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Mechanisms of carbapenemase-mediated resistance among high-risk *Pseudomonas aeruginosa* lineages in Peru

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ABSTRACT

Objectives: *Pseudomonas aeruginosa* is one of the leading causes of healthcare-associated infections globally. High-risk carbapenemase-encoding *P. aeruginosa* clones are disseminating in many regions. The aim of this study was to learn more about the lineages and mechanisms of resistance of *P. aeruginosa* circulating in Peru.

Methods: A total of 141 carbapenemase-producing isolates recovered from hospitalized and ambulatory patients in Lima were sequenced and analyzed to infer their lineages through whole-genome sequence typing (wgST) and to identify their antimicrobial resistance genes.

Results: wgST identified nine sequence types (STs); ST111 and ST357 were the most frequently encountered (44.0% and 38.3%, respectively), followed by ST179 (8.5%), with the remaining six detected only sporadically. Among ST357 isolates, 96.3% carried the novel bla_{IMP-93} allele, whereas the remainder harbored bla_{IMP-74}. 74.2% of ST111 isolates co-harbored bla_{IMP-18} and bla_{VIM-2}, while the rest carried either of these genes individually. All other ST lineages carried a single carbapenemase, which was either bla_{IMP-16}, bla_{IMP-74}, or bla_{VIM-2}.

Conclusion: Our study shows that the high-risk *P. aeruginosa* clones ST357, which harbors the novel bla_{IMP-93}, and ST111, which carries bla_{IMP-18} and bla_{VIM-2}, have apparently become endemic in the region.

1. Introduction

Pseudomonas aeruginosa, which is a non-fermentative, strictly aerobic Gram-negative bacillus, is an important cause of healthcare-associated infections worldwide [1,2]. *P. aeruginosa* strains are ubiquitous in the environment and have a complex array of virulence factors and survival strategies, in addition to multiple mechanisms of antimicrobial resistance, which include both intrinsic and acquired traits [3–5]. Since infections with *P. aeruginosa* tend to be associated with increased morbidity and mortality, prompt and appropriate antimicrobial treatment is crucial to optimize patient outcomes [6]. The World Health Organization has ranked carbapenem-resistant *P. aeruginosa* as a critical target (priority 1) for which new antimicrobial agents are needed [7]. Current antipseudomonal therapies include beta-lactams and beta-lactam/beta-lactamase inhibitor combinations, fluoroquinolones, and aminoglycosides. Combinations of antimicrobial agents are often administered because of widespread multidrug resistance [8,9]. Carbapenem resistance in *P. aeruginosa* is frequently the result of overexpression of the *MexAB-OprM* efflux pump system and mutations involving inactivation of the OprD outer membrane protein, but it can also be mediated by horizontally acquired genes that hydrolyze carbapenems [10]. *P. aeruginosa* isolates can harbor diverse types of carbapenemase genes, including bla_{VIM}, bla_{IMP}, bla_{NM}, and bla_{KPC}, either alone or in combination [5,11,12]. More recently, highly virulent strains of *P. aeruginosa,* referred to as 'high-risk clones', appear to be increasing in frequency in multiple regions around the world and many of these produce carbapenemases [13,14]. While high-risk clones of *P. aeruginosa* are known to circulate globally, little is known about the carbapenem-resistant *P. aeruginosa* strains that are present in South America, and, specifically, in Peru. The aim

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of this study was to characterize isolates of carbapenem-resistant *P. aeruginosa* recovered from patients and outpatients in Lima, Peru using phenotypic methods and whole-genome sequence analysis to better understand the strains that are circulating and the resistance mechanisms they harbor.

2. Materials and methods

2.1. Bacterial strains

A convenience sample of 141 carbapenem-resistant isolates of *P. aeruginosa* (50 collected in 2018 and 90 collected in 2021) was obtained from a reference laboratory in Lima, Peru. The isolates were identified to the species level and tested for antimicrobial resistance using Vitek 2.0 automated system (bioMérieux, Marcy Etoile, France) and/or by the disk diffusion method (for resistance to imipenem and meropenem), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [15]. Carbapenemase production was demonstrated using the Blue-Carba test, as previously described [16].

