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First Report of bla_{IMP-8} in *Raoultella planticola*

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Two carbapenem-resistant *Raoultella planticola* clinical isolates were isolated from patients with pneumonia and Port-A catheter-related bacteremia, respectively, in Taiwan. These isolates remained susceptible to fluoroquinolone, aminoglycoside, and colistin. Though the two isolates had the same antibiogram, plasmidic carbapenemase bla_{IMP-8}, class 1 integron cassette (*dfrA12*-orfF-*aadA2*), and *qnrB2*, they had different pulsed-field gel electrophoresis patterns, plasmid sizes, and outer membrane protein loss profiles. To our knowledge, this is the first report of bla_{IMP-8} found in *R. planticola*. Interestingly, bla_{IMP-8} is the most common carbapenemase found in *Klebsiella pneumoniae* in Taiwan. In the literature, carbapenemase genes in *R. planticola* in each country were also found in carbapenem-resistant *Enterobacteriaceae* in the same country.

Raoultella planticola belongs to the *Enterobacteriaceae* family, is related to *Klebsiella* spp., and is mostly found in soil and water (1). Although the reports of *R. planticola* infection are limited, this bacterium has been reported in bloodstream infection, surgical site infection, and cystitis (2–4). Bacteremia was the most common clinical manifestation in six of the 11 *R. planticola* infection cases in the literature (3). To date, there are isolates of *Raoultella ornithinolytica* and *R. planticola* which are resistant to carbapenem and carry different carbapenemases, including bla_{KPC}, bla_{OXA-48}, and $bla_{OXA-162}$ genes (2, 5, 6). However, bla_{IMP-8}-producing *R. planticola* has not been reported in the literature.

Among 411 carbapenem-resistant *Enterobacteriaceae* isolates collected from a nationwide surveillance study in Taiwan in 2012, two carbapenem-resistant *R. planticola* clinical isolates were identified from patients in the National Taiwan University Hospital. One was isolated from a sputum specimen; the other was from a blood specimen. The 16S rRNA gene sequencing confirmed identification of *R. planticola* initially with the Vitek 2 system (7).

Case 1. A 77-year-old male patient was a case of non-small-cell lung cancer. He was admitted with pneumonia in February 2012. After admission, respiratory failure and shock developed. Chest roentgenography revealed bronchopneumonia in the right lower lung field. Sputum culture yielded carbapenem-resistant *R. planticola* (isolate 139), and the patient received levofloxacin and cefepime. Due to progressive leukocytosis, unstable hemodynamics, and increased O_2 demand, meropenem and colistin were prescribed instead of cefepime. The patient eventually died of pneumonia and shock.

Case 2. A 57-year-old male patient had non-small-cell lung cancer with bilateral mediastinal lymph nodes and multiorgan metastasis. He received therapy with erlotinib and cisplatin. Port-A catheter-related bacteremia was suspected, and blood cultures grew *Acinetobacter baumannii* and carbapenem-resistant *R. planticola* (isolate 193). The patient received ceftazidime, levofloxacin, and gentamicin, and then the follow-up blood cultures were negative sterile 5 days later.

These two isolates were regarded as health care-associated pathogens and had the same antibiogram, where both were susceptible to aztreonam, piperacillin-tazobactam, aminoglycosides, fluoroquinolones, colistin, and tigecycline but resistant to imipenem, doripenem, ertapenem, ticarcillin-clavulanic acid, and trimethoprim-sulfamethoxazole (Table 1). Pulsed-field gel elec-

TABLE 1 Characterization of bla_{IMP-8}-carrying *R. planticola* clinical isolates

Drug or resistance profile	MIC[a] (μg/ml) or characteristic of *R. planticola* clinical isolate	
	Isolate 139 (case 1)	Isolate 193 (case 2)
Carbapenems		
Imipenem	4 (R)	4 (R)
Meropenem	2 (I)	2 (I)
Doripenem	>2 (R)	>2 (R)
Ertapenem	1 (R)	2 (R)
Cephems		
Cefepime	16 (I)	>16 (R)
Cefoxitin	>16 (R)	>16 (R)
Ceftazidime	>16 (R)	>16 (R)
Cefazolin	>16 (R)	>16 (R)
Cefuroxime	>16 (R)	>16 (R)
Cefotaxime	>32 (R)	>32 (R)
Aztreonam	<1 (S)	<1 (S)
Ticarcillin-clavulanic acid	>64 (R)	>64 (R)
Piperacillin-tazobactam	16 (S)	8 (S)
Trimethoprim-sulfamethoxazole	>2 (R)	>2 (R)
Tigecycline	0.5	0.5
Amikacin	<4 (S)	<4 (S)
Gentamicin	2 (S)	2 (S)
Colistin	<0.5 (S)	1 (S)
Nalidixic acid	>8	>8
Ciprofloxacin	0.5 (S)	1 (S)
Levofloxacin	1 (S)	1 (S)
Resistance profiles		
Outer membrane porins	OmpK35 (−), OmpK36 (−)	OmpK35 (−), OmpK36 (+)
Class I integron	*dfrA12*-orfF-*aadA2*	*dfrA12*-orfF-*aadA2*
PMQR[b] genes	*qnrB2*	*qnrB2*

[a] MICs were determined by the agar dilution method. R, resistant; S, susceptible; I, intermediate.
[b] PMQR, plasmid-mediated quinolone resistance genes, including *qnrA*, *qnrB*, *qnrS*, *qepA*, *aac(6′)-Ib*-cr, *armA*, and *rmtB*.

