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HEALTH SCIENCES

Differential Proteomic and Genomic Comparison of Resistance Mechanism of *Pseudomonas aeruginosa* to Cefoperazone Sodium/Sulbactam Sodium

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Abstract: The aim of this study was to determine the resistance mechanism of *Pseudomonas aeruginosa* to cefoperazone sodium/sulbactam sodium. We retrospectively analyzed the drug resistance of *P.a* isolated at the First Affiliated Hospital of Guangxi Medical University. Drug-resistant *P.a* strains were constructed, then wild-type (WT) and drug-resistant (DR) strains were compared using protein and gene microarrays to determine differences between DR and WT strains. The resistance rates of *P. aeruginosa* during 2013, 2014 and 2015 were 21.2%, 21.4%, and 24.6% respectively. Among 242 protein peaks of WT and DR bacteriophage proteins, 41 were differentially expressed between the two groups. The expression of 26 and 15 proteins were respectively upregulated and downregulated in the DR compared with the WT group. Gene microarray results revealed 679 mutant loci in the DR group, of which 42 with the top 50 Q values were found in the NCBI database. The rate of *P.a* resistance to cefoperazone sodium/sulbactam sodium remained high between 2013 and 2015. The numbers of different proteins and genetic variations in the DR strains suggested that the resistance mechanism of *P.a* to cefoperazone sodium/sulbactam sodium involves multiple genes and proteins that might be key to controlling *P.a* resistance to cefoperazone sodium/sulbactam sodium.

Key words: *Pseudomonas aeruginosa*, cefoperazone sodium/sulbactam sodium, drug resistance, mechanism.

INTRODUCTION

Pseudomonas aeruginosa is a common pathogen that causes respiratory and urinary tract infections (Cho & Lee 2021). Widespread treatment with broad-spectrum antibacterial drugs has led to an annually increasing trend of *P. aeruginosa* resistance, resulting in longer hospital stays and increased medical costs for patients (Enzor et al. 2021). Furthermore, *P. aeruginosa* can develop cross-resistance to different classes of antimicrobial drugs, rendering the treatment of infections induced by this organism increasingly difficult and often life-threatening for patients (Ibrahim et al. 2020).

Pseudomonas aeruginosa generates resistance to antimicrobial drugs through various mechanisms (Hansen et al. 2021), such as β-lactamase hydrolysis of β-lactam, and pumping antimicrobial drugs extracellularly through an active efflux system (Mularoni et al. 2021). When adsorbed to medical materials, *P. aeruginosa* can produce biofilms that can cause bacteria to adhere to each other and create tangled colonies, which subsequently prevent antimicrobial drugs from passing through the biofilm (Arca-Suarez et al. 2021). Antimicrobial

drugs also cannot attain effective bactericidal concentrations in biofilms. In addition, the continuous release of bacteria from biofilms causes repeated refractory chronic infections, particularly in patients with cystic pulmonary fibrosis. Thus, biofilm construction is an important cause of chronic infections in such patients (Cipko et al. 2021).

Among the cephalosporins that are usually applied to treat infections caused by *P. aeruginosa*, cefoperazone sodium/sulbactam sodium is the most prevalent (Millar et al. 2021). Protein expression changes during the process of resistance to antimicrobial drugs regardless of whether it is β-lactamase production, an active efflux system, biofilm construction, or other mechanisms (Sarker et al. 2021). Mutations in the nfxB gene can be induced when *P. aeruginosa* infection is treated only with quinolones. This elicits expression of the efflux pump gene, MexCD-OprJ that is not expressed in wild-type (WT) *P. aeruginosa* strains, and this causing resistance to antimicrobial drugs such as tetrasubstituted cephalosporins, chloramphenicol, and tetracyclines (O'Donnell et al. 2020). Therefore, understanding the resistance mechanism of *P. aeruginosa* using proteomic and genomic techniques should help to reduce the emergence of drug-resistant strains, which is critical for effectively treating *P. aeruginosa* infections.

MATERIALS AND METHODS

Samples

Bacteria were isolated from sputum, traumatic secretions, and urine samples collected from patients at the First Affiliated Hospital of Guangxi Medical University between January 1st, 2013 and December 31st, 2015. All patients provided written informed consent to participate in this study, which was approved by the Ethics Committee, and proceeded in accordance with the guidelines of the Federation of European Laboratory Animal Science Association. The Animal Ethics Committee of Guangxi Medical University (Nanning, China) also approved the study protocols. This project fully considered and protected the rights and interests of the study objects. It meets the criteria of Ethical Review Committee. All methods were carried out in accordance with the Declaration of Helsinki.

