Emergence of Extensively Drug-Resistant Proteus mirabilis Harboring a Conjugative NDM-1 Plasmid and a Novel Salmonella Genomic Island 1 Variant, SGI1-Z

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Abstract
Acquisition of bla_{NDM-1} in bacterial species, such as Proteus mirabilis that is intrinsically resistant to tetracycline, tigecycline and colistin, will make clinical treatment extremely difficult. Here, we characterized an NDM-1-producing clinical isolate of P. mirabilis (PMS8) that displayed an extensively drug-resistant (XDR) phenotype, susceptible only to aztreonam. Molecular analysis revealed that PMS8 harbored both a conjugative NDM-1 plasmid and a novel Salmonella genomic island 1 variant on chromosome.

Disciplines
Genetics and Genomics | Veterinary Microbiology and Immunobiology | Veterinary Pathology and Pathobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

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Emergence of Extensively Drug-Resistant *Proteus mirabilis* Harboring a Conjugative NDM-1 Plasmid and a Novel *Salmonella* Genomic Island 1 Variant, SGI1-Z

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Acquisition of *bla*\_NDM-1^ in bacterial species, such as *Proteus mirabilis* that is intrinsically resistant to tetracycline, tigecycline and colistin, will make clinical treatment extremely difficult. Here, we characterized an NDM-1-producing clinical isolate of *P. mirabilis* (PM58) that displayed an extensively drug-resistant (XDR) phenotype, susceptible only to aztreonam. Molecular analysis revealed that PM58 harbored both a conjugative NDM-1 plasmid and a novel *Salmonella* genomic island 1 variant on chromosome.

Global dissemination of New Delhi metallo-β-lactamase 1 (NDM-1), an Ambler class B metallo-β-lactamase (MBL) able to hydrolyze all β-lactams except monobactams, in Gram-negative bacteria represents a great threat for public health (1). Transmission of *bla*\_NDM-1 is of special concern with bacterial species of intrinsic antibiotic resistance, such as *Proteus mirabilis* that is intrinsically resistant to tetracycline, tigecycline and colistin, which will make clinical treatment extremely difficult.

*Salmonella* genomic island 1 (SGI1), consisting of a conserved backbone structure and a multidrug resistance (MDR) region, is integrated into the chromosome specifically at the last 18 bp of the *3′*-end of the *tdhG* gene and was initially reported in *Salmonella enterica* serovar Typhimurium DT104 (2). To date, multiple variants of SGI1 have been discovered, and they are classified from SGI1-A to SGI1-Y, corresponding to various structures of the MDR region. Since several novel variants of SGI1, such as SGI1-L and SGI1-U to SGI1-Y, have been identified in *P. mirabilis*, this species is considered an acceptor of SGI independent of *Salmonella* spp. (3–5). Recently, the *bla*\_NDM-1 gene was reportedly integrated into a novel SGI1 variant, named PGI1 (*Proteus* genomic island 1), in a *P. mirabilis* clinical isolate (6). However, the *bla*\_NDM-1-carrying PGI1 was nontransferable in the presence of helper plasmid pR55, and this PGI1-harboring isolate remained susceptible to multiple antibiotics, including meropenem, ertapenem, gentamicin, and fosfomycin (6). Here, we describe a clinical extensively drug resistant (XDR) *P. mirabilis* isolate that harbors both a conjugative NDM-1 plasmid and a novel SGI1 variant on chromosome.

A 3-year-old girl was admitted to the emergency department of The First Affiliated Hospital of Zhengzhou University on August 10, 2013, with a diagnosis of spinal cord injury (SCI) caused by a knife injury. After the surgery operation on August 11, intravenous cefathiamidine was given empirically (1 g administered 2 times/day) for 1 week to prevent bacterial infections. After receiving treatment, the patient was in a stable condition and was transferred to the rehabilitation department for restorative treatment. Unfortunately, the patient developed symptoms of frequent and urgent micturition and urodynamic on December 10, 2013, and was treated with gentamicin bladder irrigations via catheter (20 mg administered 2 times/day). On December 15, an XDR *P. mirabilis* isolate named PM58 in this study was obtained from the urine culture. According to the drug resistance pattern of PM58, antibiotic treatment was switched to aztreonam (30 mg/kg administered as an intravenous drip 2 times/day). After 1 week of treatment, the girl recovered, and the urine was negative for *P. mirabilis*.  

