside the South American continent [40]. The patient from which the isolate was recovered had an international travel history to an endemic region. Also we detected \textit{bla}\textsubscript{CTX-M-2}, one of the dominant Asian β-lactamases [41] for the first time in the Middle East. \textit{bla}\textsubscript{CTX-M-56} gene is in the same context as \textit{bla}\textsubscript{CTX-M-2} by a single nucleotide mutation (G824A), resulting in a replacement of serine by asparagine at position 275 [42]. Previously no explanation was given as to what this change means, however we propose that based on other class A β-lactamases [43,44], as this modification takes place at the C terminal of the α-11 helix it is involved in the resistance to inactivation by β-lactamase inhibitors. The isolate harbouring \textit{bla}\textsubscript{CTX-M-56} also contained \textit{qnrB1} and \textit{bla}\textsubscript{CMY-2} genes and carried IncF1 plasmids of about 97 kb and 160 kb. Production of plasmid AmpC such as \textit{cmy} genes confers resistance to all penicillins, most

![Figure 1 PFGE profiles of E. coli O25b-B2-ST131 isolates collected in this study harbouring qnr genes.](image-url)

*Figure 1* PFGE profiles of \textit{E. coli} O25b-B2-ST131 isolates collected in this study harbouring qnr genes. The degree of similarity is shown on the scale at the top left of the figure. *Isolate no. Specimen Age Gender.*
Dashti et al. BMC Microbiology 2014, 14:214
http://www.biomedcentral.com/1471-2180/14/214

We also detected the presence of qnr genes in eight other blaCTX-M-15 harbouring isolates. Although Qnr enzyme by itself produces low-level resistance to quinolones, its presence facilitates the selection of higher-level cephalosporins and currently available β-lactamase inhibitors. Therefore the emergence of a clinical isolate that contains blaCMY-2 as well as blaCTX-M-56 poses a risk to combination β-lactam/ β-lactamase inhibitor therapy.
resistance, thus contributing to the alarming increase in resistance to quinolones.

ISEcp1-blaCTX-M-15 element was located in the upstream region of 33% of isolates harbouring blaCTX-M-15.

Twenty seven per cent of which were associated with blaSHV, blaTEM as well as blaCTX-M-15. ISEcp1 plays a role in gene transfer or in providing a promoter for β-lactamase genes and supports their dissemination [45]. IncFII plasmid that also harboured blaOXA-1 and the aminoglycoside/fluoroquinolone acetyl transferase aac(6')-Ib-cr gene (aac(6')-Ib-Ib-cr) was present in 59 (71%) of isolates of which 33 (40%) contained both genes. Two isolates containing blaOXA-48 contained ISEcp1 and class 1 integrons. It has been reported [46] that a novel Tn1999 transposon inserted into a single 62-kb IncL/M-type plasmid is responsible for the dissemination of blaOXA-48 gene in E. coli strains.

The rate of carriage of MDR E. coli O25b-ST131 is estimated at 7% in healthy adults [47]; however the rate of E. coli O25b-ST131 susceptible to extended-spectrum cephalosporins has never been reported. We identified 3.6% of the E. coli O131 isolates did not contain any of the related resistance genes which reflect the infection caused by cephalosporin-susceptible clones.

**Conclusion**

We did not find any association between resistance profiles and genotypes. However; we detected for the first time the appearance blaCTX-M-2 in the Middle East and blaCTX-M-56 outside Latin America. We also identified the spread of qnrB1 and qnrS1 in isolates harbouring aac(6')-Ib-Ib-cr and blaCTX-M. The isolate harbouring blaCTX-M-56 also contained qnrB1 and blaCMY-2 genes and carried IncF1 plasmids. In conclusion the appearance of a highly virulent E. coli O25b-ST131 that is resistant to penicillins, most cephalosporins, β-lactamase inhibitors as well as flooroquinolones is a cause for concern and poses a risk to combination β-lactam/β-lactamase inhibitor therapy.

**Additional file**

*Additional file 1: Table S1.* Specimen types and Demographics of E. coli O25b-B2-ST131 isolates. Samples from pus, skin and wound have been illustrated under soft tissue.

