Comprehensive Genome Analysis of Carbapenem-Resistant Strains of Raoultella Species, an Emerging Multidrug-Resistant Bacterium in Hospitals

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ABSTRACT We report the characterization of six carbapenem-resistant Raoultella spp. (CRRS) in our hospital and a genomic analysis of 58 publicly available isolates. CRRS isolates are sporadically identified around the world, and different transposons carrying carbapenemases were the resistant mechanisms. Mobile genetic elements play an important role in acquiring antibiotic resistance genes from the hospital. An improved understanding of these transposon and targeted control measures will be very valuable to prevent CRRS dissemination.

KEYWORDS Raoultella spp., carbapenem resistance, mobile genomic elements, transposons, mechanisms of resistance, molecular epidemiology

Raoultella species, which were moved into a separate genus from genus Klebsiella in 2001, are normally found in aquatic environments (1). Due to the introduction of accurate molecular techniques in clinical microbiology laboratories, there has been an increasing trend in the number of reports of clinical infections with Raoultella species strains (2). Notably, cases of infection with carbapenem-resistant Raoultella spp. (CRRS) have been sporadically reported (3–7). In recent years, despite the high mortality in clinical infection, there has not yet been a systematic study of the mechanisms underlying carbapenem resistance in Raoultella species strains (8).

Characteristics of Raoultella species isolates. From January 2013 to December 2016, six CRRS strains were isolated in our hospital. The drug sensitivities of the CRRS are shown in Table 1. Whole-genome sequencing of these strains showed three different carbapenemase genes, including \textit{bla}\textsubscript{IMP-4} (Ro23804 and Ro24005), \textit{bla}\textsubscript{KPC-2} (Ro10311 and Ro10648), and \textit{bla}\textsubscript{NDM-1} (Ro19773 and Ro23820). The phylogenetic results of the six CRRS strains show that strains carrying the same carbapenem resistance genes cluster closely. According to the date of isolation, the 3 couples of CRRS strains appeared to be compatible with cross-transmission; however, the number of single nucleotide polymorphisms (SNPs) from the core genome does not support this speculation (Fig. 1).

Furthermore, a comparative genomics analysis of 58 Raoultella species strains whose genome sequences were publicly available (as of 30 September 2018) was performed. The basic information for the strains is provided in Fig. 2a and Table S1 in the supplemental material. In addition to the CRRS in our hospital, there were seven other CRRS strains, including two KPC-2-producing strains, three KPC-3-producing isolates, and two strains carrying \textit{bla}\textsubscript{OXA-48} (Fig. 2b). Five different carbapenemases were found in the 13 CRRS strains, suggesting no clear epidemic domain carbapenemases. In addition, the distribution of carbapenemases in CRRS isolates is consistent with the...
TABLE 1 Characteristics and antimicrobial resistance of isolates and transconjugants

| Strain ID  | Specimen | Strain      | Isolation date (yr-mo-day) | MIC (µg/ml) |
|------------|----------|-------------|---------------------------|-------------|
|            |          |             |                           | AMC | FEP | TZP | CAZ | CTX | AZM | IPM | MEM | GEN | AMK | CIP | LEV | SXT | TGC |
| Ro10311    | Sputum   | R. ornithinolytica | 2013-05-06 | >32/16 | >64 | >128/4 | >64 | >64 | >64 | >16 | >8 | <1 | <2 | 1 | 1 | <20 | <0.5 |
| Ro10648    | Sputum   | R. ornithinolytica | 2013-05-20 | >32/16 | >64 | >128/4 | >64 | >64 | >64 | >16 | >8 | <1 | <2 | 1 | 1 | <20 | <0.5 |
| Ro19773    | Sputum   | R. ornithinolytica | 2014-06-25 | >32/16 | >64 | >128/4 | >64 | >64 | >64 | >16 | >8 | <1 | <2 | 4 | 8 | >320 | <0.5 |
| Ro23804    | Sputum   | R. ornithinolytica | 2014-07-11 | >32/16 | >64 | >128/4 | >64 | >64 | >64 | >16 | >8 | >16 | >64 | 4 | 2 | <20 | <0.5 |
| Ro23820    | Feces    | R. ornithinolytica | 2014-07-12 | >32/16 | >64 | >128/4 | >64 | >64 | >64 | >16 | >8 | <1 | <2 | 4 | 8 | >320 | <0.5 |
| Ro24005    | Drainage | R. ornithinolytica | 2014-08-21 | >32/16 | >64 | >128/4 | >64 | >64 | >64 | >16 | >8 | >16 | >64 | 1 | 1 | <20 | <0.5 |
| J53-Ro19773| NA       | E. coli      | NA            | >32/16 | 4 | >128/4 | >64 | >64 | >64 | >16 | >8 | 0.5 | <2 | <0.25 | <0.25 | <0.25 | <0.25 |
| J53-Ro23820| NA       | E. coli      | NA            | >32/16 | 4 | >128/4 | >64 | >64 | >64 | >16 | >8 | 0.5 | <2 | <0.25 | <0.25 | <0.25 | <0.25 |

aID, identifier.  
bAMC, amoxicillin-clavulanic acid; AMK, amikacin; AZM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, ceftriaxone; FEP, cefepime; GEN, gentamicin; IPM, imipenem; LEV, levofloxacin; MEM, meropenem; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline; TZP, piperacillin-tazobactam.  
NA, not available.

global distribution of carbapenemases, suggesting that carbapenem resistance genes are regionally distributed.

