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Epidemiological insights into global metallo-β-lactamases-producing *pseudomonas aeruginosa*: A comprehensive analysis based on NCBI database

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ABSTRACT

This study aims to analyze the distribution of metallo-β-lactamase (MβLs) encoding genes in global *Pseudomonas aeruginosa*. Genomes of *P. aeruginosa* (21,788 in total) were obtained from the NCBI database and annotated using Prodigal to ensure consistency. Blastn analysis was conducted to compare the structured $bla_{\mathrm{M\beta L}}$ databases with annotated genomes to obtain detailed distribution of all $bla_{\mathrm{M\beta L}}$ genes in all genomes. A self-written sequence typing tool was utilized for sequence typing analysis on $bla_{\mathrm{M\beta L}}$ carrying *P. aeruginosa*, and meta-information of strains was extracted for further analysis. Out of 21,788 genomes, 2,639 (12.1%) contained 4,014 $bla_{\mathrm{M\beta L}}$, with bla_{VIM} being the most prevalent (51.2%), followed by bla_{IMP} (24.1%) and bla_{NDM} (23.4%). Twenty-four bla_{VIM} variants, with $bla_{\mathrm{VIM\text{-}2}}$ being the most common, accounting for 73.2%; There are 39 variants of bla_{IMP}, mainly $bla_{\mathrm{IMP\text{-}1}}$ and $bla_{\mathrm{IMP\text{-}7}}$, accounting for 16.1% and 14.5%, respectively. Among 2,639 *P. aeruginosa* carrying $bla_{\mathrm{M\beta L}}$, 109 distinct sequence types (STs) were identified, with ST235 (16.8%) and ST111 (16.7%) being the predominant types, followed by ST308 (372, 14.1%), ST233 (190, 7.2%), ST357 (144, 5.5%), ST1203 (108, 4.1%), and ST773 (105, 4.0%). Notably, $bla_{\mathrm{M\beta L}}$-producing *P. aeruginosa* showed a continuous increase, peaking in 2019 since first detection in 1997. These strains were predominantly isolated from human sources (1,836, 69.6%), primarily from urine samples (21.9%). The predominant metalloenzymes in global *P. aeruginosa* are $bla_{\mathrm{VIM\text{-}2}}$, $bla_{\mathrm{IMP\text{-}1}}$, and $bla_{\mathrm{IMP\text{-}7}}$, primarily associated with epidemic high-risk clones ST235, ST111, and ST308. Given the rising trend of $bla_{\mathrm{M\beta L}}$-producing *P. aeruginosa*, enhanced infection prevention and control measures are warranted.

1. Introduction

Pseudomonas aeruginosa is a ubiquitous non-fermentative gram-negative bacterium found widely distributed in various environments, with a notable presence in healthcare settings [1]. As a leading nosocomial pathogen, this bacterium poses significant challenges to healthcare systems. It is responsible for a wide array of infections, including urinary tract infections, bloodstream infections, burns, surgical site infections, and ventilator-associated pneumonia [2]. These infections are associated with high morbidity and mortality rates [3], exacerbating the burden on healthcare facilities [4].

One of the major concerns regarding *P. aeruginosa* is its increasing resistance to last-resort antibiotics, particularly carbapenems [5], which are commonly used to treat infections caused by multidrug-resistant (MDR) strains. According to the CDC's Antimicrobial Resistance Threats Report, MDR *P. aeruginosa* is classified as a serious threat to public health due to its antibiotic resistance. (https://www.cdc.gov/antimicrobial-resistance/data-research/threats/update-2022.html). This alarming trend of antimicrobial resistance has led to significant limitations in treatment options and has heightened the urgency for the development

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of novel therapeutics [6]. Recognizing the severity of the situation, the World Health Organization has designated *P. aeruginosa* as a critical priority pathogen [7], highlighting the global imperative to address this public health threat through the advancement of new treatment strategies.

Resistance mechanisms to carbapenems in *P. aeruginosa* are multifactorial and include: (1) Production of carbapenem hydrolyzing β-lactamases from various molecular classes, including Ambler class A (e.g., KPC), class B (metallo-β-lactamases such as IMP, VIM, and NDM), and class D (carbapenem-hydrolyzing oxacillinases) enzymes [8]. (2) Derepression of chromosomal AmpC cephalosporinases, often in combination with outer membrane impermeability or efflux pump overexpression. which can hydrolyze carbapenems and contribute to resistance [9]. (3) Downregulation or inactivation of *oprD*, a specific outer membrane porin responsible for the uptake of carbapenems, reduces the entry of carbapenems into the bacterial cell, thereby conferring resistance [10]. These mechanisms collectively contribute to the ability of *P. aeruginosa* to resist the action of carbapenem antibiotics, posing a significant challenge in the treatment of infections caused by MDR strains.

Epidemiological studies have revealed that metallo-β-lactamase (MβL) production is identified in over 20% of carbapenem-resistant *P. aeruginosa* isolates in various regions worldwide [4], although it remains a rare cause of carbapenem resistance in *P. aeruginosa* in the United States. Of particular concern is the predominance of MβLs among carbapenem hydrolyzing β-lactamases (CHβLs) detected in *P. aeruginosa* [11]. This trend is alarming due to the limited therapeutic options available and the associated increase in mortality and morbidity rates [3]. The prevalence of $bla_{\mathrm{M\beta Ls}}$ underscores the urgent need for novel treatment strategies and heightened surveillance efforts to mitigate the impact of carbapenem resistance in *P. aeruginosa* infections. To date, ten types of MβLs have been identified, including Central Alberta metallo-β-lactamase (CAM) [12], Dutch imipenemase (DIM), Florence imipenemase (FIM), German imipenemase (GIM), Hamburg metallo-β-lactamase (HMB), Imipenemase metallo-β-lactamase (IMP), New Delhi metallo-β-lactamase (NDM), Seoul imipenemase (SIM), Sao Paulo metallo-β-lactamase (SPM), and Verona integron-encoded metallo-β-lactamase (VIM) [13]. Among them, bla_{IMP}, bla_{VIM}, and bla_{NDM} are the most commonly encountered variants. They are frequently harbored within various mobile genetic elements, such as integrons, plasmids, or transposons, increasing the likelihood of horizontal transfer between bacterial strains [5].

The initial discoveries of bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, and bla_{GIM} within *P. aeruginosa*, coupled with current reports revealing over 32 different variants of bla_{IMP} and 23 variants of bla_{VIM} in *P. aeruginosa* [14], highlight this organism as a favorable reservoir for transferable $bla_{\mathrm{M\beta Ls}}$, posing a significant threat to antimicrobial therapy. Moreover, internationally transmitted high-risk clones, such as ST111, ST175, ST233, ST235, ST277, ST357, and ST654, are frequently associated with multidrug resistance [14]. Among them, ST733 has been identified as the origin of widespread dissemination of bla_{NDM}, bla_{VIM}, and bla_{IMP} [15]. Notably, the global high-risk clone ST235 has predominated and circulated for the past 14 years in Singapore [4]. Some reports suggest that 30.0% of MDR strains possess $bla_{\mathrm{M\beta Ls}}$. However, data on the prevalence of $bla_{\mathrm{M\beta L}}$ among *P. aeruginosa* and the main clone harboring $bla_{\mathrm{M\beta L}}$ are limited.

