Research note

\(\text{bla}_{\text{IMP-27}}\) on transferable plasmids in \(\text{Proteus mirabilis}\) and \(\text{Providencia rettgeri}\)

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Abstract

Objectives: A carbapenem-resistant \(\text{Providencia rettgeri}\) (PR1) isolate was recovered from a wound infection in Missouri, USA. This isolate possessed an EDTA-inhibitable carbapenemase that was unidentified using the Xpert CARBA-R assay. Our objective was to elucidate the molecular determinant of carbapenem resistance in this isolate. We then sought to test the transmissibility of \(\text{bla}_{\text{IMP-27}}\) loci in clinical \(\text{P. rettgeri}\) and \(\text{Proteus mirabilis}\) isolates.

Methods: In October 2016 the novel ambler Class B carbapenemase \(\text{bla}_{\text{IMP-27}}\) was reported in two different \(\text{Proteus mirabilis}\) (PM185 and PM187) isolates. Broth mating assays for transfer of carbapenemase activity were performed for the three clinical isolates with recipient sodium azide-resistant \(\text{Escherichia coli}\) J53. Antibiotic susceptibility testing and phenotypic carbapenemase activity testing were performed on the clinical isolates, J53 and transconjugants using the Kirby–Bauer disc diffusion method according to CLSI guidelines. Plasmid DNA from PM187, PR1 and their transconjugants were used as input for Nextera Illumina sequencing libraries and sequenced on a NextSeq platform.

Results: PR1 was resistant to both imipenem and meropenem. PM187 and PR1 could transfer resistance to \(\text{E. coli}\) through plasmid conjugation (pPM187 and pPR1). pPM187 had a virB/virD4 type IV secretion system whereas pPR1 had a tetrA type IV secretion system.

Conclusion: Two of three \(\text{bla}_{\text{IMP-27}}\)-bearing clinical isolates tested could conjugate resistance into \(\text{E. coli}\). The resulting transconjugants became positive for phenotypic carbapenemase production but did not pass clinical resistance breakpoints. \(\text{bla}_{\text{IMP-27}}\) can be transmitted on different plasmid replicon types that rely on distinct classes of type IV secretion system for horizontal transfer. R.F. Potter, Clin Microbiol Infect 2018; e1

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understand, with PR1, the potential for lateral transfer of this resistance gene from *Proteus mirabilis* and *Providencia rettgeri* into *E. coli*, and the associated changes in antibiotic resistance [1].

**Materials and methods**

**Bacterial isolates**

The *P. rettgeri* isolate (PR1) was recovered from a chronic foot wound infection clinical culture. The isolate received for evaluation was a de-identified strain. As a result, the study team was not able to obtain patient consent. *Proteus mirabilis* strains (PM185 and PM187) were provided by Nancy Hanson at Creighton University [1]. The sodium-azide-resistant *E. coli* J53 strain (ATCC number BAA-2730™) was used as a recipient for transconjugation experiments.

**Broth conjugation**

Colonies of PM185, PM187, PR1 and wild-type *E. coli* J53 were separately suspended in tryptic soy broth (Sigma Aldrich, St Louis, MO, USA) and diluted to 0.05 OD600. One hundred microlitres of PM185, PM187 and PR1 were separately added to 100 μL *E. coli* J53 (for a 1:1 ratio) and diluted to 5 mL with tryptic soy broth. Cultures were incubated at 37°C for 18 h without shaking for 24 h. Then, 50 μL of co-cultures were suspended onto MacConkey agar plates containing sodium azide (Thermo Fisher Scientific, Waltham, MA, USA) (150 μg/mL) and ceftriaxone (5 μg/mL) spread with glass beads, and incubated for 18 h at 37°C. Individual transconjugant colonies were propagated overnight in tryptic soy broth supplemented with 5 μg/mL ceftriaxone under shaking conditions (220 rpm).

**Susceptibility testing**

Each clinical isolate, J53, J53:pPR1 and J53:pPM187, was cultured overnight as described previously. *Escherichia coli* ATCC 25922 was used as a quality control. Susceptibility testing was performed using Kirby–Bauer disc diffusion on Mueller–Hinton agar (Hardy Diagnostics, Santa Maria, CA) in accordance with CLSI Standards [3].

**Plasmid assembly and annotation**

We used Illumina sequencing to specifically investigate bla*IMP*-27-bearing plasmids in PR1 and PM187. Plasmid DNA was obtained using a miniprep kit (Qiagen, Valencia, CA, USA). Plasmid DNA for PR1 and PM187 was processed to remove Illumina adapters (trimmomatic) and contaminating DNA (deconseq) (see Supplementary material, Table S1). Raw reads from the transconjugant minipreps were processed for quality in a Southern blot analysis indicated that PR1 has a single copy of the bla*IMP*-27 gene from these type IV secretion systems showed similarity to previously described systems from pathogenic *Proteus mirabilis* HI4320 and carbapenem-resistant *P. rettgeri* 09ACRGNY2001 [13].

