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# Microbiological Features of KPC-Producing *Enterobacter* Isolates Identified in a U.S. Hospital System

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## Abstract

Microbiological data regarding KPC-producing *Enterobacter* spp. are scarce. In this study, 11 unique KPC-producing *Enterobacter* isolates were identified among 44 ertapenem-non-susceptible *Enterobacter* isolates collected between 2009 and 2013 at a hospital system in Western Pennsylvania. All cases were healthcare-associated and occurred in medically complex patients. While pulsed-field gel electrophoresis (PFGE) showed diverse restriction patterns overall, multilocus sequence typing (MLST) identified *Enterobacter cloacae* isolates with sequence types (STs) 93 and 171 from two hospitals each. The levels of carbapenem minimum inhibitory concentrations were highly variable. All isolates remained susceptible to colistin, tigecycline, and the majority to amikacin and doxycycline. A $bla_{KPC}$-carrying IncN plasmid conferring trimethoprim-sulfamethoxazole resistance was identified in three of the isolates. Spread of $bla_{KPC}$ in *Enterobacter* spp. appears to be due to a combination of plasmid-mediated and clonal processes.

### Keywords

*Klebsiella pneumoniae* carbapenemease; *Enterobacter cloacae*; *Enterobacter aerogenes*

## 1. Introduction

*Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* has become endemic in many hospitals and long-term care facilities in the United States. The rate of



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carbapenem resistance has exceeded 10% among *Klebsiella* isolates causing healthcare-associated infections in U.S. hospitals as of 2010 (Sievert, et al., 2013). Most of this is presumed to be due to production of KPC (Kaiser, Castanheira, Jones, Tenover, & Lynfield, 2013). *Enterobacter* spp., also part of the family *Enterobacteriaceae*, ranks eighth among the most common pathogens causing healthcare-associated infections (Sievert, et al., 2013). While less common than in *Klebsiella* spp., the rate of carbapenem resistance in *Enterobacter* spp. has reached approximately 4% in U.S. hospitals in 2010 (Sievert, et al., 2013). However, microbiological data regarding KPC-producing *Enterobacter* spp. are still limited. Here, we report the microbiological characteristics of KPC-producing *Enterobacter* spp. collected at our hospitals between 2009 and 2013.

## 2. Materials and methods

### 2.1. Enterobacter clinical isolates

*Enterobacter* clinical isolates resistant to ertapenem or meropenem were collected from two clinical microbiology laboratories serving four hospitals in Pittsburgh, Pennsylvania between 2009 and 2013. The isolates were identified as *Enterobacter cloacae* or *Enterobacter aerogenes* using either MicroScan WalkAway (Siemens, Tarrytown, NY) or Vitek2 (bioMérieux, Durham, NC) automated instruments in the clinical microbiology laboratories. Only one isolate was collected per patient. De-identified medical records were provided to the investigators for review by a certified honest broker under approval from the University of Pittsburgh Institutional Review Board (PRO12060302).

### 2.2. Susceptibility testing and identification of antimicrobial resistance genes

The ertapenem-non-susceptible isolates were subjected to PCR for detection of the KPC gene $bla_{KPC}$ (Kim, et al., 2012). The PCR products were sequenced to determine the $bla_{KPC}$ allele. Antimicrobial susceptibility was determined by the broth microdilution method using Sensititre GN2XF (TREK Diagnostics, Cleveland, OH). For carbapenems, the agar dilution method was used to test a wider range of minimum inhibitory concentrations (MICs) (0.06 μg/ml to 256 μg/ml). The assays were conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and their breakpoints (Clinical and Laboratory Standards Institute, 2012a, 2014). Potential ESBL genes $bla_{CTX-M}$, $bla_{SHV}$ and $bla_{TEM}$ were sought by PCR (Kim, et al., 2012). Amplified products were sequenced to determine whether they represented ESBL genes or not. Plasmid-mediated AmpC β-lactamase genes were sought using previously described PCR primers, except for $bla_{ACT/MIR}$ which originates from the *E. cloacae* chromosome (Perez-Perez & Hanson, 2002). Plasmid-mediated fluoroquinolone resistance genes *qnrA*, *qnrB* and *qnrS* were detected by PCR (Tian, et al., 2010).

