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Emergence of Proteeae Species Isolates Carrying *bla*$_{IMP-27}$-like in United States and Mexico

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Abstract

Background: *Proteus mirabilis* (PM) and indole-positive Proteeae (IPP) species are opportunistic pathogens that cause a wide variety of infections, including nosocomial infections. Five PM and IPP isolates carrying *bla*$_{IMP-27}$-like genes were detected as part of the SENTRY Program during 2015 and were evaluated using whole genome sequencing analysis (WGS). A novel IMP-27 variant was characterized.

Methods: A total of 426 *Morganella morganii* (MM), 1,106 PM, 158 other *Proteus* spp., and 198 *Providencia* spp. were collected worldwide during 2015. Isolates were susceptibility (S) tested using CLSI broth microdilution reference methods, and 10 isolates displaying meropenem and/or doripenem MIC ≥2 µg/mL were screened for carbapenemase genes. WGS evaluated resistant genes, genetic surroundings of *bla*$_{IMP}$, and clonal relatedness. The *bla*$_{IMP-27}$ gene and its novel variant were cloned and susceptibility tested in an *Escherichia coli* host.

Results: Five isolates yielded positive PCR results for *bla*$_{IMP}$-like genes. Isolates included 1 *P. rettgeri* (Iowa, US) and 3 MM (1 North Carolina, US, and 2 Durango, Mexico) that displayed modestly elevated doripenem MIC values (2 µg/mL) and carried *bla*$_{IMP-27}$. One PM from Colorado, US, had elevated MIC values for all carbapenems (>8 µg/mL) and harbored a gene encoding an IMP enzyme displaying 1 amino acid (G19A) difference when compared to IMP-27. All *bla*$_{IMP}$ were embedded in class 2 integrons, and *bla*$_{IMP-27}$ was followed by *sat1* and aminoglycoside-modifying enzymes (*aadA1* or *aph-(3')-Ia*). The new gene, *bla*$_{IMP-64}$, was followed by *sat1, aph-(3')-Ia*, and 5 other genes and was located on an IncQ plasmid. Four isolates carried other β-lactamase genes that included *bla*$_{PER-1}$ and *bla*$_{DHA-6}$ or $_{-18}$. Two MM from Mexico carried *bla*$_{IMP-27}$ in the same plasmid structure and were identical. *E. coli* hosts carrying *bla*$_{IMP-27}$ and *bla*$_{IMP-64}$ showed similar susceptibility profiles with elevated MIC values for doripenem (>8 µg/mL) and cephalosporins and lower results for imipenem (1 µg/mL) and meropenem (4 µg/mL).

Conclusions: IMP-27- and IMP-64-producing isolates were identified from diverse geographic locations from the US and Mexico. The *bla*$_{IMP}$ genes were located in a similar genetic background that could indicate a common genetic source. Isolates producing IMP-27 exhibited low carbapenem MIC results that might be challenging to detect. The novel variant IMP-64 exhibited spectrum similar to IMP-27, but the clinical isolate carrying this enzyme was considerably more resistant when compared to the IMP-27 producers.

Introduction

- Metallo-β-lactamases (MBLs) are cation-dependent enzymes that hydrolyze virtually all β-lactam agents, sparing only monobactams
  - These enzymes are detected among gram-negative organisms worldwide, but their occurrence in the United States is scarce
- Among MBLs, IMP variants were the first described, and more than 60 variants have been reported
- Recently, *Proteus mirabilis* isolates carrying *bla*$_{IMP-27}$ have been reported as a cause of human infections (2009 and 2015) and carried by farm animals (2015) in Ohio

- We characterized 5 *P. mirabilis* and indole-positive Proteeae isolates carrying *bla*$_{IMP-27}$-like genes
  - These isolates were detected in the US and Mexico as part of the SENTRY Antimicrobial Surveillance Program during 2015 and were evaluated using whole genome sequencing (WGS) analysis
  - A novel IMP-variant, IMP-64, was characterized

Materials and Methods

- A total of 1,106 *Proteus mirabilis*, 426 *Morganella morganii*, 158 other *Proteus* spp., and 198 *Providencia* spp. were collected worldwide during the 2015 SENTRY Program
- Susceptibility testing was performed by reference broth microdilution method, according to the Clinical and Laboratory Standards Institute guidelines
  - Quality control (QC) was performed according to CLSI guidelines (M100-S27), and all QC MIC results were within acceptable ranges as published in CLSI documents
  - Categorical interpretations for all comparator agents were those found in CLSI criteria in M100-S27 (2017) or EUCAST breakpoint tables (version 7.0, January 2017) for colistin and tigecycline
- Ten *Proteus mirabilis* and indole-positive Proteeae species isolates displaying elevated meropenem and/or doripenem MIC results (≥2 µg/mL) were screened for carbapenemase genes including *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{NDM}$, *bla*$_{KPC}$, and *bla*$_{OXA-48}$-like using PCR and sequencing
- *bla*$_{IMP}$ positive isolates were submitted to WGS on a MiSeq (Illumina, San Diego, California, US) instrument targeting a 30X coverage
  - Sequences were *de novo* assembled and searched for the presence of acquired resistance genes using a curated library and applying criteria of >94% sequencing identity and 40% minimum length coverage
  - Screening of *bla*$_{IMP}$, surrounding genetic context, and clonal relatedness information was analyzed using Lasegene software (DNAStar, Madison, Wisconsin, USA)
- *bla*$_{IMP-27}$-like genes were cloned in PCRScript® Cam cloning vector per manufacturer's instructions (Stratagene, La Jolla, California, USA) and transformed in commercially available *Escherichia coli* TOP10
  - Transformants were screened by PCR and inserts were sequenced to confirm orientation
  - Recombinant isolates were susceptibility tested and the MIC profiles were compared