In this study, we investigated the distribution of $bla_{\mathrm{M\beta L}}$-encoding genes, including bla_{IMP}, bla_{NDM}, bla_{SPM}, bla_{VIM}, bla_{SIM}, bla_{GIM}, bla_{CAM}, bla_{DIM}, bla_{HMB}, and bla_{FIM}, among global *P. aeruginosa* using genomes from the GenBank database. Furthermore, we analyzed the prevalent characteristics of $bla_{\mathrm{M\beta L}}$-carrying *P. aeruginosa*, including epidemic clones, spread across countries, and other relevant factors.

2. Materials and methods

2.1. Downloading and annotating global P. aeruginosa genomes

To obtain a comprehensive dataset for global *P. aeruginosa* strains, we batch-downloaded whole-genome sequences (WGS) in GenBank format from the NCBI database using Aspera by the deadline of August 10th, 2023, resulting in a total of 21,788 genome assemblies. A custom Perl script was used to extract nucleotide sequence files from each GenBank record. To ensure consistency in gene prediction across all genomes and avoid annotation discrepancies caused by the heterogeneous annotation methods used in public databases, we re-annotated all 21,788 genomes using Prodigal (version 2.6.3) [16], a widely used microbial gene prediction tool. Prodigal provides standardized gene calling by applying consistent algorithms for open reading frame prediction, which minimizes biases introduced by differing annotation pipelines and ensures comparability across genome datasets.

2.2. Examining the distribution of blaMβLs

The ten types of resistance gene sequences from the NCBI Pathogen Resistance Gene Database were downloaded to construct a structured antimicrobial drug resistance database. The following genes were included: bla_{DIM} (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{DIM}).), bla_{GIM}, (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{GIM}).), bla_{IMP} (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{IMP}).), bla_{NDM} (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{NDM}).), bla_{SIM} (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{SIM}).), bla_{SPM} (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{SPM}).), bla_{VIM} (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{VIM}).),bla_{CAM} (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{CAM}).), bla_{FIM} (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{FIM}).), and bla_{HMB} (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#gene_family:(bla_{HMB}).)

The nucleotide coding sequence files for all genes in the 21,788 genomes were obtained from Prodigal annotation results. Blast software was then utilized to compare the structured resistance gene databases, aiming to derive a detailed distribution of all $bla_{\mathrm{M\beta L}}$ genes across all genomes. A relatively strict threshold was set, with parameters including ’Evaluate=1e-5′, ’identity ≥ 90′, ’coverage ≥ 90%’, and ’Match_Length ≥ 30′, to minimize false positive results.

2.3. Analysis of sequence typing

To determine the sequence types (STs) of the $bla_{\mathrm{M\beta L}}$-carrying *P. aeruginosa* genomes, we adopted the multilocus sequence typing (MLST) scheme based on seven housekeeping genes: *acsA, aroE, guaA, mutL, nuoD, ppsA*, and *trpE*. The allele sequences and ST profile file were downloaded from the pubMLST database (https://pubmlst.org/organisms/pseudomonas-aeruginosa). All predicted coding sequences from Prodigal annotations of $bla_{\mathrm{M\beta L}}$-positive genomes were searched using blastn, with identity and coverage thresholds both set to 100%. The best hits for each gene were compared against the pubMLST profile to assign an ST to each genome. Only isolates with complete matches for all seven loci were considered for final ST assignment to ensure accuracy.

2.4. Construction of genome phylogenetic tree for the three predominant clones

The genome phylogenetic trees for the ST111, ST235, and ST308 strains were constructed as follows: Genome nucleotide sequence

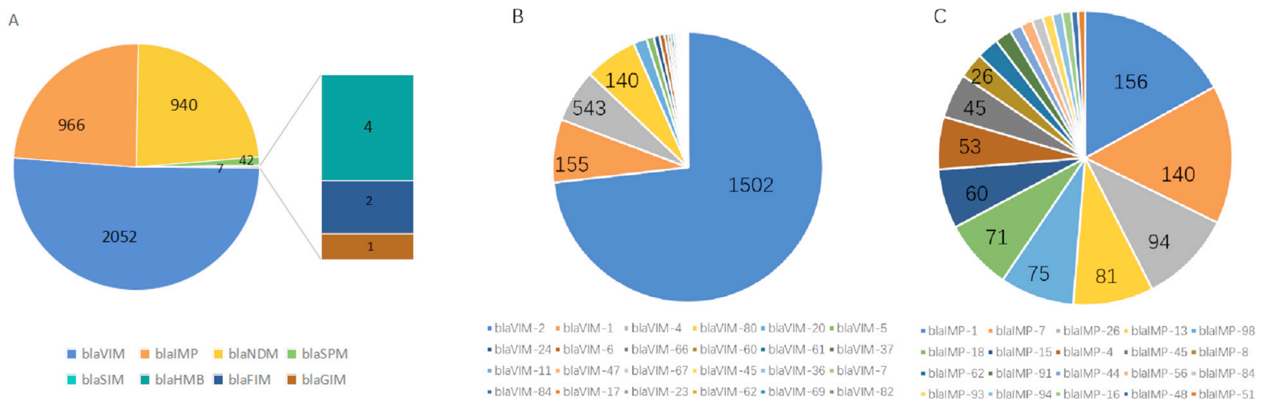

Fig. 1. The predominant metallo-β-lactmase encoding genes among global Pseudomonas aeruginosa. Figure 1A. The distribution of the global metallo-β-lactmase genome; Figure 1B. The distribution of the blaVIM variants; Figure 1C. The distribution of the blaIMP variants.

files were downloaded in bulk from the NCBI Genome Database based on genome assembly numbers. The genome sequences of the ST111 (442 strains), ST235 (444 strains), and ST308 (372 strains) were annotated using Prokka version 1.14.6 [17]. The gff files of these annotated genomes were used as input files for metagenomic analysis with Roary version 3.13.0 [17], resulting in multiple sequence alignment files of core genes: 4910 for ST111, 4705 for ST235, and 5330 for ST308 (homologous genes distributed in 99% $\leq$ strains $\leq$ 100% were designated as core genes based on Roary's default parameters). SNP sequence files were extracted from the core gene multiple sequence alignment files using SNP-sites v2.5.1 [18]. The optimal nucleotide substitution models were determined using jModelTest 2 [19]: TVM+*I* for ST111, GTR for ST235, and TPM3uf+*G* for ST308. Maximum likelihood phylogenetic trees were constructed using RAxML-NG v. 1.2.1 software [20], with the respective nucleotide substitution models and 1000 bootstrapping samplings performed to obtain the phylogenetic trees. These phylogenetic trees were imported into iTOL Version 6.5.8 software. For ST111 and ST235, branches with bootstrap values less than 50 were removed, while for ST308, no branches were removed. The SVG files were exported, and the generated heatmap phylogenetic trees were beautified using Adobe Illustrator software.

