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COMPARATIVE GENOMICS OF CARBAPENEM-RESISTANT *Pseudomonas aeruginosa* STRAINS SEQUENCE TYPE 3151 ISOLATED FROM TWO MAJOR HOSPITALS IN HANOI, VIETNAM

Nguyen Quang Huy[1,✉], Do Bich Ngoc[1], Tran Thi Thanh Tam[1], Le Thi Thu Hang[1], Doan Thi Nhung[2], Anne-Laure Bañuls[1,3]

[1]*University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam*
[2]*Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam*
[3]*UMR MIVEGEC (University of Montpellier- IRD-CNRS), Montpellier, France*

✉To whom correspondence should be addressed. E-mail: nguyen-quang.huy@usth.edu.vn

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SUMMARY

Carbapenem-resistant *Pseudomonas aeruginosa* belongs to the critical species of global priority pathogens, nevertheless, molecular mechanisms of such resistance in low- and middle-income countries are limited. In this context, we conducted a comparative genomic analysis between two clinical carbapenem-resistant *P. aeruginosa* strains VD641 and XP646 isolated from hospitals in Hanoi, Vietnam. The phenotypic-resistant profile revealed that both strains were resistant to at least 26 drugs belonging to 6 different antibiotic classes, and therefore they were identified as extensively drug-resistant bacteria. The chromosomal genome of strain VD641 was 7.1 Mb with GC a content of 65%, while the genome of XP646 consisted of an approximately 7.3 Mb with a GC content of 64.8%. The two strains belong to sequence type 3151 (ST3151). Genomic comparative analysis revealed that VD641 possessed higher numbers of antimicrobial resistance genes compared to XP646 (35 and 26 genes, respectively). This data was in accordance with their antibiotic-susceptibility profiles. Notably, the strain VD641 acquired a multidrug-resistant gene region with 223 kb in length carrying 11 antibiotic-resistant genes. Strains VD641 and XP646 acquired a *bla*IMP-15 and *bla*KPC-2 carbapenem-resistant gene, respectively. Analysis of virulent protein-protein interaction networks revealed six gene clusters involving the pathogenicity of the ST3151. Finally, four plasmids found in *P. aeruginosa* XP646 (n=1) and VD641 (n=3) carried different ARGs genes. The draft genomes and plasmid sequences of *P. aeruginosa* VD641 and XP646 were submitted to GenBank under BioSample accessions SAMN39268202 and SAMN39268203, respectively. The findings in our study underline that genomic surveillance is essential for management of carbapenem resistance emergence in healthcare setting in Vietnam.

Keywords: Carbapenem resistance, multidrug resistance, plasmidome and whole-genome sequencing, Pseudomonas aerginosa, resistome, virulome

INTRODUCTION

Pseudomonas aeruginosa, an opportunistic pathogen, is strongly associated with hospital-acquired infections (HAIs) including pneumonia, bloodstream infections, urinary tract infections, and surgical site infections, particularly dangerous for patients with chronic lung diseases (Folic *et al*., 2021). HAIs caused by *P. aeruginosa* have high levels of morbidity and mortality. The pathogen possesses many innate antibiotic resistance mechanisms including reduced permeability, antibiotic efflux, expression change, antibiotic modification and degradation, target protection and modification. In addition, *P. aeruginosa* can acquire antibiotic resistance via either horizontal gene transfers (HGTs) or mutations in targeted genes (Pang *et al*., 2019). Altogether, *P. aeruginosa* evolved quickly to multidrug-resistant (MDR) or extensively drug-resistant (XDR) forms which are extremely difficult to treat (Kunz Coyne *et al*., 2022; Pang *et al*., 2019).

Carbapenems are last-line antibiotics used to treat serious MDR infections caused by resistant Gram-negative bacteria including MDR and XDR *P. aeruginosa* (Kunz Coyne *et al*., 2022). Unfortunately, the prevalence of resistance to carbapenems has been observed in 60% of *P. aeruginosa* isolates from HAIs worldwide, and they are even resistant to almost available antibiotics (Reyes *et al*., 2023). The increased mortality associated with carbapenem-resistant *P. aeruginosa* infections highlights the therapeutic challenges posed by these organisms. In 2017, the World Health Organization (WHO) published a priority list of highly antibiotic-resistant bacteria for the research and development of new antibiotics, in which carbapenem-resistant *P. aeruginosa* ranked second in the critical, priority 1 pathogen. The major mechanisms of carbapenem resistance in *P. aeruginosa* include the loss of outer membrane porin OprD, the overexpression of multidrug efflux pumps (MexAB-OprM and MexXY–OprM) or the production of intrinsic β-lactamase AmpC protein and the production of carbapenemases (Yoon and Jeong, 2021). The acquisition of carbapenems-resistant genes via horizontal gene transfer mechanisms has accelerated the emergence and spread of the resistant strains. Presently, almost carbapenem-resistant gene variants including *bla*KPC, *bla*GES, *bla*VIM, *bla*IMP, *bla*OXA and *bla*NDM have been reported worldwide (Lee *et al*., 2022; Reyes *et al*., 2023; Yoon and Jeong, 2021). Notably, the emergence of carbapenems in carbapenem-resistant *P. aeruginosa* isolates differs according to geographical regions (Lee *et al*., 2022; Reyes *et al*., 2023).

