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SHORT REPORT
Emergence of metallo-β-lactamase IMP-14 and VIM-2 in *Pseudomonas aeruginosa* clinical isolates from a tertiary-level hospital in Thailand

C. PIYAKUL[1], R. TIYAWISUTSRI[2] AND K. BOONBUMRUNG[2]*

[1] *Graduate Programme in Clinical Biochemistry and Molecular Medicine, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand*
[2] *Department of Transfusion Medicine, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand*

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SUMMARY

Seventy-five clinical isolates of *Pseudomonas aeruginosa* collected in a tertiary teaching hospital in Thailand were investigated for susceptibility to antimicrobials including imipenem. Metallo-β-lactamase (MBL) enzymes were detected by E-test MBL assay and PCR; class 1 integron genes were also detected by PCR. Strains positive for bla_{IMP} and bla_{VIM} genes were further characterized by DNA sequencing and examined for clonality by pulsed-field gel electrophoresis. High rates of resistance to anti-pseudomonal agents were found. MBL enzymes were found in 13 (17·3%) strains and 24 (32%) carried class 1 integron genes. Twelve of the latter strains harboured the bla_{IMP-14} gene and one strain the bla_{VIM-2} gene. All of the IMP-14 strains were identical or closely related suggesting clonal dissemination of these genes.

Key words: Antibiotic resistance, *Pseudomonas*.

Pseudomonas aeruginosa producing metallo-β-lactamase (MBLs) have been recognized in several parts of the world, e.g. Poland, Southern Brazil, Taiwan, and Europe [1]. To date, at least 26 IMP and 30 VIM variants have been reported in several countries (http://www.lahey.org/Studies/other.asptable1). Genetic determinants of MBLs (*bla* genes) are mostly carried on class 1 and class 3 integrons [2] and can spread rapidly in hospitals via the integron system. In Thailand, Boonkerd *et al*. reported the first case of a bla_{IMP-1} gene in carbapenem-resistant *P. aeruginosa* in 2009; bla_{VIM}-type MBL was not found in their study [3]. We report here the prevalence of bla_{IMP} and bla_{VIM} genes associated with class 1 integrons and also investigated possible clonal dissemination of *P. aeruginosa* by pulsed-field gel electrophoresis (PFGE) based on observations in a tertiary-level teaching hospital in Thailand during March–April 2009.

Non-duplicate, clinical isolates of *P. aeruginosa* were randomly selected and species identity was confirmed by standard techniques. Isolates were tested for susceptibility to 11 antimicrobial agents [amikacin (AMK), ceftazidime (CAZ), ciprofloxacin (CIP), colistin (CL), cefepime (FEP), gentamicin (GEN), imipenem (IPM), levofloxacin (LVX), meropenem (MEM), cefoperazone/sulbactam (SCFP) and piperacillin/tazobactam (TZP) (Oxoid, UK) by the disk diffusion assay according to Clinical and Laboratory Standards Institute (CLSI) guidelines [4]. Minimum inhibitory concentrations (MICs) of IPM were determined by an IPM E-test strip and isolates were screened for the presence of MBL enzymes by the E-test MBL strip (AB Biodisk, Sweden). A MIC

* Author for correspondence: K. Boonbumrung, Ph.D., Chulalongkorn University, 154 Chulapat 1, Rama1 Road, Pathumwan, Bangkok 10330, Thailand.
(Email: khaemaporn.b@chula.ac.th)

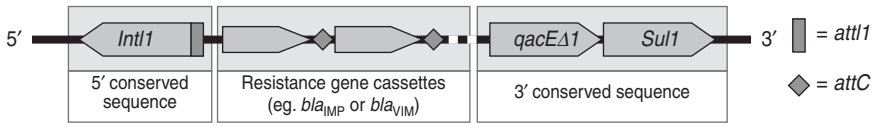

Fig 1. Schematic representation of class 1 integron.

ratio (MIC of IPM alone/MIC of IPM plus EDTA) of $\geqslant 8$ was interpreted as suggestive of MBL production. Genomic DNA of *P. aeruginosa* was extracted and purified by phenol/chloroform as described previously [5]. PCR amplification for MBL genes (bla_{IMP}, bla_{VIM}) was performed as described previously [6]. Class 1 integron genes were amplified by multiplex PCR with the following primers: *intI-1* primers (forward: 5′-GGC GCG CTG AAA GGT CTG GT-3′; reverse: 5′-CCG CTG CGT TCG GTC AAG GT-3′); *qacEΔ1* primers (forward: 5′-TTG CCC CTT CCG CCG TTG TC-3′; reverse: 5′-CCT CCG CAG CGA CTT CCA CG-3′); *sul1* primers (forward: 5′-GAC GCG AGG CCT GTA TCG CC-3′; reverse: 5′-TCC GTC GCA AGG CGG AAA CC-3′). PCR products were purified and directly sequenced using IMP-F, IMP-R, VIM-F and VIM-R primers (1st BASE Pte Ltd, Thailand). DNA sequences were analysed by the BLAST program (http://blast.ncbi.nlm.nih.gov/). *P. aeruginosa* IMP-1-producing strains were kindly provided by Dr Neil Woodford as positive controls for MBL detection. Genotyping of isolates carrying bla_{IMP} or bla_{VIM} was performed by PFGE with the restriction enzyme *Spe*I in a CHEF Mapper system (Bio-Rad Laboratories, USA).