### 2.3. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)

PFGE was conducted to determine the genomic relatedness of the KPC-producing *Enterobacter* isolates using restriction enzyme XbaI (Kim, et al., 2012). Dendrograms were generated by the weighted pair group method with arithmetic mean using Bionumerics (Austin, TX).

For the 8 *E. cloacae* isolates, the sequence types (STs) were determined by MLST (Miyoshi-Akiyama, Hayakawa, Ohmagari, Shimojima, & Kirikae, 2013). Novel STs were registered through the database (pubmlst.org/ecloacae/).

### 2.4. Transfer of $bla_{KPC}$-encoding plasmids

*E. coli* TOP10 transformants harboring $bla_{KPC}$-carrying plasmids were obtained from each clinical isolate by electroporation as described previously (Kim, et al., 2012). The transformants were selected on lysogenic agar plates containing 50 μg/ml of ampicillin or 0.5 μg/ml of ertapenem. The presence of $bla_{KPC}$ was confirmed by PCR.

### 2.5. Characterization of $bla_{KPC}$-encoding plasmids

The sizes of $bla_{KPC}$-encoding plasmids were estimated from the *E. coli* TOP10 transformants using the S1 nuclease PFGE method (Bueno, Francisco, O'Hara, de Oliveira Garcia, & Doi, 2013). Replicon typing of the plasmids was conducted as described by Carattoli *et al*. (Carattoli, et al., 2005). Susceptibility of the transformants to ertapenem, tetracycline, gentamicin, amikacin, trimethoprim-sulfamethoxazole and nalidixic acid was tested by the standard disk diffusion method (Clinical and Laboratory Standards Institute, 2012b) to confirm reduced susceptibility to ertapenem and identify co-resistance to non-β-lactams conferred by the $bla_{KPC}$-carrying plasmids.

## 3. Results

### 3.1. Identification of KPC-producing Enterobacter spp

A total of 4,687 unique *Enterobacter* isolates were identified at two clinical microbiology laboratories serving four hospitals in Pittsburgh, Pennsylvania between 2009 and 2013. Of them, 127 were non-susceptible to ertapenem or meropenem. Forty-four of them were available for testing in the research laboratory, all of which had been reported as non-susceptible to ertapenem in the clinical microbiology laboratories. Among them, 11 unique KPC-producing *Enterobacter* isolates were identified by PCR. Eight cases were due to *E. cloacae*, and the remaining 3 cases were due to *E. aerogenes*. The clinical features of the 11 cases are summarized in Table 1. All affected patients had substantial comorbidity, and had been in hospital for a median of 24 days (range, 0 to 200) before the first KPC-producing *Enterobacter* spp. isolate was identified. Six patients were deemed to be infected, and the remainder colonized, by the organism. The sources included blood (3), urine (3), post-operative drain (2), sputum (1), bronchoalveolar lavage (1), and cerebrospinal fluid (1). The antimicrobial therapy given was highly variable, both for the empiric and definitive phases. Three patients expired during the hospitalization, three were discharged to another healthcare setting (long-term acute care hospital, skilled nursing facility or hospice) and five were discharged home (Table 1).

### 3.2. Antimicrobial susceptibility

A wide range of carbapenem MICs were observed among the KPC-producing *Enterobacter* isolates (Table 2). Ertapenem MICs ranged between 0.25 and 128 μg/ml, and a similar range was observed for the other carbapenems tested as well. Overall, 7, 3 and 1 isolates were susceptible to doripenem, meropenem, imipenem and ertapenem by the agar dilution

method, respectively. As expected, most isolates were resistant to cephalosporins and β-lactam/β-lactamase inhibitor combinations. Among the non-β-lactam agents tested, all were susceptible to tigecycline and colistin. All but one isolates were susceptible to amikacin, whereas susceptibility to gentamicin, tobramycin, trimethoprim-sulfamethoxazole and ciprofloxacin was variable. Of note, 7 isolates were susceptible to doxycycline.

