An IncP-2 plasmid sublineage associated with dissemination of bla\textsubscript{IMP-45} among carbapenem-resistant \textit{Pseudomonas aeruginosa}

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\textbf{ABSTRACT}

IMP-45, a variant of IMP-9, is one of the dominant metallo-\(\beta\)-lactamases (MBLs) in clinical carbapenem-resistant \textit{Pseudomonas aeruginosa} (CRPA) isolates in China. The aim of this study was to investigate the distribution and mechanism of dissemination of \(\text{bla}_{\text{IMP-45}}\). MBL genes were detected by PCR in 173 non-duplicate CRPA isolates collected from Hospital HS in Shanghai and 605 \textit{P. aeruginosa} isolates from a multicenter surveillance of \(\text{bla}_{\text{IMP-45}}\) in China. In total, 17 IMP-45-producers (14 from Hospital HS and 3 from other hospitals) were identified. Molecular typing identified an outbreak of 11 IMP-45-producing ST508 CRPA in the ICU of Hospital HS. Conjugation assays and whole genome sequencing were conducted among IMP-45-producers. Genomic comparison revealed that 16 \(\text{bla}_{\text{IMP-45}}\)-carrying plasmids (9 from this study and 7 from GenBank) shared a similar backbone with IncP-2 \(\text{bla}_{\text{IMP-9}}\)-carrying plasmid pOZ176 but lacked \text{repA}-\text{oriV}-\text{parA} region. \text{repA}2 gene was present in pOZ176, \(\text{bla}_{\text{IMP-45}}\)-carrying plasmids (17 from this study and 7 from GenBank) and 15 megaplasmids from GenBank. Phylogenetic analysis of \text{repA}2 showed that most \(\text{bla}_{\text{IMP-45}}\)-carrying plasmids were clustered into a sublineage separate from the one containing pOZ176. This \(\text{bla}_{\text{IMP-45}}\)-plasmid sublineage contributed to the dissemination of \(\text{bla}_{\text{IMP-45}}\) among genetically diverse \textit{P. aeruginosa} and recruited multiple resistance genes during its evolution.

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\section*{Introduction}

\textit{Pseudomonas aeruginosa} is prone to be resistant to \(\beta\)-lactams, aminoglycosides and quinolones. Production of metallo-\(\beta\)-lactamases (MBLs) is one of the primary carbapenem resistance mechanisms in this species, among which IMP and VIM are the most prevalent [1]. IMP-1, IMP-4, IMP-6, IMP-8, IMP-9, IMP-10 and IMP-45 have been reported in China [2]. IMP-9 was initially identified in \textit{P. aeruginosa} isolates from Guangzhou, China [3]. Afterwards, outbreaks of IMP-9-producing \textit{P. aeruginosa} were observed in this area in 2000 and from 2005 to 2007 [3,4]. IMP-45, a single amino acid substitution variant (G214S) of IMP-9, was initially identified in \textit{P. aeruginosa} isolates from Guangzhou, China [3,9]. IncP-2 plasmids, generally >300 kb and in single copy, exhibit tellurite resistance and are narrow-host-range for \textit{Pseudomonas spp} [10]. Previous studies have characterized two \(\text{bla}_{\text{IMP-45}}\)-carrying megaplasmids from clinical isolates (\textit{P. putida} and \textit{P. aeruginosa}) in China [6,7], but information about the dissemination of \(\text{bla}_{\text{IMP-45}}\) gene remains largely limited and the role of plasmids in the dissemination of \(\text{bla}_{\text{IMP-45}}\) is poorly understood.

In this study, we report an outbreak of carbapenem-resistant \textit{P. aeruginosa} (CRPA) co-carrying \(\text{bla}_{\text{IMP-45}}, \text{qnrVC1}\) and \text{armA} in a tertiary hospital of Shanghai and the disappearance of outbreak clones after strengthened infection control measures. Subsequently, we carried out a multicenter surveillance of \(\text{bla}_{\text{IMP-45}}\) in \textit{P. aeruginosa} clinical isolates in China and explored the role of IncP-2 plasmids in the dissemination of \(\text{bla}_{\text{IMP-45}}\) among \textit{P. aeruginosa}.

