Case Report

Extensively drug-resistant *Raoultella planticola* carrying multiple resistance genes including *bla_{NDM-1}*

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Introduction: *Raoultella planticola* is an uncommon pathogen rarely associated with clinical infections. Previous studies showed that most *R. planticola* isolates were sensitive to cephalosporins, aminoglycosides, fluoroquinolones and carbapenems, and no extensively drug-resistant (XDR) strains have been reported.

Case presentation: In this study, an XDR *R. planticola* strain, RP01, was isolated from a Chinese patient with multiple chronic diseases. Antimicrobial susceptibility testing showed that RP01 was resistant to almost all clinically available antibiotics except tigecycline. PCR and sequencing revealed the presence of *bla_{NDM-1}*, encoding a metallo- β -lactamase with hydrolysing activity against carbapenems. Further genomic sequencing and ResFinder analysis identified 20 resistance genes, encoding resistance to β -lactams, aminoglycosides, sulfonamides, tetracycline, fluoroquinolones, trimethoprim and fosfomycin.

Conclusion: These results described the ability of the rare pathogen *R. planticola* to acquire multiple resistance genes.

Keywords: *Raoultella planticola*; *bla_{NDM-1}*; extensively drug-resistant; tigecycline.

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Introduction

Raoultella planticola is a Gram-negative anaerobic oxidase-negative non-motile bacillus belonging to family *Enterobacteriaceae* (Drancourt *et al.*, 2001). This bacterium used to be considered an environmental organism residing in water and soil. However, an increasing number of cases of *R. planticola* infection, including bacteraemia (Hu *et al.*, 2012), soft tissue infection (O'Connell *et al.*, 2010), pancreatitis (Alves *et al.*, 2007) and urinary tract infection (Olson *et al.*, 2012), have recently been reported. Gastrointestinal and oropharyngeal colonization of the bacterium has also been described in adults and newborns (Podschun *et al.*, 1998; Toivanen *et al.*, 1999). Although *bla_{KPC}*-producing strains with a multi-drug resistance phenotype have been reported (Castanheira *et al.*, 2009), most *R. planticola* isolates were sensitive to cephalosporins, aminoglycosides, fluoroquinolones and carbapenems. In this study, we report the first extensively drug-resistant (XDR) *R. planticola* clinical isolate expressing the *bla_{NDM-1}* gene, with resistance to almost all clinically available

antibiotics except tigecycline and colistin; the resistance gene profiles were analysed by high throughput sequencing and ResFinder analysis.

Case report

On 26 May 2012, an 82-year-old male patient was admitted to the First Affiliated Hospital of Guangzhou Medical University (FAHG) in Guangzhou, China, because of cough and shortness of breath for 10 days. He had also suffered chronic obstructive pulmonary disease, coronary disease and hypertension. A sputum sample from the patient was cultured and grew a Gram-negative bacillus with a bacterial count of 10^7 c.f.u. ml⁻¹. By using the Vitek2 Biochemical Identification system, the isolate was identified as *R. planticola* with a 99 % probability. PCR and DNA sequencing of the 16S rRNA gene confirmed the biochemical identification results. The isolate was designated RP01 and antimicrobial susceptibility testing was performed by Vitek2 Automated System and Etest (bioMérieux). The results were interpreted according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2012). RP01 exhibited an XDR phenotype. It was resistant to all β -lactams,

Abbreviations: FAHG, First Affiliated Hospital of Guangzhou Medical University; XDR, extensively drug-resistant

aminoglycosides, sulfonamides, tetracycline, fluoroquinolones, trimethoprim and fosfomycin (Table 1). The isolate was susceptible to tigecycline and colistin with MIC values of 2 and 0.125 µg ml⁻¹, respectively. After comprehensive analysis of other examination results, including normal body temperature, normal white blood cell count, and slightly elevated levels of serum procalcitonin, the responsible physicians felt the clinical scenario was more consistent with colonization rather than infection. The patient received symptomatic treatment for 2 weeks, which alleviated his respiratory distress. Further specific treatment for coronary disease was then carried out and the patient was discharged after 32 days.

