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Original Article

Prevalence and fluoroquinolone resistance of pseudomonas aeruginosa in a hospital of South China

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Abstract: *Pseudomonas aeruginosa* is an opportunistic pathogen that poses a threat in clinical settings. This study aimed to investigate the molecular characterization and epidemiology of fluoroquinolones (FQs) resistance in *P. aeruginosa* isolated from South China. A total of 256 *P. aeruginosa* strains isolated from outpatients, emergency patients and inpatients were collected from January 2010 to December 2010 in the hospital of South China. The resistance profile of all isolated strains was screened by antibiotic-susceptibility testing, and the molecular characteristics of plasmid-mediated quinolone resistance (PMQR) and the quinolone resistance determining region (QRDR) were determined using PCR in combination with DNA sequencing. The result of antibiotic-susceptibility tests showed that most strains were sensitive to polymyxin B, piperacillin, piperacillin/tazobactam, ceftazidime and amikacin. Moreover, 65 isolates were identified as resistant to ciprofloxacin. Further analysis of QRDR revealed that the resistant strains carried at least one mutation in the *gyrA* (The83Ile), *gyrB* (Ser467Phe, Gln468His) and *parC* (Ser87Leu) genes, but no mutation was detected in *parE*. For the first time, we report here that the qnrA1 gene is associated with low levels of resistance to ciprofloxacin from clinical *P. aeruginosa* isolates in South China. The mutation of *gyrA* (at position 83) is clearly linked to the FQs resistance of *P. aeruginosa*. Moreover, FQs resistance of *P. aeruginosa* may be due to the chromosome-mediated resistance mechanism rather than PMQR.

Keywords: Fluoroquinolone resistance, PMQR, QRDR, *Pseudomonas aeruginosa*

Introduction

Pseudomonas aeruginosa is one of the most common and important opportunist gram-negative pathogens causing hospital-acquired infections [1]. The pressure of antibiotics has led to the rapid development of bacterial resistance. Among these antibiotics, Fluoroquinolones (FQs) are some of the most commonly prescribed effective antimicrobials against *P. aeruginosa* infections. Unfortunately, overuse of FQs in medicine has promoted bacterial resistance to FQs in recent years, which has caused a huge challenge in the anti-infective therapy of *P. aeruginosa* [2, 3]. Therefore, the monitoring of antimicrobial susceptibility is crucial for selecting effective antimicrobial agents in the treatment of this disease.

Substantial evidences have shown that resistance to FQs is mainly due to: (i) the point mutations in the DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) genes, (ii) the presence of transferable plasmid-mediated quinolone resistance (PMQR) determinants, and (iii) mutations in genes regulating the expression of efflux pumps and decreased expression of outer membrane porins [4]. The aim of this study was to investigate the prevalence and molecular characteristics of *P. aeruginosa* in South China, including antimicrobial susceptibility, and whether they present transferable PMQR genes or mutations of quinolone resistance determining region (QRDR), in order to assess the resistance mechanisms of *P. aeruginosa* in the local area.

Materials and methods

Bacterial isolates

A total of 256 isolates of non-duplicated *P. aeruginosa* from outpatients, emergency pati-

Table 1. Distribution of specimens among 256 *P. aeruginosa* clinical isolates

Specimens	Isolates	Rate (%)
Sputum	206	80.5
Urine	18	7.0
Wound secretions	12	4.7
Bile	7	2.7
Blood	6	2.4
Other	7	2.7

ents and inpatients were collected from the Third Affiliated Hospital of Sun Yat-sen University between January 2010 and December 2010. All strains were identified by the Micro-Scan WalkAway-40 automatic microorganism analyzer.

Susceptibility testing

The antibiotics susceptibility of all *P. aeruginosa* isolates to 19 common antibiotics was determined by methods of K-B disk diffusion. Nineteen common antibiotics, including Piperacillin (PIP, 100 μg), piperacillin/tazobactam (TZP, 100 μg/10 μg), Amoxycillin/clavulanic acid (AMC, 20 μg/10 μg), Ampicillin/sulbactam (SAM, 10 μg/10 μg), Ticarcillin/clavulanic acid (TIC, 75 μg/10 μg), Aztreonam (ATM, 30 μg), Cefoperazone (CPZ, 75 μg), Cefoperazone/sulbactam (SCF, 75 μg/30 μg), Cefoxitin (FOX, 30 μg), Ceftriaxone (CRO, 30 μg), Ceftazidime (CAZ, 30 μg), Ceftne (FEP, 30 μg), Imipenem (IMP, 10 μg), Ciprofloxacin (CIP, 5 μg), Levofloxacin (LVX, 5 μg), Moxifloxacin (MXF, 5 μg), Gentamycin (GM, 10 μg), Amikacin (AK, 30 μg), and Polymyxin B (PB, 10 μg), were used. Generally, the breakpoints for the antimicrobial agents for *P. aeruginosa* were according to standards from the Clinical and Laboratory Standards Institute (CLSI). The reference strains *Escherichia coli* ATCC25922 and *P. aeruginosa* ATCC 27853 served as quality control strains for MIC determinations.