For all patients, sputum specimens were collected from those presenting with symptoms of respiratory tract infection if they were able to cough up sputum spontaneously (Dept. of Chinese Med., Respiratory Diseases Division, Geriatrics Division, Pediatric Division, etc.), and sputum was collected using bronchoscopy if they were not able to cough up sputum spontaneously (ICU-1, ICU-2, EICU, PICU, etc.). Traumatic secretions were collected from patients presenting with localized infection of skin wounds (Dept. of Hands Surgery, Dept. of Burns, etc.). Clean urine was collected from those presenting with symptoms of urinary tract infection, and urine from catheters was collected from catheterized patients for bacterial culture.

Induction of *P. aeruginosa* resistance to cefoperazone sodium/sulbactam sodium *in vitro*

M-H broth medium containing cefoperazone sodium/sulbactam sodium was mixed in sterile tubes at a 50% minimum inhibitory concentration (MIC) with bacteriophage solution (0.1 mL containing 1×10^7 CFU/mL). Nine days later, the final screened *P. aeruginosa* PAO1 (PAO1) strain resisted 50% MIC. The concentrations of the medium containing the drugs were changed to 100% MIC and 200% MIC, then all experiments were repeated two and three times. The final screen isolated drug-resistant PAO1 at MIC×100% and MIC×200%.

Protein detection

Bacteria were ultrasonicated at 120 W and lysed. Protein concentrations were adjusted to 1.5 μg/μL with buffer (50 mM NaAc, pH 4.0), then 100 μL was added to the spiked wells of a chip. Binding buffer (200 μL) was added to the spiked wells, then plates were shaken at 300 rpm for 5 min at 24°C. The wells were rinsed with 200 μL of 1-mM HEPES water (pH 4.0), then dried naturally. Thereafter, SPA (0.5 μL) was added to the spiked wells on the chip, then proteins were detected.

Data acquisition

The protein chips were analyzed by mass spectrometry using a PBS II C protein chip reader. The mass spectrometer was calibrated using a ProteinChip NP20 array (Bio-Rad Laboratories Inc., Hercules, CA, USA.)

The analytical parameters were as follows: maximum molecular weight, 50,000 Da; protein molecular weight range. 2,000–20,000 Da; the working laser power, 185; sensitivity of detection, 5. Mass spectrometry data acquired at 1×10^9 Hz/s were plotted.

Gene chip hybridization

A bacterial gDNA library (1 μL) was diluted to 1 ng/μL, then fragment sizes and concentrations of the library were determine using Agilent® High Sensitivity DNA Kits (Agilent Technologies Inc., Santa Clara, CA, USA). The library molar concentration (pM) was calculated as: library concentration (ng/μL) × 1.515 × 1,000 km library length. The library was diluted to 100 pM, the directly applied for template preparation and hybridization.

Reference sequences for two sets of *P. aeruginosa* sequences

Genomic base sequences of standard PAO1 strains were retrieved from NCBI as references for comparison with mutated base sites. The locations of mutated sites were determined based on the gene information annotated by the genomic base sequence of the standard PAO1 strain.

Statistical analysis

Data were statistically analyzed using SPSS 19.0, and data collected by ProteinChip® Software 3.2 (Bio-Rad) were analyzed using Biomarker Wizard software (Ciphergen Biosystems, Fremont, CA, USA). Mass spectrometry data were analyzed using t-tests, with $P < 0.05$, indicating a statistically significant difference.

RESULTS

Detection rates of *P. aeruginosa* of sampled obtained between 2013-2015

We detected 1,306 *P. aeruginosa* strains during 2013 at the First Affiliated Hospital of Guangxi Medical University, of which 684 (52.4%), 345 (26.4%) and 345 (21.2%) were sensitive, moderately sensitive, and resistant to cefoperazone sodium/sulbactam sodium, respectively. The *P. aeruginosa* strains were the most prevalent in the Department of Burns, the Department of Chinese Medicine, and intensive care unit (ICU)-1. Among the 1,149 *P. aeruginosa* strains detected in 2014, 621 (54%), 282 (24.6%), and 246 (21.4%) were sensitive, moderately sensitive, and resistant to cefoperazone sodium/sulbactam sodium, respectively, and were the most prevalent in the Department of Chinese Medicine, the Department of Burns, and ICU-1. We detected 1,101 P. aeruginosa strains in 2015, among which 585 (53.1%), 245 (22.3%) and 271 (24.6%) 1101) were sensitive, moderately sensitive, and resistant to cefoperazone sodium/sulbactam sodium, respectively and were most prevalent in the Department of Chinese Medicine, ICU-1, and the Department of Burns (Figure 1).