2.2. Whole genome sequence analysis

Genomic DNA was extracted from pure cultures of organisms grown overnight on blood agar plates using the Qiagen DNeasy Blood and tissue kit (Qiagen, Valencia, CA, USA). Sequencing libraries were prepared using Illumina DNA Prep Kit (Illumina, San Diego, CA, USA) and sequencing was carried out on the MiSeq instrument using Reagent Kit v2 chemistry (Illumina). De novo assemblies, multilocus sequence typing (MLST), and detection of virulence and antimicrobial resistance determinants were performed with the CLC Genomics Workbench 21.0.5 and CLC Microbial Genomics Module 21.1 (CLCbio, Denmark). DNA extraction, library preparation, and sequencing were all performed according to each manufacturer's instructions. Sequence data of representative clones of *P. aeruginosa* were deposited with links to BioProject Accession PRJNA795491 in the National Center for Biotechnology Information (NCBI) BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

2.3. Statistical data analysis

Results were compared using the Two-sample Proportions test and *P* values were calculated using Fisher's exact test (Minitab 18 Statistical Software, State College, PA, USA). A *P* value <0.05 was considered statistically significant.

3. Results and discussion

3.1. Antimicrobial susceptibility profiles

All 141 isolates were resistant to imipenem and meropenem by both Vitek minimal inhibitory concentration (MIC) testing and/or by disk diffusion, and all isolates were positive for carbapenemases using the Blue Carba phenotypic test. Furthermore, all the isolates were resistant to extended and expanded spectrum cephalosporins (ceftazidime and cefepime, respectively), and beta-lactam/beta-lactamase inhibitor combinations (including piperacillin/tazobactam, ceftolozane/tazobactam, and ceftazidime/avibactam) (Table 1). The majority (75.2%) of *P. aeruginosa* isolates were also non-susceptible to aztreonam (25.5% intermediate and 49.6 % resistant), as well as non-susceptible to amikacin and gentamicin (95.7% and 94.3%, respectively). All isolates, with one exception, were resistant to ciprofloxacin; the remaining isolate was intermediate to this fluoroquinolone.

Whole-genome sequence types (STs) were determined to ascertain whether the isolates were clonal or represented multiple strain types. ST data identified four high-risk *P. aeruginosa* clones in this collection of isolates from patients from 12 hospitals and outpatients. The results are shown by ST and year of collection in Table 2, and by ST and carbapenemase subtype in Fig. 1. Overall, nine STs were identified; ST111 and ST357 were the most frequently encountered (44.0% and 38.3%, respectively), followed by ST179 (8.5%), ST273 (4.3 %), ST235 (1.4%), and ST260 (1.4 %). There was a single isolate each of ST309 and ST244, and the remaining isolate had an inconclusive ST (closest to ST2245). Four of nine STs identified, namely ST111, ST357, ST244, and ST235, have been reported as international high-risk clones [14,17]. Urine was the most frequent specimen from which these four clones were isolated (45.4%), followed by bronchial/tracheal aspirates (37.6%).

Susceptibility to aztreonam, which can be an indicator of metallo-beta-lactamase carriage, varied by ST types; all but one of the ST357 strains were non-susceptible to aztreonam, while only 64.5% of ST111 and 16.7% of ST179 were non-susceptible to the antimicrobial agent (Supplementary Table S1). All *P. aeruginosa* isolates were intermediate or resistant to four or more classes of the antimicrobials tested. Approximately 63% of ST111 isolates were non-susceptible to all antimicrobial agents tested (six classes) compared with 96.3% of ST357 (*P* value <0.001). Other STs resistant to all antimicrobial agent classes tested were ST235, ST244, ST273, ST309, and 16.7% of ST179 (2/12).

