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bla$_{IMP-27}$ on transferable plasmids in *Proteus mirabilis* and *Providencia rettgeri*

Robert F. Potter[1], **Meghan A. Wallace**[2], **Allison R. McMullen**[2,&], **Jerome Prusa**[3], **Christina L. Stallings**[3], **Carey-Ann D. Burnham**[2,3,4,#], and **Gautam Dantas**[1,2,3,5,#]

[1]The Edison Family Center for Genome Sciences and Systems Biology, Washington University in St. Louis School of Medicine, Saint Louis, MO, USA

[2]Department of Pathology and Immunology, Washington University in St. Louis School of Medicine, Saint Louis, MO, USA

[3]Department of Molecular Microbiology, Washington University in St. Louis School of Medicine, Saint Louis, MO, USA

[4]Department of Pediatrics, St. Louis Children's Hospital, Saint Louis, MO, USA

[5]Department of Biomedical Engineering, Washington University in St. Louis, Saint Louis, MO, USA

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 mirablis* (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 and phenotypic carbapenemase activity testing was performed on the clinical isolates, J53, and transconjugants using the Kirby-Bauer Disk diffusion method according to Clinical & Laboratory Standards Institute guidelines. Plasmid DNA from PM187, PR1, and their transconjugants were used as input for Nextera Illumina sequencing libraries and sequenced on a NextSeq platform

[#]To whom correspondence should be addressed: CAB (cburnham@wustl.edu); GD (dantas@wustl.edu, Fax: (314) 362-2156, Phone: (314) 362-7238).

[&]Current affiliation -Department of Pathology, Medical College of Georgia at Augusta University, Augusta, GA, USA
Postal address: Bin Wang c/o Dantas Lab, 4524 Clayton Avenue, CB 8510, Couch Biomedical Research Building, Room 5121, St. Louis, MO 63110

Disclosures

Dr. Burnham has nothing to disclose. Dr. Dantas has nothing to disclose.

Results—PR1 was resistant to both imipenem and meropenem. PM187 and PR1 could transfer resistance to *E. coli* via plasmid conjugation (pPM187 and pPR1). pPM187 had a virB/virD4 type IV secretion system (T4SS) whereas pPR1 had traB/traD (T4SS).

Conclusion—2 of 3 $bla_{\text{IMP-27}}$ 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. $bla_{\text{IMP-27}}$ can be transmitted on different plasmid replicon types that rely on distinct classes of T4SS for horizontal transfer.

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, United States). PR1 was positive for an EDTA-inhibited carbapenemase but no gene was identified by multiplex PCR. Whole genome sequencing (WGS) and antibiotic resistance gene (ARG) identification of the PR1 draft genome identified $bla_{\text{IMP-27}}$. $bla_{\text{IMP-27}}$ was first reported in October 2016 from two *Proteus mirabilis* strains (PM185 and PM187) from the upper plains region of the United States (1). In December 2016, $bla_{\text{IMP-27}}$ was identified on IncQ plasmids from a variety of swine associated Enterobacteriaceae in the United States (2). Given these recent reports, the greater Midwest region of the United States may be endemic for $bla_{\text{IMP-27}}$, and a potential source for wider geographic dissemination. Accordingly, we acquired PM185 and PM187 to understand, with PR1, the potential for lateral transfer of this resistance gene from *P. mirabilis* and *P. rettgeri* into *E. coli*, and the associated changes in antibiotic resistance (1).

Methods

Bacterial Isolates

The *Providencia 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 wildtype *E. coli* J53 were separately suspended in Tryptic Soy Broth (TSB) (Sigma Aldrich, St. Louis, MO) and diluted to 0.05 OD_{600}. 100 μl 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 TSB. Co-cultures were incubated at 37 °C without shaking for 24 hours. 50 μl of co-cultures were suspended onto MacConkey agar plates containing sodium azide (Thermo Fisher Scientific, Waltham, MA) (150 μg/ml) and ceftriaxone (5 μg/ml), spread with glass beads, and incubated for 18 hours at 37 °C. Individual transconjugant colonies were propagated overnight in TSB supplemented with 5 μg/ml ceftriaxone under shaking conditions (220 rpm).

