Molecular characterization of plasmid-mediated quinolone resistance (PMQR) and ESBLs-producing Klebsiella pneumonia isolated from an Iranian teaching hospital

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Research

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Abstract

Background

The spread of plasmid-mediated multidrug resistance in *Klebsiella pneumonia* is a serious threat to the public health. We investigated the clinical characteristics and molecular epidemiology of *K. pneumoniae* isolated at a teaching hospital in Iran.

Methods

A total of 50 third-generation cephalosporins resistant *K. pneumoniae* strains were collected from patients’ clinical cultures. Antibiotic susceptibility testing and determination of MIC values for ceftazidime, cefotaxime and ciprofloxacin were performed. PCR and DNA sequencing were used to assess the presence of ESBL genes (*bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>) and PMQR genes (*qnrA, qnrB, qnrS, qepA, oqxA, oqxB and aac(6)-lb-cr*). Multilocus sequence typing (MLST) was performed on the strains to assess homology.

Results

Our results showed that the rates of resistance to all of antibiotics is high. All 50 *K. pneumoniae* strains harboured at least one of the ESBL resistance determinants. The *bla*<sub>CTX-M-15</sub> gene was the major ESBLs determinant found in *K. pneumoniae* (88%; 44/50). PMQR was detected in 96% of the isolates and *aac(6′)-lb-cr* was the most common (78% 39/50) followed by *oqx A* 36 (72%), *oqxB 34 (68%), qnrS 20 (40%), *qnrB 14 (28%) and *qepA 1 (2%)*. MLST identified seven sequence types (STs), with the most common being ST11 (19/39). There was a strong association between PMQR genes (especially *aac(6′)-lb-cr*) and ESBL genes.

Conclusion

The widespread detection of ESBLs-producing *K. pneumoniae* that co-carried PMQR determinants has become a threat to the treatment of infections in Isfahan Province of center Iran. Our findings suggest that *K. pneumoniae* ST11 and ST893 has a clonal distribution in our hospital. Therefore, this study highlighted the crucial need for implementing strict control measures to prevent cross transmission of these endemic clones.

Introduction

*Klebsiella pneumoniae* is a common pathogen causing nosocomial and community-acquired infections that is responsible for various infections such as pneumonia, septicemia, liver abscess, meningitis, urinary tract infections, and bacteremia, whose mortality rates are high [1]. Most of such infections are caused by multidrug-resistant (MDR) strains that interrupt the treatment processes. MDR *K. pneumoniae* acquires various resistance mechanisms that confer antibiotic resistance to commonly used antibiotics [2]. One of the important resistance mechanisms of *Enterobacterales*, including *K. pneumoniae*, is the production of extended-spectrum beta-lactamases (ESBLs), and plasmid-mediated quinolone resistance (PMQR) genes that have been detected in clinical isolates. ESBLs are plasmid-mediated enzymes that hydrolyze oxyimino-β-lactam agents such penicillins, cephalosporins, and monobactams [3, 4]. ESBL genes are classified in several types including *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>TEM</sub>-CTX-M-15-type ESBL-producing strains have especially increased in recent years [5–7]. Presence of PMQR determinants
including \( qnr \) genes, \( aac \text{ (6')}\text{-Ib-cr} \), and efflux pumps genes \( qepA, qepA2 \), and \( oqxAB \) confer reduced susceptibility to fluoroquinolones and facilitate selection of fluoroquinolone resistance in \( K. pneumoniae \) [4, 8].

In the last years, studies show that an increase in the prevalence of hospital-acquired ESBL- \( K. pneumoniae \) infections in our area [7, 9, 10]. Most of these clinical isolates harbored \( bla_{CTX-M-15} \) gene. Previous reports have identified an association between ESBL-encoding genes and genes encoding PMQR, as they can sometimes be found on the same plasmids or mobile genetic elements [3, 11]. The aim of this study was to investigate the presence of PMQR in ESBL-producing \( K. pneumoniae \) isolated from an Iranian teaching hospital. The secondary aim was to determine the genetic relatedness between these isolates by molecular typing.

**Materials And Methods**

**Identification of strains**

The 50 independently isolates of third-generation cephalosporins resistant \( K. pneumoniae \) were collected from a local general hospital. This hospital has more than 220 beds and includes a medical education center in Isfahan, Iran. The isolates were identified by use of API 20E (bioMe’rieux, Marcy-l’E’toile, France) and 5 biochemical tests which include Triple Sugar Iron test, Mannitol motility test, Simmons citrate agar test, Indole test, Urease test.