Vietnam is a hot spot of the emergence and transmission of antimicrobial-resistant (AMR) bacteria. It is estimated that 20-30% of all nosocomial infections caused by *P. aeruginosa* (Phu *et al*., 2016). Seriously, carbapenem resistance was most common in *P. aeruginosa* (55.7%) in Vietnamese patients in Intensive Care Units with HAIs. Overall, the prevalence of carbapenem-resistant *P. aeruginosa* was 45% of total *P. aeruginosa* infections in Vietnam in 2016, ranking second only after India (Vu *et al*., 2021). Unfortunately, research on genomic characteristics of carbapenem-resistant *P. aeruginosa* clones is limited in Vietnam. This knowledge gap is significant because the sequence types (STs) of *P. aeruginosa* linked to antibiotic-resistance genes vary considerably among different communities, hospitals, and countries (Miyoshi-Akiyama *et al*., 2017). A study conducted at two

hospitals in Hanoi, Vietnam revealed the dominant presence of carbapenemase-ST3151 *P. aeruginosa* strains harbouring *bla*IMP-15 and *bla*KPC-2. These strains were recognized to play an important role concerning hospital-acquired infections (Tran *et al*., 2021). To better understand patterns of molecular mechanisms of resistance to carbapenems, we performed comparative genomic analysis for two XDR *P. aeruginosa* strains sequence type 3151 (ST3151) isolated from Germany General Hospital and Saint Paul General Hospital, Hanoi, Vietnam.

MATERIALS AND METHODS

Bacterial isolation and identification

Bacterial strain VD641 was recovered from the bronchial fluid of a patient hospitalized in the Intensive Care Unit of Vietnam Germany General Hospital, while bacterial strain XP646 was isolated from a sputum sample of a pneumonia patient in Saint Paul General Hospital. These bacterial strains were primarily identified as *P. aeruginosa* using MALDI-TOF MS (Bruker, Germany) and then confirmed by 16S rRNA gene sequencing using forward primer 27F-5′-AGAGTTTGATCCTGGCTCAG-3' and reverse primer 518R-5′-CGTATTACCGCGGCTGCTGG-3' (Park *et al*., 2021). Both strains were maintained and stored in Tryptone Soya Broth (TSB, Merck) supplemented with 50% glycerol and preserved at -80°C at the hospitals. Upon usage, bacterial strains were reactivated and cultured on Tryptic soy agar (TSA, Merck) plates to serve subsequent experiments. Antibiotic susceptibility testing was conducted in biosafety laboratory level 2 at hospitals. The biomass of inactive strains was sent to University of Science and Technology of Hanoi for DNA extraction and sequencing.

Antibiotic susceptibility testing

P. aeruginosa XP646 and VD641 were tested for susceptibility against 29 recommended antibiotics using the agar disc diffusion method described in the Clinical and Laboratory Standards Institute (CLSI) guidelines 2020. The antibiotics (SirScan/i2a Diagnostics, France) were selected as follows: amikacin (30 µg), ampicillin (10 µg), fosfomycin (200 µg), gentamicin (10 µg), netilmicin (10 µg), tobramycin (10 µg), chloramphenicol (30 µg), piperacillin (30 µg) + tazobactam (6 µg), meropenem (10 µg), ertapenem (10 µg), imipenem (10 µg), cephalexin (30 µg), cefpodoxime proxetil (10 µg), cefoxitin (30 µg), cefsulodin (30 µg), ceftazidime (10 µg), cefepime (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ofloxacin (5 µg), norfloxacin (10 µg), amoxicillin (20 µg) + clavulanic acid (10 µg), piperacillin (30 µg), colistin (50 µg), nalidixic acid (30 µg), trimethoprim-sulfonamides (25 µg), aztreonam (30 µg), temocillin (30 µg) and ticarcillin (75 µg). *P. aeruginosa* ATCC 27853 was used as a control for all experiments.