### 3.4. $bla_{KPC}$ alleles, ESBL, plasmid-mediated AmpC and Qnr genes

Five and 6 isolates possessed $bla_{KPC-2}$ and $bla_{KPC-3}$, respectively, distributed in both *E. cloacae* and *E. aerogenes*. Nine of the 11 isolates co-produced ESBL. Six isolates had $bla_{SHV-5}$, $bla_{SHV-12}$ or $bla_{SHV-154}$, and 3 isolates harbored $bla_{CTX-M-15}$. Therefore, like in the case of *K. pneumoniae* (Endimiani, et al., 2009) and unlike in the case of *Escherichia coli* (Kim, et al., 2012), co-production of ESBL appeared to be a common phenomenon in KPC-producing *Enterobacter* spp. In addition, 8 isolates had $bla_{TEM-1}$, which encodes a non-ESBL, broad-spectrum β-lactamase. No plasmid-mediated AmpC genes were detected. Three and 2 isolates possessed plasmid-mediated fluoroquinolone resistance genes *qnrA* and *qnrB*, respectively. However, the $bla_{KPC}$-harboring transformants were negative for the *qnr* genes, consistent with their full susceptibility to nalidixic acid.

### 3.5. Clonality of the clinical isolates

MLST for *E. cloacae* isolates showed 6 STs, with 4 isolates sharing 2 of the STs (ST93 and ST171). Three of the STs were novel, and were assigned ST252, 253 and 254. None of the identified STs belonged to major clonal complexes that are known to date.

PFGE showed diverse restriction profiles overall (Figure). The 2 *E. cloacae* ST171 isolates (isolates 6 and 11) shared 92.7% identity, and the 2 *E. cloacae* ST93 isolates (isolates 3 and 7) shared 66.1% identity. The ST171 cases occurred at 2 hospitals 18 months apart, and the ST93 cases occurred at 2 hospitals 10 months apart. These cases were considered epidemiologically unrelated based on review of the hospitalization history. The level of clonal diversity based on PFGE was greater than that observed in a polyclonal outbreak of KPC-producing *E. cloacae* that occurred among 16 patients (6 identified by clinical cultures and 10 identified by rectal surveillance cultures) at a Canadian hospital in 2011 (Haraoui, et al., 2013).

### 3.6. Characterization of $bla_{KPC}$-carrying plasmids

The sizes of the $bla_{KPC}$-encoding plasmids were estimated to be between 30 kb to 190 kb by S1 nuclease PFGE (Table 2). The incompatibility groups could be determined for 5 of the 11 plasmids. The most common was IncN, accounting for 4 plasmids appearing in both species. Their size was approximately 90kb, and 3 of them shared an identical restriction pattern (isolates 3, 4 and 7; data not shown). Another plasmid belonged to IncFIB, but the remaining plasmids were non-typeable. Plasmid-mediated co-resistance to non-β-lactam agents was relatively uncommon, with only 3 plasmids, all IncN, conferring resistance to trimethoprim-sulfamethoxazole and one plasmid also to gentamicin.

## 4. Discussion

KPC-producing *Enterobacteriaceae* have become endemic at health care institutions in many parts of the world. Production of KPC is most commonly identified in *K. pneumoniae*, and its detection in non-*K. pneumoniae* species remains relatively rare (Munoz-Price, et al., 2013). We here studied the microbiologic characteristics of KPC-producing *Enterobacter* isolates, which were collected over five years within our hospital system. Carbapenem non-susceptibility rate was 2.7% during this period. Of the 44 unique ertapenem-non-susceptible isolates available for workup, only 11 were found to be positive for $bla_{KPC}$ (25%). Unlike in *K. pneumoniae*, *Enterobacter* spp. may become resistant to carbapenems in the absence of acquired carbapenemase by means of porin disruption and derepressed production of chromosomal AmpC (Doumith, Ellington, Livermore, & Woodford, 2009). This may account for the relative low prevalence of $bla_{KPC}$ observed among ertapenem-non-susceptible *Enterobacter* isolates in our study.

There were several interesting findings in this work. Firstly, the levels of ertapenem MICs were highly variable, including one isolate in the susceptible range (isolate 10; 0.25 μg/ml by agar dilution). The ertapenem MIC for this isolate was initially reported as 4 μg/ml by an automated instrument in the microbiology laboratory. Despite the low ertapenem MIC, this isolate was repeatedly positive for $bla_{KPC}$ by PCR, and yielded a $bla_{KPC}$-positive transformant with reduced ertapenem susceptibility. The remaining 10 isolates were resistant or intermediately resistant at least to ertapenem. It is well documented that KPC-producing *Enterobacteriaceae* may present with variable levels of carbapenem resistance (Miriagou, et al., 2010). While the number is relatively small, it appears that KPC-producing *Enterobacter* spp. testing susceptible to ertapenem under the current CLSI breakpoints is a rare event.

