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Research Article

Prevalence of Multidrug-Resistant *Pseudomonas aeruginosa* and Risk Factors for their Infections at Intensive Care Units of a Tertiary Hospital in Southern China

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Abstract

Pseudomonas aeruginosa (PA) is highly significant opportunistic pathogens causing healthcare associated infections (HAIs) in hospital settings, notably at intensive care units (ICUs). The aim of this study was to retrospectively analyze the infection status, prevalence and antimicrobial resistance (AMR) of PA at ICUs of a tertiary care hospital in southern China during a one-year period (2016) and examine the clinical risk factors for HAIs by PA. Multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA) method was employed to analyze clonality of the strains. Our results suggested that the resistance of PA in ICUs were higher than in other wards. In terms of resistance to carbapenems, the resistance gene island (*blaOXA-1+blaIMP+ant(2")-Ia+aac(6')-Ib*) carried in *IntI* was a salient feature among AMR genes. While PA infections at local ICUs seemed frequent, there were no obvious trends suggestive of outbreaks. Some epidemic strains have apparently thrived locally for substantial periods, as carriers of major AMR genes and virulence factors. For risk factors for HAIs, inappropriate treatment was found to impact empiric antibiotic therapy of PA infections, especially in the case of carbapenems, where patients often did not get proper treatment during hospitalization of more than 30 days. Multifactor analysis shows that ventilator-associated pneumonia (VAP) was an independent risk factor for increasing the 30-day mortality rate in patients. In addition, the use of antimicrobials, duration of hospitalization and use of mechanical ventilation before isolation were independent risk factors for HAIs.

Keywords: *Pseudomunas aeruginosa*; Antimicrobial resistance; Virluence; Clinical risk factors; Intensive care units (ICUs)

Abbreviations

PA: *Pseudomunas aeruginosa*; HAIs: Healthcare-Associated Infection; ICUs: Intensive Care Units; MDR PA: Multidrug-Resistant PA; AMR: Antimicrobial Resistance; TTSS: The Type III Secretion System; MV: Mechanical Ventilation; CLSI: The Clinical and Laboratory Standard Institute; VNTR: Variable-Number Tandem-Repeat; MLVA: Multiple-Locus Tandem-Repeat; UPGMA: Unweighted Pair Group Method with Arithmetic Mean; SD: Standard Deviation; HCAI: Healthcare Associated Infection; VAP: Ventilator-Associated Pneumonia

Introduction

Pseudomunas aeruginosa (PA) represents an important cause of healthcare-associated infection (HAIs) in intensive care units (ICUs) [1,2]. Owing to its extraordinary ability to form biofilm and efficiently develop resistance towards broad-spectrum antibiotics, PA has contributed to significant mortality and morbidity in HAIs and thus a heavy burden to health care systems in developed and developing countries alike, including China [3].

Prevalence of multidrug-resistant PA (MDR PA) is on the rise across the globe, with various mechanisms being attributed to the development of antimicrobial resistance (AMR) in MDR PA. The prevalence rates of MDR PA range between 15% and 30%in some geographical areas [4,5]. Of note, the genes of *ant(2")-Ia and aac(6')-Ib* carried by PA lead to increased aminoglycoside resistance [6], while the class B enzymes MBL (IMP) and class D OXA beta-lactamases were the most common ESBLs reported in PA [4,7]. *OprD2* protein forms part of the specific pathway for imipenem to enter into PA, and it has ligand specificity with loci specific binding of the imipenem [8]. Among the multitude of virulence determinants of PA, the type III secretion system (TTSS) has been identified as an important contributor to cytotoxicity and PA invasion during infections [9]. TTSS occurs as four cytotoxin genes (*exoS*, *exoU*, *exoY* and *exoT*), among which the impact of *exoS* and *exoU* on pathogen virulence is deemed crucial, whereas *exoY* and *exoT* supposedly have minor effects on virulence. The *toxA* gene is reputedly a principal virulence factor of this bacterium with ADP-ribosylation activity that could halt host protein synthesis and eventually lead to cell death [10]. The frequency of both *toxA* & *exoS* genes has been reported to be significantly higher in MDR PA strains isolates from patients with burnt injuries [11]. Additionally, genes carried by integrons usually encode molecules

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that mediate a variety of resistance mechanisms. Among integrons found in clinically important Gram negative bacteria such as PA, class 1 integron is most common [12].

In terms of risk factors for HAIs, over-prescription and inappropriate use of antimicrobials in the hospital environment clearly drive the development of antibiotic resistance [13]. Inappropriate empiric antimicrobial therapy adversely affected the outcomes of in patients diagnosed with PA infections [14,15]. In this study, we investigated the prevalence of drug-resistant PA in a coastal region (Chaoshan) of southern China, focusing on carriage status of AMR genes and virulence factors, and related clinical data including risk factors of HAIs with PA.

Materials and Methods

Patients and research settings

Databases at the Microbiology Division, Department of Clinical Laboratory, The First Affiliated Hospital of Shantou University Medical College, Shantou City, Guangdong, China, were reviewed to identify patients with PA infections in three types of intensive care units (namely, comprehensive, cardiovascular, and neurosurgery ICUs) within the period of January to December 2016. For patients with multiple episodes of PA infections, only first episodes were analyzed.

Ethical approval

The ethics committee on medical research of the First Affiliated Hospital of Shantou University had evaluated and approved the experimental design of this study.

