Analysis of integrons and associated gene cassettes of metallo-β-lactamase-positive *Pseudomonas aeruginosa* in Malaysia

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In this study, 90 non-replicate imipenem-resistant *Pseudomonas aeruginosa* (IRPA) Malaysian isolates collected between October 2005 and March 2008 were subjected to a screening test for detection of the integron and the gene cassette. Class 1 integrons were detected in 54 IRPA clinical isolates, whilst three isolates contained class 2 integrons. Analysis of the gene cassettes associated with the class 1 integrons showed the detection of *accC1* in isolates carrying bla_{IMP-7} and *aacA7* in isolates carrying bla_{VIM-2}. *aadA6* was detected in two isolates carrying bla_{IMP-4}. Using random amplification of polymorphic DNA analysis, 14 PCR fingerprint patterns were generated from the 32 isolates carrying metallo-β-lactamase (MBL) genes (35.5 %), whilst 20 patterns were generated from the 58 non-MBL gene isolates (64.4 %). Based on the differences in the fingerprinting patterns, two clusters (A and B) were identified among the MBL-producing isolates. Cluster A comprised 18 isolates (56 %) carrying the bla_{VIM} gene, whereas cluster B comprised 14 (44 %) isolates carrying the bla_{IMP} gene. The non-MBL isolates were divided into clusters C and D. Cluster C comprised 22 non-MBL isolates harbouring class 1 integrons, whilst cluster D consisted of three isolates carrying class 2 integrons. These findings suggest that the class 1 integron is widespread among *P. aeruginosa* isolated in Malaysia and that characterization of cassette arrays of integrons will be a useful epidemiological tool to study the evolution of multidrug resistance and the dissemination of antibiotic resistance genes.

INTRODUCTION

The recent emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* has become a serious problem in healthcare settings worldwide, including in Asian countries (Leung *et al.*, 2008; Raja & Singh, 2007; Sekiguchi *et al.*, 2007). The rapid emergence of antibiotic resistance among clinical isolates of bacteria is due to dissemination of antibiotic resistance genes by horizontal transfer (Leverstein-van Hall *et al.*, 2003). Carbapenems, including imipenem and meropenem, are the most potent antimicrobial agents for the treatment of *P. aeruginosa* infections (Lee *et al.*, 2011). However, carbapenem resistance due to metallo-β-lactamase (MBL) production of *P. aeruginosa* has been reported (Varaiya *et al.*, 2008). Several families of MBLs have been documented in *P. aeruginosa*, including IMP, VIM, SPM, GIM, SIM, KHM, AIM and NDM (Toleman & Walsh, 2008). The genes of both IMP- and VIM-type MBLs (bla_{IMP} and bla_{VIM}) in clinical isolates of *P. aeruginosa* are often encoded on mobile gene cassettes inserted into class 1 integrons (Pasteran *et al.*, 2005; Peleg *et al.*, 2004; Yong *et al.*, 2009; Zhao *et al.*, 2009). Integrons have been identified as a primary source of resistance genes and are suspected to serve as reservoirs of antimicrobial resistance genes within microbial populations (Xu *et al.*, 2009). They are able to capture one or more gene cassettes from the environment and incorporate them using site-specific recombination. To date, there are more than nine classes of integron, with class 1 integrons being the most documented and well characterized (Hwa *et al.*, 2009). A class 1 integron carries the integrase gene (*intI1*), which encodes the site-specific recombinase responsible for cassette insertion. It also contains an *attI1* site where the cassettes are integrated and a promoter, which is responsible for the transcription of the cassette-encoded genes (Dubois *et al.*, 2009). A gene cassette often contains a single antibiotic resistance gene and a 59-base element (or *attC* site) downstream of the gene, which is responsible for recombination events (Hu & Zhao, 2009; Jeong *et al.*, 2009; Zhao *et al.*, 2009).

Previous studies reported that the genetics of resistance in MDR *P. aeruginosa* dramatically changed during a 7 year study from 2002 to 2008 in Malaysia (Ho *et al.*, 2002;

Abbreviations: CS, conserved segment; IRPA, imipenem-resistant *Pseudomonas aeruginosa*; MBL, metallo-β-lactamase; MDR, multidrug resistant; RAPD, random amplification of polymorphic DNA.

