Emergence of an Extensive Drug Resistant *Pseudomonas aeruginosa* Strain of Chicken Origin Carrying $\text{bla}_{\text{IMP}-45}$, tet(X6), and tmexCD3-toprJ3 on an Inc$_{\text{PRBL16}}$ Plasmid

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**Abstract**

This study reports an extensively drug resistant *Pseudomonas aeruginosa* strain PA166-2 which was of chicken origin and carrying $\text{bla}_{\text{IMP}-45}$, tet(X6) and tmexCD3-toprJ3 on a single plasmid. The strain was characterized by antimicrobial susceptibility testing, resistance gene screening, conjugation assay, whole-genome sequencing, and bioinformatics analysis. Strain PA166-2 was resistant to tigecycline and carbapenems. It belonged to ST313 and carried a plasmid pPA166-2-MDR, which belongs to the incompatibility group Inc$_{\text{PRBL16}}$. pPA166-2-MDR harbored a 78 Kb multidrug resistance (MDR) region carrying an array of antimicrobial resistance genes, including $\text{bla}_{\text{IMP}-45}$, tet(X6), and tmexCD3-toprJ3. The gene $\text{bla}_{\text{IMP}-45}$ was inserted into the backbone of plasmid pPA166-2-MDR within a class 1 integron, In$_{786}$. tmexCD3-toprJ3 in plasmid pPA166-2-MDR was inserted in $\text{umuC}$, constituting the genetic context of IS$_{Cfr1-tnfxB3-tmexC3-tmexD3-toprJ3-4}\text{umuC}$. The genetic context of tet(X6) in this plasmid was identical to that of other reported plasmid-borne tet(X) variants, namely, tet(X6)-$\text{abh-guaA-ISVsa3}$. To the best of our knowledge, this is the first report of the cooccurrence of $\text{bla}_{\text{IMP}-45}$, tet(X6), and tmexCD3-toprJ3 in one plasmid in *Pseudomonas* sp. The emergence of plasmid-mediated tigecycline resistance genes tmexCD3-toprJ3 and tet(X6), as well as carbapenemase genes from chickens expanded the global transmission of vital resistance genes. Findings from us and from others indicate that plasmids of the incompatibility group Inc$_{\text{PRBL16}}$ may serve as a reservoir for carbapenem and tigecycline resistance determinants.

**Importance**

*Pseudomonas aeruginosa* is an opportunistic pathogen that causes infections that are difficult to treat. This study reported, for the first time, the occurrence of last-resort antibiotic resistance determinants $\text{bla}_{\text{IMP}-45}$, tet(X6), and tmexCD3-toprJ3 on a single plasmid in *P. aeruginosa* from chickens. The *P. aeruginosa* strain belonged to ST313 and was resistant to last-line antibiotics, namely, carbapenems and tigecycline. The plasmid carrying the last-line resistance genes belonged to the incompatibility group Inc$_{\text{PRBL16}}$, which was reported to contain different profiles of accessory modules and thus carried diverse collections of resistance genes. The emergence of plasmid-mediated tigecycline resistance genes tmexCD3-toprJ3 and tet(X6), as well as carbapenemase genes, from chickens expanded the global transmission of vital resistance genes. The results in this study highlighted that Inc$_{\text{PRBL16}}$ plasmids may serve as a reservoir for the dissemination of resistance genes. Control measures should be implemented to prevent the further dissemination of such strains.