Study design and clinical data collection

This study was designed as a retrospective study aiming to determine the prevalence, virulence genes and resistance genes in PA isolates as well as PA infection rates, based on data collected by the Department of Clinical Laboratory. Furthermore, we analyzed the impact of inappropriate therapy on patients with PA infections at the ICUs. The main outcome was patient mortality, measured on the basis of 30-day mortality rates. We also assessed secondary outcomes, including the duration of hospitalization and use of invasive procedures. For each patient studied, the following characteristics were recovered from their clinical records: age, gender, date of hospital admission, treatment outcomes as discharge or death (within 30 days from admission), length of total hospital stay, surgery, invasive procedures such as mechanical ventilation (MV), central venous line, urinary catheter, tracheostomy, haemodialysis, catheter enteral or gastric nutrition, and surgical drain during the current hospitalization, underlying conditions such as diabetes mellitus, chronic renal failure, heart failure and cancer, sources of infection, antibiotic use during the current hospitalization, and cases of inappropriate antimicrobial therapies. Antimicrobial therapy was considered to be "appropriate" if the initial antimicrobials, which were administered within 24 hours of acquisition of a culture sample, included at least one antibiotic that was active in vitro. As a universal consensus considered to be lacking, the definition of antibiotic appropriateness used in this study relies on the authoritative guidelines and previous works elsewhere [16].

Identification of isolates and antimicrobial susceptibility testing

A total of 70 non-duplicated strains of PA were used in this study. All isolates were identified by the VITEK 2 COMPACT system (BioMérieux, France) and antimicrobial susceptibility tests were performed with its assemble kit of AST 09 card with the following antibiotics: aminoglycosides (gentamicin, amikacin, tobramycin), carbapenems (imipenem, meropenem), cephalosporins (ceftazidime, cefepime), fluoroquinolones (ciprofloxacin, levofloxacin), penicillins plus β-lactamase inhibitors (piperacillin-tazobactam), monobactams (aztreonam). PA strains that showed intermediate susceptibility were considered to be resistant. Quality-control protocols were used according to the 2016 guidelines of the Clinical and Laboratory Standard Institute (CLSI). PA ATCC 27853 was used as a quality control strain.

Characterization of drug resistance phenotypes and genotypes

The presence of virulence genes (including *exoS*, *exoU*, *toxA*), class I integron gene, aminoglycoside resistance genes (including *ant(2")-Ia, aac(6')-Ib*) and β-lactamase genes (including *IMP*, *OXA*, *OprD2*) were tested by PCR. All primers were based on previously published works as summarized in Table 1. The amplified gene products were sequenced by Sanger method and compared to the sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov/gene/).

Multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA)

MLVA analysis was performed to investigate the clonal relationship between isolated PA strains. Gene amplification in MLVA was based on published primers [17] for the following variable-number-of-tandem-repeats (VNTRs): ms142, ms211, ms212, ms213, ms215, ms217, ms222 for amplifying random DNA fragments. In the clustering analysis, by applying the categorical coefficient (also called Hamming's distance), this corresponds to an interval of 85 to

Table 1: Specific primers used for PCR.

Target	Nucleotide sequence (5'→3')	Size (bp)	Source
IMP	GGAATAGAGTGGCTTAATTCTC	188	[4]
	CCAAACCACTACGTTATCT		
OXA	ACACAATACATATCAACTTCGC	813	[7]
	AGTGTGTTTAGAATGGTGATC		
OprD2	GCGCATCTCCAAGACCATG	193	[8]
	GCCACGCGATTTGACGGAG		
ant(2")-Ia	TCCAGAACCTTGACCGAAC	700	[6]
	GCAAGACCTCAACCTTTTCC		
aac(6')-Ib	GCTCTTGGAAGCGGGGACGG	300	[6]
	TCGCTCGAATGCCTGGCGTG		
exoS	TCAGGTACCCGGCATTCACTACGCGG	572	[9]
	TCACTGCAGGTTCGTGACGTCTTTCTTTTA		
exoU	CCTTAGCCATCTCAACGGTAGTC	911	[9]
	GAGGGCGAAGCTGGGGAGGTA		
toxA	GGTAACCAGCTCAGCCACAT	352	[9]
	TGATGTCCAGGTCATGCTTC		
IntI	AGTCAGCGGCTTAGATA	457	[12]
	GGTGTGGCGGGCTTCGT		

100% similarity and unweighted pair group method with arithmetic mean (UPGMA) clustering approaches within BioNumerics. A cut-off value of 50% similarity was applied to define MLVA clusters. Lineages had been arbitrarily numbered according to the order which they were listed in the clustering analysis.

Statistical analysis

Results pertaining to patients' clinical characteristics and drug resistance were expressed as a percentage of samples. Statistical differences among groups were using the χ^2 test with SPSS v.22.0 (SPSS Inc., Chicago, IL). Duration of hospitalization was analyzed by using the Mann–Whitney U-test. Multivariate analysis with binary logistic regression was conducted to examine the associations of risk factors with PA resistance, with control for potential confounders. In addition to duration of hospitalization, all variables with a P-value of <0.05 in the univariate analysis were included in the logistic regression model. A two-tailed P-value of <0.05 was considered to be statistically significant. After establishing the production of some virulence traits and resistance gene geneotypes in different clinical strains of PA, we analyzed the possible correlation between them.

