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Emergence of *Raoultella ornithinolytica* Coproducing IMP-4 and KPC-2 Carbapenemases in China

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We report here the emergence of seven IMP-4-producing *Raoultella ornithinolytica* isolates obtained from one patient. All isolates carried the bla_{IMP-4} carbapenemase gene, five isolates also carried bla_{SHV-12}, four contained bla_{TEM-1}, and one contained bla_{OXA-1}. Notably, the *R. ornithinolytica* isolate Ro25724 also expressed *Klebsiella pneumoniae* carbapenemase (KPC)-2. The bla_{KPC-2} gene was located on a Tn*3*-Tn*4401* integration structure on a plasmid of ~450 kb. This is the first description of the coexistence of bla_{KPC-2} and bla_{IMP-4} from the genus *Raoultella*.

Raoultella ornithinolytica is mostly recovered from the environment and rarely causes severe infections in humans; so far, only a few invasive *R. ornithinolytica* infections have been reported (1, 2). Carbapenem resistance in *Enterobacteriaceae* represents one of the greatest problems in the realm of antibiotic resistance, and resistance is most often mediated by carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC), IMP, NDM, and OXA-48 (3). Plasmid-mediated carbapenem resistance may further aggravate the problem (4). However, the prevalence and molecular diversity of carbapenemases in *Raoultella* are largely unknown. Until now, the emergence of carbapenemases in the genus *Raoultella* has scarcely been described (1, 5–7).

In June 2014, a 13-year-old boy was admitted to our hospital. He had suffered a sport-related injury and required an urgent operation. The patient underwent an open reduction and internal fixation surgery. Two weeks later, his clinical status worsened, with episodes of obvious wound infection. Urgent debridement and drainage were carried out for four times in the following period of 80 days, and seven samples obtained from wound fluid and necrotic tissue were sequentially collected at different time points for routine clinical microbiology investigation. The general information and phenotypic characteristics of the seven isolates are summarized in Table 1.

All seven strains were identified as *R. ornithinolytica* by 16S rRNA sequencing analysis. *In vitro* antimicrobial susceptibility testing was conducted by Vitek 2 and Etest (bioMérieux, France) and interpreted as in previous studies (5, 8). The MIC results demonstrated that all isolates were resistant to ampicillin, amikacin, ceftriaxone, imipenem, ertapenem, aztreonam, gentamicin, cefepime, tobramycin, ceftazidime, and ampicillin-sulbactam but

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TABLE 1 General features and phenotypic characteristics of the seven *R. ornithinolytica* isolates

Isolate no.	Isolation date (yr-mo-day)	Source	Antibiotic resistance gene(s)	MIC (μg/ml) for[a]: AMP	AMK	CIP	CTRX	IPM	ETP	AZT	GEN	FEP	TZP	TOB	SXT	SAM
Ro23980	2014-7-18	Exudate	bla_{IMP-4}	≥32	≥64	1	≥64	8	≥8	≥64	≥16	≥64	8	≥16	≤20	≥32
Ro24005	2014-7-19	Exudate	bla_{IMP-4}, bla_{SHV-12}, bla_{TEM-1}	≥32	≥64	1	≥64	8	≥8	≥64	≥16	≥64	8	≥16	≤20	≥32
Ro24362	2014-8-4	Necrotic tissue	bla_{IMP-4}, bla_{SHV-12}, bla_{TEM-1}	≥32	≥64	1	≥64	8	≥8	≥64	≥16	≥64	8	≥16	≤20	≥32
Ro24522	2014-8-11	Exudate	bla_{IMP-4}, bla_{SHV-12}, bla_{TEM-1}, bla_{OXA-1}	≥32	≥64	1	≥64	8	≥8	≥64	≥16	≥64	64	≥16	≤20	≥32
Ro24724	2014-8-21	Necrotic tissue	bla_{IMP-4}, bla_{KPC-2}, bla_{SHV-12},	≥32	≥64	1	≥64	8	≥8	≥64	≥16	≥64	64	≥16	≤20	≥32
Ro25277	2014-9-18	Exudate	bla_{IMP-4}, bla_{SHV-12}, bla_{TEM-1}	≥32	≥64	1	≥64	8	≥8	≥64	≥16	≥64	8	≥16	≤20	≥32
Ro25687	2014-10-9	Exudate	bla_{IMP-4}	≥32	≥64	1	≥64	8	≥8	≥64	≥16	≥64	8	≥16	≥320	≥32

[a] AMP, ampicillin; AMK, amikacin; CIP, ciprofloxacin; CTRX, ceftriaxone; IPM, imipenem; ETP, ertapenem; AZT, aztreonam; GEN, gentamicin; FEP, cefepime; TZP, piperacillin-tazobactam; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; SAM, ampicillin-sulbactam.