Results

- Five isolates yielded positive PCR results for *bla*$_{IMP}$-like genes (Table 1)
  - One *Providencia rettgeri* from Iowa (US) and 3 *Morganella morganii,* 1 from North Carolina (US) and 2 from Durango (Mexico), carried *bla*$_{IMP-27}$
  - One *P. mirabilis* from Colorado (US) harbored a gene encoding an IMP enzyme displaying 1 amino acid (G19A) difference when compared to IMP-27 and was named IMP-64

Table 1 Characteristics of indole-positive Proteeae isolates carrying *bla*$_{IMP-27}$-like

Organism	City, Country	Infection site	IMP-variant	*bla*$_{IMP}$ location	Other resistance markers
Proteus mirabilis	Aurora, Colorado, US	Pleural fluid	IMP-64	IncQ1 plasmid	*bla*$_{PER-1}$, *bla*$_{TEM-1}$, *aac(3)IId, aadA5, ant(2")-Ia, strA, msrE, erm42, catA1, catB3, cat, sul1, sul2, sul3, tetJ*
Morganella morganii	Chapel Hill, North Carolina, US	Urine	IMP-27	Chromosome	*bla*$_{DHA-6}$, *ant(3")Ia, catA2, qnrD, sul2, tetB*
Providencia rettgeri	Des Moines, Iowa, US	Wound	IMP-27	Chromosome	None detected
Morganella morganii	Durango, Mexico[a]	Blood culture	IMP-27	Chromosome	*bla*$_{DHA-18}$, *bla*$_{OXA-1/-30}$
Morganella morganii	Durango, Mexico[a]	Wound	IMP-27	Chromosome	*bla*$_{DHA-18}$, *bla*$_{OXA-1/-30}$

[a] Isolates from Mexico have identical plasmid structures carrying *bla*$_{IMP-27}$, and these isolates are clonal based on sequence analysis of 8 housekeeping genes

- All 4 isolates carrying *bla*$_{IMP-27}$ displayed modestly elevated doripenem MIC values (2 µg/mL) and low meropenem (MIC, 0.5 µg/mL) MIC results (Table 2)
  - For 3 out of 4 *bla*$_{IMP-27}$-carrying isolates, ceftazidime MIC results were also lower than expected (MIC range, 1-2 µg/mL)
- The isolate carrying *bla*$_{IMP-64}$ had elevated MIC values for all carbapenems (>8 µg/mL) and other β-lactams (Table 2)
- The 2 *M. morganii* from Durango were genetically related based on sequence analysis of 8 housekeeping genes (Table 1)
  - These isolates carried *bla*$_{IMP-27}$ in the same genetic structure and also carried *bla*$_{OXA-1/-30}$, in addition to the intrinsic cephalosporinase (*bla*$_{DHA-18}$)
- The *bla*$_{IMP-64}$-harboring *P. mirabilis* isolate carried *bla*$_{PER-1}$ and *bla*$_{TEM-1}$ among other acquired resistance genes (Table 1)
- *bla*$_{IMP-27}$ was chromosomally located in all isolates while *bla*$_{IMP-64}$ was located on an IncQ1 plasmid
- In all isolates, *bla*$_{IMP-27}$-like was embedded in class 2 integrons and this gene was followed by *sat1*
- The integrase gene (*intI2*) in all 4 *bla*$_{IMP-27}$ integrons was identical and displayed an internal stop codon
- *bla*$_{IMP-64}$ gene context was distinct from the other isolates, and the integron carrying this new gene harbored a copy of *aph-(3')-Ia* flanked on both sides by Tn*3*, followed by plasmid replication and mobilization genes (Figure 1)
  - This integron structure was similar to *P. mirabilis bla*$_{IMP-27}$ and surrounding regions from an isolate recovered from a swine operation in Ohio (GenBank number KY126032)
- *E. coli* hosts carrying *bla*$_{IMP-27}$ and *bla*$_{IMP-64}$ showed similar susceptibility profiles with elevated MIC values for doripenem (>8 µg/mL) and cephalosporins, but lower MIC values for imipenem (1 µg/mL) and meropenem (4 µg/mL; Table 2)