Results and Discussion

Sites of isolation

These 70 strains of PA were isolated from ICUs in 2016, including sputum (66 isolates; 95.78%), blood (2 isolates; 2.11%) and pus (2 isolates; 2.11%), which showed that respiratory infections were the predominant type in PA infections.

Antimicrobial susceptibility testing

The drug susceptibility rates of the isolates were indicated in Table 2. Among 70 P. aeruginosa isolates, 36 (51.43%) and 32 (45.71%) isolates were resistant to imipenem and meropenem, respectively. Thus, a total of 37 isolates (52.86%) resistant to imipenem and/or meropenem were determined to be resistant to carbapenems. 31(83.78%) of the 37 carbapenem-resistant isolates showed multidrug resistance and 25 isolates were resistant to all antimicrobials tested except polymyxins B. Among 33 carbapenem-susceptible isolates, only 4 isolates (12.12%) were MDR and just one was the XDR PA, which was significantly lower than that among carbapenem-resistant isolates (P < 0.001).

Overall, Susceptibility rates to piperacillin/tazobactam, ceftazidime were 52.86% and 58.57% , respectively, higher than those reported previously [18]. The polymyxins B remained active against all these 70 isolates. Antimicrobial susceptibility test revealed that 35(50.00%) PA isolates were MDR PA, among these, 26 isolates (37.14%) were XDRPA. Overall, the correlation could be established between the resistance profiles and the different MLVA genotypes.

Prevalence of resistance genes and class I integrons

All the resistant genes designed in the study and class I integrons was found in the experimental isolates.

19 isolates (27.14%) were simultaneously positive for both *OXA-1, IMP, ant(2'')-Ia, aac(6')-Ib* and *IntI*. The isolates with these genes were all the MDR PA, and among these, 18 isolates were XDR PA. Of these, 15 belonged to the same genotype in MLVA typing. PCR assay with primers revealed that 20 (28.57%) of the 70 P. aeruginosa strains were class I integrase positive. Analysis of the integrase PCR product by the test of gene sequencing confirmed class I integron as described previously [19]. The class I integron positive strains of multidrug resistant rate was 38.00% (19/50) and the negative was 14% (7/50), there were significant difference between the two groups (χ^2=40.146, P=0.000). The detection rate of the resistant gene of *OprD2* was 64.29% (45/70). But the detection of *OprD2* gene deletion strains in this study did not produce significant relationship with the imipenem resistance.

Among the 70 isolates tested, 37 genotypes were identified by the MLVA-PCR; and they were divided into 6 gene clusters (1-6). Cluster 4 included 22 isolates from different patients, which was the main clone type in this study. Out of them 15 isolates harboured *IntI, aac(6')-Ib, ant(2'')-Ia* and *OXA-1, IMP*.

Detection of the virluence genes

According to PCR, the T3SS effector genes contained in almost each clinical isolate, except for 4 isolates. The amplification of *exoS* and *exoU* were more variable with only 1(1.43%) isolates containing both genes. There were 49(70.00%) isolates carried *exoS* and 18(25.71%) carried *exoU*. This enabled the strains to be split into three major groups *exoS*+/*exoU*-, *exoS*-/*exoU*+ and *exoS*+/*exoU*+. The *toxA* (90.0%) was present in almost all isolates. Carriage both *toxA* and *exoS* genes were observed in 27 among 35 MDR strains. Meanwhile, there were only 6 strains harboring *toxA* and *exoU* (P<0.001), and 2 strains had none of them.