**Keywords**

*Pseudomonas aeruginosa*, $\text{bla}_{\text{IMP}-45}$, extensive drug resistance, tet(X6), tmexCD3-toprJ3
Pseudomonas aeruginosa is a leading cause of morbidity and mortality in cystic fibrosis patients and immunocompromised individuals (1). The treatment of *P. aeruginosa* infections has become a significant challenge due to its remarkable capacity to resist many of the currently available antibiotics (2). *P. aeruginosa* exploits intrinsic, acquired, and adaptive resistance mechanisms to counter antibiotic attacks (3). Efflux pumps belonging to the plasmid-mediated resistance–nodulation–division (RND) family play a prominent role in the multidrug resistance of *P. aeruginosa*. Recently, a novel RND-type efflux pump gene cluster, *tmexCD-toprJ1*, and its homologs, *tmexCD2-toprJ2* and *tmexCD3-toprJ3*, were reported to confer resistance to different classes of antibiotics, including the last-line antibiotic, tigecycline (4–7). The *tmexCD-toprJ* gene clusters were speculated to have originated from the chromosome of a *Pseudomonas* species and disseminated among diverse bacterial species, including *Pseudomonas* sp., *Klebsiella* sp., *Aeromonas* sp., *Enterobacter* sp., *Proteus* sp., and *Raoultella* sp. (4–8). The coexistence of *tmexCD-toprJ* with other antimicrobial resistance genes, such as the colistin resistance gene *mcr*, the high-level mobile tigecycline resistance gene *tet(X)*, and the carbapenemase genes *bla*$_{OXA}$ and *bla*$_{KPC}$ in single isolates, has been reported, particularly in *Klebsiella* sp. (5, 9, 10). The spread of mobile elements coexisting different last-line antimicrobial resistance determinants seriously compromises the effectiveness of clinical therapy. In this study, we report an XDR *P. aeruginosa* strain that co-harbors *bla*$_{IMP-ASG}$, *tet(X6)*, and *tmexCD3-toprJ3* on an Inc$^R_{BBL16}$ plasmid of chicken origin. Heightened efforts are needed to control the dissemination of such strains.

*P. aeruginosa* strain PA166-2 was isolated from the cloaca swab of a chicken in a poultry farm in Shanxi, China in 2019. Antimicrobial susceptibility testing was conducted via the broth dilution method, and the results suggested that PA166-2 was resistant to tetracyclines (doxycycline and minocycline), a glycylcycline (tigecycline), carbapenems (meropenem and imipenem), some β-lactams (ceftazidime, cefepime, piperacillin-tazobactam, cefoperazone-sulbactam, ceftazidime-avibactam), ciprofloxacin, and an aminoglycoside (amikacin). The strain also exhibited intermediate resistance to colistin. However, the strain remained susceptible to aztreonam. The antimicrobial resistance profiles and mechanisms of resistance of *P. aeruginosa* PA166-2 are shown in Table 1. According to the nonsusceptibility level of strain PA166-2, it was classified as an extensive drug resistant (XDR) strain which was resistant to at least one agent in all but two or fewer antibiotic categories. Carbapenem resistance in *P. aeruginosa* is frequently associated with the expression of carbapenemase genes, so genes, including *bla*$_{OXA}$, *bla*$_{NDM}$, *bla*$_{OAM}$, *bla*$_{KPC}$, and *bla*$_{OXA}$, were screened via polymerase chain reaction (PCR) and Sanger sequencing, using primers described previously (11). A *bla*$_{IMP}$ gene was detected positive. Meanwhile, strain PA166-2 was positive for the RND-type efflux pump gene cluster *tmexCD-toprJ* and for the *tet(X)* gene, both of which were recently reported to have conferred resistance to tigecycline (4, 12, 13). The antimicrobial resistance gene screening results were in line with the antimicrobial susceptibility testing (AST) results.

