Detection of NDM-1 and OXA-10 Co-Producing Providencia rettgeri Clinical Isolate

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Background: The coexistence of \textit{bla}	extsubscript{NDM-1} with other resistance determinants is rarely reported for \textit{Providencia rettgeri}. Therefore, this study investigates the phenotypic and genetic characteristics of a multidrug-resistant \textit{P. rettgeri} strain YQ150713.

Methods: \textit{P. rettgeri} YQ150713 was identified as carrying \textit{bla}	extsubscript{NDM-1}. S1-pulsed-field gel electrophoresis (S1-PFGE), Southern blotting, and conjugation experiments were used to determine plasmid characteristics. An antimicrobial susceptibility test was conducted. The complete genomic sequence of YQ150713 was obtained using Illumina NovaSeq 6000 and Oxford nanopore platforms. To further characterize the phylogenetic structure of \textit{P. rettgeri} YQ150713, average nucleotide identity (ANI) and phylogenetic analyses were conducted.

Results: The S1-PFGE, Southern blot, and conjugation assays have confirmed that the isolate \textit{P. rettgeri} YQ150713 contains the \textit{bla}	extsubscript{NDM-1} gene on a conjugative plasmid pYQ150713-NDM-1. Antimicrobial susceptibility testing has indicated that strain YQ150713 was resistant to various common antibiotics, except aztreonam and fosfomycin. Bioinformatics analysis has further shown that pYQ150713-NDM-1 was a novel plasmid with a size of 265,883 bp, and \textit{bla}	extsubscript{NDM-1} and \textit{bla}	extsubscript{OXA-10} were co-located on it. Phylogenetic analysis suggesting \textit{P. rettgeri} has spread widely throughout the world.

Conclusion: In this study, \textit{bla}	extsubscript{NDM-1} and \textit{bla}	extsubscript{OXA-10} were co-localized on a novel plasmid pYQ150713-NDM-1 with a horizontal transfer function. To reduce the risk of the dissemination of such \textit{P. rettgeri} isolates in clinical settings, more surveillance will be required in the future.

Keywords: antimicrobial resistance, \textit{bla}	extsubscript{NDM-1}, \textit{bla}	extsubscript{OXA-10}, \textit{Providencia rettgeri}, carbapenemase-producing, average nucleotide identity, phylogenetic

Introduction

\textit{Providencia rettgeri} is a Gram-negative opportunistic human pathogenic bacterium that belongs to the Proteae bacteria. It is a pathogen commonly found in the hospitals that mainly cause urinary tract infections, pneumonia, bacteremia, neonatal sepsis, eye infections, meningitis, endocarditis, and diarrhea.\textsuperscript{1–3} The pathogen \textit{Providencia rettgeri} demonstrates inherent resistance to numerous antimicrobial agents, such as ampicillin, first-generation cephalosporins, polymyxins, and tigecycline, thereby posing significant challenges in the management of infections caused by this microorganism. Moreover, the escalating prevalence of multidrug-resistant (MDR) strains of \textit{P. rettgeri} poses a global threat to public health.\textsuperscript{4} MDR bacterial infections were widely treated with carbapenems. However, a number of carbapenemase-producing \textit{P. rettgeri} isolates have been found carrying carbapenem-resistant genes like \textit{bla}	extsubscript{NDM}, \textit{bla}	extsubscript{VIM}, and \textit{bla}	extsubscript{IMP-27}.\textsuperscript{5–8} A neonate was reported to have suffered from a late-onset case of neonatal sepsis caused by MDR \textit{P. rettgeri} that was resistant to ampicillin, polymyxins, and first-generation cephalosporins.\textsuperscript{9,10} In 2014, \textit{P. rettgeri} was
first reported to produce NDM-1 in China. However, the detection rate of NDM-1 in *P. rettgeri* isolates continues to increase around the world. As far as we know, *P. rettgeri* carrying *bla*<sub>NDM-1</sub> has been reported in Israel, Brazil, Argentina, and Colombia.6,12–15