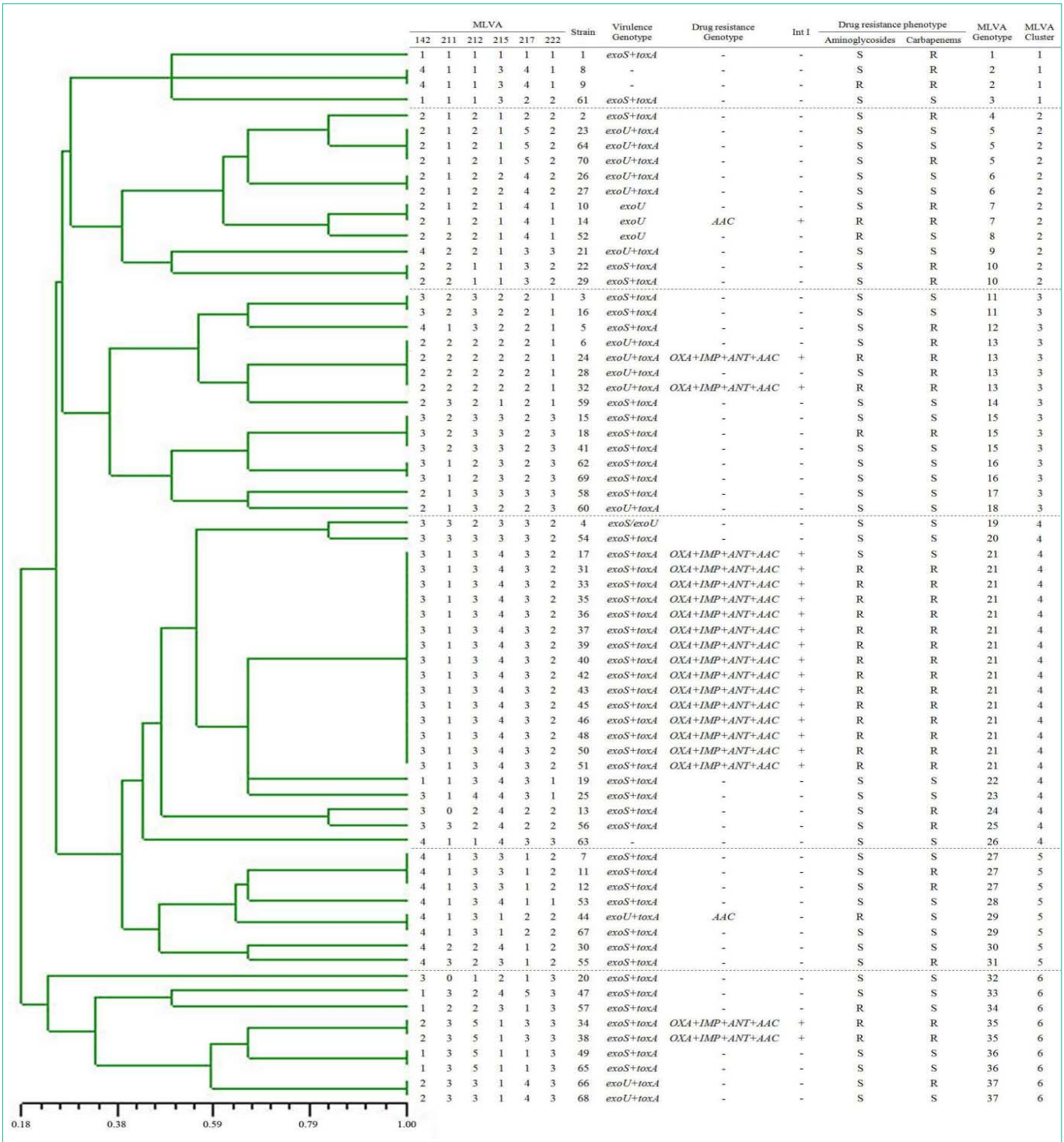
Analyzing the association of *exoU* or *exoS* with antimicrobials resistance, there was no significant difference in sensitivity to antimicrobials between the two groups. However, in the 19 strains of PA with resistance genes of *OXA-1, IMP* and *aac(6')-Ib, ant(2'')-Ia*, there were 17 isolates in *exoS*+/*exoU*- mode, but other 2 strains in *exoS*-/*exoU*+ mode were with aac(6')-Ib gene positive (P<0.001).

Molecular epidemiology

All isolates were distinguished by 37 different genotypes, 6 clusters by MLVA method (Figure 1). The largest cluster contained 22 isolates but only included genotypes 4, of which 15 isolates carried all the resistance genes (included *OXA-1, IMP*, and *aac(6')-Ib, ant(2'')-Ia)* and *IntI* detected, harboring virulence genotype *exoS*. Most

Table 2: Antimicrobial resistance rates among P. aeruginosa isolates.

Antimicrobial	Sensitive	Intermediate	Resistant
Piperacillin	50.00% (35/70)	11.43% (8/70)	38.57% (27/70)
Piperacillin/tazobactam	52.86% (37/70)	10.00% (7/70)	37.14% (26/70)
Aztreonam	44.29% (31/70)	14.29% (10/70)	41.43% (29/70)
Ceftazidime	58.57% (41/70)	1.43% (1/70)	40.00% (28/70)
Cefepime	60.00% (42/70)	2.86% (2/70)	37.14% (26/70)
Imipenem	48.57% (34/70)	22.86% (16/70)	28.57% (20/70)
Meropenem	54.29% (38/70)	4.29% (3/70)	41.43% (29/70)
Ciprofloxacin	52.86% (37/70)	5.71% (4/70)	41.43% (29/70)
Levofloxacin	58.57% (41/70)	5.71% (4/70)	35.71% (25/70)
Amikacin	68.57% (48/70)	2.86% (2/70)	28.57% (20/70)
Gentamicin	65.71% (46/70)	1.43% (1/70)	32.86% (23/70)
Tobramycin	67.14% (47/70)	1.43% (1/70)	31.43% (22/70)
Polymyxin B	100.00% (70/70)	0.00% (0/70)	0.00% (0/70)



