NDM-1 Metallo-β-Lactamase and ArmA 16S rRNA methylase producing Providencia rettgeri clinical isolates in Nepal

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Abstract

Background: Drug-resistant Providencia rettgeri producing metallo-β-lactamase and 16S rRNA methylase has been reported in several countries. We analyzed P. rettgeri clinical isolates with resistance to carbapenems and aminoglycosides in a hospital in Nepal.

Methods: Five clinical isolates of multidrug-resistant P. rettgeri were obtained in a hospital in Nepal. Antimicrobial susceptibilities were determined using the microdilution method and entire genomes were sequenced to determine drug-resistant genes. Epidemiological analysis was performed by pulsed-field gel electrophoresis.

Results: Four of the 5 isolates were resistant to carbapenems (imipenem and meropenem), with MICs ≥16 mg/L, with the remaining isolate showing intermediate resistance to imipenem, with an MIC of 2 mg/L and susceptibility to meropenem with an MIC ≤1 mg/L. All 5 isolates had blaVEB-1. Of the 4 carbapenem-resistant strains, 3 had blaNDM-1 and 1 had blaOXA-72. All isolates were highly resistant to aminoglycosides (MICs ≥1,024 mg/L) and harbored armA. As the result of pulsed-field gel electrophoresis pattern analysis in the 5 P. rettgeri isolates, 4 had identical PFGE patterns and the fifth showed 95.7% similarity.

Conclusions: This is the first report describing multidrug-resistant P. rettgeri strains harboring blaNDM-1 or blaOXA-72 and armA isolated from patients in Nepal.

Keywords: NDM-1, OXA-72, 16S rRNA methylase, Providencia rettgeri, Molecular epidemiology

Background

Providencia rettgeri has been associated with hospital acquired infections, including catheter-related urinary tract infections, bacteremia, skin infections, diarrhea, and gastroenteritis [1,2]. To date, there have been 5 reports of P. rettgeri isolates harboring metallo-β-lactamase (MBL) encoding genes, including IMP-type MBL producers in Japan [3,4]; VIM-type MBL, PER-1 extended-spectrum β-lactamase (ESBL) and 16S rRNA methylase ArmA in Korea [5]; and NDM-type MBL in Israel [6] and Brazil [7].

NDM-type MBL was initially identified in Klebsiella pneumoniae and Escherichia coli in 2009 in Sweden [8]. Since then, NDM-1-producing Enterobacteriaceae have been isolated in various parts of the world [9,10].

Exogenously acquired 16S rRNA methylase genes responsible for very high levels of resistance to various aminoglycosides are widely distributed among Enterobacteriaceae and glucose-nonfermentative microbes [11]. Gram-negative pathogens producing 16S rRNA methylase ArmA have been isolated in various countries [11].

Although co-production of several resistance determinants is not rare in Enterobacteriaceae [12-16], it is less common in P. rettgeri [5]. We describe here P. rettgeri clinical isolates from Nepal that produce carbapenemase (NDM-1 or OXA-72) and 16S rRNA methylase (ArmA).
## Table 1 Summary of the characteristics of the 5 *P. rettegeri* strains, including antimicrobial resistance profiles and resistant genes

| Strains | Tissue sources | Infection | MIC (mg/L) | Antibiotics resistant genes |
|---------|----------------|-----------|------------|----------------------------|
|         |                |           | PIP | TZP | CAZ | FEP | IPM | DPM | MEM | ATM | ABK | AMK | GEN | CIP | CST | FOF | TIG |
| IOMTU1  | Pus            | SSI       | 1,024 | 512 | >1,024 | 64 | 32 | 16 | 64 | 1,024 | >1,024 | >1,024 | 128 | >128 | 512 | 4 | blaNDM-1, blaOXA-10, blaVEB-1, blaTEM-1, blaADC-67, armA, aadA1, aadA2 |
| IOMTU4  | Sputum         | NLRTI     | 1,024 | 128 | >1,024 | 256 | 16 | 16 | 32 | 1,024 | >1,024 | >1,024 | >128 | 512 | 4 | blaOXA-72, blaOXA-10, blaVEB-1, blaTEM-1, blaADC-67, armA, aadA1 |
| IOMTU91 | Sputum         | NLRTI     | >1,024 | 1,024 | >1,024 | 1,024 | 64 | 32 | 64 | 1,024 | >1,024 | >1,024 | >1,024 | 128 | 128 | 4 | blaNDM-1, blaOXA-10, blaVEB-1, blaTEM-1, blaADC-67, armA, aadA1 |
| IOMTU94 | Pus            | SSI       | 1,024 | 4 | >1,024 | 256 | 2 | 1 | 1 | >1,024 | 1,024 | 1,024 | 1,024 | 256 | >128 | 1,024 | 4 | blaOXA-10, blaVEB-1, blaTEM-1, blaADC-67, armA, aadA1 |
| IOMTU99 | Sputum         | NLRTI     | >1,024 | 512 | >1,024 | 128 | 64 | 32 | 64 | 1,024 | >1,024 | >1,024 | >1,024 | >1,024 | >1,024 | >1,024 | 4 | blaNDM-1, blaOXA-10, blaVEB-1, blaTEM-1, blaADC-67, armA, aadA1 |

