Combination of Tigecycline and Levofloxacin for Successful Treatment of Nosocomial Pneumonia Caused by New Delhi Metallo- β -Lactamase-1-Producing *Raoultella planticola*

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Raoultella planticola is a gram-negative bacterium that rarely causes diseases in humans. Here, we present a case of hospital-acquired pneumonia caused by *R. planticola* that likely originated in the gastrointestinal tract. To the best of our knowledge, this is the second report describing the detection of the gene New Delhi Metallo- β -lactamase-1 (*bla*_{NDM-1}) in multidrug-resistant *R. planticola*. Clinical samples were collected for bacterial culture and antimicrobial susceptibility testing from a patient during hospitalization. The presence of *bla*_{NDM-1} was detected by PCR and sequencing. An NDM-1-positive *R. planticola* was isolated from the sputum and stool of the same patient. Further findings confirmed that *bla*_{NDM-1} was located on a plasmid. Isolates from the sputum and stool cultures were identical, suggesting that the *R. planticola* may have originated in the gastrointestinal tract. The patient completely recovered and was discharged after treatment with tigecycline combined with levofloxacin, for a week. In conclusion, *R. planticola* is a possibly underestimated pathogen that contributes to the spread of the *bla*_{NDM-1} gene. Early and precise identification of this pathogen can lead to better prognosis of the associated infections and an improved approach to controlling the spread of carbapenemase-resistant gram-negative bacteria.

Keywords: multi-drug resistance, NDM-1, pneumonia, *Raoultella planticola*

Introduction

RAOULTELLA PLANTICOLA, ORIGINALLY known as *Klebsiella planticola*, was first described by Bagley *et al.* as a group of organisms isolated primarily from botanical and aquatic environments.¹ The phylogenetic analyses of *Klebsiella* species were performed in 2001 by Drancourt *et al.*, after which the term *K. planticola* was replaced by *R. planticola* based on the comparative analysis of the 16S rRNA and *rpoB* genes.² *R. planticola* is a gram-negative, aerobic, oxidase-negative, nonmotile, and encapsulated bacterium commonly found in water, soil, and the aquatic environment. Occasionally, it is also found in mammalian mucosae. The first invasive infection caused by *Raoultella* spp. in humans was reported by Freney *et al.*³ More than 20 cases have been reported thereafter, and although carbapenem resistance was rarely observed, these cases had much poorer outcomes than the cases in which the infections were caused by bacteria that were susceptible to carbapenem strains. In this study, we report a

case of pneumonia associated with a New Delhi Metallo- β -lactamase-1 (NDM-1)-producing *R. planticola*. The patient recovered completely after receiving a combination therapy of tigecycline and levofloxacin.

Case Presentation

This study was performed in China, with approval from the Ethics Committee of Zhejiang University. Written informed consent was obtained from the patient not only for sampling but also for publication of the case. Medical records were reviewed for administration of antimicrobial therapy and determination of patient outcome.

The patient was a 74-year-old man from a village in Zhejiang Province. On June 16, 2014, the patient was admitted to the hospital for fever and cough. He was hospitalized for 25 days and discharged on July 11, 2014. He underwent subtotal gastrectomy and Billroth I anastomosis for perforation of a duodenal ulcer and acute peritonitis since 4 weeks. His

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sputum and stool were sampled for isolation of bacteria and for antimicrobial susceptibility testing. Carbapenem-resistant isolates were collected and investigated further.

Species identification was performed using the Micro-Scan WalkAway 40 S1 identification system (Dade Behring, Deerfield, IL). The 16s rDNA sequences were also analyzed to confirm the species identity. Susceptibility of the isolates to antimicrobial therapy was determined using the E-test methodology (BioMerieux SA, La Balme-les-Grottes, France). The modified Hodge test was performed to screen for carbapenemases. These results were interpreted according to the 2014 Clinical and Laboratory Standards Institute recommendations and guidelines. *R. planticola* isolates from the sputum and stool samples were subjected to pulsed-field gel electrophoresis (PFGE), according to previously reported protocols.⁴ The location of *bla*_{NDM-1} was determined using the PFGE-S1 nuclease method. Separation of large fragments after restriction enzyme digestion was performed using PFGE, using a Bio-Rad CHEF Mapper XA system (Bio-Rad, CA) with an initial switch time of 2.16 sec and a final switch time of 63.8 sec for a run time of 18 hr. Southern hybridization was performed with a 560-bp segment of the *bla*_{NDM-1} gene as a probe, using the Roche digoxigenin labeling and NBT/BCIP coloration system (Roche Biochemicals, Mannheim, Germany). Conjugation experiments were carried out by solid surface methods, with sodium azide-resistant *E. coli* J53 as the recipient. Transconjugants were selected on Luria-Bertani agar plates containing sodium azide (100 mg/L) and ampicillin (100 mg/L), and then confirmed by PCR amplification of *bla*_{NDM-1} for NDM-1-positive donors and *bla*_{OXA-30} gene for NDM-1-negative donors.