MLVA						Strain	Virulence Genotype	Drug resistance Genotype	Int I	Drug resistance phenotype		MLVA Genotype	MLVA Cluster
142	211	212	215	217	222					Aminoglycosides	Carbapenems		
1	1	1	1	1	1	1	*exoS+toxA*	-	-	S	R	1	1
4	1	1	3	4	1	8	-	-	-	S	R	2	1
4	1	1	3	4	1	9	-	-	-	R	R	2	1
1	1	1	3	2	2	61	*exoS+toxA*	-	-	S	S	3	1
2	1	2	1	2	2	2	*exoS+toxA*	-	-	S	R	4	2
2	1	2	1	5	2	23	*exoU+toxA*	-	-	S	S	5	2
2	1	2	1	5	2	64	*exoU+toxA*	-	-	S	S	5	2
2	1	2	1	5	2	70	*exoU+toxA*	-	-	S	R	5	2
2	1	2	2	4	2	26	*exoU+toxA*	-	-	S	S	6	2
2	1	2	2	4	2	27	*exoU+toxA*	-	-	S	S	6	2
2	1	2	1	4	1	10	*exoU*	-	-	S	R	7	2
2	1	2	1	4	1	14	*exoU*	*AAC*	+	R	R	7	2
2	2	2	1	4	1	52	*exoU*	-	-	R	S	8	2
4	2	2	1	3	3	21	*exoU+toxA*	-	-	S	S	9	2
2	2	1	1	3	2	22	*exoS+toxA*	-	-	S	R	10	2
2	2	1	1	3	2	29	*exoS+toxA*	-	-	S	R	10	2
3	2	3	2	2	1	3	*exoS+toxA*	-	-	S	S	11	3
3	2	3	2	2	1	16	*exoS+toxA*	-	-	S	S	11	3
4	1	3	2	2	1	5	*exoS+toxA*	-	-	S	R	12	3
2	2	2	2	2	1	6	*exoU+toxA*	-	-	S	R	13	3
2	2	2	2	2	1	24	*exoU+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	13	3
2	2	2	2	2	1	28	*exoU+toxA*	-	-	S	R	13	3
2	2	2	2	2	1	32	*exoU+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	13	3
2	3	2	1	2	1	59	*exoS+toxA*	-	-	S	S	14	3
3	2	3	3	2	3	15	*exoS+toxA*	-	-	S	S	15	3
3	2	3	3	2	3	18	*exoS+toxA*	-	-	R	R	15	3
3	2	3	3	2	3	41	*exoS+toxA*	-	-	S	S	15	3
3	1	2	3	2	3	62	*exoS+toxA*	-	-	S	S	16	3
3	1	2	3	2	3	69	*exoS+toxA*	-	-	S	S	16	3
2	1	3	3	3	3	58	*exoS+toxA*	-	-	S	S	17	3
2	1	3	2	2	3	60	*exoU+toxA*	-	-	S	S	18	3
3	3	2	3	3	2	4	*exoS/exoU*	-	-	S	S	19	4
3	3	3	3	3	2	54	*exoS+toxA*	-	-	S	S	20	4
3	1	3	4	3	2	17	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	S	S	21	4
3	1	3	4	3	2	31	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	33	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	35	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	36	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	37	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	39	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	40	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	42	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	43	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	45	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	46	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	48	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	50	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
3	1	3	4	3	2	51	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	21	4
1	1	3	4	3	1	19	*exoS+toxA*	-	-	S	S	22	4
3	1	4	4	3	1	25	*exoS+toxA*	-	-	S	S	23	4
3	0	2	4	2	2	13	*exoS+toxA*	-	-	S	R	24	4
3	3	2	4	2	2	56	*exoS+toxA*	-	-	S	R	25	4
4	1	1	4	3	3	63	-	-	-	S	S	26	4
4	1	3	3	1	2	7	*exoS+toxA*	-	-	S	S	27	5
4	1	3	3	1	2	11	*exoS+toxA*	-	-	S	R	27	5
4	1	3	3	1	2	12	*exoS+toxA*	-	-	S	R	27	5
4	1	3	4	1	1	53	*exoS+toxA*	-	-	S	S	28	5
4	1	3	1	2	2	44	*exoU+toxA*	*AAC*	-	R	S	29	5
4	1	3	1	2	2	67	*exoS+toxA*	-	-	S	S	29	5
4	2	2	4	1	2	30	*exoS+toxA*	-	-	S	S	30	5
4	3	2	3	1	2	55	*exoS+toxA*	-	-	S	R	31	5
3	0	1	2	1	3	20	*exoS+toxA*	-	-	S	S	32	6
1	3	2	4	5	3	47	*exoS+toxA*	-	-	S	S	33	6
1	2	2	3	1	3	57	*exoS+toxA*	-	-	R	S	34	6
2	3	5	1	3	3	34	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	35	6
2	3	5	1	3	3	38	*exoS+toxA*	*OXA+IMP+ANT+AAC*	+	R	R	35	6
1	3	5	1	1	3	49	*exoS+toxA*	-	-	S	S	36	6
1	3	5	1	1	3	65	*exoS+toxA*	-	-	S	S	36	6
2	3	3	1	4	3	66	*exoU+toxA*	-	-	S	R	37	6
2	3	3	1	4	3	68	*exoU+toxA*	-	-	S	S	37	6

Figure 1: MLVA Genotypic features of 70 Pseudomonas aeruginosa isolates. OXA: blaOXA-1; IMP: blaIMP; ANT: ant(2'')-Ia; AAC: ant(6')-Ia.

of the strains carrying multidrug resistance genes belonged to the same genotypes. Among the 6 different clusters, the strains of *toxA* and *exoS* were detected more in the cluster 4, and were statistically significant compared with other clusters (χ^2=13.555, P=0.019; χ^2=21.222, P=0.001). The strains of *exoU* were carried more in the cluster 2, which had statistical significance compared with other clusters (χ^2=25.10, P=0.000). In conclusion, there was a significant correlation between the drug-resistant genotypes and the virulence genotypes with the MLVA genotypes.

Clinical data

A total of 70 nonrepetitive patients in ICUs with PA infection were included in the study. The detailed information on factors

Table 3: Risk factors associated with infection caused by P. aeruginosa in ICUs.