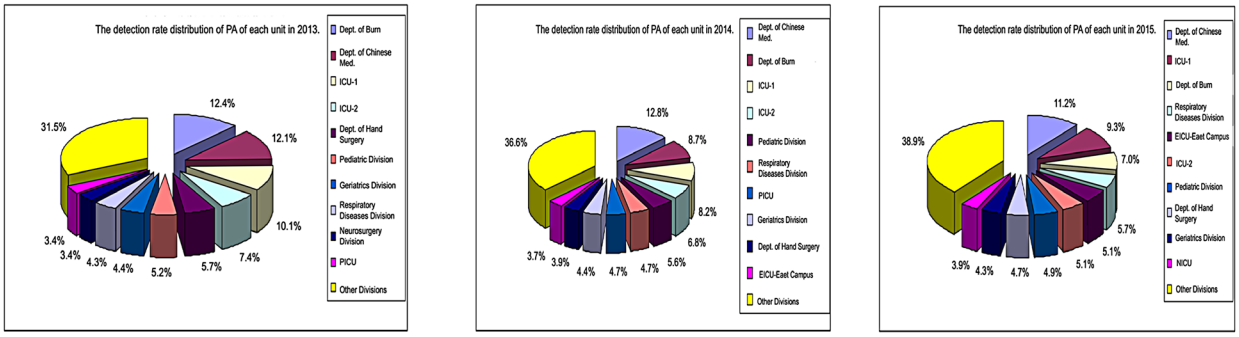

Figure 1. Detection rates of *P. aeruginosa* in hospital departments between 2013 and 2015. We detected 3,556 strains of *P. aeruginosa* between 2013–2015, mostly in Departments of Burns and Chinese Medicine, and ICU-1.

Analysis of *P. aeruginosa* hospital-wide resistance to cefoperazone sodium/sulbactam sodium from 2013–2015

As the figure 2 showed that the trend in hospital-wide resistant *P. aeruginosa* strains slightly increased (21.2%, 21.5%, and 24.6%, respectively) between 2013 and 2015. The resistance rates of P. aeruginosa strains were relatively high in the electronic ICU (EICU), Neurosurgery Division, ICU-1, ICU-2, the Department of Chinese Medicine, the Department of Burns, and the pediatric (PICU), and neonatal (NICU) ICUs.

The resistance rate of *P. aeruginosa* strains detected in the EICU during 2013 peaked at 61.9%, but decreased annually to almost the hospital-wide prevalence (24.6%) by 2015. These rates in strains detected increased from 2.3% to 37.2% between 2013 and 2015 in the PICU, but did not significantly differ in the Neurosurgery Division, ICU-1, ICU-2, and the Department of Chinese Medicine between 2013 and 2015. The detection rates of *P. aeruginosa* were below the hospital-wide average in the Pediatric Division, the Respiratory Diseases Division, the Department of Hand Surgery, and the rate in the Geriatrics Division was close to the hospital-wide level.

Protein fingerprint analysis by mass spectrometry

A total of 242 protein peaks were detected in PAO1 bacteriophage proteins in the induced resistant PAO1 in the DR and WT groups (Fig. 3a), of which 41 were differentially expressed ($P < 0.05$). The expression of 26 and 15 proteins was much higher in the PAO1, than the WT group (Fig. 3b).

Comparison of base sequences with standard strains in NCBI database

The WT group contained 679 mutant loci. Figure 4 shows that the main peak among library fragments was 330 bp, and the molar concentration was 2,999 pmol/L. Analysis of single nucleotide polymorphisms (SNPs) using Freebayes software (Biomatters Ltd., Auckland, NZ) revealed 141 (5%) and 792 (15.6%) common mutant loci in three WT (ID numbers: 011, 012, 013) and three DR (ID numbers: 014, 015, and 016) samples with Q (quality) values ≥ 20. The DR group contained 679 loci. Regardless of the numbers of common mutation sites or the total number of mutation sites, the DR group contained significantly more sites than the WT group ($P < 0.05$; Figure 5). The mutant loci were sorted by Q values, then the top 50 were queried using the NCBI bioinformatics database, which revealed found 42 of them. Table I shows biological information about the 42 mutated loci, which were located in 35 genes. Among genes with multi-locus mutations, the ID880777 gene encoded SPA. The proteins encoded by ID882269, ID880742, and ID878199 have not yet been deciphered.