Antimicrobial susceptibilities were tested by the microdilution method and the agar dilution method (for fosfomycin) following the CLSI guidelines, and the MIC results were interpreted according to the CLSI breakpoints (7). PM58 was resistant to all the tested antibiotics used for treatment of infections caused by *Enterobacteriaceae*, with the exception of aztreonam. (Table 1). The positive result for the imipenem–ethylenediaminetetraacetic acid (EDTA) double-disk synergy test indicated that PM58 produced metallo-β-lactamase (MBL). PCR and sequencing analysis identified the MBL and β-lactam genes *bla*\_NDM-1 and *bla*\_TEM-1, as well as other genes conferring resistance to aminoglycoside (*rmtB*), quinolones (*qnrA1*), tetracyclines (*tet(J), tet(C)*), chloramphenicol (*floR*), and fosfomycin (*fosA3*). Primers used for detecting these resistance genes were described in previous studies (8–11). S1-pulsed field gel electrophoresis and Southern blotting demonstrated that the *bla*\_NDM-1 gene, together with *rmtB* and *fosA3* genes, was located on an ~85-kb plasmid (see Fig. S1 in the accepted version).

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supplemental material), designated pNDM-PM58. Conjugative assay was performed using an established method to evaluate the transferability of pNDM-PM58 (8). The result showed that this plasmid was successfully transferred to *Escherichia coli* J53 at a frequency of $9.4 \times 10^{-7}$ per donor cell. In addition, all of the resistance determinants detected in PM58 except *bla*<sub>TEM-1</sub> were cotransferred with the *bla*<sub>NDM-1</sub> gene to *E. coli* J53 by the mating experiment. This was confirmed by using PCR and sequencing.

The surrounding genetic environment of the *bla*<sub>NDM-1</sub> gene on pNDM-PM58 was sequenced by a modified random primer walking strategy (12), yielding a 12,146-bp fragment (GenBank accession number KP662515; Fig. 1). The *bla*<sub>NDM-1</sub> gene was embedded in a genetic context that included *ndm-1* and *tem-1* genes, as well as other resistance determinants. The boxed arrows indicate the positions and directions of transcription of the genes. The gray-shaded areas represent regions sharing >99% DNA identity.

### TABLE 1

<table>
<thead>
<tr>
<th>Antimicrobial category</th>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>PM58</th>
<th>PM58-J53</th>
<th><em>E. coli</em> J53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins</td>
<td>Ampicillin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Penicillins + ß-lactamase inhibitors</td>
<td>Ampicillin-sulbactam</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Antipseudomonal penicillins + ß-lactamase inhibitors</td>
<td>Piperacillin-tazobactam</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>2</td>
<td></td>
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<tr>
<td>Non-extended-spectrum cephalosporins</td>
<td>Cefazolin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Extended-spectrum cephalosporins</td>
<td>Ceftazidime</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>0.5</td>
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<tr>
<td>Cephamycins</td>
<td>Cefepime</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>0.25</td>
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<tr>
<td>Carboxapenems</td>
<td>Imipenem</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>&lt;0.125</td>
<td></td>
</tr>
<tr>
<td>Monobactams</td>
<td>Meropenem</td>
<td>8</td>
<td>8</td>
<td>&lt;0.125</td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Aztreonam</td>
<td>1</td>
<td>1</td>
<td>&lt;0.125</td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Levofloxacin</td>
<td>32</td>
<td>8</td>
<td>&lt;0.125</td>
<td></td>
</tr>
<tr>
<td>Folate pathway inhibitors</td>
<td>Gentamicin</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Phenicol</td>
<td>Amikacin</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Phosphonic acids</td>
<td>Trimethoprim-sulfamethoxazole</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>1</td>
<td></td>
</tr>
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<td>Tetracyclines</td>
<td>Chloramphenicol</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>4</td>
<td></td>
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<tr>
<td>Tetracyclines</td>
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<td>&gt;1,024</td>
<td>&gt;1,024</td>
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<tr>
<td>Glycylcyclines</td>
<td>Doxycycline</td>
<td>128</td>
<td>64</td>
<td>2</td>
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<tr>
<td>Polymyxins</td>
<td>Tigecycline</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;64</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Phenicols</td>
<td>Colistin</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Phosphonic acids</td>
<td>Chloramphenicol</td>
<td>&gt;64</td>
<td>&gt;64</td>
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<td>Aminoglycosides</td>
<td>Gentamicin</td>
<td>&gt;64</td>
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<td>Penicillos</td>
<td>Chloramphenicol</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> ND, not determined; *P. mirabilis* is intrinsically resistant to tetracycline, tigecycline and colistin.