Secondly, the KPC-producing *Enterobacter* isolates were clonally diverse as defined by PFGE, whereas some of the *E. cloacae* isolates shared the same ST. While clonal outbreaks of KPC-producing *Enterobacter* isolates have been reported (Haraoui, et al., 2013; Qin, Yang, Hu, & Zhu, 2014), our findings suggested that the occurrence of KPC-producing *Enterobacter* spp. remains sporadic and generally polyclonal in the absence of a local outbreak, as was the case in our series. This is in contrast with the highly clonal and global dissemination observed in *K. pneumoniae*, where a specific MLST clone, defined as sequence type 258 (ST258), and its related STs account for the majority of KPC-producing isolates worldwide including the U.S. (Munoz-Price, et al., 2013). However, 2 of the 6 STs were identified in *E. cloacae* isolates from different hospitals in the system. Therefore, prospective surveillance and typing of KPC-producing *E. cloacae* with MLST may be helpful in detecting future expansion of certain STs at an early stage.

Finally, while many $bla_{KPC}$-carrying plasmids remained non-typeable, four were identified as IncN. Three of them shared an identical size and restriction pattern and conferred resistance to trimethoprim-sulfamethoxazole. IncN is a common incompatibility group observed among $bla_{KPC}$-encoding plasmids in *K. pneumoniae* (Gootz, et al., 2009). While resistance genes are generally sparse in fully characterized $bla_{KPC}$-encoding IncN plasmids, a recently reported IncN plasmid identified in *K. pneumoniae* from New York City also conferred resistance to trimethoprim-sulfamethoxazole (Chen, et al., 2013). All 4 $bla_{KPC}$-

encoding IncN plasmids we previously identified in *E. coli* also conferred resistance to this agent (O'Hara, et al., 2014). Therefore, resistance to trimethoprim-sulfamethoxazole may be a hallmark of $bla_{KPC}$-encoding IncN plasmids currently circulating across *Enterobacteriaceae* in the Northeast U.S.

In summary, KPC-producing isolates accounted for approximately a quarter of *Enterobacter* spp. identified as non-susceptible to ertapenem in hospitals in Pittsburgh, Pennsylvania. These isolates caused healthcare-associated infections in medically complex patients. The isolates were diverse in both *E. cloacae* and *E. aerogenes* with PFGE, but some *E. cloacae* isolates from different hospitals shared the same STs by MLST. Some of the $bla_{KPC}$-encoding plasmids were shared between the two species, including a trimethoprim-sulfamethoxazole-resistant IncN plasmid. These findings suggested a combination of plasmid-mediated and clonal processes underpinning their spread.

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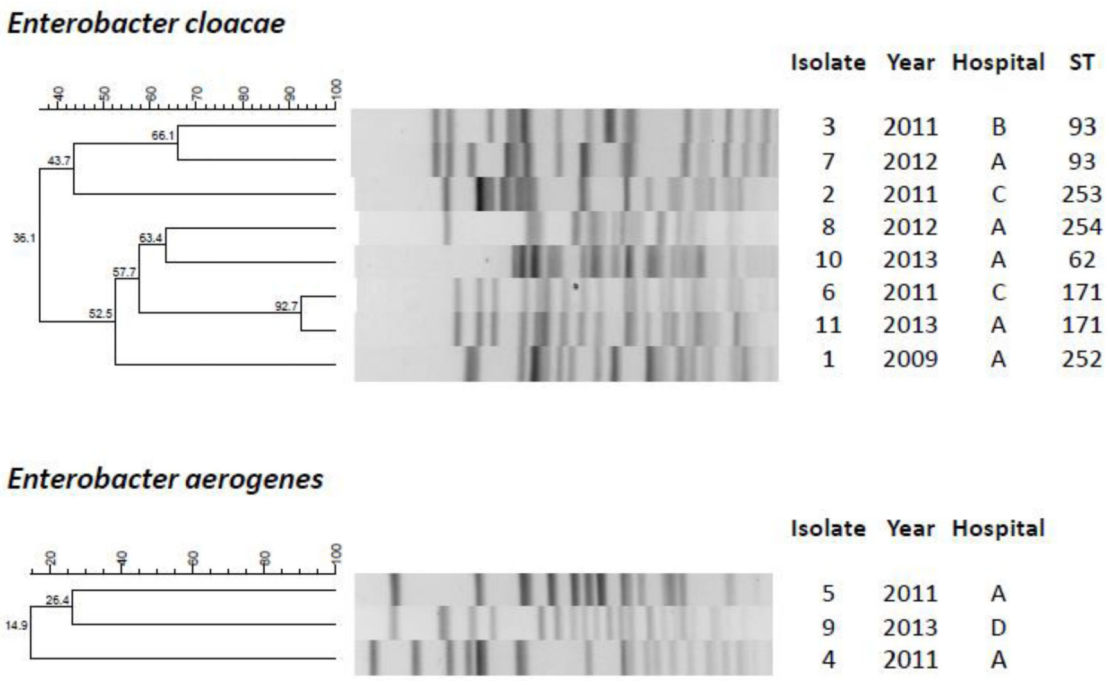