The existence of *bla*<sub>NDM-1</sub> within a bacterial isolate poses a significant challenge for clinical therapeutics. Nevertheless, the challenge becomes even more formidable when it coexists with other resistance determinants, as this leads to a more pronounced restriction of available treatment options. There are few reports about *P. rettgeri* coexisting *bla*<sub>NDM-1</sub> with other resistance determinants. A study by Piza-Buitrago et al6 reported the coexistence of β-lactamase genes including *bla*<sub>NDM-1</sub>, *bla*<sub>VIM</sub>-2, and *bla*<sub>CTX-M</sub>-15, *bla*<sub>OXA-10</sub>, *bla*<sub>CMY-2</sub> and *bla*<sub>TEM-1</sub> in *P. rettgeri* isolates from two patients in Colombia. The objective of this study was to assess the co-harboring of *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-10</sub> in *P. rettgeri* isolated from a patient with pulmonary infection in China. Furthermore, the significance of continuous surveillance of β-lactamase-carrying *P. rettgeri* strains in clinical settings was underscored.

**Materials and Methods**

**Strain Collection and Species Identification**

Strain YQ150713 was obtained from a 79-year-old patient admitted to a tertiary hospital in Zhejiang province, China with pulmonary infection. The collected sputum sample was plated on Columbia Blood agar plate (Autobio, China) and identification of isolates by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Bruker, Bremen, Germany). As described previously, *bla*<sub>NDM-1</sub> gene were detected by PCR and Sanger sequencing.16

**S1-PFGE, Southern Blot Hybridization and Conjugation Experiments**

The size and number of *P. rettgeri* YQ150713 plasmids were identified by S1-pulsed-field gel electrophoresis (S1-PFGE). The location of *bla*<sub>NDM-1</sub> was detected by Southern blotting and hybridization with DIG-labeled *bla*<sub>NDM</sub>-specific probe. To verify plasmid transferability, a conjugation experiment was conducted using rifampicin-resistant *E. coli* 600 and J53 as recipients.17 The transconjugants were selected on Mueller-Hinton agar (OXOID, Hampshire, UK) medium containing both 200 mg/L rifampin/sodium azide and 2 mg/L meropenem. Finally, a combination of MALDI-TOF-MS and PCR was conducted to ensure successful transfer of the plasmid to the recipient strain.

**Antibiotic Sensitivity Test**

Broth microdilutions were used to determine the susceptibility of *P. rettgeri* YQ150713 and transconjugants to antibiotics. The tested antibiotics were β-lactams (amoxicillin/clavulanic acid, aztreonam, ceftazidime, ceftriaxone, cefepime, cefotaxime, imipenem, meropenem and piperacillin/tazobactam), fluoroquinolones (levofloxacin, ciprofloxacin), trimethoprim/sulfamethoxazole, aminoglycosides (amikacin, gentamicin), fosfomycin. The interpretation of results was based on Clinical and Laboratory Standards Institute (CLSI) guidelines (https://clsi.org). The quality control strains were *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

**Whole Genome Sequencing (WGS) and Bioinformatics Analysis**

Genomic DNA extraction was performed using a commercial kit (QIAGEN, Gentra Puregene Yeast/Bact.Kit, Germany) following the manufacturer’s instructions. Short and long reading data were then obtained from DNA sequencing on the Illumina NovaSeq 6000 (Illumina, San Diego, California, United States) and Oxford nanopore platforms (Oxford nanopore technologies, Oxford, United Kingdom). The Unicycler v0.4.7 software was utilized for hybrid assembly in order to obtain the complete genome sequence of YQ150713.18 Subsequently, Prokka was employed to annotate the genome. ResFinder and PlasmidFinder databases were used to identify the antimicrobial resistance genes (ARGs) and replicon type of plasmids.19 Finally, we employed the BLAST Ring Image Generator (BRIG) to generate a circular representation of the plasmid, while Easyfig was utilized to produce linear comparison figures of the genetic environment encompassing the *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-10</sub> genes.20,21
Phylogenetic Analysis

