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Nosocomial spread of class 1 integron-carrying extensively drug-resistant *Pseudomonas aeruginosa* isolates in a Thai hospital

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ABSTRACT

Fifty non-duplicate multiresistant isolates of *Pseudomonas aeruginosa* from a regional hospital in Northern Thailand were investigated for their antimicrobial susceptibility, presence of class 1 integrons and arrangement of gene cassettes as well as their genetic relationships. All but one isolate were classified as extensively drug-resistant *P. aeruginosa* (XDR-PA). Forty-one isolates (82%) were found to carry class 1 integrons. Amplification of the variable regions of class 1 integrons revealed seven diverse bands ranging in size from 0.7 kb to 7.0 kb. Sequence analysis of class 1 integron variable regions revealed the presence of several gene cassettes associated with resistance to aminoglycosides (*aac*, *aad* and *aph*), including the *aac(3)-Ic* cassette reported for the first time in Thailand. Gene cassettes encoding resistance to chloramphenicol (*cmlA*), β-lactams (bla_{PSE}, bla_{OXA} and bla_{VEB}) and rifampicin (*arr*) were found. The putative small multidrug resistance protein (*smr*) and an open-reading frame with unknown function (*orfD*) were also detected. The *aadA6–orfD* cassette array was the most common integron found in this study. Integron-positive isolates had higher frequencies of antimicrobial resistance than isolates lacking integrons. Pulsed-field gel electrophoresis (PFGE) demonstrated the occurrence of horizontal gene transfer. Interestingly, a large number of XDR-PA isolates carrying identical integrons clearly exhibited the same PFGE pattern, indicating nosocomial spread of these isolates. The presence of XDR-PA carrying class 1 integrons is implicated in the possible spread of drug-resistant organisms, therefore screening for integron-positive *P. aeruginosa* might be necessary for protection against nosocomial infection.

1. Introduction

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that is an important cause of various life-threatening infections associated with hospitalisation. Nosocomial infections caused by *P. aeruginosa* are often difficult to treat because this organism displays resistance to all, or almost all, commercially available antibiotics. Furthermore, the ability of *P. aeruginosa* to live in many diverse environmental conditions and to survive on minimal nutritional requirements has caused difficulties in the control and eradication of this pathogen [1]. Infections caused by drug-resistant *P. aeruginosa* are associated with significant increases in morbidity and mortality. The rapid increase of multidrug-resistant *P. aeruginosa* (MDR-PA) during recent years has become a serious therapeutic problem worldwide. According to the National Antimicrobial Resistance Surveillance, Thailand, the rate of ceftazidime resistance in *P. aeruginosa* was relatively high during the 6-year surveillance period (2000–2005) [2]. Recently, studies in several hospitals across Thailand showed a high prevalence of carbapenem resistance in *P. aeruginosa* [3,4].

The mechanisms of resistance in *P. aeruginosa* have been extensively investigated. These are enzyme production, target mutation, outer membrane impermeability and efflux pump overexpression [1]. However, resistance determinants associated with the presence of integrons have increasingly been reported [5]. Integrons, natural genetic elements capable of capturing gene cassettes by site-specific recombination, are known to contribute to the development of multidrug resistance among several Gram-negative bacteria. Integrons are frequently located on transmissible plasmids or transposons, which facilitate their transfer among bacterial populations [5]. Several reports have demonstrated that integrons play an important role in the carriage and spread of antibiotic resistance genes among bacteria [6–8]. Many different classes of integrons have been identified, however class 1 integrons are the most commonly found in antibiotic-resistant clinical isolates of Gram-negative bacteria including *P. aeruginosa*. Typically, a class

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1 integron is composed of a 5′-conserved segment (5′CS) including an *intI1* gene coding for a site-specific integrase, and a 3′CS including the *qacEΔ1* and *sul1* genes encoding resistance to quaternary ammonium compounds and sulfonamides, respectively. Between 5′CS and 3′CS is found an internal variable region with one or more gene cassettes. Gene cassettes are mobile units composed of a gene, most often an antibiotic resistance gene, and a recombination site, the 59-base element. Several resistance gene cassettes, such as aminoglycoside-modifying enzymes (*aac*, *aad* and *aphA*) and extended spectrum β-lactamases (*bla*), are associated with class 1 integrons in *P. aeruginosa* that confer resistance to antimicrobial agents [5].

Several studies have shown that integrons are associated with multidrug resistance in *P. aeruginosa* isolates [7–10]. Although resistance to antimicrobial agents in *P. aeruginosa* is common in Thailand, published reports on the prevalence of integrons in this organism are relatively limited. In 2002, the bla_{VEB-1}-carrying class 1 integron was found in ceftazidime-resistant *P. aeruginosa* isolates collected from a university hospital in Thailand [6]. Recently, the high prevalence of class 1 integrons with a variety of gene cassettes, including bla_{IMP-14}, bla_{IMP-15} and bla_{VIM-2}, in MDR-PA isolates from Thai hospitals has been reported [11,12]. In Buddhachinaraj Hospital (Phitsanulok, Thailand), clinical isolates of *P. aeruginosa* reveal a high frequency of multidrug resistance. We previously reported the presence of a bla_{IMP-1}-carrying class 1 integron in two carbapenem-resistant *P. aeruginosa* isolates [13]. This has led to the speculation that there might be more antibiotic resistance genes that are associated with integrons among *P. aeruginosa* isolates in this hospital. Hence, this study was conducted to investigate the prevalence of class 1 integrons and their associated resistance gene cassettes of multiple antibiotic-resistant *P. aeruginosa* isolates. The genetic relationship among the integron-positive isolates was also determined.