To decipher the genomic characterization, the genome of PA166-2 was extracted from overnight cultures by using the PureLink Genomic DNA Minikit (Invitrogen, Carlsbad, CA, USA) and sequenced by using the Illumina NextSeq 500 sequencing (2 × 150 bp) platform and the Nanopore MinION sequencer platform (14). The hybrid assembly of both sequencing reads was constructed using Unicycler v0.4.9β (15). The assembled genome of PA166-2 was annotated with the rapid antimicrobial susceptibility testing (RAST) tool and edited manually (16). Multilocus sequence typing was conducted by using the mlst software package (17). Antimicrobial resistance genes were analyzed by using ResFinder 2.1 (18). The genome of strain PA166-2 contained a 431,461 bp plasmid that was designated pPA166-2-MDR and a chromosome which was assembled into two contigs with lengths of 6,438,660 bp and 116,925 bp, respectively. The overall chromosome content of strain PA166-2 was comprised of 6,732 predicted open reading frames (ORFs), with a guanine-cytosine (GC) content of 65.6%. Antimicrobial resistance genes, including *fosA*, *catB7*, *bla*$_{OXA-58}$, *aph(3’)-Iib*, and *bla*$_{PAG-1}$.
were detected on the chromosome of PA166-2. MLST analysis suggested that strain PA166-2 belonged to ST313. Plasmid pPA166-2-MDR contained 493 ORFs with a GC content of 56.3%. Two plasmids, pBM413 (CP016215) and pR31014-IMP (MF344571), both of which have similar backbones to that of pPA166-2-MDR, were retrieved from the NCBI nr database via a nucleotide Basic Local Alignment Search Tool (BLASTn) analysis (Fig. 1A). Plasmids belonging to the incompatibility group IncpRBL16 that contained diverse collections of resistance genes were recently reported in Pseudomonas spp. (19). Conserved IncpRBL16 backbone marker repAIncpRBL16 together with its iterons, parB2-parA, che, pil, and ter, were detected on pPA166-2-MDR, pBM413, and pR31014-IMP, suggesting that they all belonged to the Inc_pRBL16 plasmid. An array of different resistance genes containing tmexCD3-toprJ3, blaIMP-45, blaOXA-1, tet(C), mph(E), msr(E), armA, sul1 (2 copies), catB3, qnrVC1, arr-3, floR, strAB (2 copies), ant(3’)-ih-aac(6’)-lId, dfrA22e, aph(3’)-Vla, aph(4)-la, aac(3)-Iva, and aph(3’)-Iib were found in plasmid pPA166-2-MDR. Notably, this is the first known report of the cooccurrence of blaIMP-45, tet(X6), and tmexCD3-toprJ3 in one plasmid. The multidrug resistance (MDR) region containing all of these acquired resistance genes was 78,304 bp in length and was similar to the corresponding region in pR31014-IMP, except for the presence of a ca. 19 Kb region harboring tet(X6) in pPA166-2-MDR. As in other Inc_pRBL16 plasmids, diverse mobile genetic elements, including TrnA51, intI1 (2 copies), ISCR1, ISEC28, IS1349, ISEC29, IS6100 (2 copies), ISEC59, Tn5393, ISVasa3, ISCRf1, and IS26 (4 copies) were detected in this MDR region (Figure 1B), suggesting that it was acquired via horizontal gene transfer and that active genetic recombination could have occurred in this region. A conjugation assay was performed via the filter mating method, using E. coli EC600 and

### TABLE 1 Results of antimicrobial susceptibility tests and genetic characterization

| Antimicrobial agents | MIC (mg/L) | Interpretation | Mechanism of resistance/location of resistance gene |
|----------------------|------------|----------------|----------------------------------------------------|
| Aminoglycosides      |            |                |                                                    |
| Amikacin             | ≥128       | R              | aph(3’)-Vla, aph(3’)-Ic, aph(4)-la, armA, aac(3)-Iva, ant(3’)-ih-aac(6’)-lId, /plasmid; aph(3’)-Iib/chromosome |
| β-Lactams            |            |                |                                                    |
| Imipenem             | 4          | R              | bla_{amp-45}/plasmid                              |
| Meropenem            | 32         | R              | bla_{amp-45}/plasmid                              |
| Ceftazidime          | >128       | R              | bla_{amp-45}/plasmid                              |
| Cefepime             | 128        | R              | bla_{amp-45} and bla_{oxa-1}/plasmid              |
| Piperacillin-tazobactam | 128/4   | R              | bla_{amp-45} and bla_{oxa-1}/plasmid              |
| Cefoperazone/sulbactam | >128/64 | R              | bla_{amp-45}/plasmid                              |
| Ceftazidime-avibactam | >64/4    | R              | bla_{amp-45}/plasmid                              |
| Aztreonam            | ≤4         | S              | -                                                  |
| Fluoroquinolones     |            |                |                                                    |
| Ciprofloxacin        | 16         | R              | qnrVC1 and tmexCD3-toprJ3/plasmid                 |
| Tetracyclines        |            |                |                                                    |
| Doxycycline          | >32        | R              | Intrinsic resistance; tet(C), tet(X6) and tmexCD3-toprJ3/plasmid |
| Minocycline          | 32         | R              | Intrinsic resistance; tet(C), tet(X6) and tmexCD3-toprJ3/plasmid |
| Glycylcyclines       |            |                |                                                    |
| Tigecycline          | 16         | R              | Intrinsic resistance; tmexCD3-toprJ3 and tet(X6)/plasmid |
| Polymyxins           |            |                |                                                    |
| Colistin             | 1          | I              | -                                                  |
| Trimethoprim-sulfamethoxazole |          |                | sul1 and dfrA22e/plasmid                         |
| Not included in the AST panel | NA  | NA              |                                                    |
| Catitative antibiotics |          |                |                                                    |
| Not included in the AST panel | NA  | NA              | msr(E) and mph(E)/plasmid                         |