**Results**

PM185 and PM187 were intermediate and susceptible to meropenem and imipenem, respectively (Table 1). Only PR1 was resistant to both carbapenems. PM185 was indeterminate for the carbapenem inactivation method but PM187 and PR1 were both phenotypically positive (see Supplementary material, Table 1). Southern blot analysis indicated that PR1 has a single copy of the bla*IMP*-27 (see Supplementary material, Fig. S1), similar to PM185 [1]. In contrast, PM187 has both chromosomal and plasmid copies of the bla*IMP*-27 [1]. Transconjugants were obtained from conjugation assays of PR1 and PM187 with the *E. coli* J53 recipient but not PM185. Although conjugation did not achieve clinical resistance guidelines, the zone size for meropenem decreased from 32 mm in J53 to 25 mm in J53:pPM187 and 27 mm in J53:pPR1 (Table 1). The zone size for imipenem decreased a lesser amount, from 33 mm in J53 to 31 and 32 mm in J53:pPM187 and J53:pPR1, respectively. Both transconjugants were positive for phenotypic carbapenem production (Table 1).

The plasmid from PM187, pPM187 (GenBank NOWA01000087.1), contains a putative virB/D4 IV secretion system operon, providing a potential mechanism for horizontal dissemination (Fig. 1a). The virB4 amino acid sequence had 100% identity over its entire length with a conjugal transfer protein (WP_012368868.1) from *Proteus mirabilis* HI4320 [11]. The plasmid pPM187 has an IncX8 backbone, a newly discovered IncX family member [12]. Unlike pPM187, the assembled bla*IMP*-27-bearing plasmid, pPR1 (GenBank NOWC01000095.1) did not have a plasmid replicon identified; pPR1 also contained a putative type IV secretion system, though of the tra/trb type (Fig. 1b). The traN amino acid sequence had 100% identity across its entire length to the *traN* (WP_023159916.1) of the *bla*IMP*-1*,-bearing plasmid pYr2001 from *P. rettgeri* 09ACRGNY2001 [13].

**Discussion**

In this study, we used conjugation experiments to determine that two bla*IMP*-27-positive clinical isolates, PM187 and PR1, could transfer carbapenemase production to *E. coli*. We used Illumina sequencing of the transconjugants and clinical isolates to assemble the bla*IMP*-27-bearing plasmids, pPM187 and pPR1.

*Escherichia coli* transconjugants with these plasmids (pPR1 and pPM187) gain detectable carbapenemase activity, but this activity does shift the transconjugants past clinical breakpoints for carbapenem resistance. It is possible that regulatory or translational optimization of the conjugated *bla*IMP*-27-bearing plasmid in *E. coli* is required for clinical resistance [14]. In addition to *bla*IMP*-27 expression, it is also possible that porin mutations or efflux activity in the clinical isolates could contribute to phenotypic carbapenem resistance [15].

A previous investigation found that while *bla*IMP*-27 was plasmid-borne in swine-associated *Enterobacteriaceae*, the IncQ plasmids were not conjugatable. In contrast, the plasmids we have completely sequenced are capable of self-mobilization, probably due to a virB/virD4 type IV secretion system in *pPM187* and a trb/traA type IV secretion system in *pPR1*. The virB4 and traN gene from these type IV secretion systems showed similarity to previously described systems from pathogenic *Proteus mirabilis* HI4320 and carbapenem-resistant *P. rettgeri* 09ACRGNY2001 [11,13]. A limitation of illumina short-read sequencing is that it generally cannot enable unambiguous assembly of the chromosome and all plasmids. Further work is warranted using long-read sequencing (e.g. from PacBio, Menlo Park, CA, USA or Oxford Nanopore, Oxford, UK) on *bla*IMP*-27* isolates to unequivocally determine chromosomal sequences and compare the non-conjugatable *bla*IMP*-27* IncQ plasmids with
Implications of IMP-27 localization with a Class II integron gene cassette and type IV secretion system.