**Antimicrobial susceptibility testing and determination of MIC values**

The antimicrobial susceptibility testing for different antibiotic agents, cefotaxime (30 \( \mu g \)), ceftazidime (30 \( \mu g \)), ceftriaxon (30 \( \mu g \)), cefepime (30 \( \mu g \)), ciprofloxacin (5 \( \mu g \)), levofloxacin (5 \( \mu g \)), amikacin (30 \( \mu g \)), gentamicin (10 \( \mu g \)) (BD Diagnostics, Franklin Lakes, NJ, USA) was done by the Kirby-Bauer standard disk diffusion method. The MIC values (mg/L) of ceftazidime, cefotaxime and ciprofloxacin were determined using Etest strips (AB Biodisk, Solna, Sweden). All the values were interpreted according to CLSI guideline [12]. The reference strain \( E. coli \) ATCC 25922 was used as a control.

**Molecular detection of ESBL and PMQR genes**

Genomic DNA was extracted using the DNA genomic extraction kit (Thermo scientific, Lithuania). All isolates were screened for the presence of the of ESBL genes including \( bla_{CTX-M}, bla_{TEM}, bla_{SHV} \) and PMQR genes including \( qnrA, qnrB, qnrS, qepA, oqxA, oqxB and aac(6)-Ib-cr \), by PCR and DNA sequencing [13-15].

**Multilocus Sequence Typing (MLST)**

Genetic relatedness of the isolates was investigated by multilocus sequence typing. MLST was conducted according to previously published methods using primers of seven housekeeping genes listed in the PubMLST website (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Alleles and sequence types were assigned by using the MLST database.

**Results**

**Clinical characteristics of \( K. pneumoniae \) isolates**

A total of 50 non-reduplicate \( K. pneumoniae \) were collected from September 1st, 2019 to February 30th, 2020. Analyzing of 50 \( K. pneumonia \) isolates showed a prevalence of 27 (54%) and 23 (46%) for males and females, respectively. The mean age of participants was 52 years. Most of the strains (n = 23) were detected from tracheal
samples followed by urine (n=14), wound (n=5), CSF (n=3), blood (n=2), access (n=1), sputum (n=1), catheter (n=1) samples (Tables 1).

**Antimicrobial susceptibility testing**

All experimental strains were classified as MDR (Tables 1 and 2). All strains were resistant to cefotaxim, ceftazidim and ceftriaxone. Resistance was 98% for cefepim, 88% for gentamicin and 50% for amikacin. The resistance rates to fluoroquinolones were 88% and 78% for ciprofloxacin and *levofloxacin*, respectively. The range of MIC for ciprofloxacin was 0.064 mg/L to > 32 mg/L. Whereas, MIC against ceftazidime and cefotaxime in all isolates was > 32 mg/L.

**Detection of drug-resistant genes**

The ESBL genes were analyzed in the 50 *K. pneumonia* isolates as listed in Table 1. All of isolates harbored at least one of the ESBL genes tested in the present study. Out of the 50 strains encoding ESBL genes, 88% (44/50) carried *bla*$_{CTX-M-15}$, 78% (39/50) *bla*$_{TEM}$ and 86% (43/50) *bla*$_{SHV}$ genes. Overall 96% (48/50) isolates were confirmed to carry at least one of the PMQRs. The most common PMQR gene was *aac*(6$'$)-Ib-cr (39 (78%) followed by *oqxA* 36 (72%), *oqxB* 34 (68%), *qnrS* 20 (40%), *qnrB* 14 (28%) and *qepA* 1 (2%). None of the isolates carried *qnrA* gene (Table 2).

**Molecular characteristics of *K. pneumonia* isolates**

In our study, MLST analysis was performed for 39 isolates. Seven sequence types (STs) were identified among the 39 isolates, with nineteen (48.7%) isolates of ST11, thirteen (33.3%) isolates of ST893, two (5.1%) isolates of ST147, two (5.1%) isolates of ST16 and one (2.5%) isolate each of ST377, ST13, and ST392.