DNA extraction, whole-genome sequencing and genomic analysis

Genomic DNA (gDNA) of *P. aeruginosa* XP646 and VD641 was extracted using Norgen Bacterial Genomic DNA Isolation Kit (Norgen Biotek, Canada) following the manufacturer's guidelines. The purity of gDNA was inspected by nanodrop 20000 spectrophotometry (Thermo Fisher, USA) before being sent to the Beijing Genomics Institute (BGI, China) for sequencing on Illumina HiSeq 4000

system (Illumina, San Diego, CA, USA) with 150 bp pair-end reads. The raw reads were assessed and quality controlled by using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc) and Trimmomatic v.0.39 (Bolger *et al*., 2014). After filtering, the trimmed reads were de novo assembly by SPAdes v.3.14.1 (Bankevich *et al*., 2012). Next, the quality of each genome was compared with the reference genome of *P. aeruginosa* Pa124 (NZ_CP021774.1, GeneBank, NCBI) using QUAST v5.0.2 (Gurevich *et al*., 2013). The draft genomes of *P. aeruginosa* XP646 and VD641 were annotated using Prokka v1.14.6 (Seemann 2014). Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated genes (Cas) were also investigated with the CRISPR/Cas Finder (Couvin *et al*., 2018).

Multi-locus sequence typing and core genome-based phylogeny

The sequence type of *P. aeruginosa* XP646 and VD641 was determined according to the Multi-Locus Sequence Typing (MLST) profile on MLST v2.0 (https://pubmlst.org/). A phylogeny was constructed based on the core genome including 5104 genes of the two strains VD641 and XP646, and 18 selected *P. aeruginosa* strains retrieved from RefSeq, GenBank, NCBI using Roary pipeline along with annotation files generated by Bakta (v1.8.1) (Page *et al*., 2015; Schwengers *et al*., 2021). The phylogeny was then visualized using a Python script contributed by the scientific community (https://github.com/sanger-pathogens/Roary/), where Bootstrap support values were represented with the highest value at 1.000 (Simon, 2022). Finally, the draft genomes of *P. aeruginosa* XP646 and VD641 were submitted to GenBank, NCBI.

Identification of virulome, resistome and plasmidome

Virulent factors were searched through the functional annotation data generated from the virulence factor database (VFDB) using VFinder (Liu *et al*., 2022). ResFinder and Comprehensive Antibiotic Resistance Database (CARD) were employed to determine the presence of AMRs (Jia *et al*., 2017). Mobile genetic elements (MGEs) were predicted by the web tool Mobile Element Finder (Johansson *et al*., 2021). Plasmid sequences were extracted and predicted mobility by MOB-Suite (Robertson and Nash, 2018). The structure of draft genomes and plasmids was visualized by Proksee (Grant *et al*., 2023). The virulent protein-protein interaction networks were constructed by STRING v12.0 (Szklarczyk *et al*., 2023).

RESULTS AND DISCUSSION

Phenotypic antimicrobial resistance profiling

The analysis of the phenotypic-based resistant profile revealed that *P. aeruginosa* XP646 exhibited resistance to at least one antibiotic belonged to 7 antibiotic classes (aminoglycosides, antifolates, β-lactams, cephalosporins, phenicols, phosphonic acids, and quinolones), while still demonstrating susceptibility to polymyxins (Table 1). Specifically, out of 29 antibiotics tested, *P. aeruginosa* XP646 showed resistance to 27 antibiotics, intermediate resistance to norfloxacin, and sensitivity to colistin. On the other hand, *P. aeruginosa* VD641 displayed resistance to 23 antibiotics, while remaining

susceptible to meropenem, azetreonam, piperacillin, piperacillin-tazobactam, fosfomycin, and colistin (Table 1). Regarding resistance to carbapenems, *P. aeruginosa* XP646 were resistant to all three carbapenems tested including imipenem, meropenem and ertapenem, while *P. aeruginosa* VD641 was still susceptible to meropenem, suggesting that they might have different genetic-AMR profiles. Overall, the phenotypic-based AMR profiles revealed the XDR trait of the two strains XP646 and VD641. This underlines the emergence and spread of XDR bacteria were already complicated in healthcare settings in Hanoi. Vu *et al*. reported that the prevalence of MDR *P. aeruginosa* in Vietnam was 42% (660/1566 isolates tested) (Vu *et al*., 2021). Notably, the prevalence of carbapenem-resistant *P. aeruginosa* was increasing from 33% in the period 2012 – 2013 to 45% in 2016 – 2017 (Vu *et al*., 2021; Vu *et al*., 2019). This prevalence was even higher at 45% in the HAI group (Phu *et al*., 2016). These data alarm the rapidly increasing carbapenem-resistant *P. aeruginosa* in healthcare settings of Vietnam and genomic surveillance is urgently needed to better control the transmission of this resistant pathogen.

