Emergence of DIM-1 and KPC-1 Genes Associated Carbapenem-Resistant Pseudomonas Aeruginosa Isolates in Three Major Hospitals in Hanoi, Vietnam (2010-2015)

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Abstract

Background: Multidrug-resistant bacteria including carbapenem resistant *Pseudomonas aeruginosa* are recognised as an important cause of hospital-acquired infections worldwide. To determine the molecular characterisation and antibiotic resistant genes associated with carbapenem-resistant *P. aeruginosa*.

Methods: we conducted whole-genome sequencing and phylogenetic analysis of 72 carbapenem-resistant *P. aeruginosa* isolated from hospital-acquired infection patients from 2010 to 2015 in three major hospitals in Hanoi, Vietnam.

Results: We identified three variants of IMP genes, among which IMP-15 gene was the most frequent (n= 34) in comparison to IMP-26 (n= 2) and IMP-51 (n=12). We observed two isolates with imipenem MIC >128mg/L that co-harboured IMP-15 and DIM-1 genes and seven isolates (imipenem MIC> 128mg/L) with KPC-1 gene from the same hospital. MLST data showed that sequence types (ST) of 72 isolates were classified into 18 STs and phylogenetic tree analysis divided these isolates into nine groups.

Conclusion: Our results provide evidence that not only IMP-26, but other variants of IMPs like IMP-15 and IMP-51 genes and several STs (ST235, ST244, ST277, ST310, ST773 and ST3151) have been disseminated in health care settings in Vietnam. Also, we report the first finding in Vietnam that two isolates belonging to ST1240 and ST3340 harboured two important carbapenemase genes (IMP-15 and, DIM-1) and seven isolates belonging to ST3151 of *P. aeruginosa* carried the KPC-1 gene, which could be a potential cause of seriously restricted available treatment options in healthcare settings.

Background

Antibiotic resistance has taken centre stage as a global health issue that demands public attention and commands resources to understand where the international community will be placed in 2020–2025 [1]. Concerns have been raised due to the rapid emergence and spread of carbapenem-resistant Gram-negative bacteria resistant to the "last resort" antibiotic group in-hospital treatments. In addition, bacteria have been found to be resistant to colistin, which is recommended to be used as salvage treatment for infections caused by carbapenem-resistant bacteria [2;3] With the emergence of resistance against these drugs, there might be no effective antibiotic treatment for these bacteria in the next 5–10 years.

Multidrug-resistant *Pseudomonas aeruginosa* is recognized as an important cause of hospital-acquired infections and is listed among the WHO priority pathogens for research and development of new antibiotics [4;5]. This bacterium is highly adaptable to environmental fluctuations including low-level antibiotic exposure and many antibiotic resistance mechanisms such as reduced membrane permeability, drug efflux pumps, and enzymatic inactivation have been found. The spread of antibiotic resistance genes through mobile genetic factors greatly contributes to the formation of antibiotic resistant *P. aeruginosa* [6;7]. *P. aeruginosa* is very well known to have multiple resistance mechanisms at the same time, limiting treatment choices [8;9;10]. Epidemiological classification of *P. aeruginosa* using pulsed-field gel electrophoresis (PFGE) has been used as the gold standard for molecular epidemiology to characterize and identify the risk of transmission and spread of *P. aeruginosa* outbreaks in hospitals [11]. However, this technique has limited discriminatory capacity, high cost, complex workflow and does not provide detailed information on the evolutionary background of *P. aeruginosa*. Currently, next-generation sequencing usage is becoming broader as it provides data not only on the genetic relatedness at higher resolution but also on resistance associated genes and their relatedness and thus more insights into antimicrobial resistant bacteria. With this technique, the relatedness and transmission of hospital isolates can be assessed and used to guide infection control interventions locally. Moreover, sequence and evolutionary data contribute to enhance the global picture of AMR genes and associated bacteria [12;13].

Southeast Asia is considered as a "hot spot" of antibiotic-resistant bacteria and *P. aeruginosa* has also been identified as a common cause of hospital-acquired infections in Vietnam [14;15]. According to statistics of the Center for Disease