The GenBank/EMBL/DDBJ accession numbers for the integron sequences determined in this study are GU299868 and GU213191–GU213193.

Khosravi *et al.*, 2010a). The ratio of MDR *P. aeruginosa* (imipenem resistance) in drug-resistant *P. aeruginosa* did not change considerably, but the ratio of MBL-positive strains increased considerably for MDR *P. aeruginosa*. In 2002, most of the MDR *P. aeruginosa* did not carry MBL genes, and the resistance to imipenem could have been attributed to reduced expression of the OprD porin, combined with derepression of the chromosomal *ampC* β-lactamase gene and/or over expression of the efflux pump systems (Ho *et al.*, 2002). However, in 2008, 35.8 % of 90 imipenem-resistant *P. aeruginosa* isolates were carriers of MBL genes with four allelic variants, $bla_{\text{IMP-7}}$, $bla_{\text{IMP-4}}$, $bla_{\text{VIM-2}}$ and $bla_{\text{VIM-11}}$ (Khosravi *et al.*, 2010a). To understand further the mode of dissemination of MBL genes, possible integrons and their associated gene cassettes were characterized in this study. The genetic relatedness of the isolates was investigated using random amplification of polymorphic DNA (RAPD) analysis.

METHODS

Bacterial isolates. Ninety imipenem-resistant *P. aeruginosa* (IRPA) clinical isolates collected randomly between October 2005 and March 2008 in the University of Malaya Medical Center were used in this study. Of these isolates, 32 were classified as MDR based on resistance to more than seven antibiotics. A PCR assay showed that all 32 isolates were positive for the MBL gene, with identification of four allelic variants: $bla_{\text{IMP-7}}$ (12 isolates), $bla_{\text{IMP-4}}$ (2 isolates), $bla_{\text{VIM-2}}$ (17 isolates) and $bla_{\text{VIM-11}}$ (1 isolate) (Khosravi *et al.*, 2010a). The isolates were stored at −80 °C in Luria–Bertani medium containing 30 % glycerol.

DNA preparation. Extraction of DNA was performed according to the protocol provided with the Qiagen Mini Amp kit.

Determination of integron class using PCR-RFLP. To identify the presence of integrons in our isolates, PCR-RFLP was performed by using the degenerate primers hep35 and hep36 (Table 1). The expected amplicon size (491 bp) was ascertained by electrophoresis on a 1.5 % agarose gel with appropriate molecular size markers (100 bp DNA ladder; MBI Fermentas), and the PCR products were further restricted using *Hin*fI (MBI Fermentas) to determine the class of integron (Gu *et al.*, 2007).

Table 1. Primers used in this study

Primer	Sequence (5′–3′)	Length (nt)	Product size (bp)
5′CS	GGCATCCAAGCAGCAAG	17	Variable
3′CS	AAGCAGACTTGACCTGA	17	Variable
IMP-F	GAGGYGTTTATGTTCATAC	19	587
IMP-R	GTAMGTTTCAAGAGTGATGC	20	587
VIM-F	AATGCGCAGCACCAGGATAG	20	382
VIM-R	GTTTGGTCGCATATCGCAAC	20	382
hep35	TGCGGGTYAARGATBTKGATTT	22	491
hep36	CARCACATGCGTRTARAT	18	491
272	AGCGGGCCAA	10	Variable

Amplification and sequencing of gene cassette regions. The gene cassettes of the class 1 integron were amplified using specific primers for 5′ and 3′ conserved segments (5′CS and 3′CS; Table 1) combined with primers VIM-R/IMP-R and VIM-F/IMP-F, respectively (Pitout *et al.*, 2007). Amplification was performed in a 25 µl mixture containing 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTP (MBI Fermentas), 2.5 U *Taq* DNA polymerase (MBI Fermentas) and 2 µl boiled bacterial extract in a Bio-Rad PCR system thermal cycler. PCR was carried out using the following conditions: an initial denaturation step for 5 min at 95 °C, with 30 cycles of 1 min at 94 °C, 1 min at 61 °C (for VIM-F/3′CS), 57 °C (for VIM-R/5′CS), 60 °C (for IMP-F/3′CS) or 57 °C (for IMP-R/5′CS), and 5 min at 72 °C, followed by 10 min at 72 °C.