Conclusions

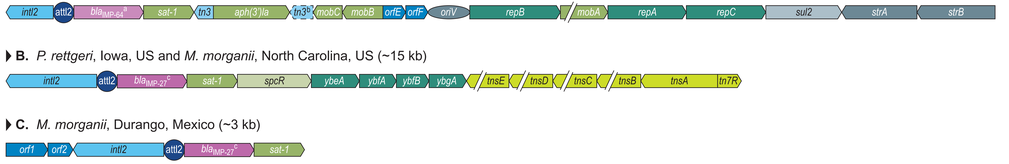
- Isolates carrying *bla*$_{IMP-27}$-like were identified among different Proteeae species in the US and Mexico
  - IMP-27 was chromosomally located and was found in a class 2 integron with a possibly defective integrase gene that might impair the gene's mobilization
  - IMP-64 detected in *P. mirabilis* has 1 amino acid change compared to IMP-27 and has been mobilized on IncQ plasmid that is generally non-conjugative but mobilizable
- The novel variant IMP-64 exhibited MIC spectrum similar to IMP-27, but the clinical isolate carrying this enzyme was considerably more resistant when compared to the IMP-27 producers
- Inherently elevated imipenem MIC values and low meropenem and doripenem results observed among the isolates carrying *bla*$_{IMP-27}$-like might cause difficulties detecting these isolates

Table 2 Susceptibility results for clinical isolates carrying *bla*$_{IMP-27}$-like and IMP-27 and IMP-64 expressed in the same background

Antimicrobial agent	Isolate; MIC value in µg/mL (CLSI interpretation)						
	P. rettgeri clinical isolate carrying *bla*$_{IMP-27}$	*M. morganii* clinical isolate carrying *bla*$_{IMP-27}$	*M. morganii* clinical isolate carrying *bla*$_{IMP-27}$	*M. morganii* clinical isolate carrying *bla*$_{IMP-27}$	*P. mirabilis* clinical isolate carrying *bla*$_{IMP-64}$	*E. coli* TOP10 carrying PCRScript-*bla*$_{IMP-27}$	*E. coli* TOP10 carrying PCRScript-*bla*$_{IMP-64}$	*E. coli* TOP10 carrying PCRScript without insert
Doripenem	2 (I)	2 (I)	2(I)	2 (I)	>8 (R)	>8 (R)	>8 (R)	≤0.06 (S)
Imipenem	1 (S)	2 (I)	1 (S)	2 (I)	>8 (R)	1 (S)	1 (S)	0.25 (S)
Meropenem	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	16 (R)	4 (R)	4 (R)	≤0.12 (S)
Ceftriaxone	8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	≤0.25 (S)
Ceftazidime	2 (S)	2 (S)	1 (S)	32 (R)	>32 (R)	>32 (R)	>32 (R)	≤1 (S)
Ceftazidime-avibactam[b]	2 (S)	2 (S)	1 (S)	16 (R)	8 (R)	>32 (R)	>32 (R)	NT
Cefepime	1 (S)	1 (S)	1 (S)	8 (I)	32 (R)	16 (R)	16 (R)	≤0.12 (S)
Aztreonam	≤0.12 (S)	≤0.12 (S)	≤0.12 (S)	2 (S)	>16 (R)	0.25	0.25	≤0.12 (S)
Ampicillin-sulbactam	1 (S)	>32 (R)	32 (R)	>32 (R)	8 (S)	8 (S)	8 (S)	8 (S)
Piperacillin-tazobactam	1 (S)	1 (S)	0.5 (S)	2 (S)	0.25 (S)	2 (S)	2 (S)	1 (S)
Amikacin	0.5 (S)	2 (S)	2 (S)	4 (S)	4 (S)	NT[a]	NT	NT
Tobramycin	1 (S)	2 (S)	1 (S)	1 (S)	>8 (R)	NT	NT	NT
Tetracycline	>16 (R)	>16 (R)	>16 (R)	>16 (R)	>16 (R)	NT	NT	NT
Tigecycline[c]	1 (S)	0.5 (S)	1 (S)	1 (S)	2 (R)	NT	NT	NT
Ciprofloxacin	>4 (R)	>4 (R)	>4 (R)	>4 (R)	>4 (R)	NT	NT	NT
Colistin[b]	>8 (R)	>8 (R)	>8 (R)	>8 (R)	>8 (R)	NT	NT	NT

[a] NT, not tested
[b] US Food and Drug Administration and EUCAST breakpoint criteria
[c] EUCAST breakpoint

Figure 1 Schematic representation of *bla*$_{IMP-27}$-like and surrounding regions observed in indole-positive Proteeae species



[a] Similar to the gene context from *bla*$_{IMP-4}$ described from a swine operation in Ohio (Mollenkopf et al, 2017)
[b] Listed as non-functional
[c] *bla*$_{IMP-27}$ gene array identical to *P. mirabilis* isolated from US patients (Dixon et al, 2016)

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