Endemicity of *Pseudomonas aeruginosa* Producing IMP-18 and/or VIM-2 Metallo-β-Lactamases from the High-Risk Clone ST111 in Central America

Lalitagauri M. Deshpande[1], Andrew Davis[1], Oscar Aspelin[2], Silvio Vega[3], Juan Carlos Tinoco[4], Mariana Castanheira[1]

[1]JMI Laboratories, North Liberty, Iowa, USA; [2]1928 Diagnostics, Gothenburg, Sweden; [3]Complejo Hospitalario Metropolitano, Panama City, Panama; [4]Hospital General 450, Secretaría de Salud, Durango, México

Objective

Pseudomonas aeruginosa is an important cause of serious nosocomial infections.

Despite the overall genetic diversity of *P. aeruginosa* isolates, highly conserved clonal complexes (CCs) have been observed among multidrug-resistant isolates.

P. aeruginosa that belong to ST235, ST357, and ST111 are common among MDR and metallo-beta-lactamase-producing (MBL) isolates.

We evaluated 5 *P. aeruginosa* isolates from Central America that carried IMP-18 and/or VIM-2 encoding genes from the SENTRY Antimicrobial Surveillance Program.

Methods

- Five extensively drug resistant (XDR; CLSI/EUCAST) *P. aeruginosa* isolates collected in 2017-2018 were investigated.
- Susceptibility testing was performed by CLSI broth microdilution.
- Whole genome sequencing was performed using MiSeq (Illumina) and MinION (Oxford Nanopore).
  - Assembled contigs from short and long reads were combined for *in silico* screening of resistance genes, multilocus sequence typing (MLST), core genome (cg)MLST, and SNP analysis using the 1928 Diagnostics platform.
  - SENTRY isolates were compared at nucleotide level to a *P. aeruginosa* AG1 (PaeAG1) ST111 strain isolated from a Costa Rican hospital in 2010 which carried $bla_{\text{VIM-2}}$ and $bla_{\text{IMP-18}}$.

Results

- The 5 *P. aeruginosa* isolates were recovered from patients with urinary tract infections or pneumonia.
- Isolates were resistant to imipenem (MIC >8 mg/L), meropenem (>32 mg/L), ceftolozane-tazobactam (>32 mg/L), tobramycin (>16 mg/L), amikacin (4 of 5; >32 mg/L), and levofloxacin (>16 mg/L) and intermediate to colistin (CLSI; 1-2 mg/L).

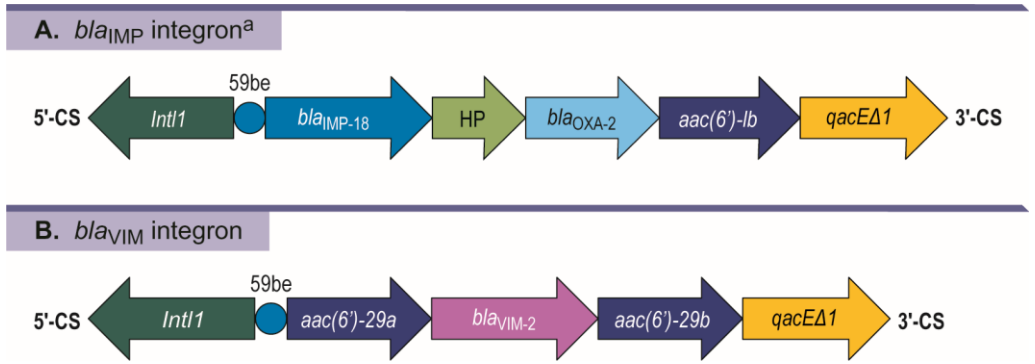
Table 1. Summary of demographic and molecular findings of ST111 *P. aeruginosa* isolates

Characteristic	1PAN	2PAN	3PAN	4MEX	5PAN
Country (Month-Year isolated)	Panama (MAR-2017)	Panama (JUN-2017)	Panama (JUL-2017)	Mexico (JUL-2017)	Panama (MAR-2018)
Specimen type	Pneumonia in hospitalized patients	Pneumonia in hospitalized patients	Urinary tract infection	Urinary tract infection	Pneumonia in hospitalized patients
Infection source	Nosocomial	Community-acquired	Nosocomial	Community-acquired	Not known
β-lactamase encoding genes	bla_{IMP-18}, bla_{OXA-2}, bla_{VIM-2}	bla_{IMP-18}, bla_{OXA-2}, bla_{VIM-2}	bla_{IMP-18}, bla_{OXA-2}	bla_{VIM-2}	bla_{IMP-18}, bla_{OXA-2}, bla_{VIM-2}
Aminoglycoside modifying enzyme encoding genes	*aac(6`)-29a*, *aac(6`)-29b*, *aac(6`)-Ib*, *aph(3`)-IIb*-like	*aac(6`)-29a*, *aac(6`)-29b*, *aac(6`)-Ib*, *aph(3`)-IIb*-like	*aac(6`)-29b*, *aac(6`)-Ib*, *aph(3`)-IIb*-like	*aac(6`)-29a*, *aac(6`)-29b*, *aph(3`)-IIb*-like	*aac(6`)-29a*, *aac(6`)-29b*, *aph(3`)-IIb*-like
Raw SNP differences compared to PaeAG1 from Colombia[a]	336	331	283	1,124	101

[a] Analysis performed on the 1928 Diagnostics bioinformatic platform. The consensus sequence from each sample was individually compared to the reference.