Table 1

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>PM185 (S)</th>
<th>PM187 (R)</th>
<th>PR1 (S)</th>
<th>J53 (S)</th>
<th>J53:pPM187 (S)</th>
<th>J53:pPR1 (S)</th>
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<tbody>
<tr>
<td>Ampicillin</td>
<td>24 (S)</td>
<td>6 (R)</td>
<td>14 (R)</td>
<td>21 (S)</td>
<td>17 (S)</td>
<td>17 (S)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>9 (R)</td>
<td>9 (R)</td>
<td>6 (R)</td>
<td>25 (S)</td>
<td>8 (R)</td>
<td>9 (R)</td>
</tr>
<tr>
<td>Cefotetan</td>
<td>11 (R)</td>
<td>14 (I)</td>
<td>6 (S)</td>
<td>33 (S)</td>
<td>11 (R)</td>
<td>10 (R)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>17 (R)</td>
<td>17 (R)</td>
<td>18 (S)</td>
<td>35 (S)</td>
<td>15 (R)</td>
<td>14 (R)</td>
</tr>
<tr>
<td>Cefazidine</td>
<td>23 (S)</td>
<td>20 (I)</td>
<td>22 (S)</td>
<td>30 (S)</td>
<td>17 (R)</td>
<td>15 (R)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>20 (I)</td>
<td>19 (I)</td>
<td>19 (SDD)</td>
<td>36 (S)</td>
<td>28 (S)</td>
<td>26 (S)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>20 (I)</td>
<td>22 (S)</td>
<td>6 (R)</td>
<td>32 (S)</td>
<td>25 (S)</td>
<td>27 (S)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>20 (I)</td>
<td>24 (S)</td>
<td>15 (R)</td>
<td>33 (S)</td>
<td>31 (S)</td>
<td>32 (S)</td>
</tr>
<tr>
<td>Pipercillin-Tazobactam</td>
<td>33 (S)</td>
<td>26 (S)</td>
<td>31 (S)</td>
<td>30 (S)</td>
<td>30 (S)</td>
<td>31 (S)</td>
</tr>
<tr>
<td>Ampicillin-Sulbactam</td>
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<td>18 (S)</td>
<td>6 (S)</td>
<td>24 (S)</td>
<td>20 (S)</td>
<td>22 (S)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<td>32 (S)</td>
<td>27 (S)</td>
<td>25 (S)</td>
<td>25 (S)</td>
<td>25 (S)</td>
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<tr>
<td>Levofloxacin</td>
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<td>30 (S)</td>
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<td>25 (S)</td>
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<td>25 (S)</td>
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<tr>
<td>Gentamicin</td>
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<td>15 (S)</td>
<td>16 (S)</td>
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<td>25 (S)</td>
<td>26 (S)</td>
</tr>
<tr>
<td>Amikacin</td>
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<td>24 (S)</td>
<td>24 (S)</td>
<td>25 (S)</td>
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<td>26 (S)</td>
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<tr>
<td>Aztreonam</td>
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<td>35 (S)</td>
<td>35 (S)</td>
<td>36 (S)</td>
<td>35 (S)</td>
<td>35 (S)</td>
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<tr>
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<td>6 (R)</td>
<td>6 (R)</td>
<td>22 (S)</td>
<td>22 (S)</td>
<td>22 (S)</td>
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<tr>
<td>Minocycline</td>
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<td>6 (R)</td>
<td>26 (S)</td>
<td>26 (S)</td>
<td>26 (S)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>18 (I)</td>
<td>23 (S)</td>
<td>20 (S)</td>
<td>29 (S)</td>
<td>29 (S)</td>
<td>30 (S)</td>
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</table>

Carbapenem inactivation method

<table>
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<tr>
<th>PM185</th>
<th>PM187</th>
<th>PR1</th>
<th>J53</th>
<th>J53:pPM187</th>
<th>J53:pPR1</th>
</tr>
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<tbody>
<tr>
<td>(Indeterminate)</td>
<td>(Positive)</td>
<td>(Positive)</td>
<td>(Negative)</td>
<td>(Positive)</td>
<td>(Positive)</td>
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</tbody>
</table>

Fig. 1. (a) Annotated plasmid diagram from DNAPlotter of pPM187 (36,843 bp) displaying blaIMP-27 co-localized with a Class II integron gene cassette and type IV secretion system. The innermost ring shows GC plot, the second ring shows GC skew, the third ring represents open reading frames in the forward direction, and the fourth ring (adjacent to the nucleotide position counter) indicates open reading frames in the reverse direction. (b) Annotated plasmid diagram from DNAPlotter of pPR1 (107,365 bp) displaying blaIMP-27 co-localized with a Class II integron gene cassette, a tra operon, and additional resistance genes.

pPM187 and pPR1. Although Southern blot analysis indicates only a single blaIMP-27 locus in PM185 and PR1, this may further enable a comparison between the chromosomal and plasmid (pPM187) platforms of blaIMP-27 in PM187.

blaIMP-27 was unidentified using the FDA-cleared Xpert CARBA-R assay but the first report of blaIMP-27 used the ARM-D™ Multiplex PCR, which indicates some commercially available platforms can assay for blaIMP-27 [11]. Therefore, further evaluations of commercial molecular diagnostic tests for blaIMP-27 are warranted.

Acknowledgements

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Transparency declaration

The authors have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2018.02.018.

References