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# Molecular Epidemiology of Carbapenem Resistance Among Multidrug-resistant *Pseudomonas aeruginosa* isolated Clinically in Tertiaries Care Centers, Southern Thailand

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## ABSTRACT

*Pseudomonas aeruginosa* is one of potent opportunistic nosocomial human pathogen that shows resistance to a multiple class of antibiotics with carbapenems being the most potent inhibitor of *P. aeruginosa*. This study aimed to investigate the prevalence of metallo-β-lactamases (MBLs) genes and antimicrobial susceptibilities of *P. aeruginosa* isolated in two tertiaries care hospitals of Songkhla Province. A total number of 209 *P. aeruginosa* isolates were collected between August 2015 and December 2016 from various clinical samples in two tertiaries care hospitals of Songkhla Province. Antibiotic susceptibility was determined using the disk diffusion method. MBLs genes were detected by multiplex-PCR and blasted. Out of 209 *P. aeruginosa*, 198 (94.7%) was carbapenem-resistant isolates. The susceptibility rate of *P. aeruginosa* toward polymyxin B and Amikacin was 100% and 48.3% respectively. The highest of *Pseudomonas* infections were found in sputum 111 (53.1%), urine 37 (17.7%) and secretions 17 (8.1%) of *P. aeruginosa* isolates.

It was also found that the rate of multidrug resistance *P. aeruginosa* infection was 36.4% in the medical ward, 20.1% in ICU and 12.9% in surgical wards. The most dominant MBLs genes among *P. aeruginosa* isolates was $bla_{DIM}$ (53.6%), while the prevalence of $bla_{VIM}$, $bla_{IMP}$ and $bla_{AIM}$ were 29.2%, 12.4% and 9.6% respectively, whereas *bla*oxa48 was detected in only 9.6% of carbapenemase-positive isolates. The results showed that the prevalence of multidrug resistance was high and the presence of more than one *bla*MBLs gene in *P. aeruginosa* isolates to emphasize the challenge to therapeutic treating emerging.

**Keywords:** Epidemiology, metallo-β-lactamase, multidrug resistance, *Pseudomonas aeruginosa*

## INTRODUCTION

*Pseudomonas aeruginosa* is one of a potent opportunistic nosocomial human pathogens that shows resistance to multiple classes of antibiotics including carbapenems, the first choice of drug for treatment serious Gram-negative bacterial infections (Riera et al., 2011). This organism trends to increase resistance to many antimicrobial agents and a high percentage of the *P. aeruginosa* clinical isolates show the multidrug resistance (MDR) phenotype (Peix, Ramírez-Bahena and Velázquez, 2018).

Metallo-β-lactamase (MBLs) are carbapenemase with a capacity of hydrolyzing the penicillin, all β-lactam antibiotics except for aztreonam and have fetched us a step closer to the encounter of extremely drug-resistant bacteria (Cornaglia, Giamarellou and Rossolini, 2011). Over the last decade, various Classes A, B, and D β-lactamases have been revealed in *P. aeruginosa* (Braun et al., 2014). The most different types of MBL are such as AIM, SIM, GIM, IMP, NDM, SPM, FIM and VIM, which their frequency has been increased in *P. aeruginosa* throughout the world (Hong et al., 2015). The emergence of MBL-producing strains makes treatment hard and occasionally inefficient that cause high mortality (Doosti, Ramazani, and Garshasbi, 2013).

Detection of MBLs gene and recognition of MBLs are important in carrying out adequate countermeasures to control the spread of these enzymes and proper treatment of infections caused by microorganisms
produced by MBLs (Rizek et al., 2014). Furthermore, molecular technic emphasized the need for laboratories to be able to detect MBL genes associated with drug-susceptible and diminish the spread of multidrug resistance within the hospital.

Therefore, it is essential to understand the epidemiology, resistance mechanism, and molecular characteristics of MBL for infection control and prevention of a possible global health crisis. This study aimed to determine the prevalence of MBL-producing *P. aeruginosa* isolates from tertiaries care hospital in southern of Thailand followed by a multiplex PCR technique.