2.5. Exploring prevalent characteristics

The Perl program was employed to extract relevant metadata of strains from the GenBank files of genomes carrying *bla*$_{\mathrm{M}\beta\mathrm{L}}$, and the results were saved in an Excel file. Concurrently, the names of *P. aeruginosa* strains were extracted from the NCBI genome database summary file 'assembly_summary_genbank.txt' and integrated with the metadata into the same file for further analysis.

3. Results

3.1. bla$_{\mathrm{VIM}}$ and bla$_{IMP}$ predominate among bla$_{\mathrm{MßLs}}$ in P. aeruginosa

Among 21,788 genomes worldwide, 2639 (12.1%) strains were found to contain 4014 *bla*$_{\mathrm{M}\beta\mathrm{Ls}}$ including 2052 (51.2%) *bla*$_{\mathrm{VIM}}$, 966 (24.1%) *bla*$_{\mathrm{IMP}}$, 940 (23.4%) *bla*$_{\mathrm{NDM}}$, 42 (1.0%) *bla*$_{\mathrm{SPM}}$, 7 (0.2%) *bla*$_{\mathrm{SIM}}$,4 (0.1%) *bla*$_{\mathrm{HMB}}$, 2 (0.1%) *bla*$_{\mathrm{FIM}}$ and 1 *bla*$_{\mathrm{GIM\text{-}1}}$ (Fig. 1A).

In detail, 24 *bla*$_{\mathrm{VIM}}$ variants were identified, with *bla*$_{\mathrm{VIM\text{-}2}}$ ($n = 1502$) being the most common, accounting for 73.2% (Fig. 1B); Additionally, 39 *bla*$_{\mathrm{IMP}}$ variants were found, with *bla*$_{\mathrm{IMP\text{-}1}}$ ($n = 156$) and *bla*$_{\mathrm{IMP\text{-}7}}$ ($n = 140$) being the most predominant, accounting for 16.1% and 14.5%, respectively (Fig. 1C). Two *bla*$_{\mathrm{NDM}}$ variants, including *bla*$_{\mathrm{NDM\text{-}1}}$ ($n = 939$, 99.9%) and *bla*$_{\mathrm{NDM\text{-}7}}$ ($n = 1$, 0.1%), were identified, along with two *bla*$_{\mathrm{SIM}}$ variants, including *bla*$_{\mathrm{SIM\text{-}1}}$ ($n = 3$, 42.9%) and *bla*$_{\mathrm{SIM\text{-}2}}$ ($n = 4$, 57.1%). Furthermore, 42 *bla*$_{\mathrm{SPM\text{-}1}}$ and 1 *bla*$_{\mathrm{GIM\text{-}1}}$ were also detected.

3.2. ST235, ST111 and ST308: major sequence types in bla$_{\mathrm{M}\beta L}$-Carrying P. aeruginosa

In total, 109 distinct STs were identified. The STs of 43 *bla*$_{\mathrm{M}\beta\mathrm{L}}$-containing *P. aeruginosa* strains remained unknown, while the STs of 16 strains were novel. The most dominant STs were ST235 ($n = 444$, 16.8%), ST111 ($n = 442$, 16.8%), and ST308 ($n = 372$, 14.1%), followed by ST233 ($n = 190$, 7.2%), ST357 ($n = 144$, 5.5%), ST1203 ($n = 108$, 4.1%), and ST773 ($n = 105$, 4.0%). Other relatively common STs included ST823 ($n = 93$, 3.5%), ST175 ($n = 68$, 2.6%), and ST654 ($n = 57$, 2.2%), while the remaining STs were relatively less common.

The global distribution of various STs of *P. aeruginosa* carrying *bla*$_{\mathrm{M}\beta\mathrm{Ls}}$ showed that in the USA, there was a diverse presence of STs, with no single type dominating significantly. Singapore showed a high prevalence of ST308. European countries such as Germany and Poland displayed a variety of STs, with no single ST overwhelmingly dominant, though certain types like ST235 appeared to be more common in Poland. In some regions like Brazil and Kenya, the data suggested a more limited diversity of STs, primarily focusing on a couple of types (Fig. 2).

Analysis of primary *bla*$_{\mathrm{M}\beta\mathrm{Ls}}$ among predominant STs showed that a very high presence of the *bla*$_{\mathrm{VIM}}$ gene were observed in ST111, ST233, ST235, and ST1203. Significant quantities of both *bla*$_{\mathrm{IMP}}$ and *bla*$_{\mathrm{VIM}}$ genes, with a minimal presence of the *bla*$_{\mathrm{NDM}}$ gene were shown in ST235. ST308 and ST773 mainly carried the *bla*$_{\mathrm{NDM}}$ gene, with smaller amounts of the *bla*$_{\mathrm{IMP}}$ and *bla*$_{\mathrm{VIM}}$ genes. ST823 only contained the *bla*$_{\mathrm{VIM}}$ gene (Fig. 3).

Interestingly, we identified 48 strains with the co-occurrence of two *bla*$_{\mathrm{M}\beta\mathrm{Ls}}$, which were assigned to 10 different STs. It was observed that the same STs predominantly co-harbored the same *bla*$_{\mathrm{M}\beta\mathrm{Ls}}$, with the exception of ST235. Specifically, 18 out of 48 *P. aeruginosa* strains co-carrying two *bla*$_{\mathrm{M}\beta\mathrm{Ls}}$ were found to simultaneously harbor *bla*$_{\mathrm{VIM\text{-}2}}$ and *bla*$_{\mathrm{IMP\text{-}18}}$. Similarly, all five ST179 strains co-carried *bla*$_{\mathrm{IMP\text{-}16}}$ and *bla*$_{\mathrm{IMP\text{-}93}}$. Additionally, seven ST644 strains co-carried *bla*$_{\mathrm{IMP\text{-}62}}$ and *bla*$_{\mathrm{NDM\text{-}1}}$ (Table 1).

