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Objective. This study aimed to detect 5 kinds of genes related to plasmid-mediated quinolone resistance in four species of nonfermenting bacteria with 2 drug resistance phenotypes (multidrug resistance and pandrug resistance), which were Acinetobacter baumannii (Ab), Pseudomonas aeruginosa (Pa), Stenotrophomonas maltophilia (Sm), and Elizabethkingia meningoseptica (Em).

Methods. The Phoenix NMIC/ID-109 panel and API 20NE panel were applied to 19 isolated strains, including 6 Ab strains (2 strains with multidrug resistance and 4 strains with pandrug resistance), 6 Pa strains (3 strains with multidrug resistance and 3 strains with pandrug resistance), 4 Sm strains (2 strains with multidrug resistance and 2 strains with pandrug resistance), and 3 Cm strains (2 strains with multidrug resistance and 1 strain with pandrug resistance). After strain identification and drug susceptibility test, PCR was applied to detect 5 genes related to plasmid-mediated quinolone resistance. The genes detected were quinolone resistance A (qnrA), aminoglycoside acetyltransferase ciprofloxacin resistance variant, acc(6′)-Ib-cr, and 3 integrons (intI1, intI2, and intI3). The amplified products were analyzed by 1% agarose gel electrophoresis and sequenced. Sequence alignment was carried out using the bioinformatics technique.

Results. Of 19 strains tested, 8 strains carried acc(6′)-Ib-cr and 6 of them were of pandrug resistance phenotype (3 Ab strains, 2 Pa strains, and 1 Sm strain). The carrying rate of acc(6′)-Ib-cr was 60.0% for strains of pandrug resistance (6/10). Two strains were of multidrug resistance (1 Ab strain and 1 Pa strain), and the carrying rate of acc(6′)-Ib-cr was 22.0% (2/9). The carrying rate was significantly different between strains of multidrug resistance and pandrug resistance (P < 0.05). The class 1 integron was detected in 11 strains, among which 6 strains were of pandrug resistance (3 Ab strains, 2 Pa strains, and 1 Sm strain). The carrying rate of the class 1 integron was 60.0% (6/10). Five strains were of multidrug resistance (3 Pa strains, 1 Ab strain, and 1 Em strain), and the carrying rate was 55.6% (5/9). The carrying rate of the class 1 integron was not significantly different between strains of multidrug resistance and pandrug resistance (P > 0.05). Both acc(6′)-Ib-cr and intI1 were detected in 6 strains, which were negative for qnrA, intI2, and intI3.

Conclusion. Quinolone resistance of isolated strains was related to acc(6′)-Ib-cr and intI1 but not to qnrA, intI2, or intI3. The carrying rate of acc(6′)-Ib-cr among the strains of pandrug resistance was much higher than that among the strains of multidrug resistance. But, the strains of two drug resistant phenotypes were not significantly different in the carrying rate of intI1. The detection rates of the two genes were high and similar in Ab and Pa strains. 1 Em strain carried the class 1 integron.

1. Introduction

Acinetobacter baumannii (Ab), Pseudomonas aeruginosa (Pa), and Stenotrophomonas maltophilia (Sm) are common nonfermenting bacteria in clinic [1–3], while Elizabethkingia meningoseptica (Em) is relatively rare [4]. Mutations in these bacteria are largely attributed to overuse and misuse of antibiotics, and drug resistance of these bacteria has become a crisis [5]. Many studies are devoted to the mechanism of quinolone resistance in Ab, Pa, and Sm, but only a few studies are published regarding quinolone resistance in Cm or genes related to plasmid-mediated quinolone resistance and their relationship with different drug resistance phenotypes [6–9]. We detected 5 genes related to plasmid-
mediated quinolone resistance in 19 isolated strains (2 Ab strains with multidrug resistance, 4 Ab strains with pandrug resistance, 3 Pa strains with multidrug resistance, 3 Pa strains with pandrug resistance, 2 Sm strains with multidrug resistance, 2 Sm strains with pandrug resistance, 2 Cm strains with multidrug resistance, and 1 Cm strain with pandrug resistance). The genes detected were quinolone resistance A (qnrA), aminoglycoside acetyltransferase ciprofloxacin resistance variant, acc(6′)-Ib-cr, and 3 kinds of integrons (intI1, intI2, and intI3) by using PCR with homology analysis. The purpose of this study was to understand the mechanism of quinolone resistance and its relationship with drug resistance phenotype in clinical strains of Ab, Pa, Sm, and Em.