IMP-9-encoding plasmid pOZ176 (500 kb), the only full sequenced IncP-2 plasmid before 2013, contained \(\text{bla}_{\text{IMP-9}}, \text{bla}_{\text{OXA-10}}\) and \text{aacA}4 genes conferring \(\beta\)-lactam and aminoglycoside resistance. Two replication genes, \text{repA} and \text{repA}2 were identified in pOZ176 [3,9]. IncP-2 plasmids, generally >300 kb and in single copy, exhibit tellurite resistance and are narrow-host-range for \textit{Pseudomonas spp} [10]. Previous studies have characterized two \(\text{bla}_{\text{IMP-45}}\)-carrying megaplasmids from clinical isolates (\textit{P. putida} and \textit{P. aeruginosa}) in China [6,7], but information about the dissemination of \(\text{bla}_{\text{IMP-45}}\) gene remains largely limited and the role of plasmids in the dissemination of \(\text{bla}_{\text{IMP-45}}\) is poorly understood.

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Material and methods

Clinical isolates and antimicrobial susceptibility testing

One hundred and seventy-three non-duplicate CRPA isolates were collected from a tertiary hospital (Hospital HS) in Shanghai between January 2015 and April 2018. CRPA was defined as *P. aeruginosa* isolate resistant to either imipenem or meropenem. Additionally, a multicenter surveillance was performed with 605 non-duplicate *P. aeruginosa* isolates collected consecutively from 11 hospitals in 8 provinces/municipalities across China, including 3 hospitals in Shanghai, 2 in Beijing and 1 in each of the other 6 provinces (July, 2018 to February, 2019, Supplementary Table S1).

Minimal inhibitory concentrations (MICs) were determined for 13 antimicrobial agents by broth microdilution method and interpretation was according to recommendations of the CLSI [11].

MBLs screening and identification

PCR amplification was performed to screen for MBL genes (*bla*IMP, *bla*VIM, *bla*NDM, *bla*SPM, *bla*SIM, *bla*GIM, *bla*DIM, *bla*AIM and *bla*FIM) [12,13]. *bla*IMP-positive isolates were further amplified with primers specific for various subtypes (Supplementary Table S2).

Molecular typing of *P. aeruginosa* isolates

Pulsed-field gel electrophoresis (PFGE) was performed using SpeI (TaKaRa Bio, Dalian, China) as the restriction enzyme and with a switch time of 2 s-40 s [14,15]. The PFGE patterns were analysed by BioNumerics (version 4.0; Applied Maths, Inc.) and a dendrogram was generated by the UPGMA method based on the Dice coefficient.

Multilocus sequence typing (MLST) was performed according to the instructions in the *P. aeruginosa* MLST website (http://pubmlst.org/paeruginosa/). STs were compared with those in the MLST *P. aeruginosa* database by goeBURST [16]. A neighbor-joining tree from concatenated seven housekeeping genes (*acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA*, and *trpE*) was generated using MEGA 7.0 [17].

Conjugation assay

Transfer of plasmid carrying *bla*IMP-45 was performed by filter mating with *P. aeruginosa* PAO1Rif (rifampin resistant) as recipient. Transconjugants were selected on LB plates supplemented with meropenem (2 mg/L) and rifampin (500 mg/L), and further confirmed by PCR amplification and sequencing of *bla*IMP-45 and *guaA*, one of the seven housekeeping genes for MLST of *P. aeruginosa*. The transconjugants harbouring *bla*IMP-45 were tested for antimicrobial susceptibility. Conjugation was also carried out using *Escherichia coli* J53 (azide resistant) as recipient.

Whole genome sequencing (WGS) and sequence assembly

Sequencing of 9 IMP-45-producers, including 6 *P. aeruginosa* clinical isolates (HS15-106, HS17-127, HS18-41, GZ18-2, KM18-18 and RJ19-28) and 3 transconjugants (TcHS15-101, TcHS15-158 and TcHS15-172) were performed on Hiseq X-ten or Novaseq platforms (Illumina Inc., San Diego, CA, USA). De novo assembly was performed using Velvet version 1.2.03 [18] or SOAPdenovo2 [19]. To obtain the complete genomic sequence, clinical isolate of *P. aeruginosa* HS17-127 was further subjected to Pacbio sequencing and assembled with HGPAP [20].

For the 8 transconjugants of outbreak isolates without WGS data, the repA2 gene and genetic context surrounding *bla*IMP-45 were amplified by PCR and sequenced with a series of primers designed according to the genome sequence of the transconjugant of *P. aeruginosa* outbreak isolate HS15-101 (Table S2).