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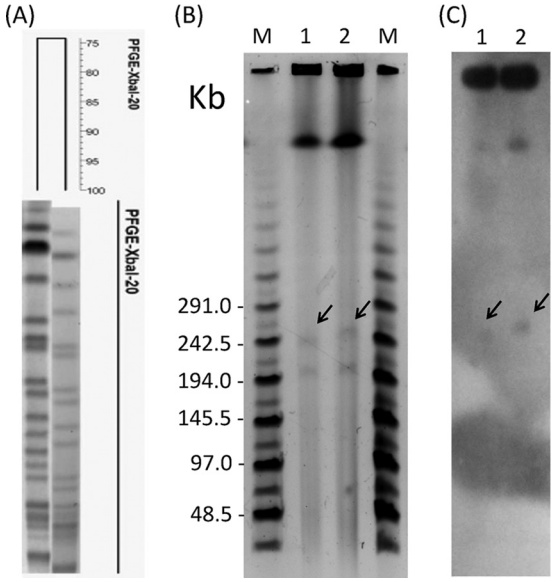

FIG 1 PFGE analysis of *R. planticola*. (A) XbaI-digested chromosome fragments were separated by PFGE, and the dendrogram was produced by BioNumerics software. (B) S1-nuclease-digested plasmid profiles separated by PFGE. (C) S1-nuclease-digested plasmid profiles hybridized with a bla_{IMP-8} probe. Lanes M, MidRange II PFG marker; lanes 1, isolate 139; lanes 2, isolate 193. The arrows show the locations of bla_{IMP-8} genes.

trophoresis (PFGE) analysis revealed that these two isolates had more than 6 bands of difference and were regarded as having different banding patterns (Fig. 1A). Detection of carbapenemases (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{NMC}, bla_{SME}, bla_{SPM-1}, bla_{GIM-1}, bla_{SIM-1}, bla_{IMI}, bla_{GES}, and bla_{OXA-48}) and extended-spectrum β-lactamases (ESBLs) and AmpC genes (bla_{SHV}, bla_{TEM}, bla_{DHA}, bla_{CMY}, $bla_{CTX-M-G1}$, $bla_{CTX-M-G2}$, and $bla_{CTX-M-G9}$) was performed, and only bla_{IMP-8} was found in these two isolates (8, 9). Both isolates contained two plasmids (ca. 204 and 239 kb in isolate 139; ca. 207 and 249 kb in isolate 193, respectively) which were identified by S1-nuclease PFGE analysis and calculated by BioNumerics GelCompar software package (version 5.0; Applied Mathematics, Sint-Martens-Latem, Belgium) (Fig. 1B).

Although plasmid transfer assays included conjugation and electroporation, using *Escherichia coli* as the receptor (DH5α and J53) did not find the successful transformants. S1-nuclease PFGE combined with Southern blot hybridization showed that bla_{IMP-8} was located on the 239-kb plasmid of isolate 139 and the 249-kb plasmid of isolate 193 (Fig. 1C). This finding suggests that bla_{IMP-8}-containing plasmids were likely to be nonconjugative, and bla_{IMP-8} was possibly acquired by mobile elements. Both isolates carried class 1 integron cassette arrays harboring *dfrA12*-orfF-*aadA2* (1.8 kb). The *dfrA12* gene, coding for dihydrofolate reductase, confers resistance to trimethoprim. The *aadA2* gene, coding for aminoglycoside-3″-adenyltransferase, confers resistance to streptomycin and spectinomycin. This integron cassette array has been reported from different organisms and become globally disseminated (10). PCR and sequencing detection of plasmid-mediated quinolone resistance determinants, including *qnrA*, *qnrB*, *qnrS*, *qepA*, *aac(6′)-Ib*-cr, *armA*, and *rmtB*, found that both isolates contained only the *qnrB2* gene (11). This gene conferred low-level resistance to all quinolones and was possibly transferred by a plasmid (12). SDS-PAGE analysis of outer membrane proteins found that loss of OmpK35 was found in isolate 193 and loss of OmpK35/36 was found in isolate 139. Previous study has shown that a double deletion of OmpK35 and OmpK36 reduced the susceptibilities of meropenem and cefepime in *Klebsiella pneumoniae* (13). This might be the reason why isolate 193 had a higher cefepime MIC than did isolate 139 (Table 1).

To our knowledge, this is the first report of bla_{IMP-8} in *R. planticola*. In the past decade, bla_{IMP-8}-producing *K. pneumoniae* isolates have been reported in Taiwan (8, 9, 14). Previous studies have reported that carbapenemases (bla_{KPC}, bla_{OXA-48}, and $bla_{OXA-162}$) could be found in *R. planticola*, *R. ornithinolytica*, and other *Enterobacteriaceae* (2, 5, 6, 9, 15). The carbapenemase genes in *R. planticola* in any specific country can be found in carbapenem-resistant *Enterobacteriaceae*, especially *K. pneumoniae*, in that country (Table 2). Our results identified a plasmid-located bla_{IMP-8} gene in *R. planticola* clinical isolates and suggested a possible association with bla_{IMP-8}-harboring *K. pneumoniae* in Taiwan.

TABLE 2 Correlation of carbapenem-resistant genes in *Raoultella* spp. and *Enterobacteriaceae* by country

Report	Carbapenemase(s)	Microorganism(s)	Country
This study	bla_{IMP-8}	*R. planticola*	Taiwan
Ma et al. (9)	bla_{IMP-8}	*K. pneumoniae*	Taiwan
Castanheira et al. (2)	bla_{KPC-2}, bla_{KPC-3}	*R. planticola*, *R. ornithinolytica*	United States
Chiang et al. (15)	bla_{KPC-2}	*K. pneumoniae*	United States
Österblad et al. (5)	bla_{OXA-48}	*R. planticola*, *E. coli*, *K. pneumoniae*	Finland
Pfeifer et al. (6)	$bla_{OXA-162}$	*R. ornithinolytica*, *E. coli*, *Citrobacter freundii*	Germany

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