3.2. Acquired carbapenemase genes identified among STss

All 141 isolates harbored metallo-beta-lactamase genes (MBL), either a bla_{IMP}, $bla_{\text{VIM-2}}$, or $bla_{\text{IMP-18}}$/$bla_{\text{VIM-2}}$ concurrently (Fig. 1,2). A novel bla_{IMP} subtype, with 99.7% identity to $bla_{\text{IMP-16}}$ and $bla_{\text{IMP-74}}$, was identified and designated as $bla_{\text{IMP-93}}$ (Fig. 3). The novel $bla_{\text{IMP-93}}$ was the most common MBL gene identified and was present in 52 of the isolates (36.9%), followed by the co-carriage of $bla_{\text{IMP-18}}$/$bla_{\text{VIM-2}}$, which was detected in 46 isolates (32.6%). The remaining 43 isolates carried either $bla_{\text{IMP-74}}$, $bla_{\text{IMP-16}}$, $bla_{\text{VIM-2}}$, or $bla_{\text{IMP-18}}$ (Fig. 2). Of 62 *P. aeruginosa* ST111 isolates, 46 harbored $bla_{\text{IMP-18}}$ and $bla_{\text{VIM-2}}$, eight isolates harbored only $bla_{\text{IMP-18}}$, and eight harbored only $bla_{\text{VIM-2}}$. Among the 54 ST357 isolates, 52 (96.3%) carried $bla_{\text{IMP-93}}$, while two isolates harbored $bla_{\text{IMP-74}}$ (Fig. 4). The remaining STs carried either $bla_{\text{IMP-16}}$ (48.0%), $bla_{\text{IMP-74}}$ (40.0%), or $bla_{\text{VIM-2}}$ (8.0%). One isolate of ST309 harbored $bla_{\text{VIM-2}}$ and a truncated $bla_{\text{IMP-18}}$-like determinant. Table 2 compares the distribution of the STs identified in 2018 to that of the STs identified in 2021. ST111 increased from 31.4% of all *P. aeruginosa* isolated in 2018 to 51.1% in 2021 (*P* value = 0.034). When we compared the isolates by year of collection, the bla_{IMP} genes were detected among 46 of 51 of the *P. aeruginosa* isolates from 2018 and in 85 of 90 of the isolates from 2021 (90.2% and 94.4%, respectively; *P* value = 0.496). However, the $bla_{\text{VIM-2}}$ gene was detected, alone or in combination with bla_{IMP}, in 12 isolates from 2018 and in 45 from 2021 (23.5% and 50.0%, respectively; *P* value = 0.002) (data not shown). Fig. 4 shows how bla_{IMP} and bla_{VIM} subtypes, or combinations of the two, were distributed across the STs in both years. ST357 harbored mostly $bla_{\text{IMP-93}}$ (89.5%) with sporadic carriage of $bla_{\text{IMP-74}}$ in 2018, but in 2021, this ST exclusively harbored $bla_{\text{IMP-93}}$. Similarly, in 2018, 43.8% of ST111 strains harbored both $bla_{\text{IMP-18}}$ and $bla_{\text{VIM-2}}$, while 25.0% carried either $bla_{\text{IMP-18}}$ or $bla_{\text{VIM-2}}$ individually. By comparison, in 2021, 84.8% of ST111 isolates carried both the $bla_{\text{IMP-18}}$ and $bla_{\text{VIM-2}}$ genes.

3.3. Virulence genes among STs

To better understand the virulence profile of the high-risk *P. aeruginosa* clones in our study, we analyzed the distribution of

Table 1
Antimicrobial susceptibility results grouped by antimicrobial class for 141 *Pseudomonas aeruginosa* isolates

Antimicrobial class tested	Antimicrobial agent	Number of isolates (%)		
		Susceptible	Intermediate	Resistant
Aminoglycosides	Amikacin	6 (4.3)	7 (5.0)	128 (90.8)
	Gentamicin	8 (5.7)	6 (4.3)	127 (90.1)
Beta-lactam/beta-lactamase inhibitor combinations	Piperacillin/tazobactam	0 (0.0)	0 (0.0)	141 (100.0)
	Ceftolozane/tazobactam	0 (0.0)	0 (0.0)	141 (100.0)
	Ceftazidime/avibactam[a]	0 (0.0)	0 (0.0)	90 (100.0)
Cephems	Ceftazidime	0 (0.0)	0 (0.0)	141 (100.0)
	Cefepime	0 (0.0)	0 (0.0)	141 (100.0)
Monobactams	Aztreonam	35 (24.8)	36 (25.5)	70 (49.6)
Carbapenems	Imipenem	0 (0.0)	0 (0.0)	141 (100.0)
	Meropenem	0 (0.0)	0 (0.0)	141 (100.0)
Quinolones	ciprofloxacin	1 (0.7)	1 (0.7)	139 (98.6)

[a] Only the 90 isolates from 2021 were tested with Ceftazidime/avibactam.