Genomic characteristics of *P. aeruginosa* VD641 and XP646

The draft genome of *P. aeruginosa* VD641 was estimated at 7,188,975 bp with a GC content of 65%, comprising 3.644 protein-coding sequences (CDS), 81 tRNAs, 10 rRNAs and four CRISPR arrays. While, the genome assembly size of the *P. aeruginosa* XP646 is 7,301,113 bp with a GC content of 64.8%, comprising 6.582 CDS, 67 tRNAs, six rRNAs and five CRISPR arrays. Analysis of MLST profile showed that both strains *P. aeruginosa* VD641 and XP646 belong to sequence type 3151 (ST3151), a sequence type was rarely reported in the world, but was commonly found in Vietnam (Tran *et al*., 2021). Furthermore, the core genome-based phylogeny demonstrated the genetic identical of the both strains (Figure 1). Previous study has confirmed that carbapenems-resistant *P. aeruginosa* strains with some dominant sequence types may efficiently spread clonally (Papagiannitsis *et al*., 2017). In concordance with previous studies, our study found the ST3151 of carbapenems-resistant *P. aeruginosa* strains from different hospitals suggesting that this genotype has widely spread in health care settings in Hanoi (Tran *et al*., 2021). The draft genome sequence and plasmid data of *P. aeruginosa* VD641 and XP646 were submitted to GenBank, NCBI under BioSample accessions SAMN39268202 and SAMN39268203, respectively.

Resistome profile of *P. aeruginosa* XP646 and VD641

Comparative genomic of *P. aeruginosa* XP646 and VD641 showed that the two strains acquired 25 similar ARGs responsible for resistance to aminoglycosides (*aad*A11, *aph*A15, *aph*(3')-IIb, *aph*(3')-XV, rmtB), β-lactams (*bla*OXA-905, *bla*PDC-36, *bla*CARB-3, *bla*CARB-2, *bla*PAO, *bla*OXA-395), bicyclomycin (*bcr*-1), quinolones (*qnr*VC1, *crp*P), fosfomycin (*fos*A), peptides (*arn*A, *bas*S, *cpr*S, *cpr*R, *bas*R), phenicols (*cat*B7, *flo*R), tetracyclines (*tet(*D), *tet*(G)) and trimethoprim (*dfr*A27). Additionally, strain XP646 acquired a *bla*KPC-2 gene responsible for carbapenem resistance, while strain VD641 possessed 10 other ARGs including *aph*(6)-Id, *aph*(3")-Ib, *aad*A9 (aminoglycosides resistance), *bla*IMP-15, *bla*OXA-573 (carbapenem resistance), *mex*M, *mex*N, *cml*A9 (phenicols resistance), *arr*-8 (rifamycin resistance), and *sul*1 (sulfonamides resistance).

Table 1. Phenotypic antibiotic-resistant profile of *P. aeruginosa* VD641 and XP646.

Antibiotic class	Antibiotic name	*P. aeruginosa* XP646		*P. aeruginosa* VD641	
		Inhibition zone (mm)	Result	Inhibition zone (mm)	Result
Aminoglycosides	Amikacin	0	R	0	R
	Gentamicin	0	R	0	R
	Netilmicin	0	R	0	R
	Tobramycin	0	R	0	R
Antifolates	Trimethoprime+sulphamedazole	0	R	0	R
β-lactams	Ampicillin	0	R	0	R
	Meropenem	0	R	20	**S**
	Ertapenem	0	R	0	R
	Imipenem	0	R	0	R
	Amoxicillin + Clavulanic acid	0	R	0	R
	Aztreonam	0	R	27	**S**
	Ticarcillin	0	R	0	R
	Piperacillin	0	R	21	**S**
	Piperacillin + Tazobactam	10	R	24	**S**
	Ticarcillin + Clavulanic acid	0	R	15	R
Cephalosporins	Cephalexin	0	R	0	R
	Cefpodoxime proxetil	0	R	0	R
	Cefoxitine	0	R	0	R
	Cefsulodine	0	R	0	R
	Ceftazidime	10	R	0	R
	Cefepime	0	R	0	R
Phenicols	Chloramphenicol	0	R	0	R
Phosphonic acids	Fosfomycin	0	R	18	**S**
Polymyxins	Colistin	20	**S**	16	**S**
Quinolones	Ciprofloxacin	15	R	0	R
	Levofloxacin	12	R	0	R
	Ofloxacin	0	R	0	R
	Norfloxacin	14	**I**	9	R
	Nalidic acid	0	R	0	R

R: Resistant; S: sensitive; I: Intermediate-resistant.