3.3. Notable genetic diversity was shown in phylogenetic trees constructed for ST111, ST235, and ST308 strains

The analysis of phylogenetic trees for ST111, ST235, and ST308 strains revealed complex branching patterns that reflect significant genetic diversity within the three clonal groups. Some clusters of strains were closely related, suggesting potential clonal expan-

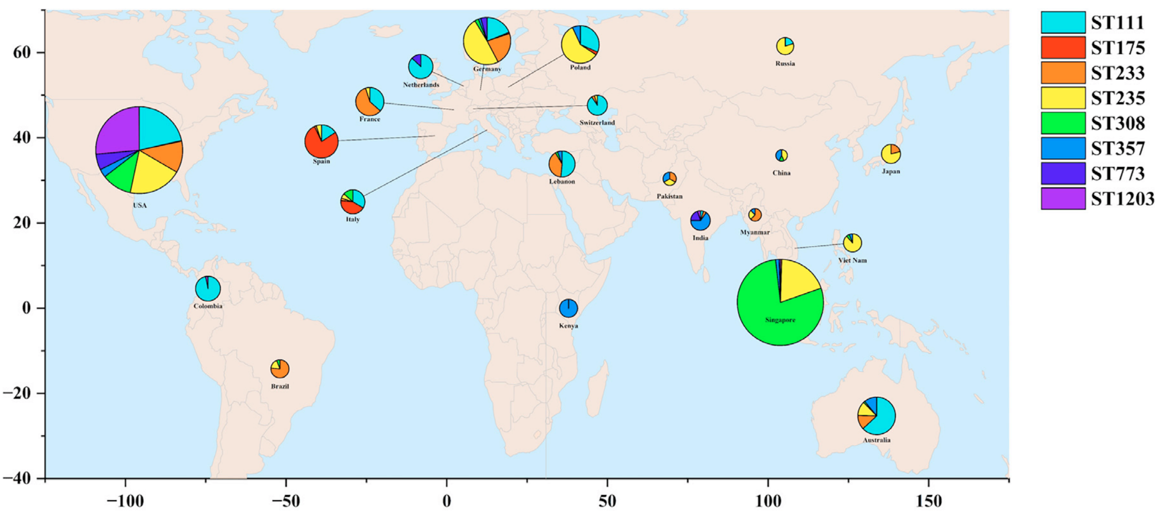

Fig. 2. The geographical distribution of predominant sequence types among global *Pseudomonas aeruginosa*. Pie charts represent the proportion of each sequence type detected in different countries, with the size of the pie reflecting the number of isolates analyzed per country. Eight major STs are indicated in different colors: ST111 (cyan), ST175 (red), ST233 (orange), ST235 (yellow), ST308 (green), ST357 (blue), ST773 (purple), and ST1203 (magenta). Countries with higher isolate counts, such as the USA and Singapore, are represented with larger pies, indicating broader ST diversity.

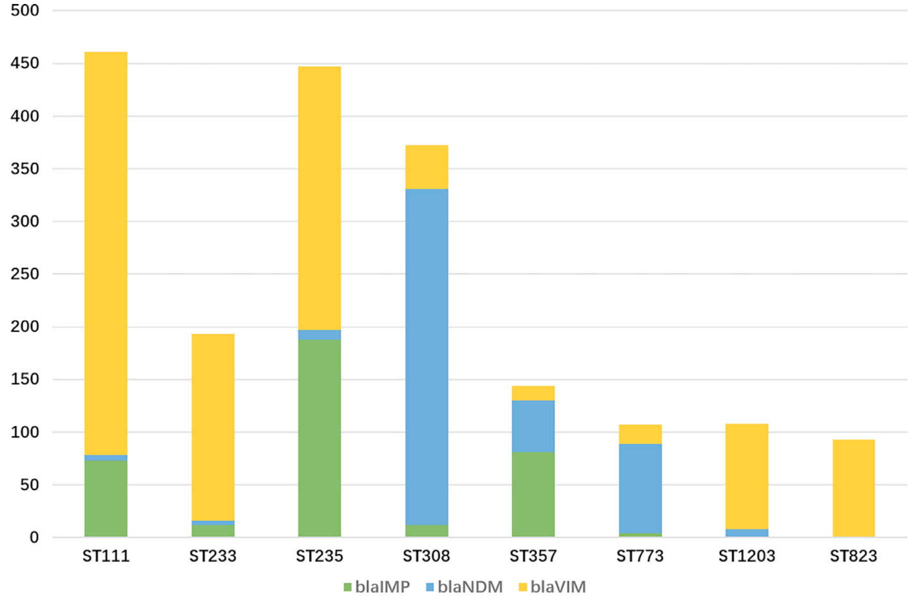

Fig. 3. The distribution of predominant metallo-β-lactmase encoding genes among the dominant high-risk clones of global Pseudomonas aeruginosa. The bar chart shows the frequency of three major blaMβL genes—blaIMP (green), blaNDM (blue), and blaVIM (yellow)—identified in the top 10 most prevalent sequence types (STs). ST235, ST111, ST308, and ST233 demonstrated the highest burden of blaMβL carriage. Notably, blaVIM was predominant in ST235, ST111, and ST1203, whereas blaNDM was most common in ST308 and ST773. ST233 and ST1203 strains primarily harbored blaVIM, while ST823 was exclusively associated with blaVIM.

Table 1
The combination of $bla_{M\beta Ls}$ among *Pseudomonas aeruginosa*.

Combination of $bla_{M\beta Ls}$		Sequence types	number
bla_{IMP-18}	bla_{VIM-2}	ST111	18
bla_{IMP-62}	bla_{NDM-1}	ST644	7
bla_{IMP-16}	bla_{IMP-93}	ST179	5
bla_{SIM-2}	bla_{VIM-2}	ST274	3
bla_{IMP-98}	bla_{NDM-1}	ST244	3
bla_{IMP-56}	bla_{VIM-2}	ST233	2
bla_{IMP-45}	bla_{VIM-2}	ST708	2
bla_{NDM-1}	bla_{VIM-5}	ST773	2
bla_{NDM-1}	bla_{VIM-2}	ST235	1
bla_{VIM-45}	bla_{VIM-2}	ST235	1
bla_{IMP-13}	bla_{VIM-2}	ST235	1
bla_{IMP-45}	bla_{VIM-2}	ST708	1
bla_{IMP-18}	bla_{VIM-2}	ST111	1
bla_{IMP-15}	bla_{NDM-1}	ST3440	1
$bla_{IMP-100}$	bla_{VIM-4}	ST233	1

sions or local outbreaks. Additionally, the branching pattern indicated both the evolutionary history and possible horizontal $bla_{M\beta Ls}$ transfer event.