On admission, the patient was coughing up large amounts of sputum and had high fever. Computed tomography (CT) imaging revealed pulmonary infiltration and pleural effusion in both sides of the chest. The patient was empirically treated with broad-spectrum antibiotics (4,500 mg of piperacillin/tazobactam Q8H) and expectorants before evaluation for pneumonia. Four days later, the results of sputum culture remained positive for *R. planticola*, which showed intermediate resis-

tance to levofloxacin, but complete resistance to ampicillin, ceftriaxone, ceftazidime, trimethoprim-sulfamethoxazole, and several other antimicrobial agents (Table 1). Based on the clinical manifestations of the patient and the results of laboratory examinations, piperacillin/tazobactam was replaced by 500 mg of levofloxacin QD. After 5 days of treatment, the symptoms had not alleviated and the body temperature of patient was fluctuating. Subsequent samples of stool and sputum continued to show *R. planticola* isolates that were susceptible to tigecycline. Therefore, 50 mg of tigecycline Q12H was added intravenously (double for the first dose) in combination with levofloxacin. The overall symptoms of the patient improved along with reduced sputum production and normal body temperature 3 days later. After treatment for a week, the neutrophil count of the patient normalized and his C-reactive protein levels decreased. CT imaging revealed significant clearing of the chest, and the patient was eventually discharged with relieved symptoms and a stable body temperature. CT imaging performed 2 weeks

TABLE 1. ANTIBIOGRAM OF *RAOULTELLA PLANTICOLA* ISOLATED FROM SPUTUM CULTURE

Antimicrobial agents	MIC (μg/ml)
Ampicillin	≥32
Ampicillin/sulbactam	≥32
Amikacin	4
Aztreonam	≥64
Ciprofloxacin	≥4
Cefotetan	≥64
Ceftriaxone	≥64
Cefazolin	≥64
Nitrofurantoin	128
Gentamicin	≤1
Imipenem/cilastatin	≥16
Levofloxacin	4
Trimethoprim-sulfamethoxazole	≥320
Ceftazidime	≥64
Tobramycin	8
Tigecycline	1
Cefepime	16

FIG. 1. PFGE analysis of *Raoultella planticola* shows (A) *Xba*I-digested chromosome fragments separated by PFGE; (B) S1-nuclease-digested plasmid profiles separated by PFGE; and (C) S1-nuclease-digested plasmid profiles hybridized with a *bla*_{NDM-1}-specific probe. Lane M: PFG marker; lane 1: sample from the sputum; lane 2: sample from the stool. The arrows show the locations of *bla*_{NDM-1} genes. PFGE, pulsed-field gel electrophoresis.