**Discussion**

Our study demonstrated that quinolone agents were not effective against ESBL-producing *K. pneumoniae* isolated inpatients in Iran. ESBL-producing *K. pneumoniae* showed high resistant rates not only to cephalosporins but also ciprofloxacin (88%) and *levofloxacin* (78%). Amikacin was indicated to be effective for about 50% of the strains in our study. In Indonesia and China, ESBL-positive *K. pneumoniae* strains were > 90% and > 70% susceptible to amikacin, respectively [16, 17]. We also found that, among the resistant strains *bla*$_{CTX-M-15}$ were the predominant ESBL gene. Therefore, we demonstrated that *bla*$_{CTX-M-15}$, which is a widespread public health problem around the world, was the most common ESBL gene in ESBL-producing *K. pneumoniae* in Iran. The finding is in agreement with recent studies in Iran and other parts of the world [11, 17, 18]. In accordance to other studies [7, 11], our results also showed that the prevalence rates of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes were high.

In the present study, a significant number of isolates (96%) carried at least one of the PMQR genes. The *aac*(6$'$)-Ib-cr gene was the most prevalent PMQR gene, in agreement with other studies[9, 19, 20]. It is suggested that *aac*(6$'$)-Ib-cr has epidemiologically strong associations with CTX-M-15 [21]. Among the *qnr* genes, *qnrS* 20 (40%) was the most predominant, followed by *qnrB* 14 (28%), the occurrence of *qnr* alleles with *aac*(6$'$)-Ib-cr gene was in accordance with previous studies [9, 20]. Analysis of the data also revealed that the prevalence of *oqxA* was highest (72%) followed by *oqxB* (68%). Frequencies of *oqxA* and *B* genes found in this study are higher than those reported by Azargun in Tabriz, Iran in 2018 which were 33.7 for *oqxA* and 20.6% for *oqxB* [9]. Previous studies have found that the prevalence of PMQR genes is more common in ESBL-producing *K. pneumoniae* [4, 9, 20]. We found that PMQR genes (*aac*(6$'$)-Ib-cr, *qnrS*, *qnrB*) were also be detected in strains containing ESBL determinants. Among the isolates
containing ESBL genes, 96% were producers of PMQR. It is to be noted that ESBLs are highly prevalent in the study isolates and could have contributed to the spread of PMQRs.

In the present study MLST was used for homology analysis. ST11 international high-risk clone, the most prevalent sequence type in this collection, has been described in outbreaks of ESBL-producing K. pneumoniae in some countries such as Iran, China, Sweden and detected in a OXA-48-producing isolates from Iran and Spain [2, 11, 22, 23]. In our study, ST11 co-carried multiple resistance determinants such as blaCTX-M-15, blaTEM, blaSHV and PMQR genes including qnrB, qnrS, oqxA, oqxB and aac(6)-lb-cr, that correlates well with earlier reports as the dominant global ESBLs are the CTX-M type beta-lactamases in K. pneumoniae [2, 22]. The presence of quinolone resistance genes aac(6)lb-cr and qnrB was recently reported in ST11 K. pneumoniae strains from Colombia [24]. The second most common endemic sequence type K. pneumoniae in our study was ST893 which co-harbored both ESBLs and PMQR genes. In recent years, the emergence of ST893 have been reported from several Iranian hospitals [11, 25] which are strongly associated with the carriage of blaNDM, blaOXA-48 and ESBLs genes. Our results suggest that, ST893 is most likely endemic in Iran. ST11 and ST893 were mainly concentrated in the ICU ward, which suggests these strains may have originated in this ward and then spread to other wards in our hospital. These results suggest more attention is required in the ICU ward to avoid dissemination outbreaks of infection.

The two K. pneumoniae isolates in our study belonged to ST16 which is one of the two isolates were positive for blaCTX-M-15, blaSHV, blaTEM, aac(6)lb-cr and qnrS genes, whereas other isolates carried blaCTX-M-15, blaSHV and blaTEM. ST16 has been reported worldwide, showing multiple resistance determinant profiles. ST16 has been identified as a carbapenemase producer in many parts of the world and reported as an ESBL producer in Iran, Denmark and Sweden [6, 11].

Another detected ST, ST147 which is an internationally successful clone, has been reported from different parts of Iran [11, 26]. In our study, ST147 (2 isolates) has been associated with blaCTX-M-15, blaSHV, blaTEM, aac(6)lb-cr and oqxB. ST147 has been described in India, Greece, and Italy [27] and has been associated with blaVIM, blaNDM-1, blaCTX-M-15, aac(6)lb-cr with qnrB and armA in that country.