Variable	*n*	Percentage (%)
Gender		
Female	15	21.43%
Male	55	78.57%
Age group		
<18y	5	7.14%
18-60y	35	50.00%
>60y	30	42.86%
Length of hospital stay (mean days)		
≤10	8	11.43%
20-Oct	7	10.00%
≥20	55	78.57%
Invasive procedures		
Mechanical Ventilation	57	81.43%
Tracheostomy	4	5.71%
Central venous catheter	70	100.00%
Surgical drain	17	24.29%
Haemodialysis	2	2.86%
Co-morbidity conditions	41	58.57%
Heart failure	35	50.00%
Cancer	3	4.29%
Diabetes mellitus	10	14.29%
Chronic renal failure	2	2.86%
COPD	8	11.43%
VAP	32	45.71%
Inappropriate therapy	35	50.00%
Antibiotic use prior to separation	42	60.00%
one kind	9	12.86%
two kinds	12	17.14%
three kinds	21	30.00%
Nosocomial infection	45	64.29%
Community infection	25	35.71%

associated with infection and the relevant demography and clinical characteristics of the study population were summarized in Table 3. Compared with the cohort of patients of the ICU, the mean age (±standard deviation [SD]) of the patients was 55.81±19.97 years, and 55(78.57%) patients were male. Of the 70 patients, 45(64.29%) had healthcare associated infection and the remaining 25 (35.71%) had community infections. The most common underlying diseases were cardiovascular disease (n=35, 50.00%). Comparing with the community infection, patients with healthcare associated infection (HCAI) prone to have a high drug resistance rate, and 40% of the strains were MDR, and even 46.67% of the strains were XDR (Table 4). The healthcare associated infection also had a higher proportion of ventilator-associated pneumonia (VAP). However, patients in ICU with HCAI would often receive empirical therapy before isolation, thus the difference of three kinds of antimicrobials use prior to separation was statistically significant. This also lead to a statistically significant increase in the proportion of patients with healthcare associated infection receiving inappropriate therapy than community infection. The hospitalization time of the patients with healthcare associated infection was significantly longer than that of the patients in the community.

Of the 70 patients with PA infection, 35 (50.00%) patients received inappropriate antimicrobial therapy. To compare the differences between the groups receiving inappropriate and appropriate antimicrobials, their clinical characteristics and the characteristics of the isolated strains' resistance were shown in Table 5. Regarding to underlying diseases and comorbid conditions, the inappropriate therapy group showed significant associations with the prior hospitalization within 30 days (all $P<0.05$). Antimicrobials used prior to separation were more frequent in the inappropriate therapy group than they were in the appropriate therapy group ($P<0.05$). The significant differences were also found with regard to the onset infection between the two groups. When assessing the clinical outcomes of PA infection, the failure rate of inappropriate experience treatment was 37.14% (13/35), and the overall 30-day mortality rate was 34.29% (24/70). The failure rate after empirical antimicrobial treatment was higher in the inappropriate treatment group than the appropriate treatment group (31.43%, 11/35). No significant difference in the 30-day mortality rates between the inappropriate therapy group and the appropriate therapy group ($P=0.615$). The multinominal logistic regression analysis showed that the use of antimicrobials before separation, prolonged hospitalization days and the use of mechanical ventilation were the independent risk factors of healthcare associated infection. There was a significant correlation between ventilator-associated pneumonia with mortality within 30 days.

In our study, carbapenems was the priority in the use of inappropriate treatment accounting for 51.43%, especially for MEM, followed by quinolones (45.71%) β-lactamase inhibitors (34.29%), amide enzyme inhibitors (17.14%), cephalosporins (5.71%). The average LOS Length of hospital stay (mean days) in the group receiving an inappropriate therapy (68.86 days±54.60 SD) was greater than the one in the group that received an appropriate therapy (45.06 days±40.07 SD), and the difference has statistically significant ($p = 0.041$).

Discussion

PA is an opportunistic human pathogen capable in causing severe infections, especially in ICUs [2,13]. CHINET surveillance of bacterial resistance across China in 2016 showed that the drug resistance rates of PA to imipenem and meropenem were 28.7% and 25.3%, respectively; the drug resistance rates to polymyxin B and amikacin were 0.5% and 8.1%, respectively; the drug resistance rate of the two enzyme inhibitor mixture, gentamicin, ciprofloxacin, ceftazidime, cefepime and piperacillin were less than 20% [18]. Compared with the results in our study, the resistance rates of most drugs were greater than the 2016 China CHINET bacterial resistance monitoring results, except for the slightly lower resistance rate of imipenem and polymyxin B. Of course, this may be related to the fact that the strains we isolated were from the patients in ICUs. It indicated the severity of the antimicrobial resistance rate of PA in ICUs of our study.

Table 4: Risk factors between HCAI and community infection.

Variable	total, n=70	The HCAI, n=45	The community infection, n=25	P
Drug resistance phenotype				
Non-MDR	35(50.00%)	18(40.00%)	17(68.00%)	0.025
MDR	35(50.00%)	27(60.00%)	8(32.00%)	0.873
XDR	26(37.14%)	21(46.67%)	5(20.00%)	0.027
Antibiotic use prior to separation	42(60.00%)	34(75.56%)	8(32.00%)	<0.001
one kind	9(12.86%)	7(15.56%)	2(8.00%)	0.366
two kinds	12(17.14%)	9(20.00%)	3(12.00%)	0.395
three kinds	21(30.00%)	18(40.00%)	3(12.00%)	0.014
Co-morbidity conditions	41(58.57%)	26(57.78%)	15(60.00%)	0.856
Charlson Comorbidity Index (median, IQR)	2.54±2.14	2.64±2.14	2.36±2.16	0.597
Surgery	17(24.29%)	11(24.44%)	6(24.00%)	0.967
Inappropriate therapy	35(50.00%)	27(60.00%)	8(32.00%)	0.025
VAP	32(45.71%)	26(57.78%)	6(24.00%)	0.007
Length of hospital stay (mean days)	56.96±49.03	68.36±50.61	36.44±39.15	0.008

Table 5: Statistical analysis of clinical factors between inappropriate and appropriate antimicrobials therapy groups.