Dynamics, Economics and Policy (CDDEP) in 2016, 36% of *P. aeruginosa* isolates in Vietnam were resistant to carbapenems, ranking second only after India [14]. A study in one hospital in Hanoi - Vietnam reported a carbapenemase-ST235 *P. aeruginosa* carrying IMP-15, IMP-26 and IMP-51 genes [16]. Although the *P. aeruginosa* ST235 isolates were identified to play an important role of concerning in relation to hospital-acquired infections, the resistance mechanisms have not yet been clearly defined, and the sequence types of *P. aeruginosa* associated with antibiotic resistance genes differ markedly among communities, hospitals, and countries [16;17;18;19]. Also, the results of our surveillance in 3 major hospitals in Hanoi between 2011 to 2015 showed that 11.5% (48/416) of carbapenem-resistant *P. aeruginosa* isolates carrying the IMP gene. That finding leads to some research questions needed to be addressed: (i) What variants of the IMP gene in the carbapenem-resistant *P. aeruginosa* isolates are circulating in healthcare settings in Vietnam, and whether they are similar to other variants, which have been reported in previous studies? (ii) What differences in molecular characterization of the IMP versus non-IMP of *P. aeruginosa* isolates? To address these questions, we conducted this study to determine the molecular characterization and antibiotic resistance genes associated with carbapenem-resistant *P. aeruginosa* isolated from three major hospitals in Hanoi between 2010 and 2015. The results provide more understanding of the genomic characteristics and the profile of antibiotic resistance genes of *P. aeruginosa*, thereby help developing appropriate strategies for treatment and prevention.

Materials And Methods

Hospital settings and isolates

Isolates were sent from three hospitals (hospital A, B and C) with high capacity, located in centre of Hanoi, the capital city of Vietnam. Hospitals A and B are reference health care settings with over 600-bed capacity each, and include many specialties such as surgery, paediatric, intensive care unit (ICU), etc. Hospital C is the largest surgical centre in Vietnam with over 1500 beds that performs different fields of surgery such as abdominal surgery, gastroenterology & hepato-biliary surgery, paediatric surgery, and urology surgery. Demographic and basic clinical information of patients whose specimens were carbapenem-resistant *P. aeruginosa* positive was collected from clinical notes including age, gender, date of admission, clinical diagnosis, the origin of collected sample, isolated bacterial strains, and date of sample collection. Treatment and clinical outcome data were not available for this study.

From the collection of carbapenem-resistant *P. aeruginosa* isolates (n = 416) collected between August 2010 and December 2015, we selected all of 48 isolates carrying IMP gene (18 isolates from hospital A, 12 from B and 18 from C), and randomly selected 24 non-carrying IMP isolates (12 isolates from hospital A, 8 from B and 4 from C). All of 72 of carbapenem-resistant *P. aeruginosa* isolates in this study were resistant to at least one antibiotic in the carbapenem group for antibiotic susceptibility by using disc diffusion testing according to international guidelines [20].

Bacterial identification and susceptibility testing

Isolates were confirmed by the MALDI Biotyper system (Brucker Daltonik, GmbH, Germany). Minimum inhibitory concentrations (MICs) of seven antibiotics which are commonly used in treatment for *P. aeruginosa* infections in Vietnam, including imipenem (IMP), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GEN), amikacin (AMK) aztreonam (AZT) and colistin (CS) (Sigma-Aldrich) were performed by agar dilutions according to Clinical and Laboratory Standards Institute (CLSI) guidelines – 2018 [20]. The broth micro-dilution of colistin susceptibility testing was analysed according to the standard of the European Committee on Antibiotic Susceptibility Testing (EUCAST).

Whole genome sequencing of P. aeruginosa

To prepare whole-genome sequencing libraries, genomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Libraries of *P. aeruginosa* strains were prepared using the Nextera XT

DNA Library Prep Kit (Illumina, San Diego, CA, USA). Multiplexed paired-end sequencing was performed using the MiSeq Reagent V3 Kit (2 × 300 cycles) on an Illumina MiSeq instrument.

Bioinformatics analysis

Whole genome sequences were analyzed using an in-house bioinformatics pipeline, which runs on a Conda environment under Linux. Briefly, we used FastQC Version 0.11.8 for quality control of raw reads. Reads were trimmed of the adaptor sequences and were subsequently *de novo* assembled into contigs using SPAdes (3.9.0) with a pre-defined Kmers set. Antimicrobial resistance genes were identified from the assembled contigs using the ABRicate program to query the Resfinder database V2.1 [21]

Multilocus sequence typing (MLST) was conducted from the Shovill-output contigs, screening seven housekeeping genes against the PubMLST database. Alleles were submitted to the PubMLST database to get the sequence type. A phylogenetic tree based on the core genome SNPs was constructed from WGS data of the 72 *P. aeruginosa* isolates using Parsnp 1.2 and IQ-TREE 1.5 [21;22].