Susceptibility Testing

Each clinical isolate, J53, J53:pPR1, and J53:pPM187 were cultured overnight as described previously. *E. coli* ATCC 25922 was used as a quality control. Susceptibility testing was performed using Kirby Bauer Disk Diffusion on Mueller Hinton Agar (Hardy Diagnostics) in accordance with CLSI Standards (3).

Plasmid assembly and annotation

We used Illumina sequencing to specifically investigate $bla_{\text{IMP-27}}$ bearing plasmids in PR1 and PM187. Plasmid DNA was obtained using a miniprep kit (Qiagen, Valencia, CA). Plasmid DNA for PR1 and PM187 was processed to remove Illumina adapters (trimmomatic) and contaminating DNA (deconseq). 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. 100% of the transconjugant reads that aligned to the clinical isolate plasmid assembly using Bowtie2 were assembled into contigs with SPAdes v3.9.0 (5) (4). Gaps were closed by PCR and Sanger-sequencing (Genewiz, South Plainfield, NJ) to yield finished plasmid assemblies (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 T4SS genes were submitted to blastp against the nonredundant protein sequence database on 12/10/17 (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 (Table 1). Southern blot analysis indicates that PR1 has a single copy of $bla_{\text{IMP-27}}$ (Figure S1), similar to PM185 (1). In contrast, PM187 has both chromosomal and plasmid copies of $bla_{\text{IMP-27}}$ (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) (27). 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 (Figure 1A). The *virB4* amino acid sequence had 100% identity over its entire length with a conjugal transfer protein (WP_012368868.1) from *P. mirabilis* HI4320 (11). pPM187 has an IncX8 backbone, a newly discovered IncX family member (12). Unlike pPM187, the assembled $bla_{\text{IMP-27}}$ 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 (Figure 1B). The *traN* amino acid sequence had 100% identity across its entire length to the *traN* (WP_023159916.1) of the $bla_{\text{NDM-1}}$ bearing plasmid pPrY2001 from *P. rettgeri* 09ACRGNY2001 (13).

Discussion

In this study, we used conjugation experiments to determine that two $bla_{\text{IMP-27}}$ 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 $bla_{\text{IMP-27}}$ bearing plasmids, pPM187 and pPR1.

E. 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 $bla_{\text{IMP-27}}$ bearing plasmid in *E. coli* is required for clinical resistance (14). In addition to $bla_{\text{IMP-27}}$ 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 $bla_{\text{IMP-27}}$ 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, likely due to a virB/virD4 T4SS in pPM187 and a traB/traA T4SS in pPR1. The *virB4* and *traN* gene from these T4SS showed similarity to previously described systems from pathogenic *P. 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 or Oxford Nanopore) on $bla_{\text{IMP-27}}$ isolates to unequivocally determine chromosomal sequences and compare the non-conjugatable $bla_{\text{IMP-27}}$ IncQ plasmids with pPM187 and pPR1. Although southern blot analysis indicates only a single $bla_{\text{IMP-27}}$ loci exist in PM185 and PR1, this may further enable a comparison between the chromosomal and plasmid (pPM187) platforms of $bla_{\text{IMP-27}}$ in PM187.