2. Materials and Methods

2.1. Strains. All 19 strains (2 Ab strains with multidrug resistance, 4 Ab strains with pandrug resistance, 3 Pa strains with multidrug resistance, 3 Pa strains with pandrug resistance, 4 Sm strains, and 3 Cm strains) were clinically isolated and preserved at our hospital. Strain No., identification code, and time of isolation are shown in Table 1.

2.2. Reagents. Phoenix NMIC/ID-109 panel (BD Corporation, BD), M-H agar medium (Oxoid, UK), and API 20NE panel/PSE5.0 susceptibility strip (bioMérieux, France) were used.

2.3. Strain Identification. Bacterial culture was prepared conventionally. The colonies were purified and subjected to the O/F test and oxidase test. The above procedures were performed using a Phoenix NMIC/ID-109 panel, API 20NE panel, and PSE5.0 susceptibility strip.

2.4. Drug Susceptibility Test. MIC was performed using an API 20NE panel, PSE5.0 susceptibility strip, and/or Phoenix NMIC/ID-109 panel according to NCCLS guidelines [10]. For quality control, Pa strain ATCC27853 was used to test the reagents every week.

2.5. Detection of Genes Related to Plasmid-mediated Quinolone Resistance. PCR was applied to 5 genes related to plasmid-mediated quinolone resistance (acc(6′)-Ib-cr, qnrA, and 3 integrons (intI1, intI2, and intI3)). The primer sequences and PCR procedures were described in the literature [11]. The amplified products were analyzed by 1% agarose gel electrophoresis.

2.6. Sequencing and Alignment. The amplified genes were purified and sequenced. Homologous sequences were searched using BLAST program in GenBank. The accession number of homologous sequence for acc(6′)-Ib-cr was EF375621.

3. Results

3.1. Drug Resistance. All 19 strains were tested by MICs for drug susceptibility using the Phoenix NMIC/ID-109 panel and PSE5.0 susceptibility strip. Ten strains were of pandrug resistance phenotype and 9 strains were of multidrug resistance phenotype (Table 1).

3.2. Carrying Rates of 5 Detected Genes in Two Drug Resistance Phenotypes. Of 19 strains detected by PCR, 8 strains carried acc(6′)-Ib-cr and 6 of them were of pandrug resistance phenotype (3 Ab strains, 2 Pa strains, and 1 Sm strain). The carrying rate of acc(6′)-Ib-cr was 60.0% for strains of pandrug resistance (6/10). Two strains were of multidrug resistance (1 Ab strain and 1 Pa strain), and they were only negative in the ciprofloxacin susceptibility test. The carrying rate of acc(6′)-Ib-cr was 22.0% (2/9). The carrying rate was significantly different between strains of multidrug resistance and pandrug resistance ($P<0.05$). The class 1 integron was detected in 11 strains, among which 6 strains were of pandrug resistance (3 Ab strains, 2 Pa strains, and 1 Sm strain). The carrying rate of the class 1 integron was 60.0% (6/10). Five strains were of multidrug resistance (3 Pa strains, 1 Ab strain, and 1 Cm strain), and they were only negative in the ciprofloxacin susceptibility test. The carrying rate was 55.6% (5/9). The carrying rate of the class 1 integron was not significantly different between strains of multidrug resistance and pandrug resistance ($P>0.05$). Both acc(6′)-Ib-cr and intI1 were detected in 6 strains, which were of pandrug resistance (3 Ab strains, 1 Pa strain, and 2 multidrug resistance strains). Five strains were found to carry only intI1, and 2 strains were found to carry only acc(6′)-Ib-cr. They were negative for qnrA, intI2, and intI3 (Table 1, Figures 1 and 2).