Results

Characterization of P. aeruginosa

In this study, 72 isolates of carbapenems resistant *P. aeruginosa* were isolated from the three hospitals between 2010 and 2015: A (n = 30), B (n = 19), and C (n = 23). The median age of patients with *P. aeruginosa* infections was 35 years (range: 1 to 85 years), ratio of male and female was 2.6. Forty-five (45/72; 62.5%) were isolated from pneumonia patients including 29 Ventilator-associated pneumonia (VAP) patients. These isolates were detected from 11 different departments, with a majority from the intensive care units (ICU) 44.4% (n = 32), paediatrics 15.2% (n = 11), and surgery 13.9% (n = 10) (Fig. 1). The *P. aeruginosa* isolates were cultured from eight types of samples, including bronchial fluid 44.4% (n = 32), sputum 18% (n = 13), surgical site fluid 13.9% (n = 10) (Fig. 1).

Profile of antibiotic resistant genes (ARGs)

P. aeruginosa clinical isolates displayed different combinations of drug resistance genes such as efflux pump and outer membrane (MexAB-OprM, MexEF-OprN, OprJ, OpmB, OpmH) genes (Fig. 2) leading to resistance to various antibiotics. All 72 *P. aeruginosa* isolates carried OXA-50, FosA (fosfomycin-resistant) and different variants of *Pseudomonas* Derived Cephalosporinase (PDC)-beta-lactamase class C genes, predominantly PDC-2 (18/72, 25%) and PDC-7 (18/72, 25%), followed by PDC-3 (17/72, 24%), PDC-5 (15/72, 21%), PDC-1 (2/72, 3%) and PDC-8 (2/72, 3%). Other genes encoding antibiotic resistance were found in this study including genes for colistin (ArnA, 69/72, 96%), CARB-3-beta-lactamase (CARB-3, 43/72, 60%), acquired fluoroquinolone resistance (QnrVC1, n/N, 51%) and Vietnamese extended-spectrum beta-lactamase (Veb-1, 3/72, 4%). Among 72 strains, 48 (67%) isolates carried one of the three variants of IMP gene, among which IMP-15 was the most prevalent (34/72, 47%), followed by IMP-51 (12/72, 17%) and IMP-26 (2/72, 3%) (Table 1).

Table 1
Distribution of antibiotic resistance genes of *P. aeruginosa* (n = 72)

Carbapenem genes	IMP variants	Some important AMR genes							
		DIM-1	OXA-50	PDC (PDC-beta-lactamase class C)	CARB-3	Veb1	QnrVC1	FosA	ArnA
IMP (n = 48)	IMP-15 (n = 34)	2/34	34/34	PDC-2 (n = 4); PDC-3 (n = 10); PDC-5 (n = 11); PDC-7 (n = 9)	32/34	-	31/34	30/34	33/34
	IMP-26 (n = 2)	-	2/2	PDC-2; PDC-3	-	-	1/2	2/2	2/2
	IMP-51 (n = 12)	-	12/12	PDC-2 (n = 12)	-	-	-	12/12	12/12
KPC-1 (n = 7)		-	7/7	PDC-7 (n = 7)	7/7	-	5/7	7/7	7/7
IMP, KPC negative isolates (n = 17)		-	17/17	PDC-1 (n = 2); PDC-2 (n = 1); PDC-3 (n = 6); PDC-5 (n = 4); PDC-7 (n = 2); PDC-8 (n = 2)	4/17	3/17	-	17/17	15/17
		2/72 (2.77%)	72/72 (100%)	72/72 (100%)	43/72 (59.72%)	3/72 (4.16%)	37/72 (51.38%)	72/72 (100%)	69/72 (95.83%)

Notably, we detected the presence of DIM-1 gene (Dutch imipenemase 1) encoding carbapenemase for the first time in Vietnam in two *P. aeruginosa* isolates. The first DIM-1 *P. aeruginosa* isolate was from urine of posterior urethral stenosis patient in Better private care department, hospital C in mid-July 2012. The second DIM-1 was isolated from a pneumonia patient in early the period of 2013 in ICU, hospital A. Noticeably, both isolates with DIM-1 genes also carried the IMP-15 gene and KPC. Patients with DIM-1 positive isolates were hospitalized in two different hospitals, and these isolates belonged to ST1420 and ST3440 (Fig. 2). The seven KPC-1 positive *P. aeruginosa* belong to ST3151 were in hospital A from 2011 to 2013 in Nurseries (n = 3); ICU (n = 3); and Ophthalmology (n = 1). The first KPC-1 was in Oct-2011 from bronchial fluid of a Ventilator-associated pneumonia patient. The second and third isolates were from blood of a septic patient and in Corneal sample of a conjunctivitis patient in November, 2011 and the fourth was in pnemonia patient in December-2011. Two positive KPC-1 were in May 2012 isolated from bronchial fluid of pneumonia patients and the last case in January 2013.