In detail, the phylogenetic tree for 442 ST111 strains showcased a complex branching pattern with notable genetic diversity (Fig. 4). The concentric rings represent the presence and number of $bla_{M\beta Ls}$, including bla_{GIM}, bla_{IMP}, bla_{NDM}, and bla_{VIM}, with bla_{VIM} and bla_{GIM} being the most dominant. The outer ring indicated the geographical origin, highlighting the international prevalence of ST111 strains. High levels of $bla_{M\beta Ls}$ were prevalent, especially in clusters from the USA, Germany, and India. The phylogenetic tree of 443 ST235 strains also demonstrated complex branching with substantial genetic diversity (Fig. 5). ARGs represented were bla_{IMP}, bla_{NDM}, and bla_{VIM}, with bla_{VIM} and bla_{IMP} being the most common. The geographical distribution showed a wide spread, with notable clusters from the USA, Germany, and

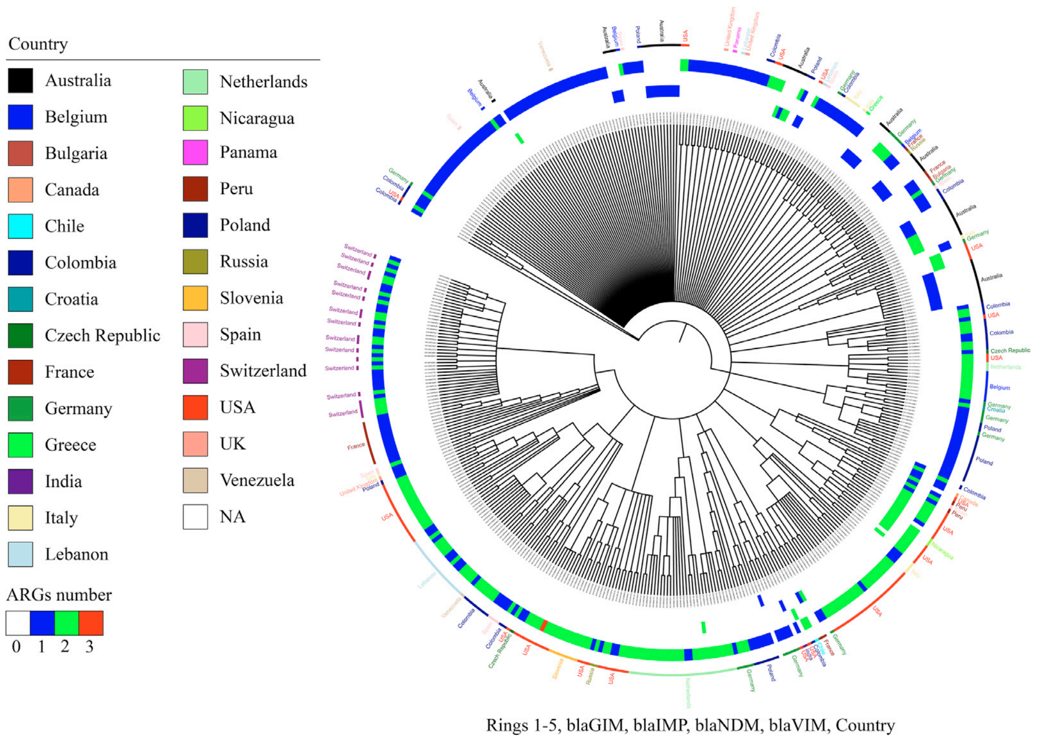

Fig. 4. Phylogenetic tree of Pseudomonas aeruginosa ST111 isolates displaying the distribution of predominant metallo-β-lactamase (blaMβL) genes and their geographic origins. The maximum-likelihood tree is based on single nucleotide polymorphisms from core genes. Concentric rings indicate the presence of blaGIM, blaIMP, blaNDM, and blaVIM (from inner to outer), followed by the number of antimicrobial resistance genes (ARGs) detected (color-coded from 0 to 3), and country of origin. Colors of the outermost ring correspond to different countries as indicated in the legend.

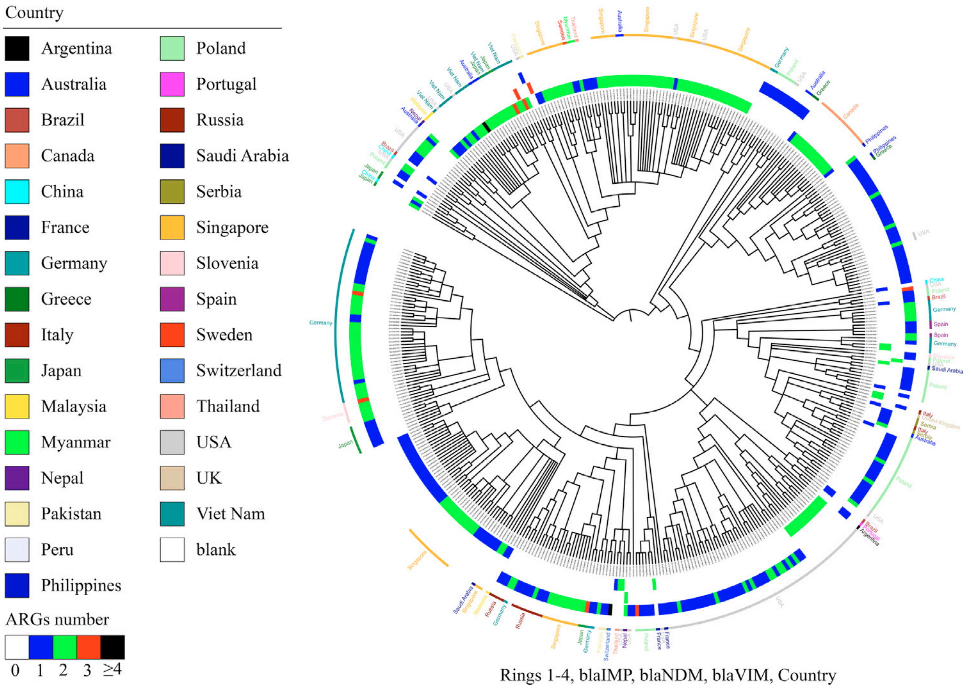

Fig. 5. Phylogenetic tree of Pseudomonas aeruginosa ST235 strains showing the distribution of predominant metallo-β-lactamase (blaMβL) genes and geographical origins. The tree was constructed based on single nucleotide polymorphisms from core genomes using a maximum-likelihood approach. Concentric rings from inside to outside represent the presence of blaIMP, blaNDM, and blaVIM, followed by the number of acquired resistance genes (ARGs) per strain (ranging from 0 to ≥ 4), and the country of isolation (color-coded as indicated in the legend).