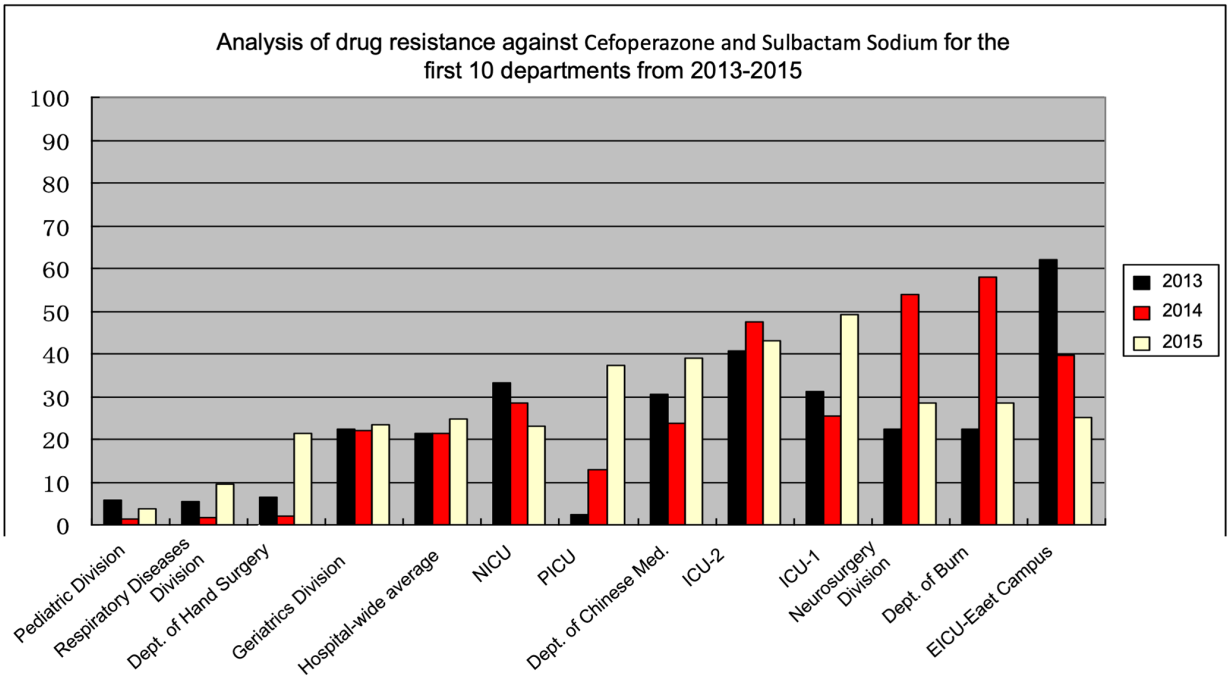

Figure 2 Analysis of *P. aeruginosa* resistance to cefoperazone sodium/sulbactam sodium of hospital-wide during 2013–2015. Hospital-wide resistance of *P. aeruginosa* strains to cefoperazone sodium/sulbactam sodium slightly increased during 2013–2015 (resistance rates: 21.2%, 21.5%, and 24.6%, respectively). Compared with hospital-wide resistant *P. aeruginosa* strains, EICU, Neurosurgery Division, ICU-1, ICU-2, Department of Chinese Medicine, Department of Burns, PICU, and NICU had relatively higher rates.

Discussion

Pseudomonas aeruginosa infections are usually treated with β-lactam or β-lactam (or penicillin) antibacterial drugs combined with β-lactamase inhibitors (Meschiari et al. 2021). The classical β-lactams cefoperazone sodium/sulbactam sodium are regarded as important and effective for treating of drug-resistant *P. aeruginosa* infections (Wei et al. 2020). However, the widespread clinical application of such drugs has led to increased resistance rates, and *P. aeruginosa* develops resistance to β-lactam antibacterial drugs through various mechanisms (Nowikiewicz et al. 2020). Antibacterial β-lactams interfere with the synthesis of *P. aeruginosa* cell wall by inhibiting penicillin-binding protein (PBP), thus killing these bacteria (Arca-Suarez et al. 2020). An altered PBP structure and quantity comprise important mechanisms of *P. resistance* to β-lactam antibacterial drugs. Jeyanathan et al. (2021) found that the MIC of the PBP-2 mutant strain of PAO1 to various β-lactam antimicrobial drugs was reduced compared with the WT strain, and that the mutant strain had lost the rod-like appearance of WT PAO1, and appeared spherical. Ceftazidime inhibits *P. aeruginosa* PBP-3. Zang et al. (2021) investigated the effect of ceftazidime on the function of PBP3 in *P. aeruginosa* and found that ceftazidime initially affects transcription, then induces expression of the DNA polymerase, DinB, then PBP-3 inhibitors upregulate the expression of several drug resistance-related genes (Calum et al. 2020). While β-lactam antimicrobial drugs act on *P. aeruginosa* through PBP, *P. aeruginosa* also constantly regulates PBP synthesis and simultaneously initiates more mechanisms to adapt to the environment where the drug is located (Abootaleb et al. 2021).