Concordance of minimum inhibition concentration (MIC) with CARG's profile of P. aeruginosa

The results of the imipenem minimum inhibition concentration (MIC) test showed that the isolates without IMP-15, IMP26, IMP-51, DIM-1 or KPC-1 genes had the lowest MICs (8–16 mg/L). Isolates carrying only IMP-51 gene had an MIC of 16–32 mg/L, and isolates carrying only IMP-15 gene had MICs of 32–64 mg/L. Noticeably, isolates with both IMP-15 and DIM-1 genes were extremely resistant to imipenem, with MICs > 128 mg/L. Similarly, isolates carrying KPC-1 gene had MIC > 128 mg/L. We also observed that isolates carrying IMP-15 + DIM-1; or only one gene among IMP-26, IMP-51, and KPC-1 genes were resistant to other five antibiotics (ciprofloxacin (CIP), ceftazidime (CAZ), gentamicin (GEN), amikacin (AK),

aztreonam (AZT) (Table 2). However, most of the isolates carrying IMP-15 gene (18/32) were still susceptible to AZT, and isolates carrying both IMP-26 + DIM-1 genes were amikacin susceptible. Among 17 isolates not harbouring acquired carbapenemase genes (IMP, DIM-1 and KPC-1), 16 isolates remained susceptible to ciprofloxacin (0.125-1 mg/L); amikacin (2–4 mg/L) and gentamicin (1–2 mg/L), 12 to aztreonam (8 mg/L) and 10 to ceftazidime (4 mg/L). Finally, we observed 8 of 72 isolates resistant to colistin with MICs ranging from 4 to 16 mg/L (Table 2).

Table 2
Antimicrobial susceptibility by minimum inhibitory concentration of *P. aeruginosa* (n = 72)

P. aeruginosa strains (n = 72)	MIC (mg/L)						
	IMP	CIP	CAZ	AK	GEN	ATZ	CS
IMP-15 (n = 32)	R (32–64)	R (4–16)	R (64–256)	S (8; n = 1); R (> 256; n = 31)	R (> 128)	S (2–8; n = 18); I (16; n = 13); R (32; n = 1)	S (0.25-2; n = 28) R (4–16; n = 4)
IMP-15 + DIM-1 (n = 2)	R (> 128)	R (16)	R (> 256)	S (16)	R (> 128)	R (128)	S (0.25-2; n = 2)
IMP-26 (n = 2)	R (> 128)	R (32)	R (> 256)	R (> 256)	R (> 128)	R (32)	S (0.5; n = 1); R (4; n = 1)
IMP-51 (n = 12)	R (16–32)	R (8–16)	R (256)	S (8–16; n = 6); R (64)	R (32–64)	I (1; n = 2); R (32–128; n = 5)	S (0.25-2; n = 11); R (4, n = 1)
KPC-1 (n = 7)	R (> 128)	R (8)	R (32)	R (> 256)	R (> 128)	R (> 128)	S (0.125-2; n = 6); R (4, n = 1)
IMP, DIM KPC negative (n = 17)	R (8–16; n = 17)	S (0.125-1; n = 16); R (64; n = 1)	S (4; n = 10); R (32–128; n = 7);	S (2–4; n = 16); R (> 256; n = 1	S (1–2; n = 16); R (32; n = 1)	S (8; n = 12); I (16; n = 3); R (> 128; n = 2)	S (0.25-2; n = 15); R (4, n = 1)

Note: S: Sensitive; I: Intermidiate; R: Resistant

Genotypic relationship of P. aeruginosa isolates.

The phylogenetic tree placed the 72 *P. aeruginosa* isolates in nine genotype groups (Fig. 3). Each group had different characteristics of antibiotic resistance genes and sequence type. Interestingly, isolates in group I were 10 isolates from hospital B from between 2012–2014 belonging to ST360 with 8/10 isolates harbouring IMP-15 and most of the isolates carrying many different resistance encoding genes: OXA-50, CARB-3, PDC (PDC-beta-lactamase class C) and gene encoding quinolone resistant (QnrVC1). Group V isolates belonged to ST3151 and harboured one or a combination of two carbapenemase genes including IMP-15, KPC-1, OXA-50, QnrVC1, CARB-3 and PCD genes. Group IX which contained only carbapenemase - ST235 isolates that originated from all three hospitals between 2012 and 2014 (Fig. 2).

Multi-locus sequence typing of *P. aeruginosa* isolates showed that 72 isolates were classified into eighteen sequence types, in which the predominant ST was ST3151 (n = 14) followed by ST235 (n = 13), ST360 (n = 10), ST310 (n = 7) and ST357 (n = 7). Remaining 13 singleton included ST179; ST244; ST245; ST277; ST 313; ST654; ST773; ST856; ST1308; ST1420, ST2166, ST3440 and ST3361. Interestingly, all ST360 (10/10) were detected from patients in hospital B. ST3151 and ST310 were found significantly more often in hospital A compared to hospital C (13/15 vs 2/15 and 5/7 vs 1/7, respectively) (Fig. 2, Fig. 3).