The 75 *P. aeruginosa* isolates were from sputum ($n=33$, 44%), urine ($n=21$, 28%), pus ($n=18$, 24%) and blood ($n=3$, 4%). Most isolates were resistant to LVX and CIP (54·7%), followed by CAZ (50·7%), SCFP (42·7%), TZP (42·7%), MEM (38·7%), and IPM (33·3%); all were susceptible to CL. Thirteen isolates (17·3%) were positive for MBLs by E-test and were multidrug resistant with a high-level of resistance to IPM (MIC $>256\,\mu$g/ml). Thirteen isolates were also positive for the bla_{IMP} allele and one was positive for the bla_{VIM} allele. Nucleotide sequencing identified the bla_{IMP} and bla_{VIM} genes to be IMP-14 (GenBank EMBL accession no. GQ302617) and VIM-2 (accession no GQ853417). Twenty-four isolates (32·0%) were positive for class 1 integron genes (*intI-1*, *qacEΔ1*, *sul1*; Fig. 1); 12 of these carried IMP-14, and one was VIM-2 positive. Another isolate with a bla_{IMP} gene was negative for class 1 integron genes. The *Spe*I-digested genomic DNA patterns of 14 isolates were classified into three types; A, B and C according to the criteria of Tenover *et al.* [7] (Fig. 2). Pattern A was dominant and consisted of five closely related restriction patterns for the 12 bla_{IMP}-producing isolates. The VIM-2 strain was unique (pattern C), and had a susceptibility profile identical with most strains of pattern A.

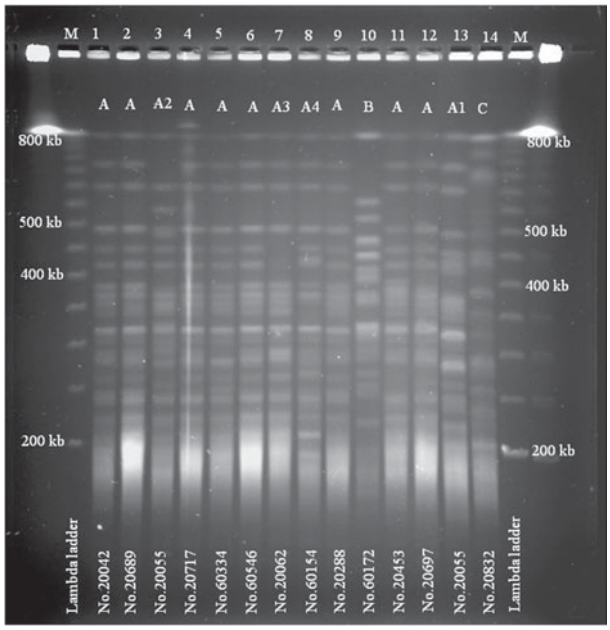

Fig 2. Pulsed-field gel electrophoresis (PFGE) pattern of some of the *P. aeruginosa* isolates carrying MBL genes after digestion with *Spe*I. Lane M, lambda ladder (Promega, USA); lanes 1-14, isolates 20 042, 20 689, 20 055, 20 717, 60 334, 60 546, 20 062, 60 154, 20 288, 60 172, 20 453, 20 697, 20 055, and 20 832, respectively.

Corresponding with data from the National Antimicrobial Resistance Surveillance Center of Thailand, clinical isolates in the present study showed high rates of resistance to most anti-pseudomonal agents especially quinolones, β-lactam/inhibitor combinations, cephalosporins and carbapenems, but no resistance to CL was found underlining its potential as a reserve antibiotic for multidrug-resistant strains of *P. aeruginosa*.

As found in a previous study [8], integron-positive strains were significantly associated with resistance to aminoglycosides, quinolones, carbapenems and cephalosporins ($P\leqslant 0{\cdot}001$). Interestingly, one strain carried the bla_{IMP} gene but not the integrase (*intI-1*)

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gene and this raises the possibility of insertion of the bla_{IMP} gene into another class of integrons [2]. Moreover, two strains which were phenotypically positive for MBLs but negative for bla_{IMP} and other MBL-encoding genes (e.g. VIM-, GIM- and SPM-type), clearly produced MBL enzymes [9], one of which was identified as VIM-2 type. By contrast, two strains harboured the genes for bla_{IMP} and class 1 integrons but were negative by the MBL E-test. This was previously observed by Collis & Hall [10] who suggested that the expression of the cassette had been affected by the position of the antibiotic resistance gene cassette which was inserted in the region of the integron.

We have shown the emergence of $bla_{IMP\text{-}14}$ and $bla_{VIM\text{-}2}$ genes in MBL-producing *P. aeruginosa* strains. The IMP-14 MBL gene was first described in a *P. aeruginosa* clinical isolate from Thailand in 2004 (GenBank accession no. GQ302617, unpublished data), and the VIM-2-type MBL gene has been reported from many countries including Japan, South Korea, Portugal, Spain, and USA [11].

PFGE showed that all isolates in pattern A were from urine samples of patients from two wards suggesting that this was the predominant clone circulating in the hospital. Although seemingly related by DNA profile the remaining four strains with the IMP-14 gene originated from various specimens and wards. Thus MBL-producing strains exhibited both clonality and diversity, which underlines that their prevalence cannot be explained solely by clonal expansion of a particular strain type.

In Thailand, the prevalence of MBL (bla_{IMP} and bla_{VIM})-producing *P. aeruginosa* strains associated with class 1 integrons has not yet been investigated in a large-scale study. Our preliminary survey showed that 17·3% of *P. aeruginosa* strains isolated over 2 months tested positive for MBL-producing genes. These data underline the importance of the emergence of MBLs associated with class 1 integrons and highlight the need for the development of infection control strategies in order to combat the spread of multidrug-resistant strains in hospitals by more efficient and appropriate prescribing of selected reserve drugs such as the carbapenems.

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DECLARATION OF INTEREST

None.

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