One isolate belonging to ST13 co-carried multiple antibiotic resistance genes such as blaCTX-M-15, blaSHV, blaTEM and aac(6)lb-cr. ST13 is a SLV of ST327, that has been reported to harbour blaNDM-1, blaCTX-M-15 and blaSHV in Iran [28]. The ST392, identified in this study in one patient, ST392 was sporadically observed in different countries related to NDM-1, OXA-48 and ESBLs in Iran[10] and to aac(6)lb-cr, oqxAB, blSHV, blacTX-M-15, blatEM-1 in Tunisia [29]. Finally, one SHV-producing K. pneumoniae in our study belonged to ST377 and were positive for qnrS, aac(6)lb-cr, oqxA and qepA genes. Previously, K. pneumoniae ST377 strain carrying blaoXA-48 and ESBLs was described in Russia and Iran [11, 30]. The complexity and diversity of ESBLs and PMQR combinations detected among K. pneumoniae isolates especially successful international clones ST11 and endemic clone ST893 in this study and their potential for spread poses a real threat to the management of infections by this species in Iran.

In conclusion, this study showed high prevalence of fluoroquinolone-resistance genes in ESBL-producing K. pneumonia strains. Clonal dissemination of ESBLs carrying K. pneumonia that co-harbour PMQR determinants have been observed. We identified the epidemiologically significant international and endemic STs of K. pneumoniae ST11, ST147 and ST893. Therefore, on the one hand, there is an urgent need for epidemiological and molecular studies to understand the dynamics of antibiotic resistance transmission and on the other hand, careful programs need to be implemented to prevent the spread of these strains in healthcare facilities.
Abbreviations

MDR: Multidrug-resistant; ESBLs: Extended-spectrum beta-lactamases; PMQR: Plasmid-mediated quinolone resistance; MLST: Multilocus sequence typing; STs: Sequence types.

Declarations

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None.

Authors’ contributions

HS and HT designed the study and drafted the manuscript. HS performed the experimental work and analyzed the data. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Tables

Table 1: Clinical characteristics, drug resistance genes and antimicrobial susceptibility testing (other than quinolones) of 50 \textit{K. pneumoniae} isolates.
| Number | No  | Isolation site(s) | Resistance genes | Ward       | Antimicrobial susceptibility testing |
|--------|-----|-------------------|------------------|------------|-------------------------------------|
|        |     |                   |                  |            | CTX  | CAZ | CRO | FEP | GM | AM |
| 1      | S15 | Urine             | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | Emergency  | R    | R   | R   | R   | R  | R  |
| 2      | S16 | Urine             | $bla_{\text{CTX-M-15}}$ | ICU        | R    | R   | R   | R   | R  | S  |
| 3      | S19 | Tracheal          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | R   | R  | S  |
| 4      | S20 | Tracheal          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | R   | R  | R  |
| 5      | S22 | Tracheal          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | Internal   | R    | R   | R   | R   | R  | R  |
| 6      | S25 | Tracheal          | $bla_{\text{CTX-M-15}}$ | Surgery    | R    | R   | R   | R   | R  | R  |
| 7      | S32 | Urine             | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}$ | ICU        | R    | R   | R   | R   | R  | S  |
| 8      | S33 | CSF               | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | S   | S  |
| 9      | S35 | Tracheal          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | R   | R  | S  |
| 10     | S36 | Tracheal          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | R   | R  | S  |
| 11     | S38 | Tracheal          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | Infectious | R    | R   | R   | R   | R  | S  |
| 12     | S39 | Tracheal          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | R   | R  |
| 13     | S42 | Tracheal          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | R   | R  |
| 14     | S43 | Urine             | $bla_{\text{CTX-M-15}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | R   | R  |
| 15     | S54 | Catheter          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | R   | R  |
| 16     | S55 | Tracheal          | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | Surgery    | R    | R   | R   | R   | R  |
| 17     | S56 | Access            | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | ICU        | R    | R   | R   | R   | R  |
| 18     | S58 | Wound             | $bla_{\text{CTX-M-15}}, bla_{\text{TEM}}, bla_{\text{SHV}}$ | Emergency  | R    | R   | R   | R   | R  |
| 19     | S61 | Urine             | $bla_{\text{TEM}}$ | Internal   | R    | R   | R   | R   | R  |