To further characterize the phylogenetic structure of NDM-1-producing *P. rettgeri*, 218 genomes of *P. rettgeri* isolates were downloaded from the National Center for Biotechnology Information (NCBI) Genome database as of March, 2022 to perform coregenome analysis. Strains’ average nucleotide identity (ANI) was analyzed by Pyani to eliminate confounding strains. Species were determined using Pyani with default settings if the genome had an ANI value greater than 95% ([https://github.com/widdowquinn/pyani](https://github.com/widdowquinn/pyani)). Roary was then used to perform phylogenetic analyses on 80 *P. rettgeri* isolates and 4 *Providencia* sp. (NCTC10286-*Providencia alcalifaciens*, NCTC6933-*Providencia rustigianii*, NCTC12003-*Providencia heimbachae*, ATCC33672-*Providencia stuartii*). Finally, MEGA X was used to generate a maximum likelihood phylogenetic tree and an Interactive Tree of Life ([https://itol.embl.de/](https://itol.embl.de/)) was used to modify the visualizations of the phylogenetic tree.

Results

Antimicrobial Susceptibility Profiles

In this study, a total of 15 antibiotics were tested (Table 1). The results revealed that isolate YQ150713 demonstrated resistance to various antibiotics, including amoxicillin/clavulanic acid (MIC>128mg/L), piperacillin/tazobactam (MIC=64mg/L), ceftazidime (MIC>128mg/L), ceftriaxone (MIC=128mg/L), cefepime (MIC=16mg/L), cefotaxime (MIC=128mg/L), ciprofloxacin (MIC=64mg/L), levofloxacin (MIC=32mg/L), imipenem (MIC=16mg/L), meropenem (MIC=4mg/L), trimethoprim/sulfamethoxazole (MIC>152mg/L), amikacin (MIC>128mg/L), gentamicin (MIC>128mg/L). However, it was sensitive to aztreonam and fosfomycin. Furthermore, the transconjugants YQ150713-NDM-EC600 and YQ150723-NDM-J53 also demonstrated resistance to imipenem and meropenem. YQ150713-NDM-EC600 was sensitive to ciprofloxacin and levofloxacin, while YQ150723-NDM-J53 was sensitive to cefepime and levofloxacin.

Characteization of Plasmid pYQ150713-NDM-1

The YQ150713 isolate was found to possess a plasmid of approximately 260kb in size, carrying the *bla*NDM-1 gene, as revealed by the results obtained from S1-PFGE, Southern blotting, and hybridization techniques. This particular plasmid was assigned the designation pYQ150713-NDM-1. (Figure 1). The presence of *bla*NDM-1 in transconjugants was