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

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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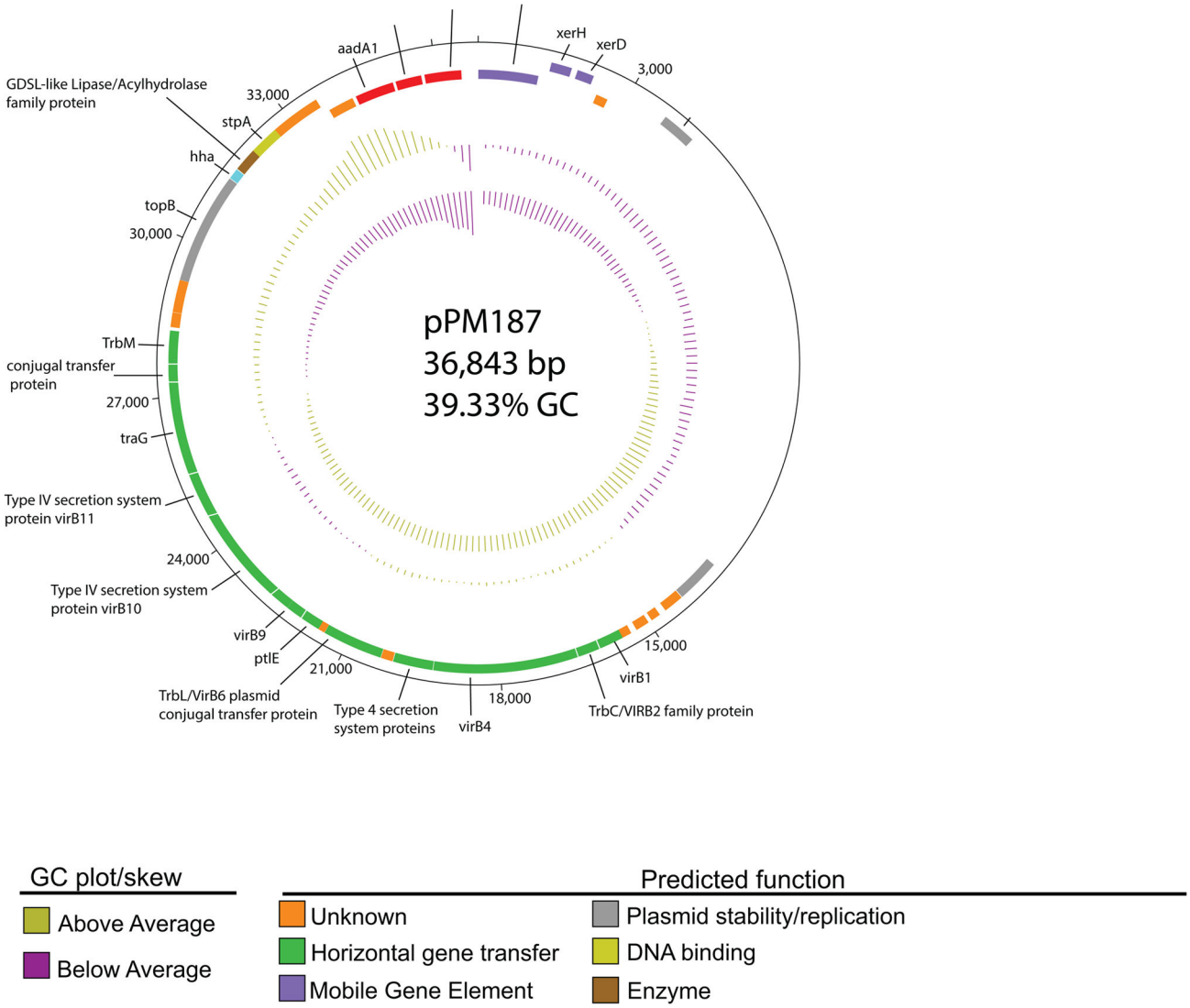

Figure 1.

(A) Annotated plasmid diagram from DNAPlotter of pPM187 (36,843 bp) displaying $bla_{IMP\text{-}27}$ co-localized with a Class II integron gene cassette and type IV secretion system. The inner most 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 $bla_{IMP\text{-}27}$ co-localized with a Class II integron gene cassette, a *tra* operon, and additional resistance genes.

Table 1

	Zone of Clearance (mm)	Interpretation										
Antibiotic	**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
Levoflocaxin	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	R	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