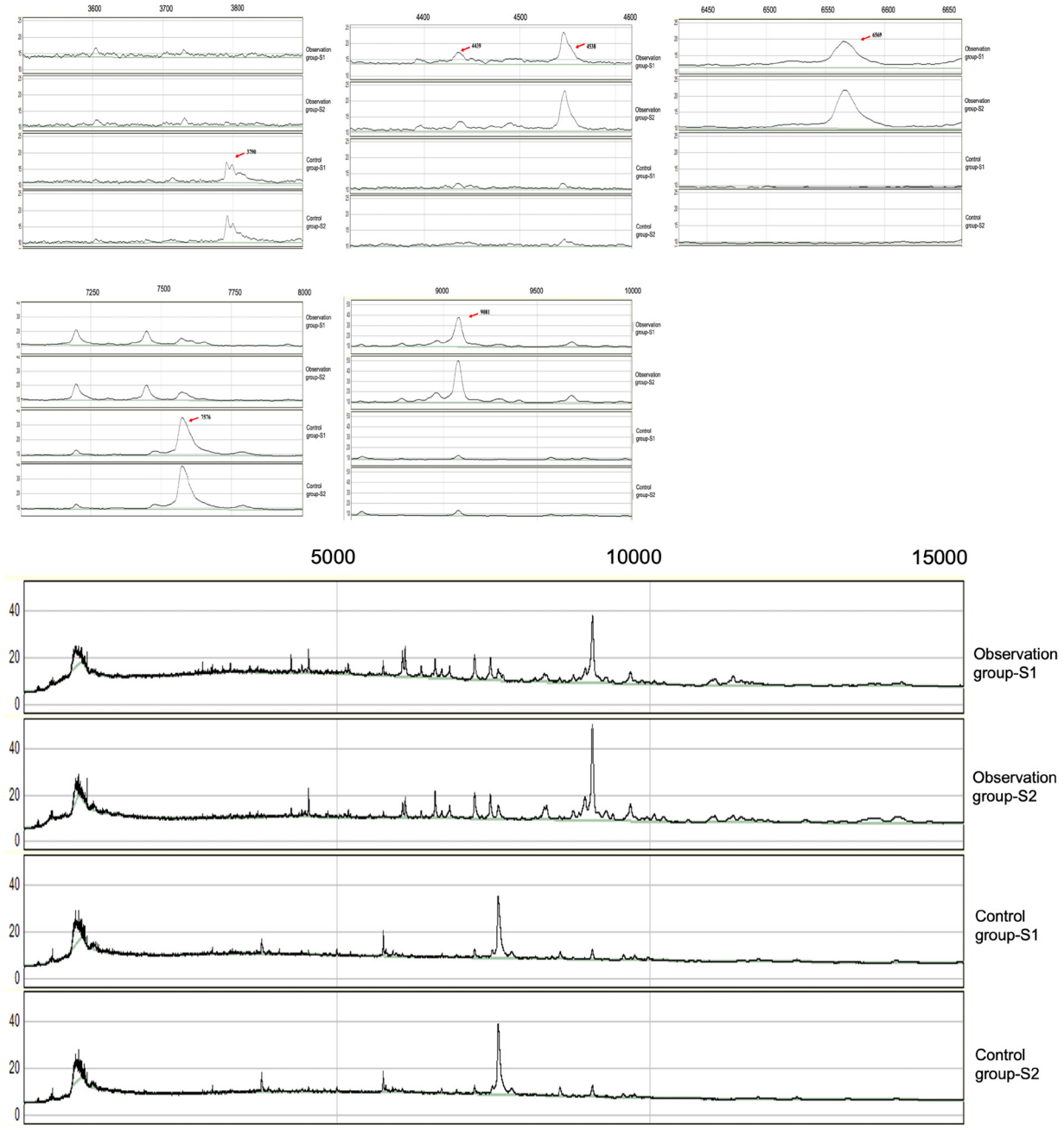

Figure 3. Protein fingerprint analysis by mass spectrometry. a) We detected 242 protein peaks for PAO1 bacteriophage proteins in induced drug-resistant (DR) PAO1 (DR) and wild-type (WT) groups ($P < 0.05$). b) Expression of 26 and 15 proteins was upregulated and downloaded, respectively, in DR, compared with WT PAO1.

These processes occur whether by mutation of genes or by changes in proteins that we directly identified, and ultimately their adaptation to the drug environment involves numerous active proteins.

The genomic bioinformatics results revealed that *P. aeruginosa* has at least 12 active efflux systems (efflux pumps), seven of which have been identified (Babich et al. 2021). The efflux pumps of *P. aeruginosa* consist of an inner membrane transporter protein, an outer membrane efflux