Discussion

In this study we characterized the antibiotic resistant profiles of 72 carbapenem-resistant *P. aeruginosa* with and without IMPs genes collected from three major hospitals in Hanoi, Vietnam. The majority of carbapenem-resistant *P. aeruginosa* infection cases in this study were from pneumonia and VAP cases and ten cases were surgical site infection. These observations suggest that potential dissemination of carbapenem-resistant *P. aeruginosa* may occur at different departments in these hospitals, either by transferring between patients or through healthcare workers.

Our study found that *P. aeruginosa* carried different combinations of antibiotic resistance genes leading to a broad-spectrum resistance to various antibiotics. Among 72 strains, 48 *P. aeruginosa* isolates carried three different IMP genes, similar to the IMP variants has been reported from several countries, including Mexico, Korean, Singapore and in Hanoi, Vietnam [16;17;18;23]. Our results showed the predominance of IMP-15 and IMP-51. These data supported the evidence that not only IMP-26, but other variants of IMP like IMP-15 and IMP-51 genes have been disseminated in health care settings in Vietnam.

The DIM-1 gene (Dutch imipenemase 1) was detected for the first time in Vietnam in two *P. aeruginosa* isolates from hospitals A and B belonging to two differences sequence types: ST1420 and ST3440. Previous studies have shown that DIM-1 gene encodes a group of B metallo-beta-lactamase enzymes capable of lysis of carbapenem antibiotics that was discovered in integron class 1 genetic element (*intI1*) of *Pseudomonas stutzeri* in the Netherlands in 2007 [24], and in *P. aeruginosa* in India (5%-2010) and Sierra Leone (46.7%-2013) [25].

We also found seven *P. aeruginosa* isolates carrying KPC-1 in Vietnam belong to sequence type ST3151. The KPC encoding gene was previously reported in *P. aeruginosa* in China [26]. We also found a plasmid carrying KPC gene in *Enterobacteriaceae* clinical isolates from hospital A in 2010 and from other hospitals of Vietnam, which are currently being characterized, suggesting that the KPC-1 encoding gene of the *P. aeruginosa* strains in the study might be acquired from *K. pneumoniae* and *E. coli* through conjugation.

The imipenem MIC values of the IMP-15, IMP-26, IMP-51, DIM-1 and KPC-1 positive *P. aeruginosa* isolates were ranging from 16 - >128 mg/L. These MIC values have been documented for carbapenemase producing *P. aeruginosa* in other studies [24;27]. The isolates without IMP-15, IMP-26, IMP-51, DIM-1 and KPC-1 had MICs of 8–16 mg/L. Previous studies showed that carbapenem resistant *P. aeruginosa* isolates without IMP-gene were likely resistant due to either active drug efflux pumps mechanisms (MexAB-OprM, MexEF-OprN, OprJ, OpmB, OpmH) or by other classes of carbapenemases OXA-50 [6;7].

Colistin is the only effective antibiotic in some cases of *P. aeruginosa* resistance to all tested antibiotics, even carbapenem. However, the emergence of colistin-resistant strains is considered a great threat for patients with severe infections [28;29;30;31]. Our study found that 11.1% *P. aeruginosa* isolates were resistant to colistin compared to 7% (3%-13%) data of CDDEP collected from the Vietnam Resistance Project (VINARES) [14]. This result indicates that Vietnam is one of the countries having a high rate of colistin resistance. Our finding suggests a cautious consideration of using colistin in treatment for *P. aeruginosa* in clinical practices.

The MLST data showed a high diversity of *P. aeruginosa* isolates. The 72 isolates were grouped into 18 different sequence types, seven of these STs (ST235, ST360, ST310, ST357, ST277, ST773 and ST2166) have also been reported in Vietnam [16;19;32;33]. We also showed the clustering of ST following the hospitals. Particularly, the fact that ST360 mainly found in hospital C and ST3151 was predominant in hospital A strongly suggest that hospital transmission may have occurred. Core genome phylogenetic and STs of the isolates were also in the same group. Some high-risk STs were found within a hospital (ST360) or in different hospitals (ST235, ST244, ST277, ST 340, ST357) and in different years (Fig. 2, Fig. 3). Interestingly, ST3151 carries different carbapenem genes: KPC-1 (n = 7), IMP-15 (n = 1), and one strain only carries OXA-50, suggested that the KPC-1 positive- *P. aeruginosa* strains in the study might be acquired from *K. pneumoniae* and *E. coli*

through conjugation transfer as mentioned above. These findings suggest that these STs have been possibly disseminated in health care settings in Vietnam and new STs could be formed under selective and antibiotic pressure.