Bioinformatics analysis

The contigs were annotated using RAST (http://rast.nmpdr.org/), screened for insertion sequences with ISfinder [21], and analysed for STs, antibiotic resistance genes and plasmid typing at the Centre for Genomic Epidemiology web site (http://www.genomicepidemiology.org/). BLASTN searches were conducted to find the complete sequenced *bla*IMP-45-harbouring plasmids in GenBank database using *bla*IMP-45 as the query sequence. BLAST Ring Image Generator (BRIG) [22] were used in the comparative analysis of the plasmids.

Nucleotide sequence accession numbers

The sequences of transconjugants TcHS15-101, TcHS15-158 and TcHS15-172, and *P. aeruginosa* clinical isolates HS15-106, HS17-127, HS18-41, GZ18-2, KM18-18 and RJ19-28 were submitted to GenBank with Bioproject ID PRJNA631492 (Supplementary Table S3). The accession numbers for the chromosome of *P. aeruginosa* clinical isolate HS17-127 and plasmid pHS17-127 are CP061376 and CP061377, respectively.

Results

Outbreak of *bla*IMP-45-bearing *P. aeruginosa* in Hospital HS

Fourteen out of 173 CRPA from Hospital HS were positive for *bla*IMP-45: 12 CRPA isolated from 2015, 1 from 2017 and 1 from 2018. All of them exhibited...
resistance to antipseudomonal β-lactams excluding aztreonam, β-lactamase inhibitor combinations and aminoglycosides. The MICs of aztreonam ranged from 4 to 16 mg/L in 7 strains whereas the remaining strains were highly resistant to aztreonam (from 64 and >128 mg/L). All the blaIMP-45 carriers were resistant to quinolones except isolate HS17-127 (Table 1).

Eleven out of 12 blaIMP-45-bearing CRPA from 2015 were isolated from ICU patients and shared the same sequence type, ST508 (Figure 1(A)). They presented indistinguishable or closely related PFGE patterns, different from that of HS15-106 (ST3014) from an outpatient. All the 12 blaIMP-45-bearing CRPA from 2015 were discovered in the first nine months this year as indicated by the timeline of the isolation date of the first blaIMP-45-carrying P. aeruginosa from each patient (Figure 1(B)). Taken together, an outbreak of blaIMP-45-bearing ST508 CRPA occurred in the ICU of Hospital HS in 2015. The other two blaIMP-45-bearing CRPA, HS17-127 (ST369) and HS18-41 (ST357), were clonally distinct from the previous outbreak blaIMP-45-carriers in Hospital HS.

### Multicenter surveillance of blaIMP-45

To investigate the prevalence of blaIMP-45, a multicenter surveillance was carried out among 605 P. aeruginosa clinical isolates collected throughout China, including 226 CRPA. Genotypic characterization found 8 isolates positive for blaIMP (4 isolates positive for blaIMP-14-like, 3 positive for blaIMP-45 and 1 for blaIMP-3) and 6 isolates carrying blavIM-2. No additional MBL-producing isolates were found. These 3 blaIMP-45-carriers belonged to ST1420, ST274 and ST708, respectively (Table 1).

### Transferability of blaIMP-45

To examine the transferability of blaIMP-45, all the 17 blaIMP-45-carrying CRPA were performed conjugation with P. aeruginosa PAO1RF as recipients. Fifteen transconjugants harbouring blaIMP-45 were obtained from the 11 ST508 outbreak strains and HS17-127, HS18-41 from Hospital HS as well as KM18-18 and RJ19-28 from another two hospitals. All the transconjugants contained the same gaaA allele with the recipient P. aeruginosa PAO1RF. Transconjugants displayed similar antimicrobial susceptibility profiles with their donors except that 3 transconjugants (TcHS15-158, TcHS15-172 and TcHS15-209) were susceptible to quinolones (Supplementary Table S4). However, transfer of blaIMP-45 to E. coli failed.

### General features of blaIMP-45-harbouring plasmids

P. aeruginosa clinical isolate HS17-127 was fully sequenced resulting in one chromosome and one
plasmid pHS17-127. The sequencing analysis revealed that repA2 and bla\textsuperscript{Imp-45} genes were on the plasmid pHS17-127 (486,963 bp), which shared a highly similar backbone with IncP-2 plasmid pOZ176 (Figure 2).