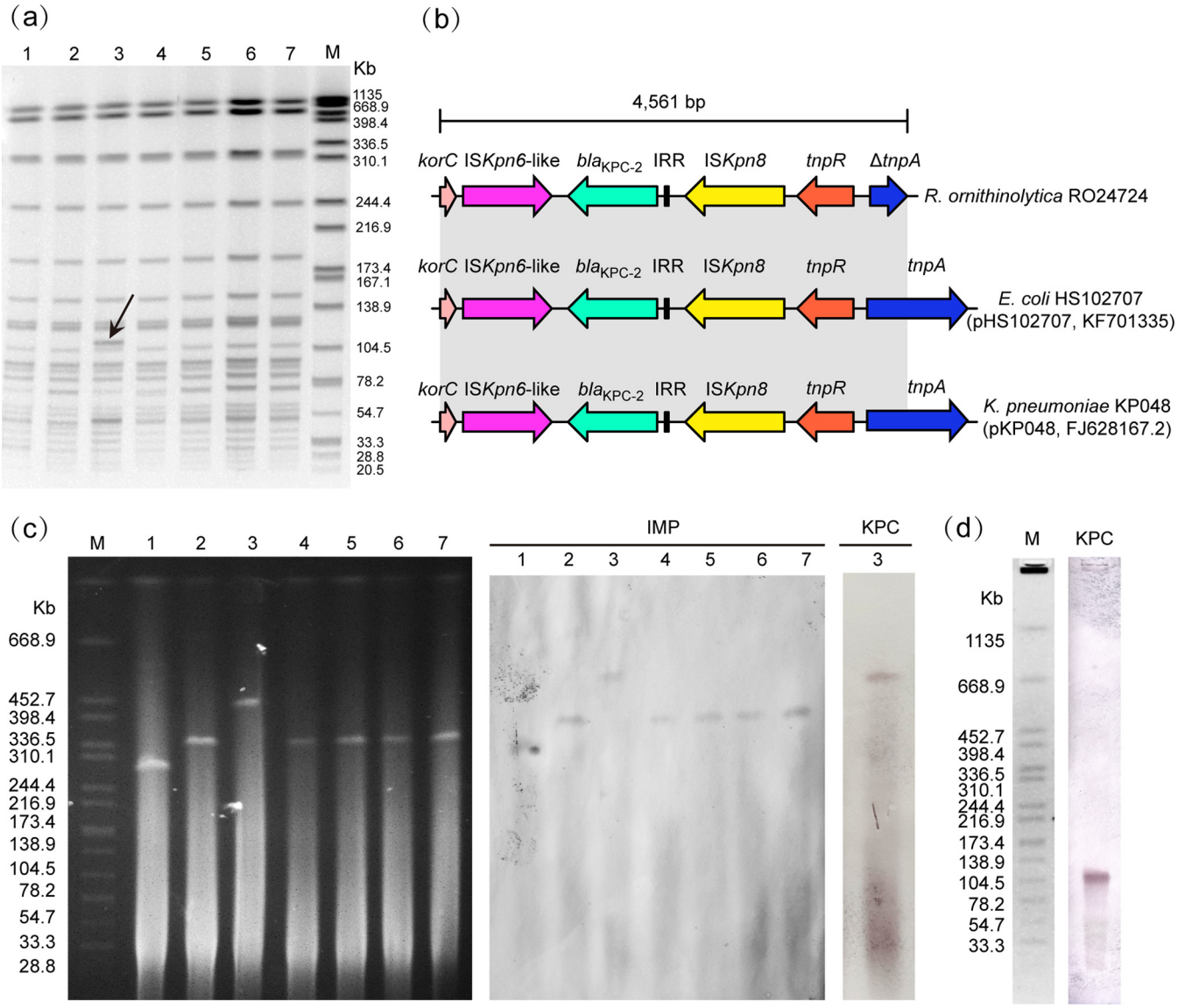

FIG 1 Molecular characterization of seven IMP-4-producing *R. ornithinolytica* strains. (a) PFGE patterns generated by XbaI restriction enzyme treatment of seven isolates. Lane 1, Ro25687; lane 2, Ro25277; lane 3, Ro24724; lane 4, Ro24522; lane 5, Ro24362; lane 6, Ro24005; lane 7, Ro23980A; M, *Salmonella enterica* subsp. *enterica* serotype Braenderup strain H9812 molecular marker. The arrow shows the band present only in strain Ro24724. (b) Schematic representation of the genetic structures surrounding the bla_{KPC-2} gene in Ro24724. Various genes and their directions of transcription are depicted as broad arrows. Regions with identical sequences are shown in gray between the different plasmids. On the right are the plasmids carried in the various strains, followed by their accession numbers. IRR, inverted repeat region. (c) S1-PFGE and Southern hybridization results with the bla_{IMP}- and bla_{KPC}-specific probes. Lane 1, Ro25687; lane 2, Ro25277; lane 3, Ro24724; lane 4, Ro24522; lane 5, Ro24362; lane 6, Ro24005; lane 7, Ro23980A; M, molecular mark. (d) PFGE followed by Southern hybridization using bla_{KPC-2} probe.

susceptible to ciprofloxacin and piperacillin-tazobactam (Table 1). It is worth noting that all isolates were susceptible to trimethoprim-sulfamethoxazole (SXT) except *R. ornithinolytica* strain Ro25687, which indicates that the patient was not infected by the same strain or that the use of antibiotics led to SXT resistance under the selective pressure of SXT usage, given that the patient received 2 weeks of SXT therapy before the isolation of Ro25687. To date, carbapenemase-resistant *R. ornithinolytica* strains have been reported only in North America (1, 9).