Our study has several limitations. Firstly, isolates might not represent for carbapenem-resistant *P. aeruginosa* in health care settings in Vietnam. Secondly, we were unable to assess the clinical significance of carbapenem-resistant *P. aeruginosa* regarding to antibiotic treatment and outcome. Therefore, we propose that future studies should incorporate clinical data to obtain a better understanding of characterization of *P. aeruginosa* infections in Vietnam.

Despite its limitations, our study shows the high diversity and different level and mechanisms carbapenem resistance among hospital *P. aeruginosa* isolates. Our data supports the evidence that not only IMP-26 but other variants of IMPs like IMP-15 and IMP-51 genes and several STs have been disseminated in health care settings in Vietnam. We also firstly reported the ST1420 and ST3340 which co-harboured IMP-15 + DIM-1, and seven ST3151 carrying KPC genes in Vietnam, which may cause a seriously restricted available treatment option in healthcare settings.

Declarations

Authors contributions: Tran Huy Hoang, Tran Nhu Duong and Rogier Van Doorn conceived the study and directed its implementation. Tran Huy Hoang, Keigo Shibayama, Anne-Laure Bañuls, Vu Thi Ngoc Bich and Dang Duc Anh designed the study. Tran Nhu Duong, Pham Duy thai, Luu Thi Vu Nga, Vu Phuong Thom, Trinh Hong Son, and Tran Huy Hoang managed the implementation of the fieldwork, and Tran Huy Hoang, Tran Hai Anh, Tran Dieu Linh, Ngo Thi Hong Hanh, Nguyen Minh Thao, Trinh Khanh Linh, Vu Thi Ngoc Bich, Pham Ha My and Tran Van Anh undertook the laboratory work. Tran Huy Hoang, Trinh Son Tung, Le Viet Thanh, Vu Thi Ngoc Bich, Ngo Thi Hong Hanh Tran Hai Anh and Lay-Myint Yoshida did analysed. Tran Huy Hoang, Tran Hai Anh, Vu Thi Ngoc Bich and Rogier van Doorn wrote the first draft of the paper. All the authors reviewed and edited drafts of the manuscript and approved the final version.

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Availability of data and materials: All data generated or analyzed during this study are included in this article [and its supplementary information files].

Ethics approval and consent to participate

Ethical approval was obtained from the Ethical Committee of the Vietnamese National Institute of Hygiene and Epidemiology for the main project “Assessing the impact and burden of antimicrobial resistance in Vietnam, genomic characterization and risk factors related to antimicrobial resistance of common bacteria in Vietnam”. Individual informed consent was waived because of the retrospective nature of this work and because no patient identifying information was collected” (IRB code: IRB-VN01057-38/2016)