Table 2
Genome STs identified in the two study periods

ST	Number (%) of isolates collected in 2018 (n=50)	Number (%) of isolates collected in 2021 (n=90)	*P* value[a]
ST111	16 (31.4%)	46 (51.1%)	0.034
ST357	19 (37.3%)	35 (38.9%)	1.000
ST179	8 (15.7%)	4 (4.4%)	0.029
ST273	6 (11.8%)	0	N/A
ST235	1 (2.0%)	1 (1.1%)	1.000
ST260	0	2 (2.2%)	N/A
ST309	0	1 (1.1%)	N/A
ST2245[b]	1 (2.0%)	0	N/A
ST244	0	1 (1.1%)	N/A

[a] *P* value was calculated using Fisher's exact test.
[b] ST was inconclusive; ST2245 was the nearest ST.

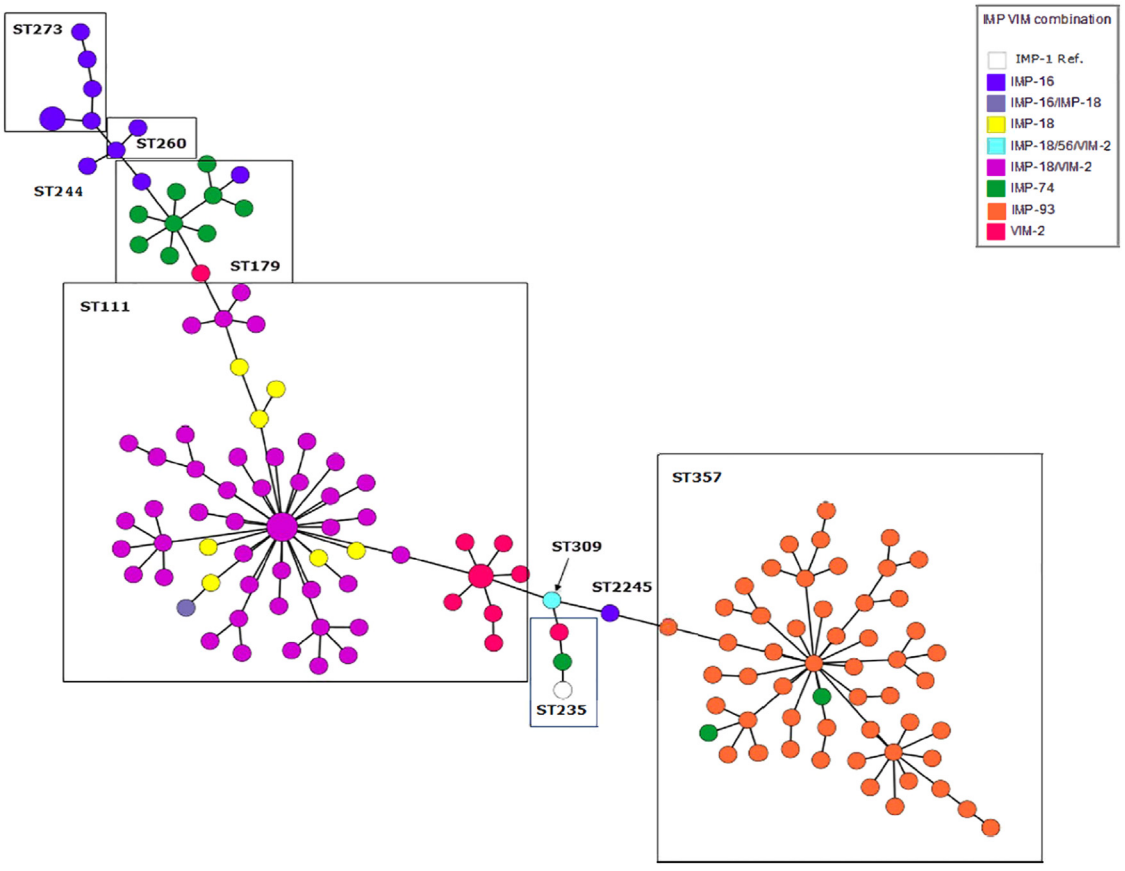