**Competing interests**

The authors declare that they have no competing interests.
34. Mazel D, Dychinco B, Webb VA, Davies J: Antibiotic resistance in the ECOR collection: Integrons and identification of a novel aad gene. *Antimicrob Agents Chemother* 2000, 44:1568–1574.

35. Sánez Y, Briñas L, Domínguez E, Zarazaga M, Vila J, Torres C: Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. *Antimicrob Agents Chemother* 2004, 48:3996–4001.

36. Kiratson P, Apisarnthanarak A, Saifon P, Laesripa C, Kitphati R, Mundy LM: The emergence of a novel ceftazidime-resistant CTX-M extended-spectrum beta-lactamase, CTX-M-55, in both community-onset and hospital-acquired infections in Thailand. *Diagn Microbiol Infect Dis* 2007, 58:349–355.

37. Ribot FM, Fair NA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ: Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157, Salmonella and Shigella for pulsenet. *Foodborne Pathog Dis* 2006, 3:59–67.

38. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KI, Threlfall EJ: Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005, 63:219–228.

39. Mshana SE, Imirzalioglu C, Hossain H, Hain T, Domann E, Chakraborty T: Conjugative IncFI plasmids carrying CTX-M-15 among *Escherichia coli* ESBL producing isolates at a University hospital in Germany. *BMC Infect Dis* 2009, 9:97. doi:10.1186/1471-2334-9-97.

40. Khan MA, Lemmens N, Riera E, Blonk T, Goedhart J, Van Belkum A, Goessens W, Hays JP, Van Westreenen M: Dominance of CTX-M-2 and CTX-M-56 among extended-spectrum beta-lactamases produced by *Klebsiella pneumoniae* and *Escherichia coli* isolated in hospitals in Paraguay. *J Antimicrob Chemother* 2009, 64:1330–1332.

41. Suzuki S, Shibata N, Yamane K, Wachino J, Ito K, Arakawa Y: Change in the prevalence of extended-spectrum-beta-lactamase-producing *Escherichia coli* in Japan by clonal spread. *J Antimicrob Chemother* 2009, 63:72–79.

42. Pallecchi L, Bartoloni A, Fiorelli C, Mantella A, Di Maggio T, Gamboa H, Gotuzzo E, Kronvall G, Paradisi F, Rossolini GM: Rapid dissemination and diversity of CTX-M Extended-Spectrum beta-Lactamase genes in commensal *Escherichia coli* isolates from healthy children from low-resource settings in Latin America. *Antimicrob Agents Chemother* 2007, 51:2720–2725.

43. Chaib EB, Sirot D, Paul G, Labia R: Inhibitor-resistant TEM beta-lactamases: phenotypic, genetic and biochemical characteristics. *J Antimicrob Chemother* 1999, 43:447–458.

44. Du bos SK, Marriott MS, Ayres SG: TEM- and SHV-derived extended-spectrum beta-lactamases: relationship between selection, structure and function. *J Antimicrob Chemother* 1995, 35:7–22.

45. Poirel L, Decousset JW, Nordmann P: Insertion sequence ISEcp1B is involved in expression and mobilization of a blaCTX-M betalactamase gene. *Antimicrob Agents Chemother* 2003, 47:2958–2945.

46. Potron A, Nordmann P, Rondinaud E, Jauregui F, Poirel L: A mosaic transposon encoding OXA-48 and CTX-M-15: towards pan-resistance. *J Antimicrob Chemother* 2013, 68:476–477.

47. Woodford N, Carattoli A, Kariuki E, Underwood A, Ellington MJ, Livermore DM: Complete nucleotide sequences of plasmids pEK204, pEK499, and pEK516, encoding CTX-M Enzymes in Three Major *Escherichia coli* Lineages from the United Kingdom, All Belonging to the International O25:H4-ST131 Clone. *Antimicrob Agents Chemother* 2009, 53:4472–4482.

doi:10.1186/s12866-014-0214-6
Cite this article as: Dashti et al.: The characterization and antibiotic resistance profiles of clinical *Escherichia coli* O25b-B2-ST131 isolates in Kuwait. *BMC Microbiology* 2014 14:214.