**Figure.**

**PFGE profiles and STs of the KPC-producing *Enterobacter* isolates**.

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**Table 1**

**Clinical features of 11 KPC–producing *Enterobacter* infections**

| Patient | Age | Year | Comorbid conditions | Admitted from | Reason for admission | Interval from admission to positive culture, days | Culture site | Infection or colonization | Empiric therapy | Definitive therapy | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 34 | 2009 | Multiple sclerosis, lacerated liver | Hospital | Liver transplant | 56 | Post-operative drainage | Likely colonization | Tigecycline, metronidazole | Meropenem, amikacin | Died |
| 2 | 69 | 2011 | COPD, bladder cancer | Home | Perforated gallbladder | 12 | Post-operative drainage | Infection | Meropenem | Tigecycline | Discharged to LTACH |
| 3 | 32 | 2011 | Acute myelogenous leukemia | Hospital | Encephalopathy | 36 | Blood | Colonization | Meropenem | Not applicable | Discharged to home hospice |
| 4 | 57 | 2011 | Peripheral vascular disease | Home | Incarcerated umbilical hernia | 42 | Sputum | Likely colonization | None | Meropenem, colistin | Discharged to home |
| 5 | 58 | 2011 | Heart transplant | SNF | Hematochezia | 200 | Blood | Infection | Doripenem, colistin | Doripenem, colistin | Died |
| 6 | 57 | 2011 | Diabetes, peripheral vascular disease | SNF | Arm abscess | 21 | Blood | Infection | Meropenem | Amikacin | Discharged to home |
| 7 | 21 | 2012 | Meningioma, complicated by pseudoaneurysm | Hospital | Mental status change | 24 | Cerebrospinal fluid | Infection | Cefepime, tobramycin | Not applicable | Died |
| 8 | 35 | 2012 | Hereditary pancreatitis, esophagectomy | Hospital | Revision of fundoplication conduit | 14 | Urine | Colonization | None | None | Discharged to home |
| 9 | 61 | 2013 | Quadriplegia | Home | Urinary tract infection | 0 | Urine | Infection | Ciprofloxacin | Amikacin, Fosfomycin | Discharged to home |
| 10 | 61 | 2013 | Alcohol abuse | Home | Encephalitis | 164 | Urine | Infection | Trimethoprim-sulfamethoxazole | Trimethoprim-sulfamethoxazole | Discharged to SNF |
| 11 | 55 | 2013 | Chronic pancreatitis, cirrhosis, diabetes | Home | Pneumonia | 21 | Bronchoalveolar lavage | Colonization | None | None | Discharged to home |

COPD, chronic obstructive pulmonary disease; LTACH, long-term acute care hospital; SNF, skilled nursing facility.