SSI, surgical site infection; NLRTI, nosocomial lower respiratory tract infection; PIP, piperacillin; TZP, piperacillin/tazobactam; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; DPM, doripenem; MEM, meropenem; ATM, aztreonam; ABK, arbekacin; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; CST, colistin; FOF, fosfomycin; TIG, tigecycline.
Methods

Bacterial strains

Five P. rettgeri clinical isolates were obtained from May to July 2012 from 5 patients at Tribhuvan University Teaching Hospital in Kathmandu, Nepal. Three isolates were from sputum and 2 from pus at surgical sites. Samples were obtained as part of standard patient care. Phenotypical identification [17] was confirmed by API 32GN (BioMérieux, Mercy l’Etoile, France) and 16S rRNA sequencing (1,497 bp) [18,19].

Antimicrobial susceptibilities

MICs were determined using the microdilution method, according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) [20]. Breakpoints to antibiotics were determined. The modified Hodge test, the meropenem-sodium mercaptoacetic acid double-disk synergy test (Eiken Chemical, Tokyo, Japan) and E-test (imipenem/EDTA) (AB Biodisk, Solna, Sweden) were performed.

Entire genome sequencing

The entire genomes of these isolates were extracted and sequenced by MiSeq (Illumina, San Diego, CA). CLC genomics workbench version 5.5 (CLC bio, Tokyo, Japan) was used for de novo assembly of reads and to search for 923 drug-resistance genes, including genes encoding β-lactamases, 16S rRNA methylases and aminoglycoside-acetyl/adenylyltransferases; point mutations in the gyrA, parC and pmrCAB operons; and point mutations in the fos genes, including fosA, fosA2, fosA3, fosC and fosC2.

Pulsed-field gel electrophoresis (PFGE) and southern hybridization

PFGE analysis was performed as described [3]. An 813 bp probe for blaNDM-1 was synthesized by PCR amplification using the primers 5’-atggaattcgcccaatattattgcacc-3’ (forward) and 5’-tcaggcagcttgctggaatgggg-3’ (reverse), and a 780 bp probe for blaOXA-72 was synthesized using the primers 5’-agtttctctcagtgcatgtcatactat-3’ (forward) and 5’-agcagccatctctcttctctacct-3’ (reverse). Southern hybridization to detect blaNDM-1 and blaOXA-72 was performed using these probes, which were detected using DIG High Prime DNA labeling and detection starter kit II (Roche Diagnostics, Mannheim, Germany).

Nucleotide sequence accession numbers

The nucleotide sequences surrounding blaNDM-1 and blaOXA-72 have been deposited in GenBank with the accession number AB828598 and AB857844, respectively.

Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board of the Institute of Medicine, Tribhuvan University (ref. 6-11-E) and the Biosafety Committee, National Center for Global Health and Medicine (approval number: 23-M-49).

Results

Antimicrobial susceptibilities

Four of the 5 isolates were resistant to carbapenems (doripenem, imipenem and meropenem) and piperacillin/tazobactam, whereas the fifth was susceptible to piperacillin/tazobactam, doripenem and meropenem and showed intermediate resistance to imipenem (Table 1). All 5 isolates were highly resistant to cephalosporins (ceftazidime and cefepime), aztreonam, aminoglycosides (arbekacin, amikacin and gentamicin), ciprofloxacin, colistin and fosfomycin, and all 5 showed intermediate resistance to tigecycline. The four isolates resistant to carbapenems were negative with the modified Hodge test, but three of the four isolates were positive with the meropenem-sodium mercaptoacetic acid double-disk synergy test and E-test/EDTA.