Screening for PMQR genes

PCR amplification of PMQR genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac (6')-Ib* and *qepA*) was performed [5, 6]. Genomic DNA templates of the 256 *P. aeruginosa* strains were prepared according to the standard boiling method [7]. Purified PCR products were cloned into pGEM-T (TAKARA, Dalian, China) and then sequenced using the Applied Biosystems ABI3730 Analyser (Applied Biosystems, Inc., USA).

Table 2. Distribution of *P. aeruginosa* isolates in the department

Department	Isolates	Rate (%)
ICU	68	26.5
Respiratory Medicine	64	25.0
Neurosurgery	39	15.2
Rehabilitation	14	5.5
Hepatobiliary surgery	13	5.1
Urinary	10	3.9
Infectious disease	8	3.1
Thoracic surgery	7	2.7
Cardiovascular	6	2.3
Emergence	6	2.3
Tumour	5	2.0
Haematology	5	2.0
Gastrointestinal surgery	4	1.6
Dermatology	4	1.6
Rheumatology	3	1.2

Determination of QRDR of gyrA, gyrB, parC and parE

To identify QRDR mutations in FQs-resistant *P. aeruginosa*, the *gyrA*, *gyrB*, *parC* and *parE* genes were amplified and then sequenced as described above [8]. To determine the mutations in these genes, the sequences were aligned with Clustal X.

Results

Bacterial isolates

Overall, 256 clinical isolates of *P. aeruginosa* were isolated from sputum (80.5%), urine (7.0%) and wound secretions (4.7%). The top three sample sources were the intensive care unit (ICU), respiratory medicine and the neurosurgery department, with proportions of 26.5% (68/256), 25.0% (64/256) and 15.2% (39/256) (**Tables 1** and **2**).

Antibiotics resistance in P. aeruginosa

The results of the antibiotic-susceptibility tests are shown in **Table 3**. Polymyxin B had the highest susceptibility rate (98.8%), while the susceptibility of piperacillin, piperacillin/tazobactam, ceftazidime, amikacin and ciprofloxacin were about 70.0%-80.0%. Lower efficacy was

Table 3. *P. aeruginosa* isolates antimicrobial resistance profiles from South China isolates

Antimicrobials	Resistant (R)		Intermediate (I)		Sensitive (S)	
	Isolates	Rate (%)	Isolates	Rate (%)	Isolates	Rate (%)
Piperacillin	75	29.3	0	0.0	181	70.7
Piperacillin/tazobactam	58	22.7	0	0.0	198	77.3
Amoxycillin/clavulanic acid	248	96.9	0	0.0	8	3.1
Ampicillin/sulbactam	250	97.7	0	0.0	6	2.3
Ticarcillin/clavulanic acid	93	36.3	0	0.0	163	63.7
Cefoperazone	69	27.0	41	16.0	146	57.0
Cefoperazone/sulbactam	48	18.7	46	18.0	162	63.3
Ceftriaxone	195	76.2	46	18.0	15	5.8
Ceftazidime	54	21.1	16	6.3	186	72.6
Cefepime	48	18.8	42	16.4	166	64.8
Cefoxitin	251	98.0	0	0.0	5	2.0
Aztreonam	78	30.5	38	14.8	140	54.7
Imipenem	70	27.4	18	7.0	168	65.6
Ciprofloxacin	65	25.4	11	4.3	180	70.3
Levofloxacin	73	28.5	21	8.2	162	63.3
Moxifloxacin	71	27.7	22	8.6	163	63.7
Gentamycin	56	21.9	28	10.9	172	67.2
Amikacin	42	16.4	11	4.3	203	79.3
Polymyxin B	3	1.2	0	0.0	253	98.8

observed in isolates when using ticarcillin/clavulanic acid, cefoperazone/sulbactam, cefepime, imipenem, gentamicin, moxifloxacin. The antibiotic-susceptibility of levofloxacin was between 60.0% and 70.0%. There were no more than 30% of isolates with resistance to cefoperazone and aztreonam. In contrast, more strains were found to be resistant to ampicillin/sulbactam, amoxicillin/clavulanic acid, ceftriaxone and cefoxitin, with less than 10% detected.

Identification of PMQR genes

PCR screening and sequence analysis showed that only one isolate presented the PMQR determinants (q*nrA1*) in 256 *P. aeruginosa*, and the ciprofloxacin MIC was 2 μg/ml. However, other PMQR genes, *qnr*B, *qnr*C, *qnr*D, *qnr*S, *aac (6')-Ib-cr* and *qep*A, were not found in all of the detected strains.