2. Materials and methods

2.1. Bacterial isolates

Between November 2007 and April 2008, 50 non-duplicate *P. aeruginosa* isolates were collected from various clinical materials at Buddhachinaraj Hospital, which is a 1000-bed teaching hospital in Phitsanulok, Northern Thailand. Isolates were recovered from hospitalised patients (sputum, 34; pus, 7; urine, 1; blood, 1; fluid, 1; wound, 2; and other, 4). Isolates were identified as *P. aeruginosa* using standard biochemical tests. All isolates were grown on *Pseudomonas* agar with C—N selective supplement (Oxoid Ltd., Basingstoke, UK) throughout the study.

2.2. Antimicrobial susceptibility testing

Susceptibility testing was performed by the disk diffusion method on Mueller–Hinton agar (Becton Dickinson and Co., Sparks, MD) and was interpreted in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [14]. Eighteen antibiotic discs from four classes of antimicrobial agents including β-lactams (ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefoperazone, cefotaxime, ceftriaxone, ceftazidime, cefepime, imipenem, meropenem, aztreonam), fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin), aminoglycosides (gentamicin, amikacin) and lipopeptide (colistin) were used. All antibiotic discs were purchased from Oxoid Ltd. Isolates showing intermediate results were considered to be resistant. Resistance patterns in *P. aeruginosa* isolates were classified according to the recently published proposed interim definitions [15]. An isolate was defined as MDR-PA if it was non-susceptible to at least one agent in three or more antipseudomonal antimicrobial categories, and as extensively drug-resistant *P. aeruginosa* (XDR-PA) if it was non-susceptible to at least one agent in all but two or less antipseudomonal antimicrobial categories.

Table 1
Primer sequences used for PCR amplification of class 1 integrons.

Primer	Sequence (5′ → 3′)	Estimated product size (bp)	Reference
Int1F	GCATCCTCGGTTTTCTGG	457	[16]
Int1R	GGTGTGGCGGGCTTCGTG		
qacEΔ1F	ATCGCAATAGTTGGCGAAGT	236	[17]
qacEΔ1R	CAAGCTTTTGCCCATGAAGC		
sul1F	CTTCGATGAGAGCCGGCGGC	437	[17]
sul1R	GCAAGGCGGAAACCCGCGCC		
In5′CS	GGCATCCAAGCAGCAAG	Variable	[18]
In3′CS	AAGCAGACTTGACCTGA		

2.3. Detection of integrons and gene cassettes

All isolates were screened for the presence of class 1 integrons by PCR using specific primers located on an integrase gene, *intI1* (Table 1). All of the *intI1*-positive isolates were investigated for the presence of typical 3′CS using primers for *qacEΔ1* and *sul1* genes (Table 1). Amplification was performed in a 50 μL mixture containing 10 μM of each primer, 1.5 mM $MgCl_2$, 200 μM dNTPs and 1 U Platinum *Taq* Polymerase together with its reaction buffer (Invitrogen, Carlsbad, CA). A portion of the bacterial colony was added to provide the DNA template. Cell lysis and DNA amplification were performed in a Hybaid PCR Sprint Thermal Cycler (Thermo Scientific, Basingstoke, UK) using the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 45 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. Class 1 integron variable regions were amplified using primers In5′CS and In3′CS (Table 1). PCR amplification was carried out as described above, with the exception that the PCR extension time was extended to 5 min. Amplicons were separated by agarose gel electrophoresis, stained with 0.5 μg/mL ethidium bromide and visualised using an ultraviolet transilluminator (Gel Documentation Systems; Bio-Rad Laboratories Inc., Hercules, CA).

2.4. Restriction fragment length polymorphisms (RFLPs)

PCR amplicons were digested by *Sma*I, *Eco*RI, *Sac*I and *Hind*III restriction enzymes (Fermentas, Hanover, MD) and the products were analysed by agarose gel electrophoresis. Amplicons with identical digested profiles were considered to contain the same gene cassettes. One representative of each RFLP type was randomly selected for DNA sequencing.

2.5. DNA sequencing and analysis of sequence data

Selected PCR products were analysed by DNA sequencing. The amplicons were purified using a DNA purification kit (GF-1 Nucleic Acid Extraction Kit; Vivantis Inc., Chino, CA) and were sent to a commercial facility for sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia). Sequences were compared with those available in the GenBank database using the BLAST algorithm available on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

2.6. Pulsed-field gel electrophoresis (PFGE)

Investigation of the genetic relationship among *P. aeruginosa* isolates was performed by PFGE according to Xiong et al. [19]. Briefly, isolates were grown overnight in Luria–Bertani broth