Variable	Inappropriate therapy (*n*=35)	Appropriate therapy (*n*=35)	P
Age (Year) (mean ± standard deviation)	57.29±19.62	54.34±20.50	0.542
Sex (Male/Female)	27/8	28/7	0.771
Charlson Comorbidity Index (median, IQR)	2.46±2.01	2.63±2.29	0.319
Underlying disease	21(60.00%)	20(57.14%)	0.808
Cardiac disease	19(54.29%)	16(45.71%)	0.473
Liver disease	1	1	-
Renal disease	1	1	-
Respiratory disease	2(5.71%)	6(17.14%)	0.133
Diabetes mellitus	7(20.0%)	3(8.57%)	0.172
Comorbid conditions			
Receipt of recent operation	8(22.86%)	9(25.71%)	0.78
Prior hospitalization within 30 days	21(60.00%)	4(11.43%)	0
Antibiotic use prior to separation	26(74.29%)	16(45.71%)	0.015
Polymicrobial infection	22(62.86%)	16(45.72%)	0.15
Indwelling urinary catheters	-	-	-
Central venous catheterization	-	-	-
Invasive procedure	27 (77.14%)	30 (85.71%)	0.356
Onset of infection			
Community-acquired	8(22.86%)	17(48.57%)	0.025
HCAI	27/35(77.14%)	18/35(51.43%)	0.025
VAP	18/35(51.43%)	14/35(40.0%)	0.337
Severity of illness			
APACHE II Score	21.06±8.61	20.66±11.11	0.387

The data emphasized the importance of establishing local monitoring for local antimicrobials guide and supported the best treatment. The polymyxin B had the lowest resistance rate in our study, followed by imipenem and aminoglycosides. However, because of the limited clinical application of polymyxin B, imipenem or aminoglycosides were preferred for empirical use, but for patients with severe infections or XDRPA infections, polymyxin B may be considered.

Our results showed considerable genetic variability among the 70 strains, with the detection of 37 distinct genetypes (0.946 of polymorphisms). The genotype cluster 4 contained *IntI*, *OXA-1, IMP*

and *aac(6')-Ib, ant(2")-Ia*, which suggested that this specific clone was endemic to the hospital. As a result of persistence over sustained periods, it had become more resistant to antimicrobials. It was speculated that *OXA-1+IMP+ant (2 ') -Ia+aac (6') -Ib+IntI* was the main resistance gene pattern in 2016. To the best of our knowledge, *IntI* was closely related to a variety of drug resistance, carrying related drug-resistant gene cassettes that might lead to transmission of resistance as often detected in clinical isolates of PA [12]. Based on previous reports that *IntI* carried related resistance gene cassettes [20], we speculated that the main resistance gene pattern (*OXA-1+IMP+ant (2 ') -Ia+aac (6') -Ib+IntI*) in this study might also be related to the relevant resistance gene cassette carried by the *IntI*, which had not been reported yet. However, the lack of sequencing of related gene cassettes and *IntI* was not enough, thus despite this speculation, there remains questions to be clarified in future studies. The *IntI* might be the main genetic elements of the global resistance transmission of PA. If there is no proper and timely monitoring, the spread of the *IntI* gene, which may be the main genetic elements of resistance global communication about PA [21], will inevitably complicate treatment of HAIs by MDR PA. Therefore, there is an urgent need not only to rationalize the use of antimicrobials, but also to monitor the dissemination and possible mutation of related resistance genes at the same time. In addition, it is known that the mutation of *OprD2* inactivation has become the main mechanism of imipenem resistance [8], but the detection of *OprD2* gene deletion strains in this study did not suggest significant relationship with the imipenem resistance.

In the 70 strains of PA clinical isolates, compared with the prevelance of the *exoS* gene (49/70, 70.00%), the detection rate of *exoU* gene was lower (18/70, 25.71%), which was similar to the previous report [22]. However, previous studies had shown that not all clinical isolates had the ability to produce *ExoS* [23], with the overall prevalence of *exoS* gene in clinical isolates being only approximately 70%. In addition, virulence gene detection rate had obvious difference from the different specimens. The production of *ExoS* can provide the advantages of PA isolates in respiratory tract colonization or persistent existence [23,24]. In the ICU, there were many cases of colonization of colonized bacteria, which might lead to a significant increase in the detection rate of *exoS*, which was also important in the mechanism of PA colonization and infection in the respiratory tract.

Interestingly, almost every isolate contained *exoS* without harboring *exoU* and vice versa. Besides, only 1 isolate carrying both genes while 4 isolates harboring none. Feltman and coworkers [22] reported these similar findings, indicating it was a nearly universal characteristic of PA isolates, which might owe to the gene altered under the corresponding environmental pressure or related to the enhancement of virulence.