TABLE 2. SUMMARY OF PREVIOUS CASE REPORTS

<i>Author (reference)</i>	<i>Age (y)/gender</i>	<i>Clinical diagnosis</i>	<i>Comorbidity</i>	<i>Invasive procedure</i>	<i>Treatment</i>	<i>Outcome</i>
Freney <i>et al.</i> ³	47/Male	Bacteremia	Mitral stenosis, infective endocarditis	Mitral valve replacement	Cefotaxime, tobramycin	Recovered
Freney <i>et al.</i> ⁵	69/NA	Bacteremia	Mitral stenosis, infective endocarditis	Mitral valve replacement	Cefotaxime, tobramycin	Recovered
Freney <i>et al.</i> ⁵	57/NA	Pneumonia, sepsis	Coronary artery disease	Postcoronary artery bypass graft	Cefotaxime	Recovered
Alves <i>et al.</i> ²¹	45/Male	Pancreatitis and retroperitoneal abscess	Alcoholism, pneumonia	Exploratory laparotomy	Imipenem, amikacin	Recovered
Castanheira <i>et al.</i> ⁶	83/Female	Pneumonia	None	NA	None	Death
Castanheira <i>et al.</i> ⁶	64/Male	Bacteremia	B lymphoblastic leukemia	Surgical abscess debridement	Daptomycin	Death
O'Connell <i>et al.</i> ¹³	30/Male	Cellulitis	None	Hammer crush injury	Benzylpenicillin, flucloxacillin, clindamycin, ciprofloxacin	Recovered
Wolcott <i>et al.</i> ¹⁴	66/Male	Surgical site infection	Coronary artery disease, hypertension, hyperlipidemia	Open reduction internal fixation	Ertapenem	Recovered
Yokota <i>et al.</i> ⁹	65/Male	Cholangitis, bacteremia	Adenocarcinoma of the neck	ERCP	Meropenem, piperacillin/tazobactam	Recovered
Hu <i>et al.</i> ¹⁰	59/Male	Cholangitis, bacteremia	Pancreatic carcinoma	ERCP	Piperacillin/tazobactam	Recovered
Kim <i>et al.</i> ¹⁵	66/Male	Necrotizing fasciitis	Coronary artery disease, diabetes	Abdominal trauma	Ceftriaxone, levofloxacin, tigecycline	Recovered
Teo <i>et al.</i> ¹⁶	62/Female	Cholecystitis	Cellac disease, IBD	None	Co-amoxiclav, surgery	Recovered
Olson <i>et al.</i> ¹⁸	89/Male	Cystitis	Coronary artery disease, hypertension	None	Ciprofloxacin	Recovered
Puerta-Fernandez <i>et al.</i> ⁷	63/Male	Bacteremia, gastroenteritis	Posterior pituitary adenoma	None/Ate fish	Cefotaxime	Recovered
Tseng <i>et al.</i> ⁸	77/Male	Pneumonia, septic shock	Nonsmall-cell lung cancer	None	Levofloxacin, meropenem, colistin	Death
Tseng <i>et al.</i> ⁸	57/Male	Catheter-related bacteremia	Nonsmall-cell lung cancer, chemotherapy	Catheterization	Ceftazidime, levofloxacin, gentamicin	Recovered
Zuberbuhler <i>et al.</i> ²²	58/Female	Bacterial conjunctivitis	None	None	Chloramphenicol	Recovered
Ershadi <i>et al.</i> ¹⁷	49/Male	Cholecystitis, severe sepsis	Alcohol abuse, alcoholic cirrhosis, diabetes	None	Vancomycin, piperacillin/tazobactam, tigecycline	Recovered
Lam <i>et al.</i> ¹¹	56/Female	Cholangitis	Nonsmall-cell lung cancer, chemotherapy	Seafood consumption	Ceftriaxone, ciprofloxacin	Recovered
Koukoulaki <i>et al.</i> ¹⁹	67/Male	Cystitis, prostatitis	Immunoglobulin-A nephropathy	Renal transplant	Ciprofloxacin	Recovered
Salmaggi <i>et al.</i> ¹²	70/Male	Cholangitis, sepsis	Pancreatic adenocarcinoma, chemotherapy	Biliary stenting	Ciprofloxacin, metronidazole	Recovered
Xu <i>et al.</i> ⁴	60/Male	Pneumonia Acute myeloid leukemia	Septic shock and respiratory failure	None	Cefoperazone/sulbactam, imipenem/cilastatin, levofloxacin, tigecycline	Death
Yoon <i>et al.</i> ²⁰	16 m/Male	Cystitis	Rhabdomyosarcoma of the bladder neck,	None	Cefotaxime, ampicillin/sulbactam	Recovered

NA, not available.

after discharge revealed complete absorption and resolution of effusions. 16S rDNA sequence analysis confirmed that the isolated bacteria were *R. planticola*.