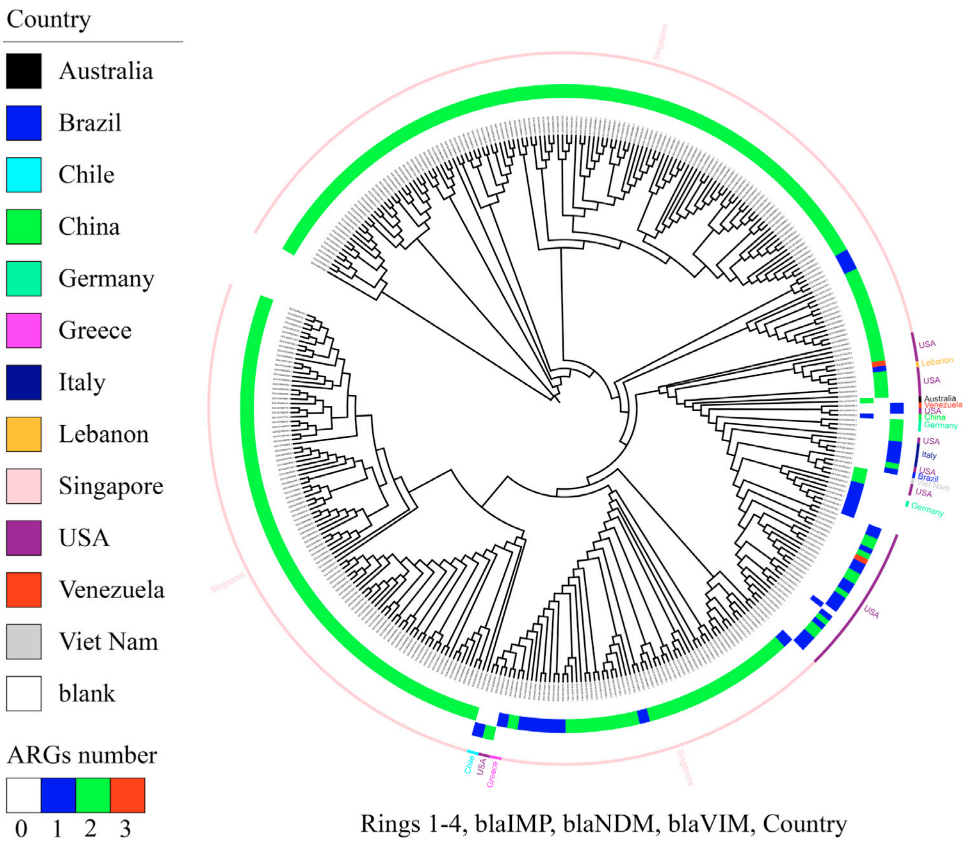

Fig. 6. Phylogenetic tree of Pseudomonas aeruginosa ST308 strains displaying the distribution of predominant metallo-β-lactamase (blaMβL) genes and their geographic origins. The tree was constructed using a maximum-likelihood method based on single nucleotide polymorphisms from core genes. Concentric rings from inside to outside indicate the presence of blaIMP, blaNDM, and blaVIM genes, followed by the number of acquired resistance genes (ARGs) per isolate (0–3), and the country of isolation (color-coded as shown in the legend).

Singapore. Regions like Thailand and the USA had a higher prevalence of multiple $bla_{M\beta Ls}$, indicating higher resistance levels. The analysis of 372 ST308 strains revealed a high prevalence of bla_{NDM} and bla_{VIM}, with strains from the USA frequently carrying bla_{VIM} and those from Singapore predominantly harboring bla_{NDM} (Fig. 6). The tree illustrated significant genetic diversity and regional clustering, particularly from Singapore and the USA.

3.4. Prevalence of $bla_{M\beta L}$-Carrying *P. aeruginosa* in the USA, Singapore, Germany, Poland and China

Geographically, the 2639 $bla_{M\beta L}$-carrying strains originated from 62 countries across five continents, although the isolation countries of 395 strains were unclear. The United States ($n = 527$, 20.0%) and Singapore ($n = 400$, 15.2%) contributed the most predominant strains, followed by Germany ($n = 151$, 5.7%), Poland ($n = 134$, 5.1%), China ($n = 109$, 4.1%), and Australia ($n = 104$, 3.9%) (Fig. 7). Other countries contributed relatively fewer strains (Fig. 7). These strains were mainly isolated from Homo sapiens (1836, 69.4%), with urine samples (403, 15.2%) being the main resource (Table 2).

3.5. Geographical distribution of $bla_{M\beta Ls}$

The distribution of $bla_{M\beta Ls}$ exhibited geographical variation (Fig. 7). In the USA and European countries, bla_{VIM} was the predominant $bla_{M\beta L}$, while in Oceania, such as Australia, bla_{IMP} predominated. In South America, such as Brazil, $bla_{SPM\text{-}1}$ was the main variant, while in North America, specifically Canada, bla_{IMP} prevailed. However, in Asian countries, the prevalence of $bla_{M\beta Ls}$ varied. In Singapore, India, and Myanmar, bla_{NDM} was predominant, while in China, Thailand, and Vietnam, bla_{IMP} dominated. Conversely, in Japan, Pakistan, Nepal, and Saudi Arabia, bla_{VIM} was the major variant.

Table 2
The distribution of global metalloenzyme-producing *Pseudomonas aeruginosa*.

Host	Isolation source (Number)
Homo sapiens (1836)	Blank (408), Urine (403), clinical sample (239), blood (178), wound (136), rectal swab (102), Colonization (101), tissue (75), bronchoalveolar lavage (68), aspirate (48), other (20), catheter (19), body fluid (14), Associated Infection (10), Pus (9), feces (6).
Environment (129)	Hospital (59), stool (18), Microbial mat material (1), wastewater (23), soil (2), sink trap (14), screen (1), river (2), bedpan washer (9)
Chicken (4)	cloaca swab (1), intestine (3)
Canis lupus familiaris (2)	Ear (1), Oral swab (1)
Dog (1)	Rectal swab (1)
Swallow (1)	feces swab (1)
Blank (666)	blood (6), blank (466), rectal swab (38), infector (19), sputum (19), throat swab (45), urine (29), wound swab (33), bronchoalveolar lavage (5), catheter tip (3), tissue (3)

3.6. Rising trends in the number and variety of $bla_{m\beta l}$ variants over time

The temporal distribution of $bla_{M\beta L}$-carrying *P. aeruginosa* revealed that bla_{SPM} was the first $bla_{M\beta L}$ identified in the year 1997, followed by the isolation of the first bla_{VIM} in 1999. Overall, there had been an increasing trend in the number of $bla_{M\beta Ls}$ detected over the years (Fig. 8A), accompanied by an increase in the variety