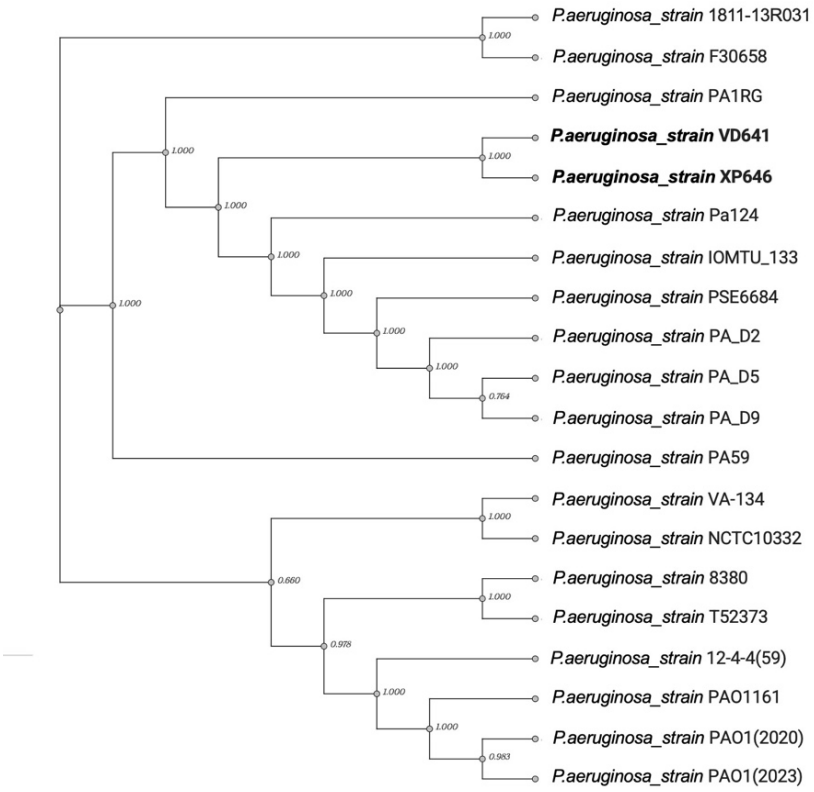

Figure 1. Core genome-based phylogeny of two strains VD641 and XP646, and 18 selected *P. aeruginosa* strains retrieved from GenBank.

This result demonstrated the phenotypic-XDR trait of the two strains VD641 and XP646. Overall, the ARGs were distributed along the chromosome of the two strains XP646 and VD641 (Figure 2). Notably, the strain VD641 acquired a multidrug-resistant gene cluster with 223 kb in length carrying 11 ARGs responsible for resistance to six antibiotic groups including aminoglycosides, β-lactams, quinolones, tetracyclines, trimethoprim and carbapenems (Figure 2).

Although these strains belonged to the same ST3151, nevertheless their antibiotic resistance evolutions were independent. This probably was the drug selection pressure and the diversity and abundance of bacterial population in these hospitals.

Regarding the carbapenem resistance, strain XP646 carried *bla*KPC-2, while VD641 possessed *bla*IMP-15, suggesting that these strains acquired carbapenem-resistant genes in different pathways. Globally, it has been previously reported that the most common carbapenem-resistant genes in *P. aeruginosa* are *bla*VIM and *bla*IMP variants, while the *bla*KPC variants are often found in *Klebsiella pneumonia* (Lee *et al*., 2022; Reyes *et al*., 2023; Tada *et al*., 2016). A study in one hospital in Hanoi, Vietnam reported a carbapenemase-ST235 *P. aeruginosa* carrying *bla*IMP-15, *bla*IMP-26, and *bla*IMP-51 genes (Tada *et al*., 2016). Nevertheless, a recent study from Vietnam showed that seven strains of *P. aeruginosa* ST3151 isolated in Saint Paul hospital carried the *bla*KPC-1 gene (Tran *et*