- When the consensus sequence of each isolate was individually compared against the published sequence of the ST111 isolate PaeAG1 (CP045739.1), between 101 and 1,124 differences were recorded (Table 1).
- All isolates belonged to ST111 but carried different combinations of resistance encoding genes, mostly aminoglycoside-modifying, enzyme-encoding genes.
  - Transposon-associated MBL genes, bla_{VIM-2} and/or bla_{IMP-18}, were chromosomally located.
  - bla_{OXA-2} was detected in the bla_{IMP-18} integron. bla_{VIM-2} was in an In59-like integron.

Figure 1. Schematic diagram of bla_{IMP-18} and bla_{VIM-2} integrons in ST111 *P. aeruginosa* isolates



Results

- Based on cgMLST analysis performed on the 1928 Diagnostics platform, these isolates were closely related, with average allele differences of just 2 to 34 (Figure 2).
- SNP analysis using Illumina data and SNP exclusion parameter 0 (which considered all SNPs between sample to sample and sample to reference) showed between 5 to 423 differences among the SENTRY isolates.
- When the SNP exclusion parameter of 10 was used (excluding all SNPs within a distance of 10 nucleotides to one another) the isolates from Panama and Mexico showed between 5 and 180 SNP differences (Table 2B).

Figure 2. cgMLST - UPGMA clustering output of SENTRY *P. aeruginosa* ST111 isolates on the 1928 Diagnostics platform

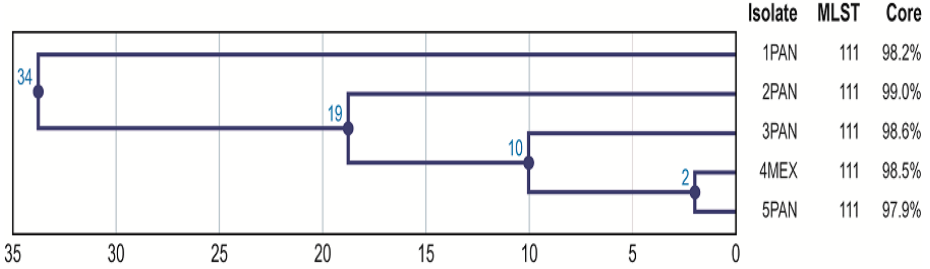


Table 2. SNP analysis distance matrix of SENTRY isolates compared to *P. aeruginosa* AG1 from Colombia

A. Distance matrix using exclusion parameter 0[a]

	1PAN	2PAN	3PAN	4MEX	5PAN	PaeAG1
1PAN	0	5	58	422	169	155
2PAN	5	0	59	423	170	156
3PAN	58	59	0	404	137	123
4MEX	422	423	404	0	423	427
5PAN	169	170	137	423	0	38
PaeAG1	155	156	123	427	38	0

B. Distance matrix using exclusion parameter 10[a]

	1PAN	2PAN	3PAN	4MEX	5PAN	PaeAG1
1PAN	0	5	33	179	56	42
2PAN	5	0	34	180	57	43
3PAN	33	34	0	178	49	35
4MEX	179	180	178	0	173	177
5PAN	56	57	49	173	0	38
PaeAG1	42	43	35	177	38	0

[a] Exclusion parameter 0 considered all possible SNPs between the samples and the reference. Exclusion parameter 10 used less stringent criteria to account for possible recombination events or other factors that might cause SNPs to accumulate in certain areas.

Results

- We excluded of SNPs within 10 nucleotides of one another to account for possible recombination events or other factors that might cause the SNPs to accumulate in certain areas.
- Compared to the PaeAG1, the clinical isolates exhibited 35 to 177 SNPs.
- The isolate from Mexico was the most distinctive isolate in the Panama cluster (Table 2A).
- The 2018 Panama isolate was the most similar to PaeAG1, as it had just 38 SNPs.
- The isolate from Mexico had 427 SNPs, the most of all investigated isolates.

Conclusions

- Metallo-β-lactamase-producing ST111 *P. aeruginosa* were originally reported in Central America in 2010 from isolates recovered in Costa Rica.
- These isolates became endemic in Panama and may have spread to Mexico via clonal dissemination.
- The isolates from Panama were more closely related to the Costa Rican isolate than the isolate from Mexico.
- Recombination events were apparent in the evolution of this clonal complex in this region.
- Surveillance is warranted to track the expansion and movement of this clone, as two classes of metallo-β-lactamases are associated.

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Contact

Mariana Castanheira, PhD

mariana-castanheira@jmilabs.com