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Research note

bla_{IMP-27} on transferable plasmids in *Proteus mirabilis* and *Providencia rettgeri*

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ABSTRACT

Objectives: A carbapenem-resistant *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 bla_{IMP-27} loci in clinical *P. rettgeri* and *Proteus mirabilis* isolates.
Methods: In October 2016 the novel ambler Class B carbapenemase bla_{IMP-27}, was reported in two different *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 *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 *E. coli* through plasmid conjugation (pPM187 and pPR1). pPM187 had a virB/virD4 type IV secretion system whereas pPR1 had a traB/traD type IV secretion system.
Conclusion: Two of three bla_{IMP-27}-bearing clinical isolates tested could conjugate resistance into *E. coli*. The resulting transconjugants became positive for phenotypic carbapenemase production but did not pass clinical resistance breakpoints. bla_{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;▪:1**

Introduction

In January 2016, we isolated a carbapenem-resistant *Providencia rettgeri* (PR1) from a foot wound infection of a patient who visited an outside hospital affiliate of Barnes-Jewish Hospital (Missouri, USA). PR1 was positive for an EDTA-inhibited carbapenemase but no gene was identified by multiplex PCR. Whole genome sequencing and antibiotic resistance gene identification of the PR1 draft genome identified bla_{IMP-27}. bla_{IMP-27} was first reported in October 2016 from two *Proteus mirabilis* strains (PM185 and PM187) from the Upper Plains region of the USA [1]. In December 2016, bla_{IMP-27} was identified on IncQ plasmids from a variety of swine-associated *Enterobacteriaceae* in the USA [2]. Given these recent reports, the greater Midwest region of the USA may be endemic for bla_{IMP-27}, and a potential source for wider geographic dissemination. Accordingly, we acquired PM185 and PM187 to

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understand, with PR1, the potential for lateral transfer of this resistance gene from *Proteus mirabilis* and *P. 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 OD_{600}. 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. Co-cultures were incubated at 37°C 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, technical appendix and methods/results). The paired reads were assembled into contigs with SPAdes v3.9.0 [4]. Raw reads from the transconjugant minipreps were processed for quality in a similar manner. All of of the transconjugant reads that aligned to the clinical isolate plasmid assembly using Bowtie2 were assembled into contigs with SPAdes v3.9.0 [4,5]. Gaps were closed by PCR and Sanger sequencing (Genewiz, South Plainfield, NJ, USA) to yield finished plasmid assemblies (see Supplementary material, Table S1). Open reading frames were annotated for coding sequence using prokka [6]. Antibiotic resistance genes were additionally annotated with Resfams and the ResFinder web server (https://cge.cbs.dtu.dk/services/ResFinder/) [7,8]. pPM187 and pPR1 plasmid maps were made by viewing the gff3 files in DNAplotter and manually annotated for putative open reading frame function [9]. Select type IV secretion system genes were submitted to blastp against the non-redundant protein sequence database on 12 October 2017 [10].

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 bla_{IMP-27} (see Supplementary material, Fig. S1), similar to PM185 [1]. In contrast, PM187 has both chromosomal and plasmid copies of 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_{NDM-1}-bearing plasmid pPrY2001 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 traB/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

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Table 1
Zone of clearance (mm) and phenotypic resistance determination for bla_{IMP-27} positive clinical isolates, wildtype *E. coli* J53, and obtained transconjugants.

	PM185	PM187	PR1	J53	J53:pPM187	J53:pPR1
Ampicillin	24 (S)	6 (R)	14 (R)	21 (S)	17 (S)	17 (S)
Cefazolin	9 (R)	9 (R)	6 (R)	25 (S)	8 (R)	9 (R)
Cefotetan	11 (R)	14 (I)	6 (R)	33 (S)	11 (R)	10 (R)
Ceftriaxone	17 (R)	17 (R)	18 (R)	35 (S)	15 (R)	14 (R)
Ceftazidime	23 (S)	20 (I)	22 (S)	30 (S)	17 (R)	15 (R)
Cefepime	20 (I)	19 (I)	19 (SDD)	36 (S)	28 (S)	26 (S)
Meropenem	20 (I)	22 (S)	6 (R)	32 (S)	25 (S)	27 (S)
Imipenem	20 (I)	24 (S)	15 (R)	33 (S)	31 (S)	32 (S)
Pipercillin-Tazobactam	33 (S)	26 (S)	31 (S)	30 (S)	30 (S)	31 (S)
Ampicillin-Sulbactam	23 (S)	18 (S)	6 (R)	24 (S)	20 (S)	22 (S)
Ciprofloxacin	36 (S)	32 (S)	27 (S)	25 (S)	25 (S)	25 (S)
Levofloxacin	35 (S)	30 (S)	26 (S)	25 (S)	25 (S)	25 (S)
Gentamicin	23 (S)	15 (S)	16 (S)	25 (S)	25 (S)	26 (S)
Amikacin	22 (S)	24 (S)	24 (S)	25 (S)	25 (S)	26 (S)
Trimethoprim-sulfamethoxazole	30 (S)	23 (S)	6 (R)	35 (S)	25 (S)	6 (R)
Colistin	6 (R)	6 (R)	6 (R)	16 (S)	24 (S)	16 (S)
Aztreonam	38 (S)	35 (S)	35 (S)	36 (S)	35 (S)	35 (S)
Doxycyline	6 (R)	6 (R)	6 (R)	22 (S)	22 (S)	22 (S)
Minocycline	11 (I)	10 (R)	6 (R)	26 (S)	26 (S)	26 (S)
Tigecycline	18 (I)	23 (S)	20 (S)	29 (S)	29 (S)	30 (S)
Carbapenem inactivation method	(Indeterminate)	(Positive)	(Positive)	(Negative)	(Positive)	(Positive)