al., 2021). Although the *bla*KPC-2 variant has been found in other genotypes of *P. aeruginosa*, our study for the first time detected this variant in carbapenems-resistant *P. aeruginosa* strain in Saint Paul hospital. It is worth noting that the *P. aeruginosa* ST3151was found significantly more often in Saint Paul Hospital compared with Viet Duc Hospital, therefore this genotype could play important epidemiology in the Saint Paul Hospital (Tran *et al*., 2021). Our results provide a snapshot of the resistome diversity of carbapenems-resistant *P. aeruginosa* in hospitals in Hanoi which is extremely important for a better understanding of the emergence and spread of drug resistance in Vietnam. Finally, *P. aeruginosa* XP646 and VD641 also possessed various genes encoding for multidrug efflux pumps (Figure 2) which play important role in the innate resistance of the pathogen. Of note, we found four RND family efflux pumps (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM) are responsible for antibiotic resistances including β-lactams, quinolones and aminoglycosides (Pang *et al*., 2019). Therefore, inhibiting the efflux pumps is a potential therapeutic strategy for increasing the effectiveness of antibiotic drugs and for better controlling *P. aeruginosa* infections.

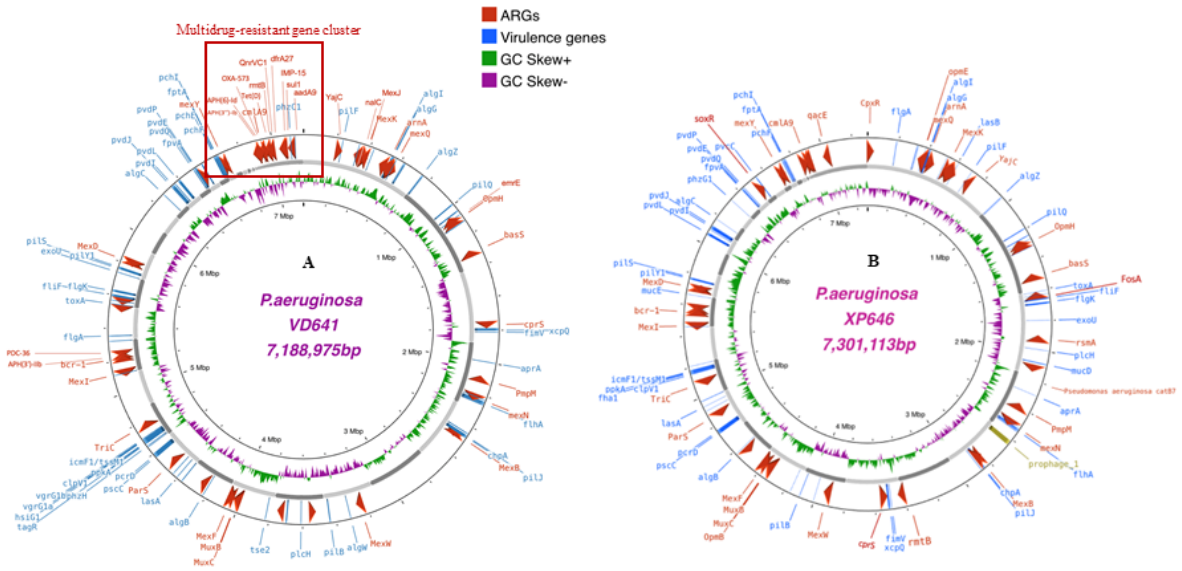

Figure 2. Circular genome of *P. aeruginosa* VD641 (**A**) and XP646 (**B**). The 223 kb multidrug-resistant gene cluster within the genome of *P. aeruginosa* VD641 is shown in the red box.

Virulomes and pathogenicity

Analysis of the genome of the two strains VD641 and XP646 showed that the virulent genes were distributed thought out the chromosome (Figure 2). Overall, the two strains belonged to the same ST3151, and therefore they possessed identical virulent genes responsible for important virulent factors including flagella protein synthesis (adherence and motility, 45 genes), endotoxin (3 genes), ion uptake (12 genes), anti-phagocytosis (22 genes), secretion systems type II (14 genes), type III (32 genes) and type VI (26 genes). All these molecular genetic determinants

enhance the infection, pathogenesis and persistence in host cells as well as play a critical role in competition and adaptation in various environments (Hwang and Yoon, 2019; Qin *et al*., 2022). In addition, both strains possessed twelve genes of the *alg*A-*alg*D operon responsible for alginate synthesis, which is an important component of the biofilm formation to prevent antibiotics diffusion into the cells and prevent phagocytosis by the host immune cells (Liao *et al*., 2022; Madaha *et al*., 2020). The genomes of VD641 and XP646 were also possessed *rhl*I, *las*I and *pvd*Q genes acting as quorum sensing systems. These quorum quenchers generate two toxins, namely elastase (protease) and pyocyanin (syderophore) as virulent factors that damage the host (de Kievit *et al*., 2002; Yang *et al*., 2021). Analysis of virulent protein-protein interaction networks exhibited six gene clusters having strong interactions (Figure 3). These data underline the high pathogenicity ability of the ST3151 of *P. aeruginosa*.