To confirm the clonality of the isolates, pulsed-field gel electrophoresis (PFGE) and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) were therefore conducted according to published protocols (8, 10). All isolates shared identical or highly similar PFGE patterns (Fig. 1a) and ERIC-PCR profiles (see Fig. S1 in the supplemental material), indicating that all isolates were closely related. Notably, *R. ornithinolytica* strain Ro24724 yielded a different PFGE and ERIC-PCR genotype, with a difference of only one band.

Screening for the presence of carbapenemase and extended-spectrum β-lactamase genes was carried out as described previously (11–15). The primers are detailed in Table S1 in the supplemental material. PCR screening revealed the presence of bla_{IMP-4} in all strains. All isolates were negative for other metallo-β-lactamase (MBL) or KPC-type β-lactamase genes, except strain Ro24724, which also carried bla_{KPC-2} (Table 1). In addition, there were 5 isolates positive for bla_{SHV-12}, 4 isolates contained bla_{TEM-1},

and one isolate contained bla_{OXA-1}. Of note, the bla_{OXA-1} gene was first identified in the genus *Raoultella*. Interestingly, in China, KPC-2 was first reported in our hospital and is now rapidly disseminated throughout China (8, 16). Additionally, bla_{KPC-2} genes are largely disseminated worldwide among *K. pneumoniae* isolates but less frequently among other *Enterobacteriaceae* (8). Until now, the coexistence of bla_{KPC-2} and bla_{IMP-4} had been described in *K. pneumoniae* and in China only (17–19). The clinical isolate Ro24724 reported here is the first documented KPC- and IMP-producing *R. ornithinolytica*.

The genetic environment of bla_{KPC-2} was sought by long-PCR mapping based on previously described primers (8). PCR amplification generated a 4,561-bp segment, which suggested the bla_{KPC-2}-flanking region in Ro24724 shared 100% identity with bla_{KPC-2}-carrying plasmid *K. pneumoniae* pKP048 and *Escherichia coli* pHS102707 (Fig. 1b). The bla_{KPC-2} gene in *R. ornithinolytica* is also located on a Tn*3*-Tn*4401* integration structure, as previously described from *K. pneumoniae* and *E. coli* (8, 20). Of note, the bla_{KPC-2} gene has been detected in *K. pneumoniae*, *E. coli*, *Enterobacter cloacae*, *Morganella morganii*, and even *Pseudomonas aeruginosa* isolates from Zhejiang province (16, 21–23). Our observation further documents the transmission of this structure in different *Enterobacteriaceae* in China.

The *in vitro* conjugation experiments were unsuccessful. We also tried to transfer plasmids extracted from seven isolates by transformation experiments. However, repeated transformation methods failed to move the plasmids to recipient *E. coli* DH5α cells. This suggests that plasmids were likely to be nonconjugative, and this may primarily be due to the large size of the plasmids, which may limit the efficiency of transformation.

Plasmid sizes were then determined using an S1-nuclease PFGE (S1-PFGE) method (24). S1-PFGE revealed that all isolates contained a single plasmid, with sizes of ~300 to 450 kb (Fig. 1c). Of note, strain Ro24724 contained a larger plasmid than that of other isolates, while isolate Ro25687 carried the smallest plasmid, which is in agreement with their phenotypic and molecular differences. The hybridization results showed that the bla_{KPC-2} and bla_{IMP-4} genes were located on a plasmid of ~450 kb in strain Ro24724. The bla_{IMP-4} gene was present on a plasmid of ~300 kb in strain Ro25687 and on plasmids of ~340 kb in *R. ornithinolytica* strains Ro23980, Ro24005, Ro24362, Ro24522, and Ro25277. PFGE followed by Southern blotting revealed that bla_{KPC-2} was located on the additional band observed in PFGE, which suggested that this band is part of a large plasmid (Fig. 1a and d). Previous studies showed that bla_{KPC} was localized on an 11-kb plasmid in *R. ornithinolytica* and *Raoultella planticola*, and bla_{IMP-8} was carried on a 249-kb plasmid in *R. planticola* (1, 6). Our observations further exhibited that the bla_{KPC-2} and bla_{IMP} genes have the potential to spread widely among members of the genus *Raoultella*.

In conclusion, we provide here the first report of the sequential isolation of plasmid-medicated IMP-4-producing *R. ornithinolytica* and the first description of the coexistence of bla_{KPC-2} and bla_{IMP-4} in *Raoultella*. This study reinforces the idea of a rapid dissemination of the bla_{IMP-4} and bla_{KPC-2} genes in clinical isolates of *Enterobacteriaceae* in China. Despite being only one case, our study is of great concern for its epidemic potential, since the emergence of KPC-2 is a severe threat in China. Further research to explore the detailed genetic features of these plasmids and their evolution in different clinical isolates is now under way.

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