Whole genome sequences of 3 transconjugants and 5 clinical isolates in this study (Supplementary Table S5) were compared to the fully sequenced plasmid pHS17-127 and the 7 completely sequenced bla\textsuperscript{Imp-45}-harbouring plasmids in GenBank (up to April, 2020). The 7 plasmids, varying in size from 374 kb to nearly 514 kb, were isolated in China and from P. aeruginosa except for 1 from P. putida [6] (Table 2). Comparative genome analysis revealed that these 16 bla\textsuperscript{Imp-45}-carrying plasmids (Table 2) shared an overall similar backbone, including genes essential for replication (repA2, 1188 bp), partition (par) and conjugal transfer (tra) (Figure 2). Moreover, they contained an operon terABCDEZ conferring tellurite resistance, which is a uniform property of IncP-2 plasmids. Virulence factors, such as pilus biogenesis gene pilD and chemotaxis gene cluster, cheABRWXZ, were also identified in the backbones.

An IncP-2 plasmid sublineage associated with dissemination of bla\textsuperscript{Imp-45}

In order to explore the mechanism underlying dissemination of bla\textsuperscript{Imp-45}, 16 bla\textsuperscript{Imp-45}-carrying plasmids were compared with IncP-2 bla\textsuperscript{Imp-9}-harbouring plasmid pOZ176. Comparative analysis revealed that they shared similar plasmid backbones (Figure 2). However, the IncP-2 repA-oriV-parAB region of pOZ176 was absent in all bla\textsuperscript{Imp-45}-harbouring plasmids except that pHS18-41 and pGZ18-2 contained a region with only about 84% identity. On the contrary, a second replication/partitioning system repA2/parAB-parB2 was shared by both bla\textsuperscript{Imp-9} and bla\textsuperscript{Imp-45}-harbouring plasmids with an identity of >98%.

When BLASTN with the repA2 gene of pOZ176 was performed for its homologs (100% query coverage), a total of 23 fully sequenced megaplasmids were identified in NCBI database, including pOZ176 and the above 7 bla\textsuperscript{Imp-45}-harbouring plasmids (Figure 3). They shared a similar backbone with pOZ176 as well as the bla\textsuperscript{Imp-45}-carrying plasmids in this study, even though these megaplasmids were absent of a large fragment containing repA-oriV-parAB of pOZ176 (Figure 2). Additionally, repA2 genes were confirmed by PCR and sequencing in the remaining 8 transconjugants of outbreak strains without WGS data in this study (Supplementary Table S2). Phylogenetic analysis of the 40 repA2 genes from 17 bla\textsuperscript{Imp-45}-carrying CRPA in this study and 23 megaplasmids in GenBank revealed 4 distinct subgroups (Figure 3). All the bla\textsuperscript{Imp-45}-carrying plasmids with the exception of RJ19-28 were in a sublineage separate from the one containing bla\textsuperscript{Imp-9}-harbouring pOZ176. IncP-2 plasmids and other plasmid lineages, such as IncP-7 and IncP-9, were phylogenetically analysed on the basis of replication genes, revealing that IncP-2 plasmid lineage was clearly separated from other plasmid lineages (Figure S2).
Coexistence of \( \text{bla}^{\text{IMP-45}} \), \( \text{armA} \) and \( \text{qnrVC1} \) or \( \text{qnrVC6} \)

Apart from 1 plasmid carrying only \( \text{bla}^{\text{IMP-45}} \) and 1 with both \( \text{bla}^{\text{IMP-45}} \) and \( \text{armA} \), including 9 together with \( \text{qnrVC1} \) and 3 with \( \text{qnrVC6} \) (Table 2). The genetic structures harbouring these resistant determinants were confirmed by WGS analysis or PCR and sequencing (Supplementary Table S2). As in the previously reported \( \text{IMP-45} \)-producers [5,8], \( \text{bla}^{\text{IMP-45}} \) was located in the variable region of In786, adjacent to a \( \text{Tn}^{1548} \)-derivative containing \( \text{armA} \) with or without \( \text{qnrVC6} \) (Figure S1). \( \text{qnrVC1} \) was in class 1 integron In1237 that coexisted with the \( \text{Tn}^{548} \)-derivative on the mega-plasmids. However, In1237 was not transferred to the recipient strain together with \( \text{bla}^{\text{IMP-45}} \)-harbouring plasmids from HS15-158, HS15-172 and HS15-209.