Discussion

To understand the mechanisms of carbapenem resistance, PCR and sequencing assays were performed to identify carbapenemase genes (*bla_{KPC}*, *bla_{IMP}*, *bla_{VIM}*, *bla_{NDM}*, and *bla_{OXA-48}*). The *bla_{NDM-1}* gene was identified in isolates from sputum and stool samples of the same patient. PFGE analysis of these isolates showed identical gel patterns, suggesting that they were derived from the same strain (Fig. 1A). S1-nuclease PFGE analysis showed that isolates from both the samples contained the same plasmid of ca. 50 kb (Fig. 1B). Furthermore, Southern blot hybridization confirmed that the *bla_{NDM-1}* gene was located on this plasmid (Fig. 1C).

In this report, we described a case of pneumonia caused by *R. planticola* in a 74-year-old man with a history of duodenal ulcer perforation and subtotal gastrectomy. The patient eventually recovered after receiving intravenous tigecycline and levofloxacin. After a thorough literature review, we found only 23 cases of *R. planticola* infections from 1984 to 2015 (Table 2). These included five cases of bacteremia,^{3,5-8} four cases of cholangitis,⁹⁻¹² three cases of soft tissue infections,¹³⁻¹⁵ four cases of pneumonia,^{4-6,8} two cases of cholecystitis,^{16,17} three cases of cystitis,¹⁸⁻²⁰ one case of pancreatitis,²¹ and one case of conjunctivitis.²² The ages of patients who developed a clinically relevant *R. planticola* infection ranged from 30 to 89 years. In addition, we found that patients in a large proportion of the reported cases had a history of recent trauma, namely surgery or invasive procedures (59.1%). Several patients also reported a history of tumors, recent chemotherapy, or immunosuppressive treatment (43.5%).

In the current case, the elderly patient had a history of gastroduodenal ulcer perforation, gastrectomy, and use of broad-spectrum antimicrobials. This factor may have led to an imbalance of the intestinal flora, which facilitated the emergence of multidrug-resistant bacteria. Laboratory tests showed that both the sputum culture and stool culture were NDM-1 positive for *R. planticola*, which was confirmed by the PFGE assay. This finding suggested a scenario of possible intestinal colonization of *R. planticola* in this patient. His pneumonia might be attributed to bacterial translocation or aspiration, accompanied by risk factors such as previous gastrointestinal operation, older age, a feeding tube procedure, or treatment with histamine H-2 antagonists.

To the best of our knowledge, this is the second reported case of *bla_{NDM-1}* being detected in *R. planticola*. The first case was reported in a *Klebsiella pneumoniae* strain that caused a urinary tract infection in a Swedish patient who had travelled to New Delhi, India, in 2009.²³ The resistance gene NDM-1 can be produced by various pathogens and can be transmitted via a plasmid to other members of the same species or to different bacterial species. The NDM-1 gene has been previously described in *E. coli*, *Enterobacter cloacae*, *Acinetobacter baumannii*, and *Citrobacter rodentium* across several countries. One study reported that five NDM-1 carbapenemase-producing isolates were identified from 186 carbapenem-resistant Enterobacteriaceae isolates recovered from our hospital between January and September 2013.²⁴ NDM-1-producing pathogens are resistant to ~200

kinds of antimicrobial agents. Antimicrobial susceptibility tests indicate that NDM-1 is somewhat sensitive to polymyxin and tigecycline, and that some strains are sensitive to aztreonam, gentamicin, or ciprofloxacin. It has been reported that polymyxin and tigecycline inhibit NDM-1 with ~80–90% efficiency.²⁵ Current research and meta-analysis of randomized controlled trials have suggested the use of combination therapy for a curative effect, but the mortality rate is not superior to that observed when monotherapy is used. However, an observational study of carbapenem-resistant *Klebsiella pneumoniae* indicated that the use of combination therapy appears to be associated with improved survival in bacteremia due to KPC-producing *K. pneumoniae*.²⁶ The patient in the current case recovered after receiving a combination therapy of tigecycline and levofloxacin, confirming the results of the susceptibility tests and the effectiveness of combination therapy.

In conclusion, we present a case of *bla_{NDM-1}* in *R. planticola*, which was cured by treatment with tigecycline. *R. planticola* is a rare and possibly underestimated pathogen in terms of its pathogenicity, and with potential gain of the *bla_{NDM-1}* gene, it might produce “superbugs” that can pose a threat to public health. Early detection of this pathogen is, therefore, critical to the selection of effective therapies.

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Disclosure Statement

No competing financial interests exist.

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