Drug-resistant genes

All 5 isolates tested had several genes associated with β-lactam and aminoglycoside-resistance (Table 1). These isolates had blaVEB-1, blaOXA-10, blaTEM-1, blaADC-67 (ampC), armA and adaA1; 3 had blaNDM-1; and 1 had blaOXA-72. None of these isolates had any other β-lactamase encoding genes, including the class A genes blasHV5, and blaCTX-Ms; the class B genes blasAIM, blasDIM, blasGIM, blasMPWII, blasNDM3, blasKHM, blasSIM, blasSMB, blasSPM, blasTMBr and blasVIM; or the class D gene blasOXA5 except for blaOXA-10 and blaOXA-72. None had other genes encoding 16S rRNA methylases or aminoglycoside acetyl/adenylyltransferases. All 5 isolates had point mutations in the quinolone-resistance-determining regions of gyrA and parC, with amino acid substitutions of S83I and D87E in GyrA and S80I in ParC.

Figure 1 PFGE profiles obtained following SfiI digestion of P. rettgeri chromosomes.
PFGE and southern hybridization
Of the 5 P. rettgeri isolates, 4 had identical PFGE patterns and the fifth showed 95.7% similarity (Figure 1). Three of these isolates had a plasmid harboring blaNDM-1 and one had a plasmid harboring blaOXA-72 with plasmid sizes ranging from 9.42 to 23.1 kbp (data not shown).

Genomic structures surrounding blaNDM-1 and blaOXA-72
The genetic environments surrounding blaNDM-1 (Accession no. AB828598) was blaNDM-1-bleMBL-trpF-dsbC-cutA1. All 3 isolates harboring blaNDM-1 (JOMTU1, 91 and 99) had the same genetic environments. The blaOXA-72 gene was flanked by conserved inverted repeats at the XerC/XerD binding sites [21], indicating mobilization by site-specific recombination mechanisms. The rep1 gene was located downstream of blaOXA-72 (Accession no. AB857844).

Discussion
The relatively high MICs to piperacillin/tazobactam and carbapenems of the five P. rettgeri isolates are likely due to the presence of blaNDM-1 or blaOXA-72. The enzymatic activities of metallo-β-lactamasas, including NDM-1, were not inhibited by tazobactam [22], a β-lactamase inhibitor, in agreement with the MIC profiles of these isolates to piperacillin/tazobactam. The high MICs of all 5 isolates to ceftazidime, ceftipime and aztreonam were likely due to the presence of blaVEB-1 [23], and the presence of armA in these isolates is likely associated with their extremely high resistance to all aminoglycosides tested [11]. Point mutations in the quinolone-resistance-determining regions of gyrA and parC have been associated with high resistance to quinolones [24]. Point mutations in pmrCAB operon have been associated with the resistance of Acinetobacter spp. [25] and Pseudomonas aeruginosa [26] to polymyxin and colistin; and the presence of fos genes, including fosA, fosA2, fosA3, fosC and fosC2, has been associated with resistance to fosfomycin in Gram-negative bacteria [27-29].

Plasmids containing blaNDM-1 or blaOXA-72 may be disseminated among Gram-negative pathogens in Nepal. The genetic environments surrounding blaNDM-1 in our P. rettgeri strains (blaNDM-1-bleMBL-trpF-dsbC-cutA1) were also observed in other plasmids, including A. baumannii plasmid pAbNDM-1 from China (Accession no. JN377410), Citrobacter freundii plasmid pVE315203 from China (Accession no. JX254913), E. coli plasmid pNDM1022337 from Canada (Accession no. JF714412), K. pneumoniae plasmid pKP-NCGM18-1 from Nepal (Accession no. AB824738) [30], K. pneumoniae plasmids pKPx1, pKPN5047 and pNDM-HN380 from China (Accession nos. AP012055, KC311431 and JX104760, respectively), and P. rettgeri plasmid pF9R90 (Accession no. JQ362415) from China. In addition, the genetic structure of OXA-72 producing Acinetobacter spp [31-34] and K. pneumoniae (Accession no. JX268653 and AB825955 deposited in 2012 and 2013, respectively) had the same genetic structure (blaOXA-72-rep1) as our strain of P. rettgeri.

Conclusions
To our knowledge, this is the first report describing P. rettgeri strains harboring blaNDM-1 or blaOXA-72 and armA isolated from patients in Nepal. These 5 strains were highly resistant to both β-lactams and aminoglycosides and expanded in a clonal manner in the hospital.

Competing interests
The authors declare that they have no competing interest.

Authors’ contributions
TT: Performed PCR and sequencing, analyzed data and drafted the manuscript. TMA: Performed entire genome sequencing. RKO and MKS: Performed drug susceptibility tests. HD: Supervised this study. KS: Performed pulsed-field gel electrophoresis and its pattern analysis. TK and BMP: Designed protocols and supervised this study. All authors read and approved the final manuscript.

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