Investigations

Although the modified Hodge test for RP01 showed a negative result, it was positive by the imipenem/EDTA double-disc synergy test, indicating the presence of metallo-β-lactamase genes. Multiplex PCR detection for 11 acquired carbapenemase genes was carried out as described previously (Poirel *et al.*, 2011) and identified the *bla*_{NDM-1} gene in RP01. The PCR product was further sequenced to confirm the *bla*_{NDM-1} gene sequence. RP01 was the first clinical isolate producing *bla*_{NDM-1} identified in FAHG, and no *bla*_{NDM-1}-positive strains were detected from other hospitalized patients during the patient's stay.

Attempts to transfer the *bla*_{NDM-1} gene to donor strain *E. coli* J53 by conjugation failed. To investigate the molecular mechanism for the XDR phenotype, high-throughput

DNA sequencing was carried out by the Ion Torrent personal genome machine (Life Technologies). Total DNA of RP01 was prepared by DNA extraction kits (Qiagen) and sequenced following the manufacturer's protocols. A total of 3 294 078 reads with a mean length of 199 bp were obtained, which represented about 79 × coverage data for the RP01 genome. *De novo* assembly was performed using the MIRA assembler (version 3.4.0.1) and 170 large contigs (>500 bp) were obtained. Antibiotic-resistant genes were analysed in the ResFinder database (Zankari *et al.*, 2012) using the assembled contigs. A total of 20 resistance genes were identified on different contigs, including 5 for β-lactam resistance (*bla*CTX-M-3, *NDM-1*, *OXA-30*, *SHV-12* and *TEM-122*), 4 for aminoglycoside resistance [*aac*(6')*Ib-cr*, *aadA16*, *aadA5* and *armA*], 3 for macrolide resistance [*mph*(A), *mph*(E) and *msr*(E)], 2 for quinolone resistance [*aac*(6')*Ib-cr* and *qnrB6*], 2 for trimethoprim resistance (*dfrA1* and *dfrA27*), 1 for fosfomycin resistance (*fosA*), 1 for phenicol resistance (*catB3*), 1 for rifampicin resistance (*ARR-3*), 1 for sulfonamide resistance (*sul1*) and 1 for tetracycline resistance (*tetD*). The presence of *aac*(6')*Ib-cr* contributes to both aminoglycoside and quinolone resistance (Strahilevitz *et al.*, 2009). The *bla*_{NDM-1} gene was located on a 5.8 kb contig with 100 % sequence homology to the counterparts of pNDM-HN380, an IncX3 plasmid isolated from *Enterobacteriaceae* strains carrying *bla*_{NDM-1} in China (Ho *et al.*, 2012). Alignment of pNDM-HN380 sequence to the sequencing reads identified all functional regions for the plasmid (data not shown), suggesting that the gene environment of *bla*_{NDM-1} in RP01 was likely identical to pNDM-HN380, a resistance plasmid distributed in *Enterobacteriaceae* isolates in China (Ho *et al.*, 2012).

Discussion

Clinical isolates carrying *bla*_{NDM-1} have been associated with serious infection and high mortality, and the co-production of *bla*_{NDM-1} with other resistance genes always leads to the failure of antimicrobial treatment. Based on high throughput genome sequencing and ResFinder resistance gene analysis, the current report presents a description of a *bla*_{NDM-1} gene co-harboured with other 19 resistance genes in the rare pathogen *R. planticola*, highlighting the ability of *bla*_{NDM-1} to spread to unusual pathogens.

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Table 1. Antimicrobial susceptibility of *R. planticola* strain RP01

Antibiotic(s)	MIC (µg ml ⁻¹)	Interpretation*
Imipenem	≥16	R
Meropenem	16	R
Ertapenem	≥8	R
Ampicillin	≥32	R
Ampicillin/sulbactam	≥32	R
Piperacillin/tazobactam	≥128	R
Ceftriaxone	≥64	R
Ceftazidime	≥64	R
Cefepime	≥64	R
Amikacin	≥64	R
Gentamicin	≥16	R
Tobramycin	≥16	R
Ciprofloxacin	≥4	R
Levofloxacin	≥8	R
Fosfomycin	≥1024	R
Rifampicin	≥32	R
Tetracycline	≥16	R
Tigecycline	2	S
Colistin	0.125	S

*MIC results were interpreted by CLSI standards for *Enterobacteriaceae*, 2012.

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