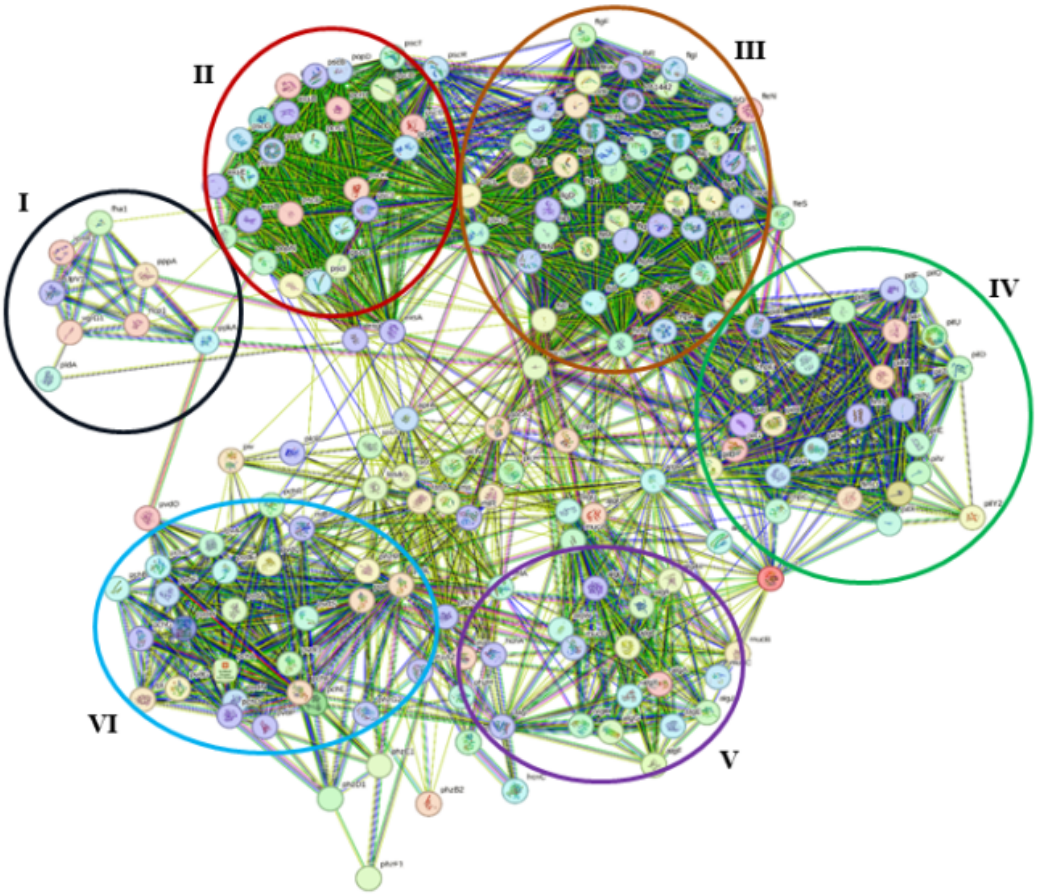

Figure 3. Virulent protein-protein interaction networks of *P. aeruginosa* ST3151 strains. Cluster 1 - type VI secretion system (I, dark circle), cluster 2 - type III secretion system (II, red circle), cluster 3 – adherence factors: flagella and type IV pili twitching motility (III, orange circle), cluster 4 - adherence factors: Type IV pili biosynthesis (IV, green circle), cluster 5 – antiphagocytosis (V, purple circle) and cluster 6: biosurfactant and iron uptake (VI, light blue circle).