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|---|---|---|---|---|---|---|---|---|---|
|   |   |   |   |   |   |   |   |   |   |
| 20 | S68 | Tracheal | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | S |
| 21 | S69 | Tracheal | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | S |
| 22 | S70 | CSF | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | S |
| 23 | S72 | Urine | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | S |
| 24 | S73 | Wound | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | S |
| 25 | S74 | Tracheal | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | S | S |
| 26 | S75 | Blood | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | R |
| 27 | S79 | Tracheal | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | R |
| 28 | S80 | Tracheal | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | R |
| 29 | S81 | Tracheal | **bla**$_{\text{CTX-M-15}}$ | ICU | R | R | R | R | R | S |
| 30 | S83 | Tracheal | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | S | R | S | S |
| 31 | S84 | Urine | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | R |
| 32 | S86 | Urine | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | R |
| 33 | S92 | Tracheal | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | S |
| 34 | S94 | Blood | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | Surgery | R | R | R | R | R | S |
| 35 | S97 | Urine | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$ | Surgery | R | R | R | R | R | R |
| 36 | S98 | Urine | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | Nephrology | R | R | R | R | R | R |
| 37 | S105 | Sputum | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | Internal | R | R | R | R | R | S |
| 38 | S107 | Tracheal | **bla**$_{\text{CTX-M-15}}$, **bla**$_{\text{TEM}}$, **bla**$_{\text{SHV}}$ | ICU | R | R | R | R | R | S |
|   | Sample Type | Location | Genes | Isolation Site | Resistance | Table 2: Antimicrobial susceptibility testing of quinolones and PMQR of 50 K. pneumoniae isolates. |
|---|-------------|----------|-------|----------------|-------------|------------------------------------------------------------------------------------------------|
| 39 | S109 Wound |           | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | Surgery       | R R R R R R S |                                                                                                  |
| 40 | S111 Tracheal |         | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | ICU           | R R R R R R R |                                                                                                  |
| 41 | S112 CSF |          | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | Emergency     | R R R R R S S |                                                                                                  |
| 42 | S115 Tracheal |        | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | Emergency     | R R R R R S S |                                                                                                  |
| 43 | S116 Urine |          | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | ICU           | R R R R R R R |                                                                                                  |
| 44 | S119 Wound |          | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | Surgery       | R R R R R R R |                                                                                                  |
| 45 | S120 Urine |          | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | ICU           | R R R R R R R |                                                                                                  |
| 46 | S122 Tracheal |        | $bla_{SHV}$ | ICU           | R R R R R R S |                                                                                                  |
| 47 | S124 Urine |          | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | Surgery       | R R R R R S S |                                                                                                  |
| 48 | S132 Urine |          | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | Infectious    | R R R R R R R |                                                                                                  |
| 49 | S133 Tracheal |          | $bla_{CTX-M-15}, bla_{TEM}, bla_{SHV}$ | ICU           | R R R R R S S |                                                                                                  |
| 50 | S134 Wound |          | $bla_{SHV}$ | ICU           | R R R R R R S |                                                                                                  |

