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# Identification and characterization of class 1 integrons among *Pseudomonas aeruginosa* isolates from patients in Zhenjiang, China

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**KEYWORDS**
*Pseudomonas aeruginosa*;
Integrons;
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**Summary**
*Objectives:* The role of integrons in the spread of antibiotic resistance has been well established. The aim of this study was to investigate the resistance profiles of *Pseudomonas aeruginosa* isolated from patients in Zhenjiang to 13 antibiotics, and to identify the structure and dissemination of class 1 integrons.
*Methods:* The Kirby—Bauer disk diffusion assay was used to determine the rate of *P. aeruginosa* resistance. Class 1 integrons from multidrug-resistant isolates were amplified by PCR, and their PCR products were sequenced. We also analyzed the integron structures containing the same gene cassettes by restriction fragment length polymorphism (RFLP). Isolates were genotyped by pulsed-field gel electrophoresis (PFGE).
*Results:* The resistance rates were between 29.6% and 90.1%. The prevalence of class 1 integrons was 38.0%. These integrons included five gene cassettes (*aadB*, *aac6-II*, *blaPSE-1*, *dfrA17*, and *aadA5*). The *dfrA17* and *aadA5* gene cassettes were found most often.
*Conclusions:* Class 1 integrons were found to be widespread in *P. aeruginosa* isolated from clinical samples in the Zhenjiang area of China. The antibiotic resistance rates in class 1 integron-positive strains of *P. aeruginosa* were noticeably higher than those in class 1 integron-negative strains. PFGE showed that particular clones were circulating among patients.
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## Introduction

The heavy selective pressure of antibiotics has promoted the rapid development of bacterial resistance. This bacterial resistance has become a severe and universal problem, leading to ineffective clinical treatment and increased economic burden, and also directly threatens the life of the patient.

*Pseudomonas aeruginosa* is an important opportunistic nosocomial pathogen, particularly evident in hospital-acquired pneumonia, especially in immunocompromised patients and those with a tracheal cannula, tracheotomy, or under mechanical ventilation.[1] Its resistance mechanism is extremely complicated. Generally, bacterial resistance is caused by chromosomal gene mutations or plasmid acquisition, however, recently, more and more bacterial resistance has been found to be associated with integrons.[2] Integrons, which consist of a 5′ conserved segment, a 3′ conserved segment, and a variable region between the two conserved segments, have been found to be mobilizable elements that capture external drug resistance gene cassettes and mediate bacterial resistance or multidrug resistance. These have caused the rapid spread of resistance in bacteria, especially among the Gram-negative.[3]

To date, four classes of integrons have been described in Gram-negative bacterial isolates, however class 1 integrons have been found to be the most prevalent in clinical isolates, carrying single or multiple gene cassettes, which confer resistance to aminoglycosides, β-lactams, chloramphenicol, carbapenems, and macrolides.[4–7]

In this study, we investigated the prevalence of resistance in 71 *P. aeruginosa* isolates to 13 antibiotics, and sought to identify the characteristics of class 1 integrons. At the same time, we also analyzed whether a particular clone was circulating among patients.

## Materials and methods

### Bacterial isolates

From April 2006 to March 2007, 71 *P. aeruginosa* isolates were collected from different patients hospitalized at the Affiliated People's Hospital of Jiangsu University, a hospital with in excess of 900 beds. Identification of isolates was carried out using the VITEK32 GNI card (bioMérieux, Hazelwood, MO, USA). Bacterial isolates were stored as suspensions in a 10% (wt/vol) sterilized milk solution containing 10% (vol/vol) glycerol at −20 °C until tests were performed.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined according to the Clinical and Laboratory Standards Institute (CLSI) using the Kirby–Bauer disk diffusion assay on Mueller–Hinton agar (Oxoid, UK).[8] The susceptibility profiles were determined for 13 antibiotics: piperacillin (PIP, 100 μg), piperacillin–tazobactam (TZP, 100/10 μg), ceftriaxone (CRO, 30 μg), cefepime (FEP, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), gentamicin (GEN, 10 μg), amikacin (AMK, 30 μg), tobramycin (TOB, 10 μg), levofloxacin (LVX, 5 μg), ciprofloxacin (CIP, 5 μg), imipenem (IPM, 10 μg), and trimethoprim–sulfamethoxazole (SXT, 12.5/23.7 μg). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as controls.

### DNA extraction

Genomic DNA used as template was obtained from bacterial suspensions grown overnight in Luria broth with shaking, suspended in 100 μl of sterile water, and boiled for 10 min.[9]

### PCR amplifications and sequencing analysis

PCR was used to amplify the class 1 integrons, and the primers were designed as follows: forward primer: 5′-GGCATCCAAGCAGCAAG-3′, and reverse primer: 5′-AAGCAGACTTGACCTGA-3′.[10] Primers were synthesized by Shanghai GeneCore BioTechnologies Co., Ltd. A Mastercycler instrument (Eppendorf, Hamburg, Germany) was used with the following reaction conditions: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 2 min, and finally, 72 °C for 10 min. The amplicons were separated on 1% agarose gel prepared in TAE buffer and visualized using ultraviolet light after staining with ethidium bromide. PCR positive products were directly sequenced on both strands by the primer walking sequence strategy using the BigDye Terminator Cycle Sequencing Kit on an ABI PRISM 3730 automated DNA sequencer. The sequences were aligned in GenBank (http://www.ncbi.nlm.nih.gov/BLAST).

### PCR-restriction fragment length polymorphism (RFLP)

To determine whether different isolates carried identical integrons, the amplicons of similar sizes were compared by RFLP typing using the restriction endonucleases HinfI and EcoRV. If the amplicons from two strains yielded the same RFLP pattern, the two integrons were considered to be identical. If the PCR products contained a different RFLP pattern, the new product was sequenced as well.

### Pulsed-field gel electrophoresis (PFGE)

*P. aeruginosa* isolates carrying class 1 integrons were genotyped by PFGE with the restriction enzyme SpeI. DNA-PFGE marker (Amersham) was used as the size marker. The DNA fragments were separated on 1.0% agarose gels in 0.5× Tris–borate–EDTA buffer with a CHEF Mapper system (Bio-Rad Laboratories, Hercules, CA, USA) at 6 V/cm for 20 h. Clonal relatedness based on the PFGE patterns was interpreted according to the criteria proposed by Tenover et al.[11]

### Nucleotide sequence accession numbers

GenBank accession numbers are as follows: *aadB–aac6-II–pse-1*, **DQ266447**; *dfrA17–aadA5*, **DQ838665**; *aac6-II*, **EU723083**.

## Results

### Antimicrobial susceptibility of *P. aeruginosa* isolates

Susceptibility testing of the 71 *P. aeruginosa* isolates showed that all the isolates were resistant to at least one of the