Plasmidomes in *P. aeruginosa* XP646 and VD641

Analysis of the draft genomes of *P. aeruginosa* XP646 detected a plasmid similar to the core structure of plasmid pAA359 originated from *Klebsiella oxytoca* strain KOX18040 (Figure 4), suggesting that *P. aeruginosa* XP646 had acquired the plasmid pAA359 thought horizontal gene transfer. Notably, this plasmid had a size of 9,984 bp and carried two ARGs including *bla*KPC-2 and *tet*(A) associated with carbapenems and tetracyclines resistance, respectively. Meanwhile, *P. aeruginosa* VD641 possessed three plasmids with the size from 9,261 bp to 90,585 bp (*aph*(6)-Id Figure 4). The largest plasmid pAF876 carried gene blaOXA-573 (β-lactams resistance) was almost identical to the plasmid pY89 of *P. aeruginosa* strain Y89 isolated from the patient sputum at the Yonsei University Severance Hospital (Hwang and Yoon, 2019). The second plasmid pAC411 is highly identical to a plasmid pEC3587 from *Escherichia coli* pER24y-8ksm which carried four ARGs including *qnr*VC1, *add*A11, *aph*(3'')-Ib and *aph*(6)-Id responsible for resistant to quinolones and aminoglycosides. The smallest plasmid pAD091 also carried three AGRs including *tet*(D), *rmt*B and *sul*1 responsible to the resistant to tetracyclines, aminoglycosides and sulphonamides, respectively, and *cml*A9 encoding for a multidrug-efflux pump. All these data underline that the two strains *P. aeruginosa* VD641 and XP646 had acquired ARGs-carried plasmids independently, which potentially increase the risk of emergence and dissemination of multidrug-resistant bacteria in healthcare settings.

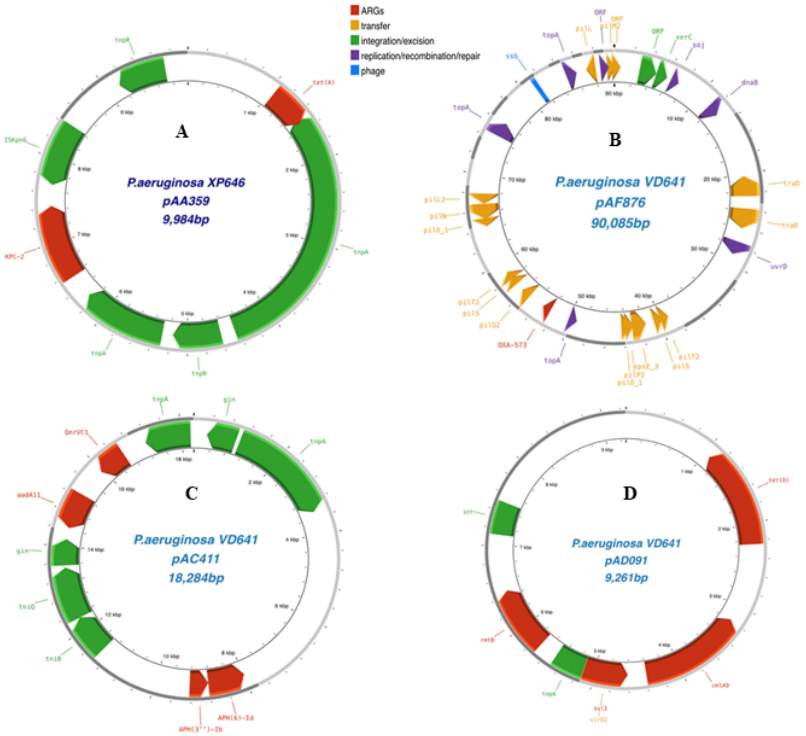

Figure 4. The distribution of antibiotic-resistant genes (in red) and integration factors (in green) along the plasmids found in *P. aeruginosa* XP646 (A) and VD641 (B, C, D).

CONCLUSION

In conclusion, our study sheds light on the genomic characteristics and antibiotic resistance mechanisms of carbapenem-resistant *P. aeruginosa* strains isolated from two major hospitals in Hanoi, Vietnam. Through comparative genomic analysis, we identified resistomes which are specific for each strain, underling that the antibiotic resistance acquisition of the strains sequence type 3151 was different in healthcare settings. Additionally, analysis of virulent protein-protein interaction networks revealed gene clusters associated with the pathogenicity of *P. aeruginosa* sequence type 3151. The presence of plasmids carrying antibiotic-resistant genes further underscores the complexity of antimicrobial resistance dissemination in these hospitals. Our findings emphasize the importance of genomic surveillance in healthcare settings to effectively manage the emergence of carbapenem resistance in *P. aeruginosa*, contributing to enhanced infection control strategies and antibiotic usage in Vietnam.

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