Consent for publication

Not applicable

Competing interests

The authors have no competing interests

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Figures

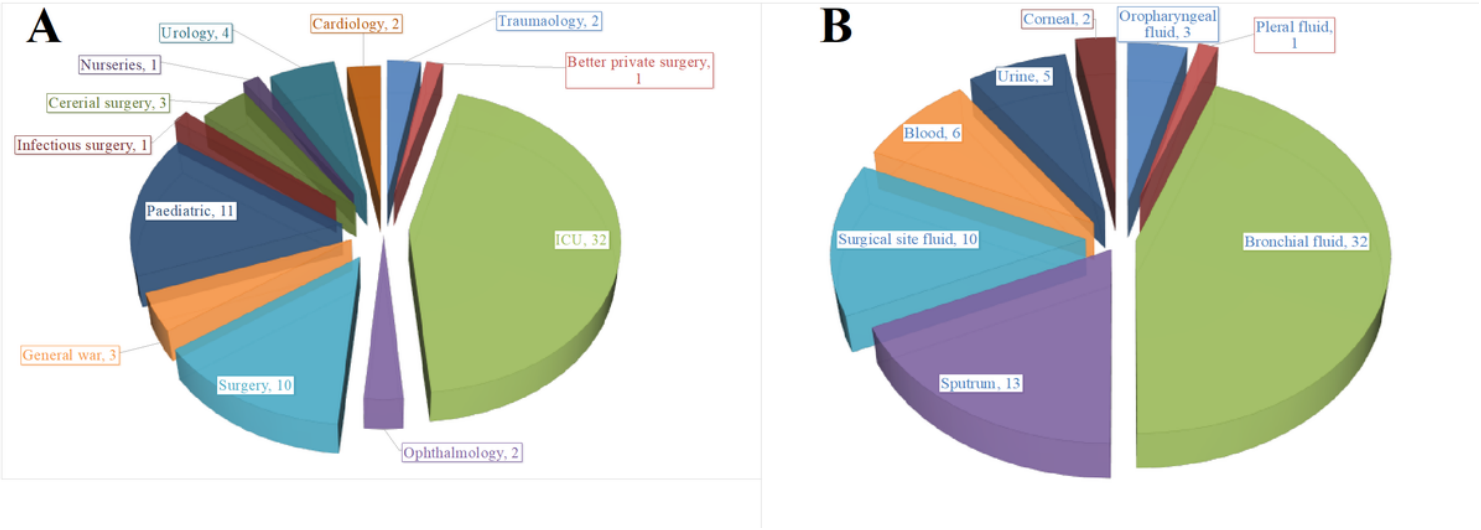

Figure 1

Distribution by department (A) and Source and type of P. aeruginosa isolates (B) in three hospitals of Hanoi (n=72)

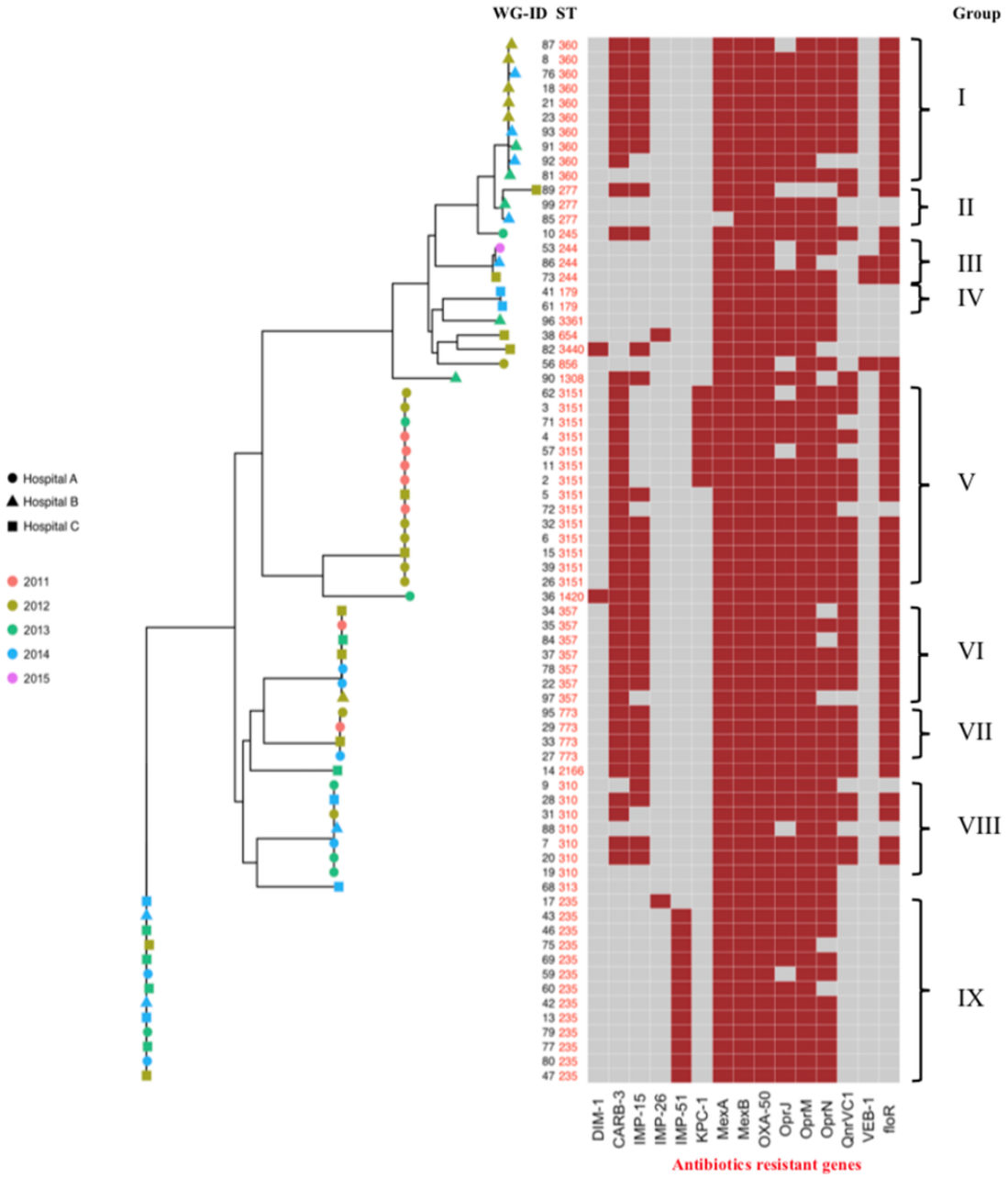

Figure 2

Core genome phylogenetic tree of the 72 P. aeruginosa isolates of the three hospitals associated with sequence types and antibiotic resistant genes. The shapes stand for hospitals ; colors of the shapes stand for collection years of isolates; each red square indicates the presence of AMR genes in isolates.