Analysis of the mutations in QRDR

Mutations in the QRDR of *gyrA*, *gyrB*, *parC* and *parE* were identified; the results are presented in **Table 4**. Among the 65 ciprofloxacin-resistant *P. aeruginosa* clinical isolates, 49 strains (75.4%) showed a missense mutation of Thr (ACC)→Ile (ATC) at the 83rd codon of the gyrA gene. Two strains (3.1%) reported the presence of an independent missense mutation in the *gyr*B gene of Ser467 (TCC)→Phe (TTC) and Gln468 (CAG)→His (CAT). Fifteen strains (23.1%) presented a missense mutation from Ser (TCG)→Leu (TTG) at the 87th codon of the *parC* gene. Moreover, strains carrying either *gyr*B or *par*C gene missense mutations were all accompanied by a *gyr*A gene missense mutation as well. However, none of the mutations mentioned above in were found in the 10 strains of ciprofloxacin-sensitive *P. aeruginosa* that were randomly drawn from 180 clinical isolates. The MIC values of ciprofloxacin were significantly higher in the strains with double mutations of either *gyr*A plus *gyr*B or *gyr*A plus *par*C than in those with only a *gyr*A gene mutation. In contrast, no missense mutation was found in the *par*E gene.

Discussion

P. aeruginosa has been recognized as a major pathogen that can caused healthcare-associated infection (HCAI), especially ventilator-associated pneumonia. However, increasing antimicrobial resistance among *P. aeruginosa* has become a major concern when managing its associated infections [9]. It has been reported that most of the *P. aeruginosa* samples were isolated from lower respiratory tract secretions [10]. Similarly, in this study, isolates were mainly isolated from the sputum, urine and puriform secretions, supporting the suggestion that *P. aeruginosa* has held a nearly unchanged position in the rank order of pathogens causing ICU-related infections [11, 12].

Table 4. Mutations in the QRDRs and the MIC of CIP in *P. aeruginosa* isolates

Mutations in QRDRs				Number of strains	MIC (μg/ml)
gyrA	gyrB[a]	*parC*	*parE*[b]		
Thr83Ile	—	—	—	32	4~256
Thr83Ile	Ser467Phe	—		1	128
Thr83Ile	Gln468His		—	1	256
Thr83Ile	—	Ser-87→Leu	—	15	32~256

[a]Other mutations in *gyr* B: GAA-457→GAG, GCG-459→GCA, GGC-482→GGT, ACG-472→ACT, GAA-485→GAG, ACC-475→ACT. [b]Mutations in *parE*: AAC-374→AAT, GTG-465→GTA, GGT-472→GGC, AGT-474→AGC, GCC-477→GCT, GGG-494→GGC, CGC-507→CGT.

Due to the presence of several drug efflux systems and porins, *P. aeruginosa* is intrinsically resistant to a wide range of antimicrobials. Our results also confirmed the low occurrence of cefoperazone/sulbactam, ceftazidime, amikacin and ciprofloxacin resistance among *P. aeruginosa* in South China, and showed a relatively high susceptibility to beta-lactam, aminoglycosides and polymyxin. With increasing utilization of fluoroquinolones in both human and veterinary medicine, emerging resistance has become a significant concern. However, in this study, more than 60% of *P. aeruginosa* showed susceptibility to ciprofloxacin, levofloxacin and moxifloxacin. Moreover, 70.3% of isolates were susceptible to ciprofloxacin. Other reports showed that FQs resistance of *P. aeruginosa* was related to the widespread use of levofloxacin rather than ciprofloxacin [13]. Therefore, ciprofloxacin should be preferred when treating *P. aeruginosa* infections in clinical scenarios.

To date, PMQR genes (q*nrA*, *qnrB*, *qnrS*, *qnrD* and *qepA*) have been reported in veterinary clinical isolates in China [14]. In 2008, Libisch et al. found that the *aac (6')-Ib-cr* gene was present in the PER-1 and ESBLs-positive *P. aeruginosa* clinical isolates [15]. However, for the first time, we isolated one *P. aeruginosa* strain with *qnr*A1 gene, and the MIC of CIP was 2 μg/ml, suggesting that *qnr*A1 may mediate the low-level resistance to CIP.

The presence of mutations is associated with resistance; for example, mutations in gyrA (Thr83→Ile, Asp87→Asn), gyrB (Ser464→Phe), and parC (Ser87→Leu) were related to the FQs resistance of *P. aeruginosa* [9]. It is worth noting that *gyrA* (at codon 83) was present in 75.4% strains, and 15 (23.1%) of isolates had mutations in parC (at codon 87), suggesting that *gyrA* mutations are closely correlated with FQs resistance in *P. aeruginosa*. Moreover, some other mutations, including those in *gyrA,* gyrB and parC, were found in these strains, which need further verification.

In summary, our work provided novel data on the molecular epidemiology of antimicrobial resistance in *P. aeruginosa*. Moreover, for the first time, we described *P. aeruginosa* containing *qnrA1* gene in South China. Our study revealed that multiple target gene mutations play an important role in the FQs resistance of *P. aeruginosa*, not only providing a scientific basis for further studying the mechanism of FQs resistance, but also highlighting its usefulness in the treatment and control of this infection.

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Disclosure of conflict of interest

None.

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