protein, and a membrane fusion protein. The specificity of the substrate against the inner membrane transporter protein has been determined. The outer membrane efflux protein consists of a membrane fusion protein that links the inner membrane transporter protein to an outer membrane exocytosis protein, and an exocytosis pump comprising these three proteins discharges the antimicrobial drug outside to reduce the drug concentration (Blomquist & Nix 2021). We identified the expression of 41 differentially expressed proteins between the DR and WT groups; 26 and 15 of ~ 1,235 and 3,790 Da, respectively, were upregulated and downregulated in PAO1. This suggested that the resistance of PAO1 to cefoperazone sodium/sulbactam sodium is mediated by many mechanisms and that these differentially expressed proteins function in identified resistance mechanisms. However, others await investigation. Here, we induced PAO1 resistance to cefoperazone sodium/sulbactam sodium *in vitro*. We compared the base sequences of genomic DNA between the DR and WT groups, and with those of standard strains within the NCBI database. We found 679 mutant loci only in the DR group, and among high-quality mutant loci with top 50Q values, 42 genes with detailed about information mutant loci were retrieved from the NCBI database. These 42 mutant loci were located in 35 protein-encoding genes, of which 22 have been deciphered. The ID880777 gene encodes phage SPA. Thirteen genes, including ID882269, ID880742, and ID878199, encode proteins that have not been deciphered. The present study found that the gene 606739 with a shift mutation encodes a CdhR family transcriptional regulator, which can directly affect gene expression and acts as a negative feedback regulator of population effects. Gene 882583 with a shift mutation (wbpM), encodes a nucleotide sugar isomerase (dehydratase) involved in the metabolism of bacterial cells or organelle membranes. Gene 881860, with a shift mutation (fliM), encodes the metabolism of protein flagella. Tamma et al. (2021) reported that the expression of both genes was downregulated when *P. aeruginosa* was exposed to ciprofloxacin, suggesting that these two genes are involved in the resistance of *P. aeruginosa* to antimicrobial drugs. The gene 879479 has a shift mutation that was sequenced in 1986 and its encoded product is the tryptophan synthase alpha (trpA) subunit (Palmer). The expression of trpA is downregulated when *P. aeruginosa* is cultured not only in the sputum of patients with cystic pulmonary fibrosis, but also in a nitrate-free environment (Filiatrault MJ). Gene 880777 has base substitution mutations at three loci, and the encoded protein is phage coat protein A. The mutations are mostly in the enzymes and transcriptional regulators required for metabolism, suggesting that the process of *P. aeruginosa* resistance to antimicrobial drugs is a metabolic alteration that requires multiple mechanisms. Some of the products encoded by genes at the mutated loci identified in this study have been deciphered, but their roles remain unclear and await elucidation by further studies. Proteins that have not yet been deciphered should be investigated in depth together with proteomics techniques. Although the genomic DNA of P. *aeruginosa* is relatively simple, its regulatory mechanism is complex, especially during resistance to antimicrobial drugs, and it can adapt well to various unfavorable environments through precisely regulating the expression of its genetic information. Genomic studies of *P. aeruginosa* drug resistance processes will help to further clarify the underlying mechanisms and make way for the selection of action sites for treatment with novel antibacterial drugs.

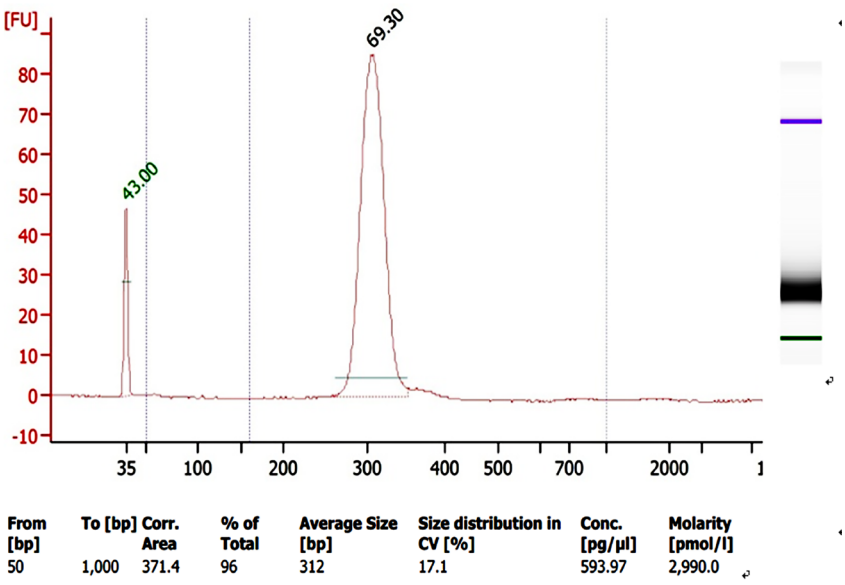

Figure 4. Distribution of library fragment peak. The WT group contained 679 mutant loci. The Figure shows that the main peak among library fragments was 330 bp, and the molar concentration was 2,999 pmol/L.