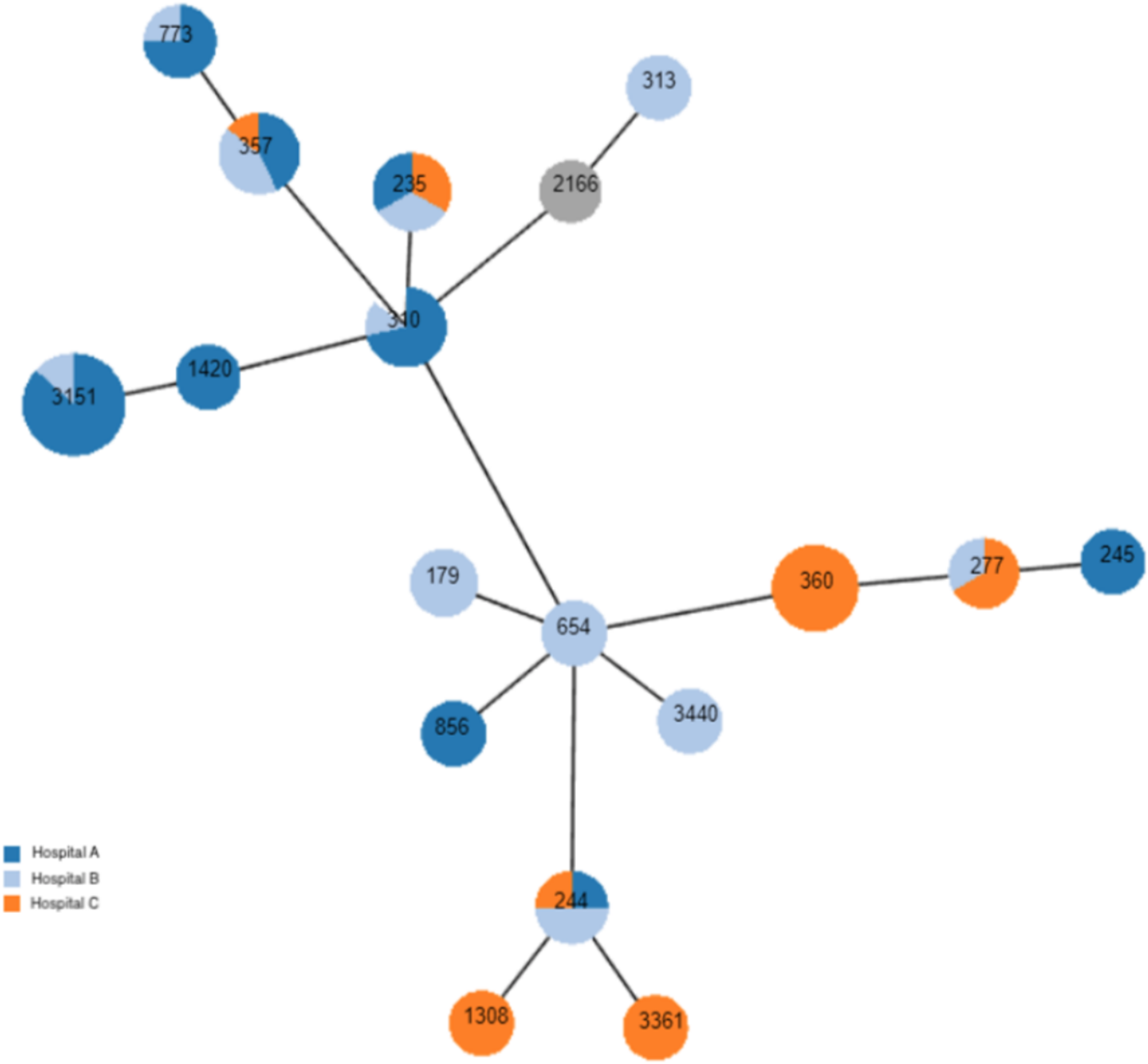

Figure 3

Spanning tree of P. aeruginosa using MLST data reported in this study. Each circle and number represent one ST; The color stand isolates from each hospital.

Supplementary Files

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- 4Suplement311220.docx