The S1-pulsed-field gel electrophoresis (PFGE) showed that the six CRRS strains carried different numbers of plasmids. Southern hybridization results showed that even the same carbapenemase is located on different-sized plasmids (see Fig. S1). The blaNDM-1 gene was successfully transferred to Escherichia coli J53 via conjugation, with a conjugation efficiency of 0.5 × 10⁻². The drug sensitivities of the transconjugants are shown in Table 1. Conjugation experiments with strains carrying blaKPC-2 and blaIMP-4 were unsuccessful. We also tried to transfer plasmids extracted from isolates by chemical and electrical transformation. However, repeated transformation methods failed to move the plasmids to recipient E. coli DH5α cells. This may have primarily been due to the large size of the plasmids, which may limit the efficiency of transformation.

Carbapenemase gene environments. The analysis showed that the blaIMP-4 gene environments of Ro24005 and Ro23804 were the same, and this environment was similar to the structure of plasmid p19051-IMP (MF344565) which was carried by a

<FIG 1 Phylogeny and carbapenemases of six carbapenem-resistant R. ornithinolytica strains. The tree on the left was constructed based on core genome SNPs. The second column shows the carbapenemases carried by these strains. The numbers in the figure indicates the number of SNPs: 15,752 SNPs between Ro23804 and Ro10311, 15,106 SNPs between Ro10648 and Ro10311, 15,444 SNPs between Ro23804 and Ro24005, 16,183 SNPs between Ro23804 and Ro19773, and 6 SNPs between Ro19773 and Ro23820.>
FIG 2 Distribution and characteristics of 58 Raoultella spp. with publicly available genomic data. (a) Distribution of Raoultella species isolates in the world; the numbers in the figure represent the numbers of isolated strains. Green circles represent the countries where CRRS strains are isolated.

(Continued on next page)
Klebsiella pneumoniae strain isolated in Ningbo, China, except for an insertion sequence common region (ISCR) element (Fig. 3a). The \( \text{bla}^{\text{IMP-4}} \) gene is located in a complete class I integron In2, and this integron is inserted into a Tn\text{1696}-like transposon bounded by IS\text{5075} elements. A special insertion sequence, ISCR, which is similar to the IS\text{91} family transposons, lies downstream of this transposon. In this study, insertion of this integron constituted the main structure of a Tn\text{1696}-like transposon, which is the main trans-
poson involved in bla_{IMP-4} dissemination in China (9). Therefore, we speculate that this transposon will lead to broad bla_{IMP-4} dissemination in the future. In addition, the class 1 integron In2 can capture drug resistance genes, and one or more ISCR sequences carrying multiple drug resistance genes are often inserted into the sequence downstream of the transposon (Fig. 3a, inserts show two ISCR sequences), which further increases the resistance of the strain. Since Tn1696-like transposons belong to the Tn3 transposon family which are commonly reported in \textit{K. pneumoniae} and \textit{Enterobacter cloacae} (10), carbapenemases in CRRS may be obtained from these strains via transposon transposition. In the future, we must pay more attention to the role of Tn1696-like transposons in the transmission of bla_{IMP-4} carbapenem genes.

The bla_{KPC-2} gene is located between ISKpn8 and ISKpn6 insertion sequences. This region, “ISKpn8-bla_{KPC-2}-ISKpn6,” is inserted downstream of the Tn3 transposase and forms a Tn1721-based transposon that includes the korC and klcA genes (Fig. 3b). It is demonstrated that the Tn1721-based transposon is the main bla_{KPC-2}-carrying transposon in \textit{Enterobacteriaceae} bacteria (11, 12) and can mediate the transfer of the bla_{KPC-2} gene between different bacteria (12). Therefore, Tn1721 may be further disseminated to other strains, including \textit{Raoultella} spp., in the future. Interestingly, as shown in Fig. 1, IMP-4-producing strains clustered closer to KPC-2-producing strains, possibly because these two carbapenemase genes were located on similar mobile elements, i.e., Tn3 family transposons.

The annotation of the bla_{NDM-1} gene environment is based on that of JX296013, which was the first NDM-1 carbapenemase-producing \textit{E. coli} strain isolated in Beijing. The bla_{NDM-1} gene is located between the ISAba125 and bleMBL genes. The main structure of the Tn125 transposon consists of the insertion of the “ISAba125-bla_{NDM-1}-bleMBL region” and the “grol-groS-cutA-nagA region” downstream of ISCR27 in CRRS strains. The ISAba125-bla_{NDM-1}-bleMBL gene structure is relatively stable in the Tn125 transposon (Fig. 3c). Currently, almost all of the globally reported bla_{NDM-1} genes exist in this structure, and the Tn125 transposon is often located on a conjugable plasmid, further leading to easy transmission of the bla_{NDM-1} gene (13, 14). It is thought that Tn125 originated in \textit{Acinetobacter baumanii} and was disseminated in this strain (15). Later, because of an insertion of a mobile element sequence in the initial segment of this transposon, e.g., the insertion of an ISCR sequence, the resulting novel Tn125 transposon was mainly disseminated in \textit{Enterobacteriaceae}, especially \textit{K. pneumoniae} and \textit{E. coli} (16). Because the structure of the Tn125 in the \textit{Raoultella} spp. was similar to this new type of Tn125, the \textit{Raoultella} spp. may have acquired the resistance gene from \textit{Enterobacteriaceae} family isolates.