FIG 1 Genetic environment of the *bla*<sub>NDM-1</sub> gene in the pNDM-PM58 of *P. mirabilis* 58 and structural comparison with *Klebsiella pneumoniae* plasmid pLK78 (Genbank accession number KJ440075), *E. coli* plasmid pECNDM1-4 (Genbank accession number JX469383), pRPNDM1-1 (Genbank accession number JX515588) from *Raoultella planticola*, and the MDR region of SGI1-O from *P. mirabilis*. The boxed arrows indicate the positions and directions of transcription of the genes. The gray-shaded areas represent regions sharing >99% DNA identity.
in a complex class 1 integron, flanked by two copies of ISCR1 elements (ISCR1-1 and ISCR1-2) located in the same orientation. Similar structures were reported in *E. coli* and *Raoella planticola* isolates from China in a very recent study (13). However, the resistance gene cassettes and genetic arrangement within the complex class 1 integron in PM58 were different from those of pEcNDM1-4, a 58-kb plasmid carrying blaNDM-1 in *E. coli,* and pRPNDM1-1, a 280-kb plasmid carrying blaNDM-1 in *R. planticola* (13) (Fig. 1). In PM58, a classical class 1 integron carrying a dfrA1-orfC cassette array was located upstream of the ISCR1-1 (immediately downstream of the *trpF* gene) and exhibited 99.74% (3,519 of 3,528 bp) nucleotide sequence identity to a corresponding segment of InSGI1-O, an In4 type integron that has been previously identified in *P. mirabilis* 58, and comparison of the MDR region of SGI1-Z (InPm58) with an In4-like backbone structure on *E. coli* plasmid pBDE0502 (EU056266). The boxed arrows indicate the positions and directions of transcription of the genes. The gray-shaded areas represent regions sharing >99% DNA identity. DR-L and DR-R represent the 18-bp direct repeats at the ends of SGI1. CS, conserved segment; IR, inverted repeat.

![Diagram](https://example.com/diagram.png)

**FIG 2** Schematic view of SGI1-Z integrated into the chromosome of *P. mirabilis* 58, and comparison of the MDR region of SGI1-Z (InPm58) with an In4-like backbone structure on *E. coli* plasmid pBDE0502 (EU056266). The observations of an InSGI1-O-like structure on pNDM-PM58 and InSGI1-Z corresponding to an In4-type integron on pBDE0502 suggest the exchange of gene cassettes between plasmid and chromosome via integron-mediated recombination.

In conclusion, our study represents the first report of coexistence of a self-transferable NDM-1 plasmid and a novel chromosomal SGI1 variant in an XDR *P. mirabilis* isolate that is susceptible only to aztreonam. It is unclear how this XDR isolate originated in the patient. It could have originated from the treatment process due to overuse or inappropriate use of antibiotic therapy. In this case, the patient received intravenous cefathimidine for a week to prevent bacterial infection, but this strategy is non-evidence based and not plausible, which might have served as a selection pressure for the emergence of the XDR isolate. Alternatively, the patient could have been infected by the XDR *P. mirabilis* isolate from other sources. Regardless, rational and evidence-based use of antibiotics should be emphasized in clinical therapy. A recent review recommended a combination of two or even three antibiotics among aminoglycoside, fosfomycin, high-dose tigecycline, and colistin for the treatment of infections caused by carbapenem-resistant *Enterobacteriaceae* with MICS for carbapenems higher than 8 μg/ml (19). Since *P. mirabilis* is intrinsically resistant to tigecycline and colistin, the emergence and spread of XDR *P. mirabilis* carrying plasmid-mediated resistance to carbapenems (blaNDM-1), fosfomycin (fosA3), and aminoglycosides (rmtB) will severely compromise the utility of combination regimens in treating infections attributed to *P. mirabilis.* Thus,
detection and surveillance of XDR \textit{P. mirabilis} and its resistance elements are urgently warranted to control their further spread.

**Nucleotide sequence accession number.** The sequence of the novel complex class 1 integron carrying \textit{bll}_{NDM-1}, and the complete SGI1-Z sequence were submitted to GenBank under accession numbers KP662515 and KP662516, respectively.

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