**Table 2**

**Antimicrobial susceptibility and plasmid characteristics of 11 KPC–producing *Enterobacter* isolates**

| # | Year | Species | Source | Minimum inhibitory concentrations (MICs; μg/ml) | | | | | | | | | | | | | | | | KPC type | ESBL | *bla*KPC plasmid | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ETP | IPM | MEM | DOR | TIM | TZP | FEP | CTX | CAZ | ATM | AMK | GEN | TOB | SXT | CIP | DOX | TGC | CST | | | Size (Kb) | Inc type | Co-resistance |
| **1** | 2009 | *E. cloacae* | Post-operative drainage | **128** | **32** | **64** | **64** | **>128/2** | **>64/4** | **>16** | **>32** | **>16** | **>16** | ≤4 | **>8** | **>8** | **>4/76** | **>2** | ≤2 | 0.5 | 0.25 | KPC-3 | SHV-5 | 90 | N | None |
| **2** | 2011 | *E. cloacae* | Post-operative drainage | **32** | **8** | **16** | **8** | **>128/2** | **>64/4** | **8** | **16** | **16** | **>16** | ≤ | **>8** | 2 | ≤0.5/9.5 | ≤0.25 | ≤2 | ≤0.25 | ≤0.25 | KPC-2 | None | 30 | FIB | None |
| **3** | 2011 | *E. cloacae* | Blood | **64** | **16** | **32** | **16** | **>128/2** | **>64/4** | **>16** | **>32** | **>16** | **>16** | ≤4 | **>8** | **>8** | **>4/76** | ≤0.25 | ≤2 | ≤0.25 | 1 | KPC-3 | CTX-M-15 | 90 | N | GEN, SXT |
| **4** | 2011 | *E. aerogenes* | Sputum | **4** | **2** | **2** | **2** | **64/2** | 16/4 | **16** | **>32** | **16** | **>16** | ≤4 | **>8** | **8** | **>4/76** | **2** | **8** | ≤0.25 | 1 | KPC-3 | CTX-M-15 | 90 | N | SXT |
| **5** | 2011 | *E. aerogenes* | Blood | **2** | 1 | 1 | 0.5 | **>128/2** | **32/4** | **4** | **32** | **>16** | **>16** | 16 | **>8** | **>8** | **1/19** | **>2** | ≤2 | ≤0.25 | ≤0.25 | KPC-3 | SHV-154 | 190 | NT | None |
| **6** | 2011 | *E. cloacae* | Blood | **2** | 1 | 0.5 | 0.25 | **>128/2** | **64/4** | **8** | **16** | **>16** | **>16** | 8 | **>8** | **>8** | **>4/76** | **>2** | **8** | 0.5 | ≤0.25 | KPC-2 | SHV-12 | 40 | NT | None |
| **7** | 2012 | *E. cloacae* | Cerebrospinal fluid | **4** | **2** | **2** | 1 | **>128/2** | **>64/4** | **>16** | **>32** | **>16** | **>16** | ≤4 | **>8** | **>8** | **>4/76** | **>2** | **>16** | 2 | 1 | KPC-2 | CTX-M-15 | 90 | N | SXT |
| **8** | 2012 | *E. cloacae* | Urine | **2** | **2** | 1 | 0.5 | **>128/2** | 16/4 | <2 | **2** | 2 | **8** | ≤4 | ≤1 | ≤1 | ≤0.5/9.5 | ≤0.25 | ≤2 | ≤0.25 | 1 | KPC-3 | None | 40 | NT | None |
| **9** | 2013 | *E. aerogenes* | Urine | **4** | **2** | 1 | 1 | **>128/2** | **64/4** | **8** | **16** | **>16** | **>16** | **32** | **>8** | **>8** | 2/38 | **>2** | **4** | ≤0.25 | 0.5 | KPC-3 | SHV-12 | 120 | NT | None |
| **10** | 2013 | *E. cloacae* | Urine | 0.25 | 0.5 | 0.25 | 0.12 | **>128/2** | 16/4 | ≤2 | **8** | **16** | **>16** | ≤4 | **8** | **8** | ≤0.5/9.5 | ≤0.25 | ≤2 | ≤0.25 | 0.5 | KPC-2 | SHV-5 | 160 | NT | None |
| **11** | 2013 | *E. cloacae* | Bronchoalveolar lavage | **4** | **2** | 1 | 0.5 | **>128/2** | **>64/4** | **8** | **32** | **>16** | **>16** | ≤4 | ≤1 | ≤1 | <0. 5/9 . 5 | **>2** | **8** | 0.5 | ≤0.25 | KPC-2 | SHV-5 | 40 | NT | None |

ETP, ertapenem; IPM, imipenem; MEM, meropenem; DOR, doripenem; TIM, ticarcillin-clavulanic acid; TZP, piperacillin-tazobactam; FEP, cefepime; CTX, cefotaxime; CAZ, ceftazidime, ATM, aztreonam; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin; DOX, doxycycline; TGC, tigecycline; CST, colistin; NT, non-typeable. The carbapenems were tested by the agar dilution method. The remainder was tested by the broth dilution method. Bold numbers indicate MICs in the non-susceptible or susceptible-dose dependent range. Plasmid sizes were estimated by S1 nuclease PFGE.