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg/L)</th>
<th>YQ150713</th>
<th>YQ150713-NDM-EC600</th>
<th>YQ150723-NDM-J53</th>
<th>J53</th>
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</thead>
<tbody>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>64/R</td>
<td>64/R</td>
<td>2/S</td>
<td>64/R</td>
<td>2/S</td>
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<td>Ceftazidime</td>
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<td>&gt;128/R</td>
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<td>&gt;128/R</td>
<td>0.25/S</td>
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<tr>
<td>Ceftriaxone</td>
<td>128/R</td>
<td>&gt;128/R</td>
<td>0.125/S</td>
<td>&gt;128/R</td>
<td>0.125/S</td>
</tr>
<tr>
<td>Cefepime</td>
<td>16/R</td>
<td>16/R</td>
<td>0.06/S</td>
<td>0.5/S</td>
<td>0.03/S</td>
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<tr>
<td>Cefotaxime</td>
<td>128/R</td>
<td>&gt;128/R</td>
<td>0.125/S</td>
<td>&gt;128/R</td>
<td>0.06/S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>64/R</td>
<td>0.25/S</td>
<td>0.03/S</td>
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<td>Levofloxacin</td>
<td>32/R</td>
<td>0.5/S</td>
<td>0.5/S</td>
<td>0.06/S</td>
<td>0.06/S</td>
</tr>
<tr>
<td>Imipenem</td>
<td>16/R</td>
<td>8/R</td>
<td>0.25/S</td>
<td>4/R</td>
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</tr>
<tr>
<td>Meropenem</td>
<td>4/R</td>
<td>4/R</td>
<td>0.03/S</td>
<td>4/R</td>
<td>0.03/S</td>
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<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>&gt;152/R</td>
<td>&gt;152/R</td>
<td>≤2.375/S</td>
<td>&gt;152/R</td>
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<tr>
<td>Gentamicin</td>
<td>&gt;128/R</td>
<td>&gt;128/R</td>
<td>2/S</td>
<td>&gt;128/R</td>
<td>2/S</td>
</tr>
<tr>
<td>Aztreonam</td>
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<td>0.5/S</td>
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<td>0.5/S</td>
<td>0.5/S</td>
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Notes: Broth microdilutions were used to determine the susceptibility of *P. rettgeri* YQ150713 and transconjugants to antibiotics. The interpretation of results was based on Clinical and Laboratory Standards Institute (CLSI) guidelines ([https://clsi.org](https://clsi.org)). The quality control strains were E. coli ATCC 25922 and *P. aeruginosa* ATCC 27853.

Abbreviations: S, susceptible; R, resistant; I, intermediate.
Figure 1 The identification of plasmid size using S1-PFGE (left) and southern blot and hybridization (right). (M) XbaI digested total DNA of Salmonella enterica serotype Braenderup H9812 as a size marker. Southern blotting hybridization with a bla<sup>NDM</sup>-specific probe.
confirmed by PCR and Sanger sequencing. WGS revealed that bla<sub>NDM-1</sub> and bla<sub>OXA-10</sub> were co-localized on the plasmid pYQ150713-NDM-1 with a size of 265, 883 bp. Interestingly, a comparison of the full plasmid BLAST showed that pYQ150713-NDM-1 had very low similarity to other plasmids (Figure 2), indicating that it is a novel bla<sub>NDM</sub>-carrying plasmid. The bla<sub>NDM-1</sub> and bla<sub>OXA-10</sub> genes were found to be co-located on a Tn3 transposon element in pYQ150713-NDM-1 with the following linear structure (Figure 3): TnAs2-hin-xerC-dhfrI-ORF-bla<sub>OXA-10</sub>-ant1-emrE-ORF-ORF-trpF-ble-bla<sub>NDM-1</sub>-cat-ORF-emrE-ORF-ORF-ISEc35. NCBI BLAST analysis revealed that the Tn3 transposon element environment of pYQ150713-NDM-1 was genetic similarity with Proteus mirabilis plasmid pDY.F1.2 (CP046050), Proteus mirabilis plasmid pSNYG35 (CP047590), and Escherichia coli plasmid pEC8-NDM-1(CP060954).

**Strain Identification and Phylogenetic Analysis**

After ANIb analysis based on BLAST, strain YQ150713 was finally identified as *P. rettgeri* (Figure S1). According to the result of ANIb analysis, 80 strains downloaded from NCBI assemble database and were used together with YQ150713 for phylogenetic analysis (Figure 4, Table S1). All specimens were obtained from a clinical setting. The results illustrated that the 81 strains aggregated into three clusters, namely cluster I, cluster II, cluster IIIa, cluster IIIb (Figure 5). YQ150713 was belonged to cluster II, and it was close to GCA 006351125.1, GCA 016512695.1, GCA 020564615.1, GCA 021869825.1, and GCA 020683065.1. Among them, GCA 006351125.1 was collected from patient’s urine in

![Image](https://doi.org/10.2147/IDR.S418131)

Figure 2 Circular map of the pYQ150713-NDM-1 plasmid. The outer circle showed the genes of pYQ150713-NDM-1. The two inner circles were GC content and GC skew of pYQ150713-NDM-1. The plasmid was visualized by BLAST ring image generator (BRIG) software.
Sichuan province, China, GCA 016512695.1 was collected from a COVID-19 patient’s blood in the USA. GCA 020564615.1, GCA 021869825.1, and GCA 020683065.1 were from Bogota, Colombia, with two of them were collected from urine on April, 2019, suggesting *P. rettgeri* spread widely in the world.