*R*, resistant; *S*, susceptible; *I*, intermediate; *NA*, not applicable; -, none.

were detected on the chromosome of PA166-2. MLST analysis suggested that strain PA166-2 belonged to ST313. Plasmid pPA166-2-MDR contained 493 ORFs with a GC content of 56.3%. Two plasmids, pBM413 (CP016215) and pR31014-IMP (MF344571), both of which have similar backbones to that of pPA166-2-MDR, were retrieved from the NCBI nr database via a nucleotide Basic Local Alignment Search Tool (BLASTn) analysis (Fig. 1A). Plasmids belonging to the incompatibility group Inc_pRBL16 that contained diverse collections of resistance genes were recently reported in Pseudomonas spp. (19). Conserved Inc_pRBL16 backbone marker repAIncpRBL16 together with its iterons, parB2-parA, che, pil, and ter, were detected on pPA166-2-MDR, pBM413, and pR31014-IMP, suggesting that they all belonged to the Inc_pRBL16 plasmid. An array of different resistance genes containing tmexCD3-toprJ3, blaIMP-45, blaOXA-1, tet(X6), tet(C), mph(E), msr(E), armA, sul1 (2 copies), catB3, qnrVC1, arr-3, floR, strAB (2 copies), ant(3’)-ih-aac(6’)-lId, dfrA22e, aph(3’)-Vla, aph(4)-la, aac(3)-Iva, and aph(3’)-Iib were found in plasmid pPA166-2-MDR. Notably, this is the first known report of the cooccurrence of blaIMP-45, tet(X6), and tmexCD3-toprJ3 in one plasmid. The multidrug resistance (MDR) region containing all of these acquired resistance genes was 78,304 bp in length and was similar to the corresponding region in pR31014-IMP, except for the presence of a ca. 19 Kb region harboring tet(X6) in pPA166-2-MDR. As in other Inc_pRBL16 plasmids, diverse mobile genetic elements, including TrnA51, intI1 (2 copies), ISCR1, ISEC28, IS1349, ISEC29, IS6100 (2 copies), ISEC59, Tn5393, ISVasa3, ISCRf1, and IS26 (4 copies) were detected in this MDR region (Figure 1B), suggesting that it was acquired via horizontal gene transfer and that active genetic recombination could have occurred in this region. A conjugation assay was performed via the filter mating method, using E. coli EC600 and
fosfomycin-resistant *P. aeruginosa* PAO1 as the recipients. Transconjugants were selected on LB agar plates containing 1 mg/L meropenem and 600 mg/L rifampicin or 150 mg/L fosfomycin, respectively. Plasmid pPA166-2-MDR could not be transferred to *P. aeruginosa* and *E. coli* via direct conjugation under laboratory conditions.