Fig. 1. Minimum spanning tree (MST) of 141 *Pseudomonas aeruginosa* isolates grouped by STs and showing metallo-beta-lactamase (MBL) subtype carriage. ST235 strain AP0122280, harboring bla_{IMP-1}, was used as reference. The MST was created with CLC Microbial Genomics Module 22.0.1 Create MLST Scheme 1.4 using the default parameters, and included MLST type, whole genome, antimicrobial resistance database ResFinder (2022-02-16), and virulence database VFDB Nucleotide Database (2019-05).

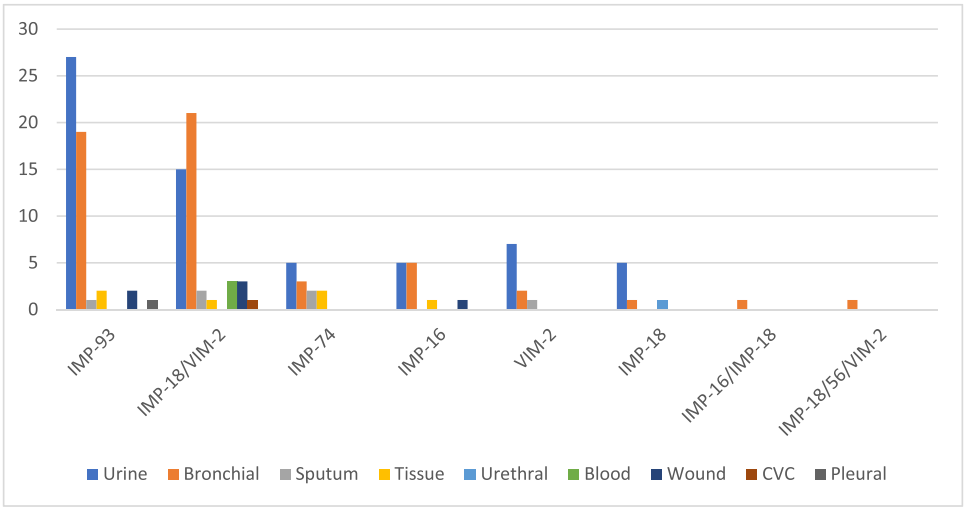


Fig. 2. Carbapenemase genes identified in 141 *Pseudomonas aeruginosa* isolates by specimen type.