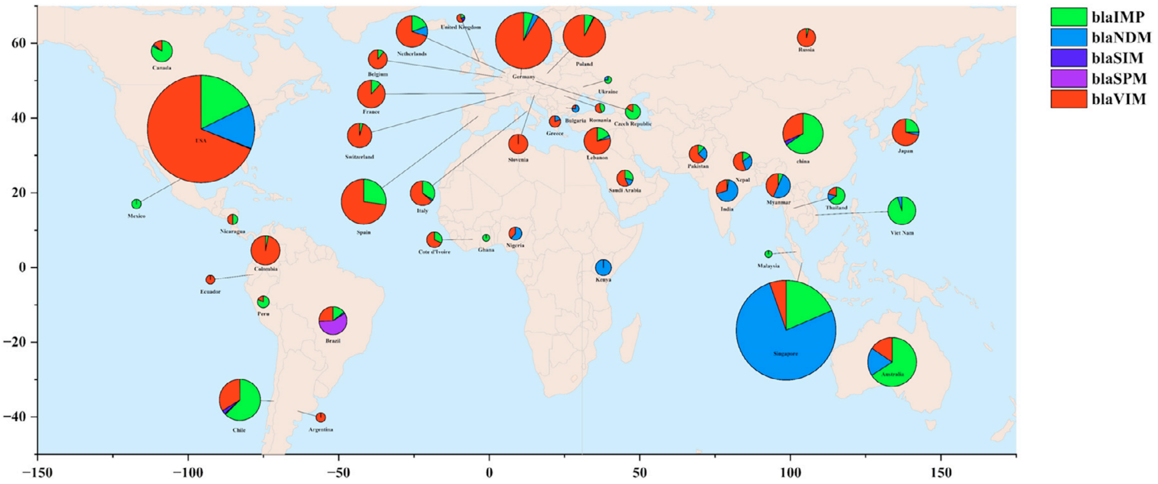

Fig. 7. Geographical distribution of predominant metallo-β-lactamase (blaMβL) encoding genes among global Pseudomonas aeruginosa isolates. Pie charts represent the relative abundance of blaIMP (green), blaNDM (blue), blaSIM (purple), blaSPM (magenta), and blaVIM (red) genes in isolates from each country. The size of each pie chart corresponds to the number of blaMβL-positive strains identified. Notably, blaVIM was dominant in Europe and the USA, blaIMP was prevalent in Asian countries including China and Vietnam, and blaNDM predominated in Singapore.

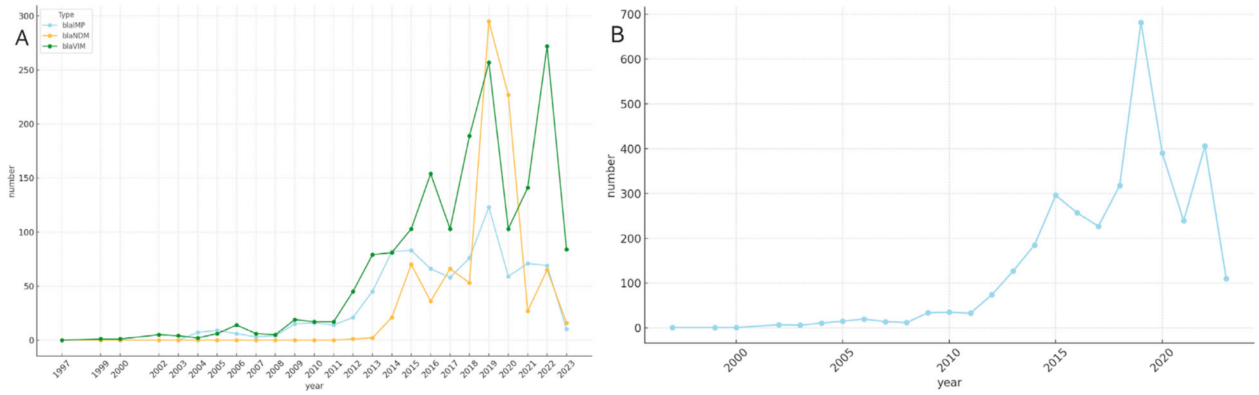

Fig. 8. Yearly distribution of predominant metallo-β-lactamase (blaMβL) encoding genes among global Pseudomonas aeruginosa isolates. Figure 8A. Line chart showing annual trends in the number of isolates carrying blaIMP, blaNDM, and blaVIM from 1997 to 2023. The data indicate a gradual increase from the early 2000s, with a sharp rise in blaVIM and blaNDM detections after 2015, peaking around 2019. Figure 8B. Line chart representing the overall yearly count of all blaMβL genes, highlighting the increasing trend in variant diversity and total gene counts over time, with notable fluctuations in recent years.

of variants (Fig. 8B). Initially, there was only one $bla_{\mathrm{M}\beta\mathrm{L}}$ identified during the period 1997–2000, followed by two during 2001–2009, three during 2010–2018, and four during 2019–2020.

Based on the distribution over time, bla_{SPM} emerged as the earliest $bla_{\mathrm{M}\beta\mathrm{L}}$ variant in 1997, displaying an intermittent and sporadic presence over the subsequent 27 years. bla_{VIM} was initially identified in strains isolated in 1999, followed by bla_{IMP} in 2004 and bla_{NDM} in 2012. The detection of these variants has been continuously increasing, and reaching a peak in 2019.

3.7. $bla_{\mathrm{M}\beta L}$-Carrying P. aeruginosa: predominantly derived from homo sapiens with urine as the primary sample source

Analysis of sample sources revealed that $bla_{\mathrm{M}\beta\mathrm{L}}$-carrying *P. aeruginosa* strains were predominantly isolated from Homo sapiens (1836, 69.6%), with urine being the primary sample source. Additionally, these strains were isolated from environmental sources (129, 4.9%), mainly hospitals and wastewater, as well as animals (8, 0.3%) (Table 2).

4. Discussion

In the current landscape of escalating MDR *P. aeruginosa* infections, the distribution and diversity of $bla_{\mathrm{M}\beta\mathrm{Ls}}$ pose a significant threat to global health. Our large-scale genomic analysis of 21,788 *P. aeruginosa* genomes represents one of the most comprehensive investigations to date into the global landscape of these resistance determinants. The identification of 2639 $bla_{\mathrm{M}\beta\mathrm{L}}$ -carrying strains (12.1%) sheds light on the dynamics of antimicrobial resistance dissemination across clonal lineages and geographic regions.

In this study, the 12.1% prevalence of $bla_{\mathrm{M}\beta\mathrm{L}}$ in *P. aeruginosa* is markedly lower than the alarmingly high rates in Iran (76.8%) and Pakistan (76.0%) [13,21]. This contrasted sharply with the 16.4% reported in southern China [22] and 15.0% in Ghanpur, Medchal, India [23], and was significantly higher than the less than 1.0% distribution rate found in our current study of blood-cultured *P. aeruginosa* from 2013 to 2023 (data unpublished). Given that strains carrying $bla_{\mathrm{M}\beta\mathrm{L}}$ were predominantly isolated from urine samples, continuous monitoring of urine-cultured *P. aeruginosa* is crucial to track prevalence of $bla_{\mathrm{M}\beta\mathrm{L}}$. Furthermore, bla_{IMP} emerged as the common $bla_{\mathrm{M}\beta\mathrm{L}}$ variant, aligning with the epidemiological studies that noted its widespread distribution in *P. aeruginosa* [8]. Notably, our study identified multiple bla_{IMP} variants, highlighting the evolving nature of antimicrobial resistance in *P. aeruginosa*. Significantly, among the 29 bla_{IMP} variants identified, $bla_{\mathrm{IMP\text{-}91}}$, $bla_{\mathrm{IMP\text{-}94}}$, $bla_{\mathrm{IMP\text{-}86}}$ and $bla_{\mathrm{IMP\text{-}37}}$ appear to be previously unreported, demonstrating ongoing evolution. Additionally, we discovered 24 variants of bla_{VIM}, with five of these variants-$bla_{\mathrm{VIM\text{-}61}}$, $bla_{\mathrm{VIM\text{-}67}}$, $bla_{\mathrm{VIM\text{-}23}}$, *bla*$_{\text{VIM-69}}$, and *bla*$_{\text{VIM-82}}$ being newly identified. These findings provide new entries to the known resistome of *P. aeruginosa* and reinforce the utility of continuous global surveillance.