CSF, Cerebrospinal fluid; CTX, Cefotaxime; CAZ, Ceftazidime; CRO, Ceftriaxon; FEP, Cefepime; GM, Gentamicin; AM, Amikacin; R, Resistance; S, Sensitive
| Number | No | Antimicrobial susceptibility testing | PMQR | qnrA | qnrB | qnrS | aac(6')-Ib-cr | oqxA | oqxB | qepA | MLST |
|--------|----|-------------------------------------|------|------|------|------|---------------|------|------|------|-------|
|        |    |                                     | CIP  | LEV  |      |      |               |      |      |      |       |
| 1      | S15 | R                                   | R    | R    | 1    | -    | -             | -    | +    | -    | 147   |
| 2      | S16 | R                                   | R    | R    | 4    | -    | +             | -    | +    | +    | 893   |
| 3      | S19 | R                                   | R    | R    | 3    | -    | +             | -    | +    | -    | ND    |
| 4      | S20 | R                                   | R    | R    | 4    | -    | -             | +    | +    | +    | 11    |
| 5      | S22 | R                                   | S    | R    | 3    | -    | +             | +    | +    | -    | 11    |
| 6      | S25 | R                                   | R    | R    | 4    | -    | +             | -    | +    | +    | 893   |
| 7      | S32 | R                                   | R    | R    | 2    | -    | -             | -    | +    | -    | ND    |
| 8      | S33 | R                                   | R    | R    | 3    | -    | -             | +    | +    | -    | 893   |
| 9      | S35 | R                                   | R    | R    | 3    | -    | -             | +    | +    | -    | 893   |
| 10     | S36 | R                                   | S    | R    | 2    | -    | +             | -    | -    | -    | ND    |
| 11     | S38 | R                                   | R    | R    | 5    | -    | +             | +    | +    | +    | 893   |
| 12     | S39 | R                                   | R    | R    | 4    | -    | -             | +    | +    | +    | 11    |
| 13     | S42 | I                                   | S    | R    | 2    | -    | +             | +    | -    | -    | ND    |
| 14     | S43 | R                                   | R    | R    | 3    | -    | +             | -    | -    | -    | ND    |
| 15     | S54 | R                                   | R    | R    | 4    | -    | -             | +    | +    | -    | 11    |
| 16     | S55 | R                                   | R    | R    | 4    | -    | -             | +    | +    | -    | 11    |
| 17     | S56 | R                                   | R    | R    | 3    | -    | -             | +    | +    | -    | ND    |
| 18     | S58 | R                                   | R    | R    | 3    | -    | +             | -    | -    | +    | 15    |
| 19     | S61 | R                                   | R    | R    | 4    | -    | -             | +    | +    | +    | 11    |
| 20     | S68 | I                                   | S    | R    | 1    | -    | -             | +    | -    | -    | 11    |
| 21     | S69 | R                                   | S    | R    | 1    | -    | -             | -    | +    | -    | 11    |
| 22     | S70 | R                                   | R    | R    | 4    | -    | -             | +    | +    | -    | 893   |
| 23     | S72 | R                                   | R    | R    | 4    | -    | -             | +    | +    | +    | 893   |
| 24     | S73 | R                                   | R    | R    | 3    | -    | -             | +    | -    | +    | ND    |
| 25     | S74 | R                                   | S    | R    | 1    | -    | -             | -    | +    | -    | 915   |
| 26     | S75 | R                                   | R    | R    | 4    | -    | -             | +    | +    | +    | 11    |
| 27     | S79 | R                                   | R    | R    | 3    | -    | -             | +    | +    | -    | 11    |
| 28     | S80 | R                                   | R    | R    | 3    | -    | -             | +    | +    | -    | 11    |
| 29     | S81 | R                                   | R    | R    | 3    | -    | -             | +    | +    | -    | 893   |
|   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 31 | S83 | R | R | 4 | - | - | + | + | + | + | - | 893 |
| 32 | S84 | R | R | 3 | - | - | - | + | + | + | - | 11 |
| 33 | S86 | R | R | 3 | - | - | - | + | + | + | - | 142 |
| 34 | S92 | I | S | 2 | - | - | - | - | + | + | - | 893 |
| 35 | S94 | R | R | 3 | - | - | - | + | + | + | - | 893 |
| 36 | S97 | R | R | 2 | - | - | + | + | - | - | - | 16 |
| 37 | S98 | I | S | 1 | - | - | + | - | - | - | - | ND |
| 38 | S105 | R | R | 4 | - | + | - | + | + | + | - | 893 |
| 39 | S107 | R | R | 4 | - | + | - | + | + | + | - | 893 |
| 40 | S109 | R | R | 2 | - | - | - | + | + | - | - | ND |
| 41 | S111 | R | R | 3 | - | + | - | + | + | - | - | ND |
| 42 | S112 | R | R | 4 | - | - | + | + | + | + | - | 11 |
| 43 | S115 | R | R | 2 | - | - | - | - | + | + | - | 11 |
| 44 | S116 | R | R | 3 | - | - | - | + | + | + | - | 11 |
| 45 | S119 | R | R | 4 | - | + | - | + | + | + | - | 11 |
| 46 | S120 | R | R | 4 | - | - | + | + | + | - | + | 377 |
| 47 | S122 | R | R | 4 | - | + | - | + | + | + | - | 11 |
| 48 | S124 | S | S | 0 | - | - | - | - | - | - | - | 16 |
| 49 | S132 | R | R | 2 | - | - | - | + | - | + | - | 147 |
| 50 | S133 | R | S | 2 | - | - | - | - | + | + | - | 11 |
| 51 | S134 | S | S | 0 | - | - | - | - | - | - | - | ND |

CIP, Ciprofloxacin; LEV, Levofoxacin; R, Resistance; S, Sensitive; I, intermediate; ND, Not-determined