Discussion

In this study, 17 \( \text{IMP-45} \)-producers were discovered and belonged to 7 dissimilar STs. All these STs were different from previously reported \( \text{IMP-45} \)-producers, such as ST308, ST235 and ST389 [5,7,8], demonstrating the diverse population structure of \( \text{IMP-45} \)-producing \( \text{P. aeruginosa} \).

Outbreaks of MBL-producing \( \text{P. aeruginosa} \) have been reported in hospitals worldwide [23–25]. Recently, an outbreak of \( \text{IMP-19} \)-producing ST235 and \( \text{IMP-29} \)-producing ST111 of clinical \( \text{P. aeruginosa} \) was reported in France [26]. Here we report an outbreak caused by \( \text{IMP-45} \)-producing \( \text{ST508} \) CRPA isolates in Hospital HS from January to September, 2015. Since around September, 2015, strengthened infection control measures were implemented in the ICU, including improved hospital-wide sanitation, hand and environmental hygiene, contact precautions, changing disinfection to sterilization for reusable ventilator accessories (exhalation valve and respiratory humidifier) and using disposable ventilator circuits instead of recycled ones. The outbreak clone subsequently disappeared and distinct clonal complex lineages carrying \( \text{bla}^{\text{IMP-45}} \) emerged sporadically in 2017 and 2018, suggesting a shift of
Table 2. Characteristics of 16 bla<sub>IMP-45</sub>-carrying plasmids (9 in this study and 7 in GenBank).

| Plasmid   | Host strain | ST  | Location      | Resistance gene<sup>c</sup> | Size (bp)<sup>d</sup> | Resource | Accession number |
|-----------|-------------|-----|---------------|-----------------------------|----------------------|----------|-----------------|
| pHS15-101 | *P. aeruginosa* HS15-101 | ST508 | Shanghai, China | bla<sub>IMP-45</sub>, bla<sub>QEXA</sub>1, armA, qnrVC1 | ND | This study | N |
| pHS15-106 | *P. aeruginosa* HS15-106 | ST3014 | Shanghai, China | bla<sub>IMP-45</sub>, bla<sub>QEXA</sub>1, armA, qnrVC1 | ND | This study | N |
| pHS15-158 | *P. aeruginosa* HS15-158 | ST508 | Shanghai, China | bla<sub>IMP-45</sub>, bla<sub>QEXA</sub>1, armA | ND | This study | N |
| pHS15-172 | *P. aeruginosa* HS15-172 | ST508 | Shanghai, China | bla<sub>IMP-45</sub>, bla<sub>QEXA</sub>1, armA | ND | This study | N |
| pHS17-127 | *P. aeruginosa* HS17-127 | ST369 | Shanghai, China | bla<sub>IMP-45</sub>, bla<sub>QEXA</sub>1, bla<sub>PEB</sub>1, bla<sub>AMM</sub>-1, armA, qnrVC6 | 486,963 | This study | CP061377 |
| pHS18-41  | *P. aeruginosa* HS18-41 | ST357 | Shanghai, China | bla<sub>IMP-45</sub>, bla<sub>QEXA</sub>1, armA, qnrVC1 | ND | This study | N |
| pGZ18-2   | *P. aeruginosa* GZ18-2 | ST1420 | Guangzhou, China | bla<sub>IMP-45</sub>, bla<sub>QEXA</sub>1, armA, qnrVC1 | ND | This study | N |
| pKM18-18  | *P. aeruginosa* KM18-18 | ST274 | Kunming, China | bla<sub>IMP-45</sub>, bla<sub>QEXA</sub>1, armA, qnrVC1 | ND | This study | N |
| pRJ19-28  | *P. aeruginosa* R19-28 | ST708 | Shanghai, China | bla<sub>IMP-45</sub>,bla<sub>QEXA</sub>1, bla<sub>QFA</sub>1, qnrVC6 | ND | This study | N |
| pR31014-IM | *P. aeruginosa* | unknown | China | bla<sub>IMP-45</sub>,bla<sub>QEXA</sub>1, armA, qnrVC1 | 374,000 | GenBank | MF344571 |
| p727-IMP  | *P. aeruginosa* | unknown | China | bla<sub>IMP-45</sub>,bla<sub>QEXA</sub>1, armA, qnrVC1 | 430,173 | GenBank | MF344568 |
| pSY153- MDR | *P. aeruginosa* | unknown | Hainan, China | bla<sub>IMP-45</sub>,bla<sub>QEXA</sub>1, armA, qnrVC1 | 468,170 | GenBank | KY883660 |
| pA681-IMP | *P. aeruginosa* | ST274 | China | bla<sub>IMP-45</sub>,bla<sub>QEXA</sub>1, armA, qnrVC6 | 397,519 | GenBank | MF344570 |
| pBM413    | *P. aeruginosa* | ST389 | Guangzhou, China | bla<sub>IMP-45</sub>,bla<sub>QEXA</sub>1, armA, qnrVC6 | 395,774 | GenBank | MF344526 |
| pAGS      | *P. aeruginosa* | unknown | China | bla<sub>IMP-45</sub>,bla<sub>QEXA</sub>1, armA, qnrVC1 | 513,322 | GenBank | MF345003 |
| pOZ176    | *P. aeruginosa* | unknown | China | bla<sub>IMP-45</sub>,bla<sub>QEXA</sub>1, armA, qnrVC6 | 423,017 | GenBank | CP016215 |
| a         | *P. aeruginosa* | M140A | unknown | unknown | ND | GenBank | KJ510410 |
| b         | *P. aeruginosa* | unknown | 14.1819 | unknown | ND | GenBank | KJ984333 |