Sequence analysis of genes encoding MBL gene cassettes in class 1 integrons. Amplified products were purified using a PCR purification kit (Geneall Biotechnology) and sequences were determined using an automated DNA sequencer (ABI PRISM DNA sequencer; Perkin Elmer) by the dideoxy chain-termination method. Nucleotide sequences were analysed and compared using BLAST software (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov).

Genotyping techniques. Determination of fingerprinting patterns of *P. aeruginosa* was carried out by RAPD analysis using primer 272 (Riccio *et al.*, 2005). The RAPD mixture (25 µl) was composed of 100 ng genomic DNA, 0.5 µM primer, 1.25 U *Taq* polymerase (MBI Fermentas), 0.2 mM each dNTP (Vivantis Technologies), 1× *Taq* buffer with KCl (pH 8) and 2 mM $MgCl_2$. Primer 272 was used with the following protocol: four cycles of 5 min at 94 °C, 5 min at 36 °C and 5 min at 72 °C; 30 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C; and a final extension step at 72 °C for 10 min. The RAPD products (one-ninth of each reaction mixture) were separated by electrophoresis on 1.5 % agarose gels with 1× TBE running buffer at 90 V cm^{-1} for 2 h. Molecular size standards were included on all gels (VC 1 kb ladder; Vivantis Technologies). The RAPD fingerprints were analysed using the Gel Compare II version 4.0 software package (Applied Maths).

RESULTS

Presence and class distribution of integrons in *P. aeruginosa*

The presence of integrons was confirmed in 57 (63 %) of the isolates, of which 54 (94.7 %) and three (5.3 %) were identified as class 1 and class 2 integrons, respectively. No class 3 integron was detected in this study. All 32 MBL-producing isolates had class 1 integrons (Fig. 1).

Characteristics of class 1 integron-associated MBL genes

Class 1 integron cassette regions were amplified by combining primers 5′CS with VIM-R/IMP-R and 3′CS with VIM-F/IMP-F. Upon sequencing the entire integrons, seven different gene cassettes were identified, including genes encoding resistance to β-lactam antibiotics ($bla_{\text{IMP-4}}$, $bla_{\text{IMP-7}}$, $bla_{\text{VIM-2}}$ and $bla_{\text{VIM-11}}$) and aminoglycosides (*aadA6*, *accC1* and *aacA7*). Partial fragments of *aacA7* and $bla_{\text{VIM-2}}$ were detected in the 1115 bp amplicons of a class 1 integron harbouring $bla_{\text{VIM-2}}$ (GenBank accession

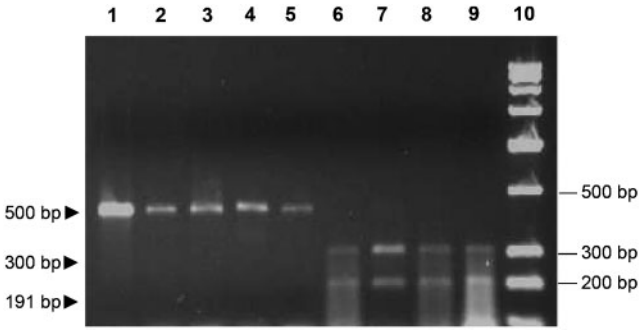

Fig. 1. PCR-amplified integrase gene digested with *Hin*fI restriction enzyme showing distinct PCR/RFLP patterns. Lanes: 1–4, *Hin*fI-treated products representative of class 1 integrons in *P. aeruginosa* strains; 5, class 1 integron-positive control strain of *Acinetobacter* sp. (YMC 05/4/P488); 6–8, *Hin*fI-treated products representative of class 2 integrons in *P. aeruginosa* strains; 9, class 2 integron-positive control strain of *Acinetobacter* sp. (P5570); 10, 100 bp ladder (MBI Fermentas).

no. GU299868). Partial fragments of *accC1* and $bla_{\text{IMP-7}}$ genes were identified in the 806 bp amplicon of a class 1 integron harbouring $bla_{\text{IMP-7}}$ (GenBank accession no. GU213192). Sequence analysis of the 656 bp amplicons derived from two $bla_{\text{IMP-4}}$ isolates revealed the presence of the gene *aadA6* (GenBank accession no. GU213193) (Table 2). A 674 bp amplicon was obtained from the single isolates of *P. aeruginosa* carrying $bla_{\text{VIM-11}}$. No gene other than $bla_{\text{VIM-11}}$ was detected in this isolate (GenBank accession no. GU213191); this result has been published elsewhere (Khosravi *et al.*, 2010b).