Studies on PA in European and American populations had suggested was the *exoU* genotype is significantly associated with multidrug resistance and fluoroquinolones resistance compared with the *exoS* genotype [25,26]. However, there was no significant difference in the resistance between *exoU*+ genotype with *exoS*+ genotype in our study, which might be related to the different distribution of population and area. Nevertheless, comparing with the *exoU*+ genotype, *exoS*+ genotype strains carried a significantly higher rate of drug resistance, which was different from the previous funding reports. Previous fundings had shown that from a clinical point of view, detection of *toxA* and *eoxS* genes in PA clinical isolates might be more significant in drug resistance [11,27]. In our study, 27 strains of MDR (27/35, 77.14%) contained both *toxA* and *eoxS* genes at the same time. Compared with non-MDR (22/35, 62.86%), there was not any significant differences could be found in gene prevalence, which was different from other studies [27]. Therefore, we should consider that pathogenicity of PA was multifactorial.

Multi-factor analysis showed that the use of antimicrobials before separation, prolonged hospitalization and mechanical ventilation were independent risk factors for HAIs. At the same time, some studies suggested that the use time of antimicrobials, hospitalization time and tracheal intubation were the main risk factors of healthcare associated infection in PA [26]. These risk factors were interacted to each other. The results of this study showed that the use of antimicrobials before separation, prolonged hospitalization, and mechanical ventilation were risk factors for the occurrence of PA infection in the hospital. The prolonged hospitalization time, the use of antimicrobials, the screening of PA lacking routine ESBL production and the colonization of multidrug-resistant strains in the environment might be the reason of the high resistance rate of PA.

Inappropriate antimicrobials therapy was significantly associated with increased mortality, morbidity, and length of hospital stay [28]. Furthermore, inappropriate empirical antimicrobials therapy was independently associated with higher mortality in patient and inappropriate initial antimicrobials therapy had an adverse effect on survival in patients with gram-negative sepsis [29], however, our study did not find a clear association with mortality. But the results showed that inappropriate treatment significantly increased the mean length of hospital stay compared with those who started receiving appropriate treatment. We observed that 50% of the strains isolated in the ICUs were treated inappropriately. And in contrast to community infections, patients with healthcare associated infections receiving inappropriate treatment was significantly higher with a high increasing incidence of VAP. Multifactor analysis showed a significant correlation between VAP and mortality within 30 days, and VAP was an independent risk factor for increased mortality in 30 of the patients. The use of Mechanical Ventilation as a means of rescue in ICU had also greatly increased the use of ventilator, thus got a high risk of patients with VAP. Current literature suggests that when the infection was effectively controlled along with improved ventilation, the time to use the ventilator should be reduced as much as possible, and the use of non-invasive ventilation can reduce the occurrence of VAP [30].

It was pointed out that the impact of inappropriate empirical antimicrobials therapy depended on the site of the infection. For patients with high risk of infection, inappropriate empiric antimicrobials therapy was identified as an independent risk factor for death [31]. Although most of the specimens were sputum from lower respiratory tract, no mortality was found to be associated with inappropriate treatment. In our study, the most inappropriate empirical antimicrobials was meropenem, which was consistent with a report from Italy showed the highest rate of drug use of meropenem

for inappropriate therapy [19]. We suggested that the use of carbapenems and aminoglycoside antimicrobials should be used with caution in the empirical treatment of antimicrobials, especially in the consideration of patients with HCAIs or MDRPA infections. However, no significant statistical significance was found in the use of different kinds of drugs between the inappropriate drug treatment group and the appropriate group which indicated that we still need to guide the application of clinical antimicrobials strictly according to the drug sensitivity report. The appropriateness of empirical treatment cannot be guaranteed. Based on the results of the study, we did not recommend the use of quinolones as a priority in empiric therapy in the study area, which can easily lead to inappropriate therapeutic use. Especially when infected with MDRPA, inappropriate empirical treatment may aggravate the change of drug resistance.

A lack of significant association between inappropriate initial antimicrobial therapy and the outcome of patients with a low-risk source of bacteremia may have been due to a high proportion of catheter removal or early intervention for decompression of biliary or urinary obstruction in the majority of patients. It suggested that nonmedical interventions such as decompression of obstruction or the removal of infection foci were also important aspects of the treatment of infection.

Conclusion

The resistance rates of most drugs in our research were higher than the 2016 China CHINET bacterial resistance monitoring results, except for the slightly lower resistance rate of imipenem and polymyxin B. The drug resistance in this area was severe, and there seems to be a long-term process of colonization or transmission leading to infections at the ICUs with an extensively drug resistant clone type. The resistance genes like *OXA-1 IMP, ant(2")-Ia, aac(6')-Ib* and *IntI* were correlated with MLVA gene cluster. It is speculated that in 2016, at the ICUs, the related resistance gene island (*OXA-1+IMP+ant(2")-Ia+aac(6')-Ib*) carried in *IntI* was the predominant pattern of drug resistance genes. In the clinical data analysis, multifactor analysis shows that the VAP was an independent risk factor for increasing the mortality rate of 30 days in patients. The use of antimicrobials, length of hospitalization and the use of mechanical ventilation before isolation were independent risk factors for HAIs.

Inappropriate antimicrobials therapy seemed to substantially influence the empiric therapy of PA infections; In particular, use of carbapenems was notably frequent. At the same time, our research showed that patients who usually hospitalized for more than 30 days often did not receive proper treatment. This indicated that clinicians must strictly comply with the drug sensitivity test results in the use of antimicrobials, and use carbapenems with caution in empirical treatment, especially for patients with PA infection who have been hospitalized more than 30 days in the study area.

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