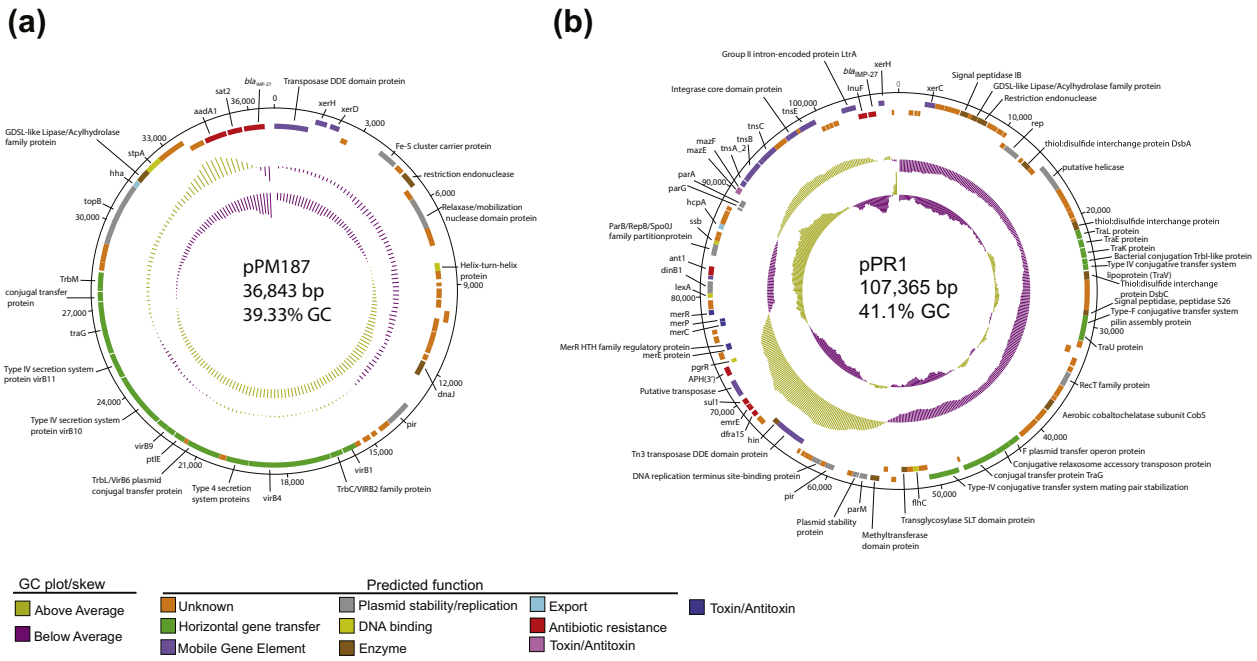

Fig. 1. (a) Annotated plasmid diagram from DNAPlotter of pPM187 (36 843 bp) displaying bla_{IMP-27} co-localized with a Class II integron gene cassette and type IV secretion system. The innermost ring shows GC plot, the second ring shows GC skew, the third ring represents open reading frames in the forward 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 (107365 bp) displaying bla_{IMP-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 bla_{IMP-27} locus in PM185 and PR1, this may further enable a comparison between the chromosomal and plasmid (pPM187) platforms of bla_{IMP-27} in PM187.

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

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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

[1] Dixon N, Fowler RC, Yoshizumi A, Horiyama T, Ishii Y, Harrison L, et al. IMP-27, a unique metallo-β-lactamase identified in geographically distinct isolates of *Proteus mirabilis*. Antimicrob Agents Chemother 2016;60:6418–21.

[2] Mollenkopf DF, Stull JW, Mathys DA, Bowman AS, Feicht SM, Grooters SV, et al. Carbapenemase-producing enterobacteriaceae recovered from the environment of a swine farrow-to-finish operation in the United States. Antimicrob Agents Chemother 2017;61.

[3] Institute CLS. Performance standards for antimicrobial susceptibility testing: Twenty-third Informational Supplement M100–S232013.

[4] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.

[5] Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods 2012;9:357–9.

[6] Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics 2014;30:2068–9.

[7] Gibson MK, Forsberg KJ, Dantas G. Improved annotation of antibiotic resistance determinants reveals microbial resistomes cluster by ecology. ISME J 2015;9:207–16.

[8] Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–4.

[9] Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J. DNAPlotter: circular and linear interactive genome visualization. Bioinformatics 2009;25:119–20.

[10] Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. BMC Bioinform 2009;10:421.

[11] Pearson MM, Sebaihia M, Churcher C, Quail MA, Seshasayee AS, Luscombe NM, et al. Complete genome sequence of uropathogenic *Proteus mirabilis*, a master of both adherence and motility. J Bacteriol 2008;190:4027–37.

[12] Guo Q, Su J, McElheny CL, Stoesser N, Doi Y, Wang M. IncX2 and IncX1-X2 hybrid plasmids coexisting in a FosA6-producing *Escherichia coli* strain. Antimicrob Agents Chemother 2017;61.

[13] Mataseje LF, Boyd DA, Lefebvre B, Bryce E, Embree J, Gravel D, et al. Complete sequences of a novel blaNDM-1-harbouring plasmid from *Providencia rettgeri* and an FII-type plasmid from *Klebsiella pneumoniae* identified in Canada. J Antimicrob Chemother 2014;69:637–42.

[14] Zeng X, Lin J. Beta-lactamase induction and cell wall metabolism in Gram-negative bacteria. Front Microbiol 2013;4:128.

[15] Potter RF, D'Souza AW, Dantas G. The rapid spread of carbapenem-resistant *Enterobacteriaceae*. Drug Resist Updat 2016;29:30–46.

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