Molecular typing of IRPA strains by RAPD

A total of 14 PCR fingerprint patterns was generated for the 32 isolates carrying a class 1 integron encoding MBL genes (35.5 %), whereas 20 PCR fingerprint patterns were generated for the 25 non-MBL isolates carrying class 1 and 2 integrons (28.9 %). Based on the numbers of band differences, two clusters (A and B) were identified in the MBL gene-positive isolates, all carrying class 1 integrons, and two clusters (C and D) in the non-MBL isolates. Cluster A of the MBL isolates comprised 18 (56.25 %) isolates carrying bla_{VIM} genes, whereas cluster B comprised 14 (43.75 %) isolates carrying bla_{IMP} genes. The non-MBL isolates were divided into clusters C and D. Cluster C comprised 22 non-MBL isolates carrying class 1 integron and cluster D contained three isolates carrying class 2 integrons. The fingerprinting patterns of cluster D isolates did not show any similarity to the other three clusters (Table 2, Fig. 2).

DISCUSSION

The use of broad-spectrum antimicrobial agents has had a profound effect on the emergence of antibiotic resistance. Resistance to various classes of antibiotics, including recently developed ones, has been reported in Gram-negative bacteria through different mechanisms, especially carbapenem-hydrolysing MBLs (Strateva *et al.*, 2007). The presence of populations of MDR strains among clinical isolates is a cause for concern for physicians applying empirical treatment, especially in serious cases of *P. aeruginosa* infections in Asian countries such as Japan (Sekiguchi *et al.*, 2007), Taiwan (Leung *et al.*, 2008), India (Navaneeth *et al.*, 2002) and Iran (Japoni *et al.*, 2006).

In this study, we confirmed the presence of class 1 integrons in 54 IRPA (32 MBL gene-positive and 22 non-MBL gene) clinical isolates in the University of Malaya Medical Center. We suggest that the number of class 1 integrons is increasing, as a previous study in 2002 at the same hospital showed that, among 50 IRPA clinical isolates, only one carried the MBL gene and harboured a class 1 integron (Ho *et al.*, 2002). However, in 2005, the presence of class 1 integrons was reported in 79.5 % of imipenem-resistant *Acinetobacter baumannii* isolates from Malaysia, with two of them having MBL genes (Hwa *et al.*, 2009). This is not surprising, as a study from Italy demonstrated that acquired class 1 integrons of MBL can emerge rapidly (Lagatolla *et al.*, 2006).

In addition to the presence of class 1 integrons, we also identified class 2 integrons in three of our isolates, which, to the best of our knowledge, is the first report of class 2 integrons in *P. aeruginosa*. Normally, class 2 integrons are most frequently associated with members of the family *Enterobacteriaceae*, such as *Escherichia coli* and *Salmonella enterica*, and are also commonly found in *A. baumannii* and *Burkholderia cepacia* (Ahmed *et al.*, 2005; Barlow *et al.*, 2004; van Essen-Zandbergen *et al.*, 2007). Thus, from these results, we suggest that the class 1 integron is the most abundant type of integron present among the clinical isolates in this hospital. Similar observations were also noted in a clinical setting in Japan (Sekiguchi *et al.*, 2007).

In the present study, 17 isolates were found to contain *aacA7* and $bla_{\text{VIM-2}}$ at the 5′CS of the class 1 integron. This result is consistent with findings from Japan (Zhao *et al.*, 2009) and Tunis (Hammami *et al.*, 2010) in which the integron carrying *aacA7* and $bla_{\text{VIM-2}}$ at the 5′ end did not possess the 3′CS. In addition, similar results have been reported in *P. aeruginosa* isolates (strain RON-1) isolated in Paris (Poirel *et al.*, 2006), Portugal (Quinteira & Peixe, 2006) and India (Toleman *et al.*, 2007). However, the downstream regions of the $bla_{\text{VIM-2}}$ gene of these isolates were found to be different when compared with the *P. aeruginosa* isolate used in our study.