**Discussion**

This study presents a description of a clinical strain of *P. rettgeri* in China, which carries a novel *bla*NDM-1 plasmid named pYQ150713-NDM-1. The plasmid co-localized β-lactamase genes *bla*NDM-1 and *bla*OXA-10.

Besides being found in environmental settings, *P. rettgeri* species have also been reported as opportunistic human pathogens in clinic. The first isolation of *bla*NDM-1 producing *P. rettgeri* was reported in Israel in 2013. Since then, *bla*NDM-1 producing *P. rettgeri* has been reported worldwide, including Asia (Israel, India, Korea, Nepal, China, Pakistan), South America (Brazil, Colombia, Argentina, Ecuador), North America (Mexico), South Africa (Nigeria) and South East Europe (Bulgaria). Most *bla*NDM genes are present in conjugative plasmids while the characteristics of a host plasmid can differ greatly in terms of size, incompatibility group, gene content, and organization. A previous study reported that *bla*NDM is carried in a great variety of plasmids belonging to diverse Inc groups (FII, FIB, A/C2, HI1A, HI1B, L/M, N, N2, X3, R, T as well as unclassified plasmids). In this study, the PlasmidFinder tool was employed to determine the compatibility group of the plasmid. However, the plasmid under investigation could not be classified into any known incompatibility group, suggesting that the plasmid type of pYQ150713-NDM-1 is novel. Interestingly, there was another β-lactamase gene *bla*OXA-10 located on pYQ150713-NDM-1 plasmid. The antimicrobial resistance tests indicated that strain YQ150713 exhibited resistance to a wide spectrum of antimicrobials, which was found to be in concordance with the presence of acquired antibiotic resistance genes in its genomic composition. This implies that the presence of genetically resistant genes in pYQ150713-NDM-1 could significantly contribute to the multidrug resistance observed in isolate YQ150713. The results of conjugation experiments showed that *bla*NDM-1 was successfully transmitted from *P. rettgeri* YQ150713 to *E. coli* 600 and *E. coli* J53. The pYQ150713-NDM-1 plasmid has horizontal metastatic ability and is capable of expressing drug resistance. These results indicated that the transferability of the plasmid increases the risk of drug-resistant bacteria and poses a great challenge to clinical treatment.

Strain YQ150713 demonstrated resistance to the carbapenems imipenem and meropenem, likely attributable to the presence of *bla*NDM-1 and *bla*OXA-10 genes. While Shen et al and Piza-Buitrago et al reported the co-existence of

![Figure 3](https://doi.org/10.2147/IDR.S418131)

