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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 *Klebsiella pneumoniae* carbapenemase (KPC)–producing *Enterobacter* spp. are scarce. In this study, 11 unique KPC-producing *Enterobacter* isolates were identified among 44 ertapenem-nonsusceptible *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 showed diverse restriction patterns overall, multilocus sequence typing identified *Enterobacter cloacae* isolates with sequence types 93 and 171 from 2 hospitals each. The levels of carbapenem minimum inhibitory concentrations were highly variable. All isolates remained susceptible to colistin and tigecycline, and the majority, to amikacin and doxycycline. A bla_{KPC}-carrying IncN plasmid conferring trimethoprim-sulfamethoxazole resistance was identified in 3 of the isolates. Spread of bla_{KPC} in *Enterobacter* spp. appears to be due to a combination of plasmid-mediated and clonal processes.

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 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 et al., 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 2 clinical microbiology laboratories serving 4 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, USA) or Vitek2 (bioMérieux, Durham, NC, USA) automated instruments in the clinical microbiology laboratories. Only 1 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-nonsusceptible 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, USA). For carbapenems, the agar dilution method was used to test a wider range of MICs (0.06–256 μg/mL). The assays were conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and their breakpoints (CLSI, 2012a, 2014). Potential extended-spectrum β-lactamase 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 and Hanson, 2002). Plasmid-mediated fluoroquinolone resistance genes *qnrA*, *qnrB*, and *qnrS* were detected by PCR (Tian et al., 2010).

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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, USA).

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

2.4. Transfer of bla_{KPC}-encoding plasmids

Escherichia 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 et al., 2013). Replicon typing of the plasmids was conducted as described by 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 (CLSI, 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 4687 unique *Enterobacter* isolates were identified at 2 clinical microbiology laboratories serving 4 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 nonsusceptible 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, 0–200) 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, 3 were discharged to another healthcare setting (long-term acute care hospital, skilled nursing facility, or hospice), and 5 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 from 0.25 to 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 1 isolates were susceptible to amikacin, whereas susceptibility to gentamicin, tobramycin, trimethoprim-sulfamethoxazole, and ciprofloxacin was variable. Of note, 7 isolates were susceptible to doxycycline.

3.3. 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 *E. 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.

3.4. 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 (Fig. 1). 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).

3.5. Characterization of bla_{KPC}–carrying plasmids

The sizes of the bla_{KPC}-encoding plasmids were estimated to be between 30 kb and 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 90 kb, 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 healthcare 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 5 years within our hospital system. Carbapenem nonsusceptibility rate was 2.7% during this period. Of the 44 unique ertapenem-nonsusceptible isolates available for workup, only 11 were found to be positive for bla_{KPC} (25%). Unlike in *K. pneumoniae*, *Enterobacter* spp. may become