Previously, we identified two isolates with $bla_{\text{IMP-4}}$ among 14 isolates with bla_{IMP} (Khosravi *et al.*, 2010a). Here, we demonstrated that these $bla_{\text{IMP-4}}$ genes were harboured by class 1 integrons that also carried the additional gene cassette *aadA6* conferring aminoglycoside resistance. Similarly, $bla_{\text{IMP-4}}$-harbouring class 1 integrons have been reported in clinical isolates of *P. aeruginosa* in Hong Kong (Chu *et al.*,

Table 2. Origin, resistogram, MBLs and RAPD of the 57 integron-positive IRPA clinical isolates

Trac/sec, tracheal section; R, resistant; I, intermediate; S, sensitive; CN, gentamicin; Net, netilmicin; AK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; IMP, imipenem; PRL, piperacillin; CFP, cefoperazone.

Strain ID	Site of isolation	Antimicrobial susceptibility testing								MBL (PCR)	Integron	Cassette	RAPD pattern
		CN	Net	AK	CAZ	CIP	IMP	PRL	CFP				
Ps1	Urine	R	S	R	R	R	R	R	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps2	Trac/sec	R	R	R	R	R	R	R	R	IMP-4	Class 1	*aadA6*	B
Ps3	Urine	R	R	R	R	R	R	R	R	IMP-4	Class 1	*aadA6*	B
Ps4	Trac/sec	R	S	R	S	S	R	S	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps5	Trac/sec	R	R	R	R	R	R	S	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps6	Wound	R	R	R	R	R	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps7	Trac/sec	R	S	R	S	R	R	S	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps8	Trac/sec	R	R	R	R	R	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps9	Trac/sec	R	R	R	R	R	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps10	Trac/sec	R	R	R	S	R	R	S	S	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps11	Urine	R	R	R	R	S	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps12	Tissue	R	R	R	R	R	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps13	Trac/sec	R	R	R	R	I	R	S	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps14	Trac/sec	R	R	S	R	R	R	R	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps15	Trac/sec	S	I	S	S	R	R	S	S	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps16	Trac/sec	R	S	S	S	R	R	S	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps17	Trac/sec	R	R	R	R	R	R	R	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps18	Trac/sec	R	R	R	R	R	R	R	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps19	Urine	R	R	R	R	R	R	R	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps20	Urine	R	R	R	R	R	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps21	Trac/sec	R	R	R	R	S	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps22	Trac/sec	R	R	R	R	R	R	S	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps23	Trac/sec	R	R	I	I	I	R	R	I	VIM11	Class 1	bla_{VIM11}	A
Ps24	Wound	R	S	S	R	R	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps25	Trac/sec	R	R	R	R	R	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps26	Urine	R	R	R	R	S	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps27	Urine	R	R	R	R	R	R	R	R	VIM-2	Class 1	*aacA7*, bla_{VIM2}	A
Ps28	Tissue	R	R	I	R	R	R	R	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps29	Trac/sec	R	S	R	R	R	R	S	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps30	Trac/sec	R	S	R	S	R	R	S	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps31	Urine	R	S	S	R	R	R	R	R	IMP-7	Class 1	bla_{IMP-7}, *accC1*	B
Ps32	Urine	R	R	R	R	R	R	R	R	IMP-7	Class 1	*accC1*, bla_{IMP-7}	B
Ps36	Tissue	R	S	R	R	R	R	S	R	–	Class 1	*aadA6*	C
Ps38	Trac/sec	R	R	R	R	R	R	R	S	–	Class 1	*aadA6*	C
Ps39	Trac/sec	S	R	R	R	R	R	R	S	–	Class 1	*aadA6*	C
Ps44	Sputum	R	R	R	R	R	R	R	S	–	Class 1	*aadA6*	C
Ps47	Trac/sec	S	S	S	R	S	R	R	R	–	Class 1	*aadA6*	C
Ps48	Trac/sec	R	S	S	S	S	R	S	S	–	Class 1	*aadA6*	C
Ps50	Trac/sec	S	S	R	R	S	R	R	R	–	Class 2	*aadA6*	D
Ps52	Trac/sec	S	R	R	R	S	R	R	R	–	Class 1	*aadA6*	C
Ps53	Wound	R	S	S	S	S	R	S	R	–	Class 2	*aadA6*	D
Ps54	Urine	S	S	S	R	R	R	R	R	–	Class 1	*aadA6*	C
Ps55	Trac/sec	S	S	S	R	S	R	S	R	–	Class 2	*aadA6*	D
Ps56	Urine	R	R	R	R	R	R	R	S	–	Class 1	*aadA6*	C
Ps58	Trac/sec	S	R	R	S	R	R	R	S	–	Class 1	*aadA6*	C
Ps60	Trac/sec	S	R	R	R	R	R	R	S	–	Class 1	*aadA6*	C
Ps63	Wound	S	S	R	R	S	R	S	R	–	Class 1	*aadA6*	C
Ps64	Trac/sec	R	S	R	R	S	R	R	R	–	Class 1	*aadA6*	C
Ps70	Trac/sec	R	R	R	R	R	R	R	R	–	Class 1	*aadA6*	C
Ps71	Trac/sec	R	S	R	R	S	R	R	R	–	Class 1	*aadA6*	C
Ps76	Trac/sec	R	S	S	R	S	R	S	R	–	Class 1	*aadA6*	C