<sup>a</sup>b<sub>IMP-45</sub> was located on the chromosome.

<sup>b</sup>Detailed information of plasmid carrying b<sub>IMP-45</sub> in *P. aeruginosa* 14.1819 was not available.

<sup>c</sup>Resistance genes referring to β-lactamase genes, armA and qnrVC here.

<sup>d</sup>N: Putative plasmids in this study without complete sequence. NA: Not available.

<sup>e</sup>N: Plasmids in this study without complete sequence.

Although at least 14 incompatible groups (IncP-1–IncP-14) of plasmids have been identified in *Pseudomonas* species, there is no well-established scheme for the plasmid typing of this species as of Enterobacteriaceae [27,28]. Similar to IncP-2 plasmids reported previously [9,10], the bla<sub>IMP-45</sub>-carrying plasmids are tellurite resistant, conjugative, and transfer between *P. aeruginosa* and *P. putida* but not to *E. coli*. Plasmids usually undergo continuous rearrangement and mutations, sometimes occurring in regions for plasmid typing [29], resulting in novel untypeable plasmids or new plasmid lineages evolving from currently well-studied plasmid types. The incompatibility group of bla<sub>IMP-45</sub>-carrying plasmids has not been fully clarified since they lack the region containing IncP-2 repA-oriV-parAB [9]. A previous study reported that IncP-2 repA-oriV-parAB, an auxiliary replicon, was located in an integrative and conjugative element Tn6398a [30]. Moreover, homologs of IncP-2 repA gene were seldom present in *Pseudomonas* spp., but more frequently found in putative genomic islands on the chromosome of a variety of other species, such as *Azotobacter*, *Burkholderia*, *Stenotrophomonas* and *Xanthomonas* [9].

In contrast, IncP-2 repA2 gene and its close relatives (>96%) have been exclusively discovered on plasmids from *Pseudomonas* spp., indicating that the repA2 gene is probably the actual one encoding the IncP-2 replication initiator protein and contributing to the narrow-host-range of IncP-2 plasmids for *Pseudomonas* spp.

Based on the phylogenetic analysis of repA2 and the comparative genome analysis in this study, all the plasmids involved are closely related genetically, belonging to the same plasmid family as IncP-2 pOZ176. The phylogenetic tree of repA2 grouped almost all bla<sub>IMP-45</sub>-carrying plasmids into a different subgroup from the one containing pOZ176.

In summary, clonal diversity was observed in the 17 IMP-45-producing CRPA isolates identified in this study except for outbreak clone ST508 from Hospital HS. All the bla<sub>IMP-45</sub>-carrying plasmids were related to IncP-2 plasmid pOZ176, and contributing to the dissemination of bla<sub>IMP-45</sub>. Moreover, this IncP-2 plasmid sublineage has undergone multiple evolutionary events, recruiting bla<sub>IMP-45</sub>, armA and qnrVC1/qnrVC6, thus acting as a vehicle for the dissemination of carbapenem, aminoglycoside and quinolone resistance among *Pseudomonas* spp., with consequent compromise of therapeutic options.
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Disclosure statement
No potential conflict of interest was reported by the author(s).

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