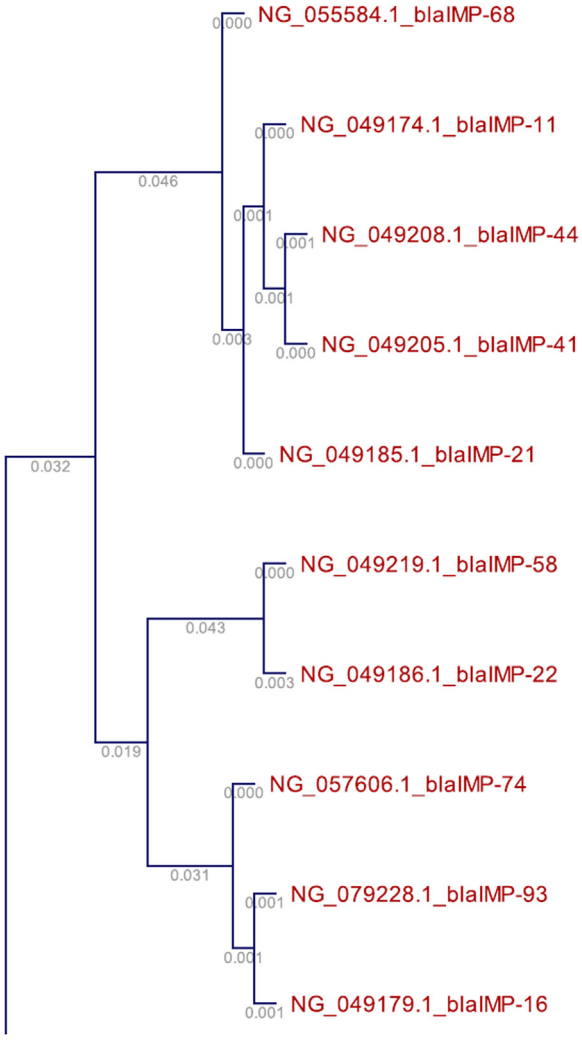


Fig. 3. Zoomed-in view of the bla_{IMP} phylogenetic tree, showing bla_{IMP-16}, bla_{IMP-93}, bla_{IMP-74}, and closely related alleles. The phylogenetic tree of 90 nucleotide sequences of bla_{IMP} (downloaded from the NCBI Reference Gene Catalog: https://www.ncbi.nlm.nih.gov/pathogens/refgene/) was constructed based on Neighbor-Joining method using CLC Genomics Workbench 22.0.1. The number below each branch corresponds to branch length.

the type 3 secretion system (T3SS) effectors (Supplementary Table S2). The *exoS*+/*exoT*+/*exoU*+/*exoY*+ virulence profile, containing all four known effectors, was only found in ST273 isolates. All but one of ST357, which was *exoU*–, and all ST235 isolates, were *exoT*+/*exoU*+/*exoY*+. ST111, ST179, ST244, and ST260 isolates all shared the *exoS*+/*exoT*+/*exoY*+ virulence profile. The non-typeable isolate closest to ST2245 carried only *exoT*+, and the ST309 clone had the *exoT*+/*exoU*+ profile.

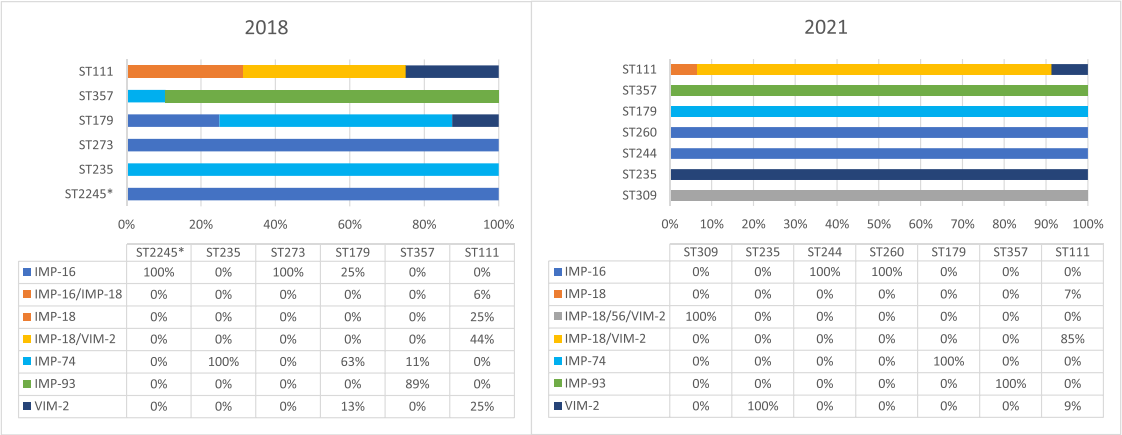
4. Discussion

In our study, ST111 and ST357 were the predominant clones identified among the isolates of carbapenem-resistant *P. aeruginosa* from Peru. Both STs have a long history as international high-risk lineages [14,17]. ST111 is a very successful clone reported worldwide and is primarily associated with carriage of bla_{VIM-2} and less frequently associated with carriage of bla_{IMP} or both genes concurrently [18]. The combination of bla_{IMP-18} and bla_{VIM-2} in ST111 was first reported in Costa Rica in isolates collected between 2004 and 2005 [19]. On the other hand, the ST357 clone has been described as a frequent carrier of bla_{IMP} since 2009, when it was reported to harbor bla_{IMP-1} in Japan [20].