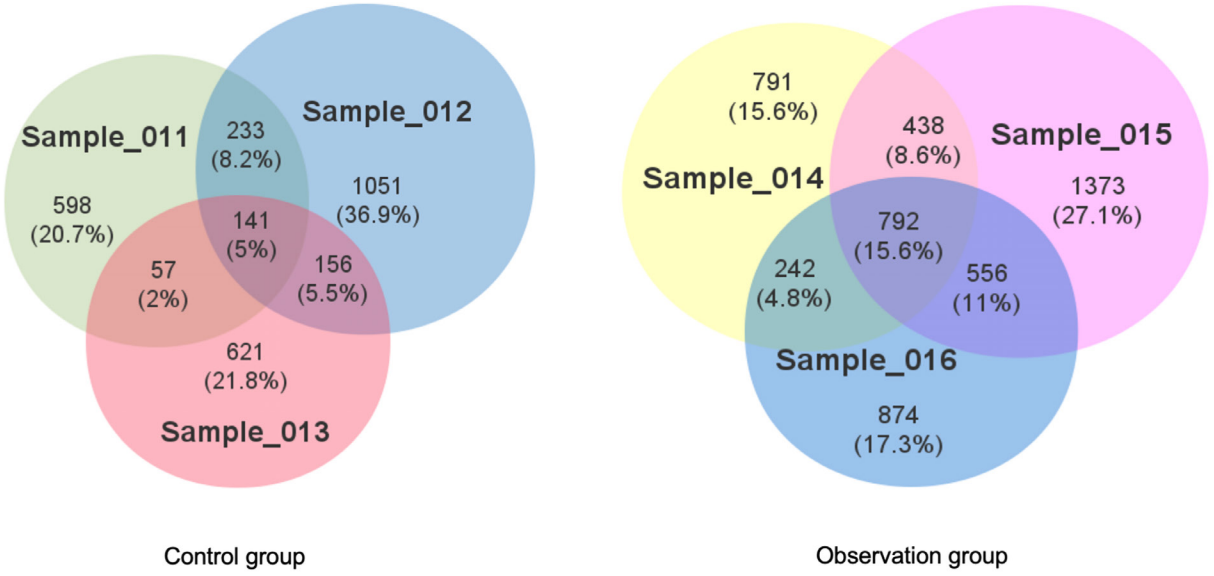

Figure 5. Mutant loci distribution in WT and DR *P. aeruginosa* samples. Analysis of single nucleotide polymorphisms (SNPs) using Freebayes software (Biomatters Ltd., Auckland, NZ) revealed 141 (5%) and 792 (15.6%) common mutant loci in three WT (ID numbers: 011, 012, 013) and three DR (ID numbers: 014, 015, and 016) samples with Q (quality) values ≥ 20. The DR group contained 679 loci. Regardless of the numbers of common mutation sites or the total number of mutation sites, the DR group contained significantly more sites than the WT group ($P < 0.05$).

Table I. Mutated genes in *P. aeruginosa* are resistant to cefoperazone sodium/sulbactam sodium.