Carbapenem gene structures of the seven publicly available CRRS strains were also explored. The two bla_{KPC-2} genes in one \textit{Raoultella ornithinolytica} and one \textit{Raoultella planticola} isolated from China is similar to Ro10311 found in our hospital, located on the Tn1721-based transposon (Fig. 3d). The bla_{KPC-3} gene in two \textit{R. planticola} and one \textit{R. ornithinolytica} isolate from Canada was located in the Tn4401b transposon with the TnpR gene replaced by IS15DIV (Fig. 3e). The bla_{OXA-48} in \textit{R. ornithinolytica} (one from Lebanon and one from the United Kingdom) was located on the Tn1999 transposon as reported by Poirel et al. (17). Downstream of bla_{OXA-48}, was a dmlR gene encoding an HTH-type transcriptional regulatory protein. The Tn1999 transposon inserted into the CRH gene, suggesting that this gene could possibly be a hot spot for integrating the Tn1999 transposon (Fig. 3f).

The analysis of the carbapenemase gene environments showed that these carbapenem resistance genes were located on transposons. The same carbapenemases were located in the same transposon structure, suggesting that transposons play an important role in the dissemination of carbapenem resistance genes. In any case, due to the low clinical isolation rate of \textit{Raoultella} species isolates, it is therefore very likely that these mobile elements were acquired from other strains in the \textit{Enterobacteriaceae} family. Thus, this possible outcome requires significant attention and the implementation of targeted control measures.
Genomic characteristics and drug resistance genes. After removing repetitive strains, 51 of 58 Raoultella species strains were included for further analysis. The core genome of the 51 strains consisted of 1,627 genes and accounted for 29.99% (1,627/5,424) of the average total genome size of the various Raoultella spp. The relationship between the number of strains and the numbers of core genes or total genes showed that the Raoultella species strains contained an open pan-genome. Of the 51 strains, 20 were isolated from the environment and only one was a CRRS strain, while there were 10 CRRS in 31 strains which were isolated from hospitals. The carbapenem resistance rate in the hospital-isolated strains was significantly higher than that in the environmental strains (chi-square statistics, $P = 0.009$). This observation suggests that hospitals are the main source of carbapenem resistance genes in Raoultella spp. The average number of drug resistance genes isolated from hospital strains was 11.78, while only 5.35 were found in the environmental strains ($P < 0.05$). This difference indicates that the hospitals might have been the source of the drug resistance genes in these Raoultella species isolates. In addition, the average numbers of plasmid replicons carried by the hospital and environmental isolates were 3.13 and 2.08, respectively (Fig. 2b), suggesting that the main strategy for Raoultella spp. to acquire drug resistance genes in hospitals is via plasmid-mediated horizontal transfer.

Currently, most of the known genes that mediate carbapenem resistance in Raoultella species isolates are carried on plasmids, and we have observed the dynamic process of the plasmid-mediated acquisition of a KPC-2 gene in an R. ornithinolytica isolate in vivo (3, 5-7). In this study, we found that two NDM-1-producing R. ornithinolytica isolates were recovered from different samples (sputum and feces) from the same patient. Interestingly, the number and size of the plasmids carried by these two strains differed (Fig. S1, lanes 5 and 6). This observation further reflects the variability of the Raoultella spp. genome. Therefore, combined with our findings, these data further indicate that Raoultella spp. have an open pan-genome and that they can easily acquire exogenous genes, such as various kinds of antibiotic resistance genes shown in Fig. 2b.

For the first time, we comprehensively analyzed the Raoultella species and showed carbapenemases in CRRS strains correspond with the prevalent carbapenemases in the isolated region; a variety of transposons carrying different carbapenemases is the main mechanism for CRRS. The open pan-genome of Raoultella spp. may be associated with acquisition of drug resistance genes in hospitals via mobile genetic elements. An improved understanding of these transposon and targeted control measures will be very valuable to prevent the dissemination of CRRS.

SUPPLEMENTAL MATERIAL
Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01367-19.
SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.
SUPPLEMENTAL FILE 2, XLSX file, 0.1 MB.

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Y. Xiao conceived and designed the study. H. Xu, J. Zhang, Y. Zhou, and S. Zhang performed experiments described in this study. Y. Zhu and Y. Wang analyzed the whole-genome sequencing data. X. Yu and T. Xiao wrote the draft, and B. Zheng revised it. All authors approved the final version.
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