Furthermore, our findings indicate a significant diversity among the STs of *P. aeruginosa* containing *bla*$_{\text{M}\beta\text{L}}$. Compared with previous pangenome-based studies [24], our findings corroborate and extend earlier observations. For instance, Freschi et al. analyzed the pangenome of over 1000 *P. aeruginosa* genomes and identified high-risk clones such as ST235 and ST111 as major carriers of resistance genes [25], especially *bla*$_{\text{VIM}}$ and *bla*$_{\text{IMP}}$. Our study reinforces this, showing ST235 and ST111 as dominant STs among *bla*$_{\text{M}\beta\text{L}}$-positive strains, now confirmed with far greater strain numbers and geographic coverage. Importantly, we detected all of the top 10 global high-risk clones of *P. aeruginosa*, including ST235, ST111, ST233, ST244, ST357, ST308, ST175, ST277, ST654, and ST298 [14], many of which have been previously implicated in regional or international outbreaks [15,26]. This suggests that these STs may have particular genetic or phenotypic traits that confer a survival advantage, possibly related to antibiotic resistance or virulence, which facilitates their proliferation and persistence in various environments [27]. Of great concern, the strong geographic clustering of ST308 in Singapore and widespread presence of ST235 and ST111 across continents mirror findings in prior epidemiological and pangenomic studies, underscoring their adaptability and transmissibility.

Noteworthily, endemic *bla*$_{\text{IMP-18}}$ carrying *P. aeruginosa* strains in ST111 have been identified in Panama [28], with potential clonal spread to Mexico among patients with urinary tract infections or pneumonia. Additionally, interregional clonal outbreaks involving ST235, ST111, ST273, and ST654 occurred in Poland between 2005 and 2015 [15], with *bla*$_{\text{VIM}}$ and *bla*$_{\text{IMP}}$ as the predominant *bla*$_{\text{M}\beta\text{Ls}}$. Travel-associated cases involving *bla*$_{\text{IMP-1}}$, *bla*$_{\text{VIM-2}}$, and *bla*$_{\text{NDM-1}}$ positive *P. aeruginosa* strains in ST235 and ST308 were reported in Malaysia [29]. Moreover, ST1203, ST773, and ST823 were also observed at relatively high frequencies, underscoring the need to monitor these clones due to their previously reported presence in *bla*$_{\text{M}\beta\text{L}}$-carrying strains. Of significant concern, the epidemic clone ST235 is known for its high virulence and mortality rates, often attributed to the production of the ExoU cytotoxin. Clones such as ST111 and ST233 are widely disseminated and often exhibit MDR or extensively drug-resistant (XDR) profiles, particularly associated with *bla*$_{\text{VIM-2}}$ [30,31]. Conversely, ST244, while prevalent, is not consistently linked to MDR or XDR profiles [32]. Clones such as ST357, ST308, and ST298, all carrying the *exoU* gene, may exhibit potentially higher virulence [14,33]. In contrast, ST175, prevalent in certain European countries, typically displays an MDR or XDR phenotype due to specific chromosomal mutations and is generally associated with lower virulence [34]. Lastly, ST277, highly prevalent in Brazil [4], is specifically linked to the *bla*$_{\text{SPM}}$. Thus, establishing and enhancing control measures targeting these bacteria is crucial to slow to slow the development of resistance and prevent outbreaks of nosocomial infections.

Additionally, studies by Mosquera-Rendón et al. and Kung et al. emphasized that *P. aeruginosa* possesses a highly open pangenome and that the acquisition of mobile elements like integrons plays a key role in the spread of carbapenemases [35]. Our phylogenetic analysis of ST111, ST235, and ST308 further reveals complex evolutionary branching, likely shaped by both clonal expansion and horizontal gene transfer [36], consistent with the open genome model.

This study has several limitations. First, the genomic data were exclusively sourced from the NCBI database, which may not fully represent the global diversity of *P. aeruginosa* strains. Some countries may maintain national genomic repositories and do not submit data to public databases, leading to potential geographic bias. Second, due to limited metadata availability, detailed clinical information such as patient demographics, infection types, or treatment outcomes could not be assessed. Third, antibiotic susceptibility profiles, especially against carbapenems, were largely unavailable for most genomes, making it difficult to correlate the presence of *bla*$_{\text{M}\beta\text{L}}$ genes with phenotypic resistance. Lastly, although multiple novel *bla*$_{\text{M}\beta\text{L}}$ variants were identified, their functional roles in resistance were not experimentally validated and warrant further investigation.

5. Conclusion

In summary, this study provides a comprehensive overview of the global distribution, diversity, and evolutionary trends of *bla*$_{\text{M}\beta\text{L}}$ among *P. aeruginosa* isolates. *bla*$_{\text{VIM}}$ and *bla*$_{\text{IMP}}$ were identified as the most prevalent *bla*$_{\text{M}\beta\text{L}}$ types, with ST235, ST111, and ST308 serving as major international high-risk clones. Importantly, the predominance of *bla*$_{\text{M}\beta\text{L}}$-positive isolates in clinical samples—particularly from urine—highlights the significant challenge these strains pose in healthcare settings. The identification of *bla*$_{\text{M}\beta\text{L}}$-carrying isolates from environmental sources such as wastewater further raises concerns about the potential for dissemination. Although rare in this dataset, the presence of such strains in food-producing animals or related environments suggests a possible One Health dimension that cannot be overlooked. These findings emphasize the need for integrated genomic surveillance across clinical, environmental, and food-related sectors, to better monitor and control the spread of carbapenem-resistant *P. aeruginosa*.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from GenBank and the accession number and web link to datasets for the provided name of these strains were shown in table S1.xlsx, The core genome, accessory genome, and MBL gene distributions of ST111, ST235 and ST308 were provided in the Table S2, Table S3 and Table S4.

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CRediT authorship contribution statement

Junbin Zhai: Writing – original draft, Visualization, Validation, Methodology, Investigation. **Ruyu Yan:** Validation, Methodology, Investigation, Data curation. **Xiaoli Cao:** Writing – review & editing, Methodology, Investigation, Formal analysis, Funding acquisition, **Chang Liu:** Supervision, Methodology, Investigation. **Fengyan Li:** Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Han Shen:** Project administration, Methodology, Investigation, Funding acquisition, **Xuejing Xu:** Conceptualization, Writing – review & editing, Formal analysis, Supervision, Resources.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of Laboratory Medicine, Nanjing Drum Tower Hospital, the affiliated Hospital of Nanjing University Medical School, Nanjing, 210,003, China.

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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.2025.05.023.

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