Our findings of bla_{IMP}, bla_{VIM}, and bla_{IMP}/$_{VIM}$ co-production in *P. aeruginosa* are similar with those reported by Mayta-Barrios and colleagues in Peru, although we did not detect any bla_{NDM} among our isolates [21]. In the same 2019 study, 23.0% of the carbapenemase-producing *P. aeruginosa* carried both bla_{IMP} and bla_{VIM}, which corroborates the upward trend in co-carriage of the genes we observed between 2018 and 2021(from 13.7% to 44.4%; *P* value <0.001).

All the *P. aeruginosa* isolates in the present study were multidrug resistant (MDR, non-susceptible to ≥4 antimicrobial classes) [22]. A 2019 study in Peru by Horna and colleagues reported the T3SS *exoU*+ genotype to be associated with MDR and XDR phenotypes more strongly than the *exoU*– genotype [23]. Consistent with the findings by Horna et al., we observed that strains that are *exoU*+ have more resistant phenotypes than the *exoU*– genotype (85.5% resistant to six antimicrobial agent classes vs. 20.3%, respectively; *P* <0.001).

The population structure of *P. aeruginosa* has been investigated extensively and described as a nonclonal epidemic population, with no distinction between clinical and environmental isolates noted. It can respond and adapt to selection pressures, such as antimicrobial treatment, rapidly [24,25]. Despite the large strain di-



	ST2245*	ST235	ST273	ST179	ST357	ST111
IMP-16	100%	0%	100%	25%	0%	0%
IMP-16/IMP-18	0%	0%	0%	0%	0%	6%
IMP-18	0%	0%	0%	0%	0%	25%
IMP-18/VIM-2	0%	0%	0%	0%	0%	44%
IMP-74	0%	100%	0%	63%	11%	0%
IMP-93	0%	0%	0%	0%	89%	0%
VIM-2	0%	0%	0%	13%	0%	25%

	ST309	ST235	ST244	ST260	ST179	ST357	ST111
IMP-16	0%	0%	100%	100%	0%	0%	0%
IMP-18	0%	0%	0%	0%	0%	0%	7%
IMP-18/56/VIM-2	100%	0%	0%	0%	0%	0%	0%
IMP-18/VIM-2	0%	0%	0%	0%	0%	0%	85%
IMP-74	0%	0%	0%	0%	100%	0%	0%
IMP-93	0%	0%	0%	0%	0%	100%	0%
VIM-2	0%	100%	0%	0%	0%	0%	9%

Fig. 4. Distribution of sequence types (STs) and bla_{IMP}/bla_{VIM} subtypes among the *Pseudomonas aeruginosa* isolated in 2018 and 2021.

versity that characterizes susceptible isolates of *P. aeruginosa* (with >3000 STs described to date), we found MDR/XDR isolates limited to just a few lineages [14]. A similar MDR/XDR clustered population structure in MBL-producing *P. aeruginosa* was reported among intensive care unit patients in a study in Iran [26], especially in hospital burn units, where appropriate and prompt antibiotic therapy is critical for successful wound management and patient survival [27]. Also consistent with the nonclonal epidemic population structure, the isolates in our study, which were collected three years apart, showed two predominant high-risk international clones, each with their preferred resistance and virulence repertoires, but without an obvious association with a hospital or sample type. Nonetheless, resistance was increasing and is of concern given the lack of effective antimicrobial agents available for treatment.

This study has several limitations. First, the 141 isolates characterized represent a convenience sample from different hospitals and outpatient settings, and from two separate time periods. Furthermore, testing for susceptibility to colistin may have provided additional insight into the lineages we suspect to be XDR. Nonetheless, the data are consistent with the evolving resistance profiles of high-risk *P. aeruginosa* clones that are present in multiple healthcare institutions in Peru.

In conclusion, our study shows that the two high-risk clones, ST357 harboring the novel bla_{IMP-93} and ST111 carrying bla_{IMP-18} and bla_{VIM-2}, appear to have become endemic in Peru. The fact that these successful clones have acquired resistance to most antimicrobial classes used to treat *P. aeruginosa* infections is cause for concern.

Competing interests

Isabella A. Tickler and Fred C. Tenover are employees of Cepheid. Anne E. Obradovich has received research funding from Cepheid. The other authors report no conflicts of interest.

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Ethical approval

Not required

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.08.018.

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