Table 2. cont.

Strain ID	Site of isolation	Antimicrobial susceptibility testing								MBL (PCR)	Integron	Cassette	RAPD pattern
		CN	Net	AK	CAZ	CIP	IMP	PRL	CFP				
Ps77	Urine	R	R	R	R	S	R	R	S	–	Class 1	*aadA6*	C
Ps78	Tissue	S	S	R	R	S	R	S	S	–	Class 1	*aadA6*	C
Ps82	Trac/sec	R	R	R	R	R	R	R	R	–	Class 1	*aadA6*	C
Ps83	Trac/sec	R	R	S	IN	R	R	R	S	–	Class 1	*aadA6*	C
Ps84	Trac/sec	R	S	R	R	R	R	R	S	–	Class 1	*aadA6*	C
Ps86	Trac/sec	R	S	S	R	R	R	S	S	–	Class 1	*aadA6*	C

2001), Japan (Zhao *et al.*, 2009) and Australia (Peleg *et al.*, 2004), and in a patient in France who was sent back from Malaysia (Bert *et al.*, 2007). A bla_{IMP-4}-harbouring class 1 integron was also identified in a clinical isolate of *A. baumannii* in Singapore, although it carried different gene cassettes, i.e. *aacA4* (aminoglycoside gene), and *catB3* (chloramphenicol acetyltransferase gene) (Koh *et al.*, 2007). In our study, 12 isolates were found to contain the gene cassettes *accC1* and bla_{IMP-7} at the 3′ CS of the class 1 integron. An integron with a similar structure has been reported in a *P. aeruginosa* isolate (GenBank accession no. AY625685) from Singapore (Koh, 2008). In summary, these results indicate a wide distribution of antimicrobial-resistance-encoding integrons with similar structures in