3.3. Distribution of Drug Resistance-Related Genes in 4 Bacterial Species. Of 6 Ab strains, 4 strains carried both acc(6′)-Ib-cr and intI1 (3 strains with pandrug resistance and 1 strain with multidrug resistance) and none carried only one drug resistance gene. Of 6 Pa strains, 3 strains carried acc(6′)-Ib-cr, 5 strains intI1, and 2 strains both two genes. No. 5 Sm strain was positive for acc(6′)-Ib-cr, and No. 7 Sm strain was positive for intI1. No. 19 Cm strain, which was of multidrug resistance, carried intI1. Other Cm strains were negative for intI1 and positive for qnrA, intI2, and intI3.

3.4. Homology Analysis. The amplified product of acc(6′)-Ib-cr was sequenced, and it contained 519 nucleic acids. By alignment with sequence (accession number EF375621) in GenBank, the identity was above 99% without sense mutation. This sequence was verified as acc(6′)-Ib-cr. The accession number of acc(6′)-Ib-cr from No. 5 Sm strain was EF210035. DNA and amino acid sequence alignment was two acc(6′)-Ib sequences (AY866525 and EU090799) and one acc(6′)-Ib cr sequence (EF375621) (Figure 3).
4. Discussion

Chromosomally mediated mechanisms mainly underlie quinolone resistance, such as change of the target site induced by drugs, decreased permeability of outer membrane porin, and active pumping of efflux pump [12]. Drug resistance in Pa may be mediated by plasmids (involving 4 genes, qnr (protection protein), qepA, oqxAB (efflux pump), and acc(6’)-Ib-cr (quinolone-modifying enzyme)) [8]. As the issue of drug resistance is intensifying, the pandrug resistance phenotype has emerged [5]. Genes related to plasmid-mediated quinolone resistance may be engaged in dissemination of genes related to drug resistance mediated by other plasmids [6-9]. Therefore, different genes are involved in quinolone resistance for different drug resistance phenotypes (multidrug or pandrug resistance).

qnrA belongs to the qnr families that protect DNA gyrase from quinolones; qnrB and qnrA are the main genes related to plasmid-mediated quinolone resistance carried by bacteria [6]. However, qnrA was not detected in any of the 19 strains, suggesting that this gene was not related to quinolone resistance in the 4 species of strains tested. Touati et al. [13] and Guler and Eraç [14] also reported that qnr is not the main mechanism of quinolone resistance in Ab strains.

| No. | No. of sample | Type of specimens | Germ name | API ID card | BD ID % | Isolated time | Results of AST | qnrA aac(6’)-Ib-Cr int11 intl2 intl3 |
|-----|--------------|------------------|-----------|------------|---------|---------------|----------------|-----------------|
| 1   | 91112-2      | Sputum           | Ab        | 204042     | 90      | 2006-9-14     | PR             | − − − − − − − − |
| 2   | 90911        | Sputum           | Ab        | 204042     | 99      | 2006-9-12     | PR             | + + + + + + + + |
| 3   | 82826-2      | Sputum           | Ab        | 204042     | 90      | 2006-8-26     | PR             | − − − − − − − − |
| 4   | 101907       | Sputum           | Ab        | NT         | 99      | 2006-10-19    | PR             | + + + + + + + + |
| 5   | 1370         | Blood            | Ab        | 204051     | 99      | 2003-8-28     | MR/CIP R       | + + + + + + + + |
| 6   | 1532         | Sputum           | Ab        | 204042     | 96      | 2003-9-5      | MR/CIP S       | − − − − − − − − |
| 7   | B736         | Blood            | Pa        | 1054555    | 99      | 2003-7-4      | MR/CIP R       | − − − − − − − − |
| 8   | 92010        | Sputum           | Pa        | 1154575    | 99      | 2006-9-20     | MR/CIP R       | − − − − − − − − |
| 9   | 90708        | Sputum           | Pa        | 1050075    | 95      | 2006-9-7      | MR/CIP R       | + + + + + + + + |
| 10  | 102021       | Sputum           | Pa        | NT         | 99      | 2006-10-20    | PR             | − + + + + + + + |
| 11  | 50407-1      | Wound pus        | NT        | 99         | 2005-5-4  | PR             | + + + + + + + + |
| 12  | 90821        | Sputum           | Pa        | 1154475    | 99      | 2006-9-8      | PR             | − − − − − − − − |
| 13  | 52616        | Sputum           | Sm        | 1472344    | 99      | 2006-5/26     | PR             | − + + + + + + + |
| 14  | 92930-2      | Sputum           | Sm        | 1472341    | 99      | 2006-10/2     | PR             | − − − − − − − − |
| 15  | 1689         | Blood            | Sm        | 1472345    | 95      | 2003/8/18     | MR             | − − − − − − − − |
| 16  | 51108        | Sputum           | Sm        | 1432355    | 99      | 2006/5/11     | MR             | − − − − − − − − |
| 17  | 61424        | Sputum           | Em        | 2476004    | 99      | 2006-6/14     | PR             | − − − − − − − − |
| 18  | 287          | Blood            | Em        | 1610204    | NT      | 2004-3-12     | MR/LEV R       | − − − − − − − − |
| 19  | 2700         | Sputum           | Em        | 2442244    | NT      | 2004-12-9     | MR/LEV R       | − − − − − − − − |