**Figure 3** Comparison of genes surrounding *bla*NDM-1 on pYQ150713-NDM-1, pDY.F1.2 (CP046050), pSNYG35 (CP047590) and pEC8-NDM-1(CP060954). Open reading frames (ORFs) are represented by arrows and colored in accordance with their putative functions: red arrows indicate antimicrobial resistance genes *bla*NDM-1 and *bla*OXA-10. Regions with a high degree of homology between plasmids are shown by Grey blue shading.
bla\textsubscript{NDM-1} and bla\textsubscript{OXA-10} in \textit{P. rettgeri}, their study only clarified the transmission of \textit{bla}_{NDM-1} through plasmids, but did not explain whether \textit{bla}\textsubscript{OXA-10} co-existed with \textit{bla}\textsubscript{NDM-1} on the same plasmid. Zhang et al\textsuperscript{35} provided a comprehensive analysis of the genetic context of a circular plasmid measuring 273.2 Kbp, which harbored \textit{bla}\textsubscript{NDM-1}, \textit{bla}\textsubscript{OXA-10}, and \textit{bla}\textsubscript{PER-4} genes in \textit{P. rettgeri}. Horizontal transmission of interbacterial resistance can occur by conjugating plasmids and integrating conjugative elements. Our study corroborates these findings. We further describe the characterization of pYQ150713-NDM-1 plasmid, where the \textit{bla}_{NDM-1} and \textit{bla}_{OXA-10} genes were found to be co-located on a Tn3 transposon element in pYQ150713-NDM-1 with the following linear structure: TnAs2-hin-xerC-dhfrI-ORF-bla\textsubscript{OXA-10}-ant1-emrE-ORF-ORF-trpF-ble-bla\textsubscript{NDM-1}-cat-ORF-emrE-ORF-ORF-ISEc35. Furthermore, the presence of mobile elements TnAs2 and ISEc35 in close proximity to pYQ150713-NDM-1 enhances the potential for dissemination of drug resistance genes.
Figure 5 The phylogenetic tree of 81 completed *P. rettgeri* genome. Maximum-likelihood phylogeny of 81 representative global *P. rettgeri* isolates. The trees were constructed using Roary software. The tips of branches are colored according to sources, countries, and year.
Consequently, it is imperative to prioritize routine testing and administer suitable treatment to patients. NCBI BLAST analysis revealed that the complete sequence of pYQ150713-NDM-1 had genetic similarity with *Proteus mirabilis* plasmid pDY.F1.2 (CP046050), *Proteus mirabilis* plasmid pSNYG35 (CP047590), and *Escherichia coli* plasmid pEC8-NDM-1(CP060954). It is noteworthy that all four plasmids were identified from isolates in China. *Escherichia coli* plasmid pEC8-NDM-1 was identified from a clinical urine sample. However, *Proteus mirabilis* plasmid pDY.F1.2 were identified from Swine and *Proteus mirabilis* plasmid pSNYG35 were from broiler chicken. These data indicate that *bla*<sub>NDM-1</sub>-carrying plasmid identified in this study might disseminate from food-producing animals to humans, and the proliferation of plasmids represents a potential public health risk. In conclusion, to curb further spread of plasmids bearing *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-10</sub>, it is imperative that an effective prevention strategy should be adopted.

In order to examine the genetic relatedness of *P. rettgeri* YQ150713 with previously documented strains, a core genome phylogeny analysis was conducted. As showed in Figure 5 and Table S1, *P. rettgeri* strains were mainly isolated from clinical samples, with few strains from environments. Further, *P. rettgeri* strains were mainly found in USA, and Nigeria. *P. rettgeri* YQ150713 had a close relationship with GCA 006351125.1 from China, GCA 016512695.1 from USA, GCA 020564615.1, GCA 021869825.1 and GCA 020683065.1 from Colombia. All of these strains were obtained from clinical samples, suggesting a widespread presence of *P. rettgeri* in clinical settings. In order to mitigate the potential dissemination of these *P. rettgeri* isolates within clinical environments, enhanced surveillance is imperative in forthcoming studies.

**Nucleotide Sequence Accession Numbers**

A complete sequence of the nucleotides described in this paper has been deposited in GenBank under accession number SAMN27400906.

**Conclusion**

In summary, we found *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-10</sub> were co-localized on a novel plasmid pYQ150713-NDM-1 which has a horizontal transfer function and a certain ability to spread. To reduce the risk of the dissemination of such *P. rettgeri* isolates in clinical settings, more surveillance is needed in the future.

**Ethical Approval**

Ethics Committee of First Affiliated Hospital of Zhejiang University has approved the protocol (no. 2021–631). The patient provided written informed consent.

**Disclosure**

The authors of this article have no conflict of interest.

**References**


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