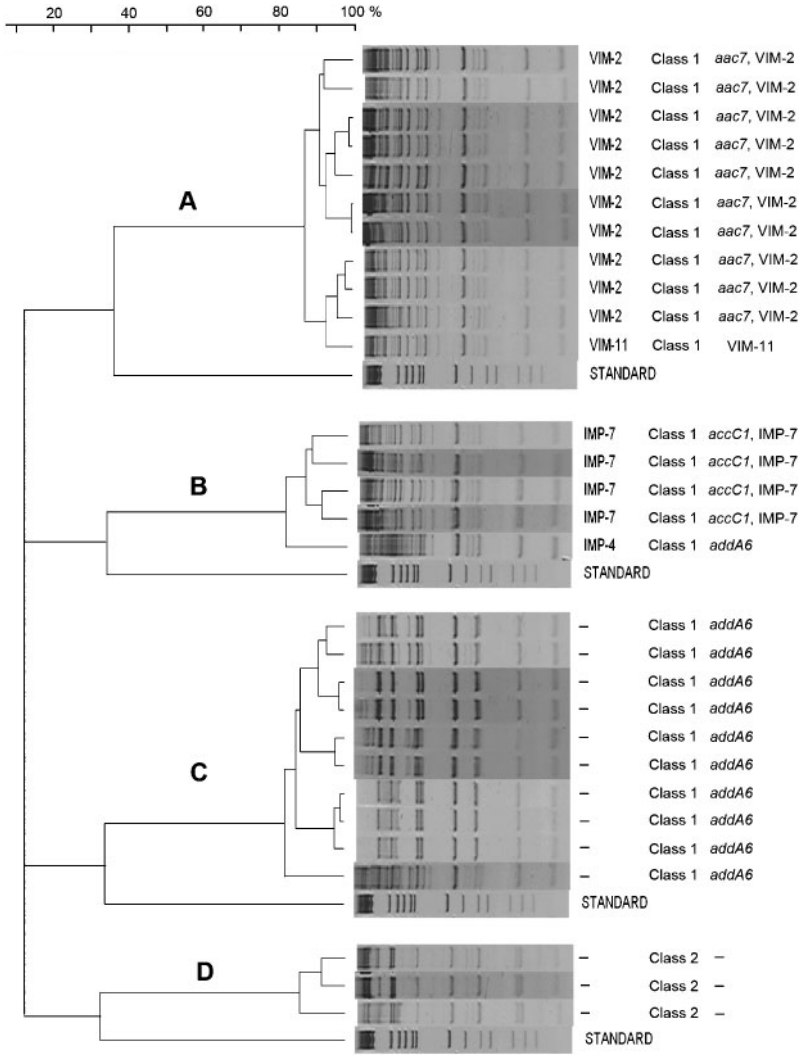

Fig. 2. RAPD polymorphisms of IRPA clinical isolates amplified by primer 272. The representative fingerprint patterns of four RAPD clusters are shown: cluster A (bla_{VIM}); cluster B (bla_{IMP}); cluster C (non-MBL gene, class 1) and cluster D (non-MBL gene, class 2). STANDARD, Molecular size markers.

various geographical regions. This suggests the possibility of international spread of the resistance determinants, possibly through travelling, and warrants continuous surveillance to control the further spread of resistance due to this mechanism.

A clonality study carried out in this work also showed that all MBL producers harbouring class 1 integrons could be divided into two clusters, A and B, whilst the non-MBL producers could be divided into clusters C and D. When correlated with the resistance patterns (Table 2), the data suggested that those strains with the same antibiotic susceptibility demonstrated similar RAPD patterns. Thus, it could be suggested that isolates carrying MBL genes may be detected in different clusters from the non-MBL isolates. These results are in agreement with the results of a study in Canada, which also suggested three clusters for *P. aeruginosa* isolates: cluster 1 (bla_{VIM} producing), cluster 2 (bla_{IMP} producing) and cluster 3 (non-MBL) (Pitout *et al.*, 2007). Our findings also suggest that PCR-RAPD can be used for routine application to investigate the clonality of isolates, which may be cheaper and easier than other methods such as PFGE. The identified diversity of RAPD types in this study suggests the spread of bla_{VIM} and bla_{IMP} genes among genetically distinct *P. aeruginosa* strains. This phenomenon is probably facilitated by carriage of the bla_{VIM} and bla_{IMP} genes by integrons, which, although not mobile themselves, are frequently parts of transposons and/or transferable plasmids (Juan *et al.*, 2009). To the best of our knowledge, this is the first report of the detection of cassette arrays of class 1 integrons (bla_{IMP-7} and *accC1*, *aacA7* and bla_{VIM-2}, and *aadA6*) from IRPA clinical isolates in Malaysia. This study highlights the resistance to imipenem due to IMP- and VIM-producing *P. aeruginosa* and their associated class 1 integrons. Horizontal dissemination of the class 1 integron-associated MBL genes may contribute to the further emergence of carbapenem resistance in other Gram-negative bacteria. Therefore, appropriate surveillance and control measures are essential to prevent the further spread of MBL-producing organisms in hospitals. Further studies similar to this one should be carried out for a better understanding of the impact of integrons on the dissemination of antimicrobial resistance in clinical practice.

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