Ab, Acinetobacter baumannii; Pa, Pseudomonas aeruginosa; Sm, Stenotrophomonas maltophilia; Em, Elizabethkingia meningoseptica; NT, undetected; PR, pandrug resistant; MR, MDR; R, resistance; S, sensitive. CIP, ciprofloxacin; LEV, Levofloxacin; ”+,” positive; and ”−,” negative.

Figure 1: Electrophogram of acc(6’)-Ib-Cr genes by PCR. S, positive sample; N, negative control; P, positive control; M, DNA marker.

Figure 2: Electrophogram of int11 genes by PCR. S, positive sample; N, negative control; P, positive control; M, DNA marker.
acc(6′)-Ib-cr participates in plasmid-mediated quinolone resistance, encoding for the acc(6′)-Ib enzyme that leads to resistance to norfloxacin and ciprofloxacin [6–9]. Of 19 strains tested, 8 genes carried this gene and 6 of them showed p andraug resistance phenotype. The remaining 2 strains were of multdrug resistance phenotype, and they were only negative in the ciprofloxacin susceptibility test. This means acc(6′)-Ib-cr is involved in the quinolone resistance of Ab, Pa, Sm, and Cm. The carrying rate of this gene was higher for the p andraug resistance phenotype than for the multdrug phenotype. The detection rate of acc(6′)-Ib-cr was higher and similar in Ab and Pa strains.

Integrons are associated with plasmid-mediated quinolone resistance, and class 1, 2, and 3 integrons are the most common, the genetic markers being intI1, intI2, and intI3, respectively. Of 19 strains, intI1 was detected in 11 strains in all species of Ab, Pa, Sm, and Em, among which 6 strains were of p andraug resistance phenotype and 5 strains of multdrug resistance phenotype. Thus, the class 1 integron was related to intI1, but no significant difference was found in the carrying rate between the p andraug resistance phenotype and multdrug resistance phenotype ($P > 0.05$). Class 2 and 3 integrons were not detected in any of the strains, which agreed with previous reports [15–18]. Therefore, class 2 and 3 integrons were not related to quinolone resistance in the tested strains. intI1 had a high and similar detection rate in Ab and Pa strains. Few reports were on the class 1 integron in EM [19, 20]; in this paper, 1 Em strain carried the class 1 integron.

Both acc(6′)-Ib-cr and intI1 were detected in 6 strains (4 p andraug resistance strains (3 Ab strains and 1 Pa strain) and 2 multdrug resistance strains). Five strains carried only intI1, and 2 strains carried only acc(6′)-Ib-cr. The strains carrying both acc(6′)-Ib-cr and intI1 mostly belonged to p andraug resistance phenotype. Moreover, it is proved that acc(6′)-Ib-cr exists in intI1 [6–9].

To conclude, the mechanism of plasmid-mediated quinolone resistance is mainly related to acc(6′)-Ib-cr and intI1, which have high and similar detection rates in Ab and Pa strains, but not to qnrA, intI2, or intI3. The carrying rate of acc(6′)-Ib-cr was higher in the p andraug resistance phenotype than in the multdrug resistance phenotype. However, the two phenotypes did not differ significantly in the carrying rate of intI1, and acc(6′)-Ib-cr exists in intI1. 1 Em strain carried the class 1 integron. Also, further studies are needed to investigate the resistance mechanisms, ESBLs, MBLs, OXA-type carbapenemases, and aminoglycoside resistance determinants in the 19 strains.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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