tet(X6), which is a variant of the tet(X) gene that confers high-level tigecycline resistance, was first reported on an SXT/R391 element, ICE *Pgs6Chn1*, in *Proteus* sp. (20). Previous studies have demonstrated that tet(X6) is frequently associated with the genetic
context of tet(X6)-abh-guaA-ISVsa3, which is highly similar to that of other reported plasmid-borne tet(X) variants that are flanked by one or two ISVsa3 elements (20, 21). Likewise, pPA166-2-MDR carried tet(X6)-abh-guaA-ISVsa3 genetic content, and the ISVsa3 element upstream of tet(X6) was absent. Highly similar to that in ICEPgs6Chn1, the tet(X6) in pPA166-2-MDR was downstream of a truncated Tn5393 (Figure 1D). A BLASTn search in NCBI suggested that the tet(X) genes were absent on the Inc<sub>P</sub>plasmids in the database. tmexCD3-toprJ3 was also first reported in Proteus sp. on an SXT/R391 element, ICEPmiChnRGF134-1 (7). Previous studies have shown that most transposition units containing the tmexCD3-toprJ3-like gene clusters inserted into a similar site in the umuC gene (7, 22). In line with these findings, tmexCD3-toprJ3 in plasmid pPA166-2-MDR was found to be inserted in umuC, constituting the genetic context of ISCrl1-tmxrB3-tmxrC3-tmxrD3-toprJ3-ΔumuC. A BLASTn search with this genetic element in the NCBI nr database returned 8 hits (MF344570, KY883660, CP016215, CP086014, MF344568, CP073081, MN208062, and MF344571) with >98.5% identity at 100% coverage. All 8 of the sequences were from plasmids that belonged to the incompatibility group Inc<sub>P<sub>bl</sub> (Figure 1C). The bla<sub>IMP-45</sub> gene in pPA166-2-MDR was located directly downstream of the transposable element TnAs1 and in a class 1 integron, ln786, with the gene arrangement intl1-aacA4-bla<sub>IMP-45</sub>-bla<sub>QAB</sub>-catB3. In786 was located within a Tn6485b transposon in pPA166-2-MDR. Similar genetic contexts were detected or reported in several other Inc<sub>P</sub>plasmids, including pBM413 and pR31014-IMP (Figure 1B) (19, 23, 24). Our findings suggested that Inc<sub>P</sub>plasmids were an important vector for the dissemination of last-line antibiotic resistance genes in <i>Pseudomonas</i> sp. The spread of plasmids like pPA166-2-MDR is of great concern for public health.

<i>P. aeruginosa</i> strains belonging to ST313 were widely disseminated across different continents (25). They have been described as intestinal colonizers in healthy individuals but were rarely reported from the poultry farm environment (26). The detection of such a strain in a chicken in this study suggested that this poultry could have been contaminated by human activities. Infections caused by <i>P. aeruginosa</i> are challenging to treat due to the intrinsic resistance of this bacterium to many antimicrobial agents as well as its ability to acquire resistance determinants (2). ST313 <i>P. aeruginosa</i> were frequently reported to be associated with antimicrobial resistance genes, such as the carbapenemase genes <i>bla</i><sub>AVM</sub> and <i>bla</i><sub>SQAC</sub> (27). However, the presence of Inc<sub>P</sub>plasmids in ST313 <i>P. aeruginosa</i> was not reported previously. The acquisition of the Inc<sub>P</sub>plasmid carrying last-resort antimicrobial resistance genes <i>bla</i><sub>IMP-45</sub>, tet(X6), and tmexCD3-toprJ3 by <i>P. aeruginosa</i> pose considerable threats to public health.

In conclusion, this study reported, for the first time, the occurrence of last-resort antibiotic resistance determinants <i>bla</i><sub>IMP-45</sub>, tet(X6), and tmexCD3-toprJ3 on a single plasmid in <i>P. aeruginosa</i> from a chicken. The results of this study highlighted that Inc<sub>P</sub>plasmids may serve as a reservoir for the dissemination of resistance genes. Control measures, such as strict supervision, the application of laws to control antibiotic use, and timely screening, should be implemented to prevent the further dissemination of such strains.

**Data availability.** The complete genome sequence of strain PA166-2 has been deposited in the NCBI GenBank database under the BioProject accession number PRJNA798590.

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