Gene ID	Name	Type of mutation	Sequence (Control group)	Sequence (Observation group)
880778	coaB	Substitution mutation	5'-TCCTGGCCGTTGCCGGCCTGA-3'	5'-TCCTGGCCGTCGCCGGCCTGA-3'
878952	lipB	Frameshift mutation	5'-TCCCGGCGTAGCCGCAGGGAT-3'	5'-TCCCGGCGTAGCGCAGGGAT-3'
879090	PA3789	Frameshift mutation	5'-TGGCGCGGATCGTCGGGGAAC-3'	5'-TGGCGCGGATCTCGGGGAAC-3'
880817	tyrZ	Frameshift mutation	5'-CTTCCGCAAGGACGTCGAGGC-3'	5'-CTTCCGCAAGGCGTCGAGGC-3'
881949	PA1576	Substitution mutation	5'-GCAAGGGCGCGGCCCAGTCCT-3'	5'-GCAAGGGCGCCGCCCAGTCCT-3'
877727	mtr	Substitution mutation	5'-TGAGCAACGCTTTCAGCGAAT-3'	5'-TGAGCAACGCGTTCAGCGAAT-3'
878655	corA	Frameshift mutation	5'-TGGCGGATGCAGCGGTTCTCG-3'	5'-TGGCGGATGCACGGTTCTCG-3'
880657	betC	Frameshift mutation	5'-GAAGTCCAGCTGGTTGGTGCG-3'	5'-GAAGTCCAGCTGTTGGTGCG-3'
881860	fliM	Frameshift mutation	5'-TGGCCAACATCGTCAGTCCCA-3'	5'-TGGCCAACATCTCAGTCCCA-3'
878041	PA0793	Frameshift mutation	5'-CCGGCTGGCTGCTCTTCGAGA-3'	5'-CCGGCTGGCTGTCTTCGAGA-3'
880269	PA0726	Substitution mutation	5'-GCCAGCATCGTTCCGGCAGCG-3'	5'-GCCAGCATCGCTCCGGCAGCG-3'
880742	PA0727	Substitution mutation	5'-CGACCCTGAACGGGGATCCGT-3'	5'-CGACCCTGAATGGGGATCCGT-3'
878199	PA5475	Frameshift mutation	5'-AGACCCTGGTAGTCGTCGGCG-3'	5'-AGACCCTGGTATCGTCGGCG-3'
882536	trkH	Frameshift mutation	5'-TCCTGCCGGGACGTCCCGAGC-3'	5'-TCCTGCCGGGAGTCCCGAGC-3'
880269	PA0726	Substitution mutation	5'-GGCTGCTCCAACGCGTCCTGC-3'	5'-GGCTGCTCCAGCGCGTCCTGC-3'
880742	PA0727	Substitution mutation	5'-TGGAAACGGTCTGGCGGCTCG-3'	5'-TGGAAACGGTGTGGCGGCTCG-3'
878199	PA5475	Frameshift mutation	5'-TCTGGCGGAAGCCGTTGCGCC-3'	5'-TCTGGCGGAAGCGTTGCGCG-3'
882583	wbpM	Frameshift mutation	5'-TGCACAGCAGAGTACCAGTCT-3'	5'-TGCACAGCAGATACCAGTCT-3'
880269	PA0726	Substitution mutation	5'-TCCGCAGACGCACCGGCCAGG-3'	5'-TCCGCAGACGTACCGGCCAGG-3'
880777	PA0724	Substitution mutation	5'-CAGTTGCTATCTGGTCAAAGG-3'	5'-CAGTTGCTATTTGGTCAAAGG-3'
880749	cdhR	Frameshift mutation	5'-AGCCATCGACGCCTTCCAGGA-3'	5'-AGCCATCGACGCTTCCAGGA-3'
879629	PA5078	Frameshift mutation	5'-ACGAAGCGGTAGGCGCCGGTG-3'	5'-ACGAAGCGGTAGCGCCGGTG-3'
880269	PA0726	Substitution mutation	5'-CGGGAGGCAGCCCGGCGCGGT-3'	5'-CGGGAGGCAGGCCGGCGCGGT-3'
880777	PA0724	Substitution mutation	5'-GCGGTGCCGATTCCTACACCT-3'	5'-GCGGTGCCGACTCCTACACCT-3'
880393	PA0446	Frameshift mutation	5'-GGCGAACTTGCGGAACTGGCT-3'	5'-GGCGAACTTGCGAACTGGCT-3'
879479	trpA	Frameshift mutation	5'-TTCGCCTCGGCGATGAAGCGT-3'	5'-TTCGCCTCGGCATGAAGCGT-3'
879244	dnaN	Frameshift mutation	5'-CCGAGCAGGACGGCGAAGTCG-3'	5'-CCGAGCAGGACGCGAAGTCG-3'
880777	PA0724	Substitution mutation	5'-CGCTGCTGCTGATGGCCGCGC-3'	5'-CGCTGCTGCTAATGGCCGCGC-3'
879590	PA5545	Frameshift mutation	5'-CGTCGTAGGTGCCGGCGGGAA-3'	5'-CGTCGTAGGTGCGGCGGGAA-3'
880803	PA0690	Frameshift mutation	5'-CCGGCGAGGTCGGCGCGCTGG-3'	5'-CCGGCGAGGTCGCGCGCTGG-3'
881861	rpsC	Frameshift mutation	5'-GCAGGGGCACACGACCTTCGC-3'	5'-GCAGGGGCACAGACCTTCGC-3'
881508	PA4326	Frameshift mutation	5'-CTCCAGCCGCGCCTTGGCACG-3'	5'-CTCCAGCCGCGCTTGGCACG-3'
882154	PA0644	Frameshift mutation	5'-TGACAGGGAACGTCTCAGCGA-3'	5'-TGACAGGGAACTCTCAGCGA-3'
878727	hpaA	Frameshift mutation	5'-CGTCAGCGGCGCCAAGGTGGT-3'	5'-CGTCAGCGGCGCAAGGTGGT-3'

Table I. Continuation.

878660	PA5507	Frameshift mutation	5'-TGCCTGGAAGCGATCAAGGGC-3'	5'-CGTCAGCGGCGCAAGGTGGT-3'
878952	lipB	Frameshift mutation	5'-CCGGCTACGACGGCGACCGCG-3'	5'-CCGGCTACGACGCGACCGCG-3'
881415	PA4675	Frameshift mutation	5'-GCTGACGAAGTCGATCTGCTT-3'	5'-GCTGACGAAGTGATCTGCTT-3'
881340	PA4391	Frameshift mutation	5'-GCCGCCATCGACGATCCGCAC-3'	5'-GCCGCCATCGAGATCCGCAC-3'
881700	PA4746	Frameshift mutation	5'-CACGGAGAATGCCTTGGTAGT-3'	5'-CACGGAGAATGCTTGGTAGT-3'
880435	PA5475	Frameshift mutation	5'-GGTGGAACAGCGTACCTGGAA-3'	5'-GGTGGAACAGCTACCTGGAA-3'
882067	nadB	Frameshift mutation	5'-AGCCACTGGATCGCTTCGCGG-3'	5'-AGCCACTGGATGCTTCGCGG-3'
878522	PA4913	Frameshift mutation	5'-CTCGTCGTAGACCTCGGATGC-3'	5'-CTCGTCGTAGACTCGGATGC-3'

Acknowledgments

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