## MATERIAL AND METHODS

### Sample collection

Two hundred and nine of *P. aeruginosa* isolates were collected from patients admitted in two tertiaries care hospitals of Songkhla Province, Thailand (August 2015 to December 2016). Ethical approval was accepted from the Ethics Committees at Faculty of Medicine, Prince of Songkla University (REC58-183-04-8).

### Bacterial identification and antimicrobial susceptibility testing

All bacterial strains isolates were grown on Tryptic Soy Agar plates at 37°C overnight in order to identify species using routine biochemical test. Antimicrobial susceptibility tests were performed using the disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI) (CLSI, 2015). Antibiotic susceptibility test were carried out at Microbiology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University.

Drug-resistant categories and interpretation were based on *in vitro* antimicrobial susceptibility test results, and performed by Magiorakos et al. (Magiorakos et al., 2012) as follows: 1) Multidrug-resistant (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, 2) extensive drug-resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories), and 3) pan drug-resistant (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories.

### DNA Extraction

Chromosomal DNA was extracted from 209 species that were resistant to vancomycin with the GF-1 bacterial DNA extraction kit (Vivantis) according to the manufacture's procedure. The DNA was measured by spectrophotometry at 260 nm and the ratio of the absorbance at 260 and 280

nm (A260/A280) was used to assess the purity of DNA. The quality of DNA was evaluated using agarose gel electrophoresis.

**Detection of metallo-β-lactamase genes by multiplex-PCR**

The presence of MBL genes were demonstrated by multiplex PCR using eleven pairs of specific primers (Poirel et al., 2011), 3 set of multiplex reactions were defined, with no. 1 including detection of $bla_{IMP}$, $bla_{VIM}$, and $bla_{SPM}$, no. 2 including detection of $bla_{NDM}$, $bla_{KPC}$, and $bla_{BIC}$, and no. 3 including detection of $bla_{AIM}$, $bla_{GIM}$, $bla_{SIM}$, and $bla_{DIM}$ (Table 1) with modification the condition as follows: initial denaturation at 94°C for 10 min, 39 cycles of 94°C for 30 s, 52°C for 40 s and 72°C for 50 s, followed by a single, final, elongation step at 72°C for 5 min.

**Table 1.** List of oligonucleotide primers used in the genetic profiling of metallo-β-lactamase genes among *P. aeruginosa* isolates in this study (Poirel et al., 2011).

| Primer | Nucleotide sequence | Amplicon size (bp) | gene |
|---|---|---|---|
| IMP-F | GGAATAGAGTGGCTTAAYTCTC | 232 | $bla_{IMP}$ |
| IMP-R | GGTTTAAYAAAACAACCACC | | |
| SPM-F | AAAATCTGGGTACGCAAACG | 271 | $bla_{SPM}$ |
| SPM-R | ACATTATCCGCTGGAACAGG | | |
| AIM-F | CTGAAGGTGTACGGAAACAC | 322 | $bla_{AIM}$ |
| AIM-R | GTTCGGCCACCTCGAATTG | | |
| VIM-F | GATGGTGTTTGGTCGCATA | 390 | $bla_{VIM}$ |
| VIM-R | CGAATGCGCAGCACCAG | | |
| OXA-F | GCGTGGTTAAGGATGAACAC | 438 | $bla_{OXA48}$ |
| OXA-R | CATCAAGTTCAACCCAACCG | | |
| GIM-F | TCGACACACCTTGGTCTGAA | 477 | $bla_{GIM}$ |
| GIM-R | AACTTCCAACTTTGCCATGC | | |
| BIC-F | TATGCAGCTCCTTTAAGGGC | 537 | $bla_{BIC}$ |
| BIC-R | TCAATTGGCGGTGCCGTACAC | | |
| SIM-F | TACAAGGGATTCGGCATCG | | $bla_{SIM}$ |
| SIM-R | TAATGGCCTGTTCCCATGTG | 570 | |
| NDM-F | GGTTTGGCGATCTGGTTTTC | | $bla_{NDM}$ |
| NDM-R | CGGAATGGCTCATCACGATC | 621 | |
| DIM-F | GCTTGTCTTCGCTTGCTAACG | | $bla_{DIM}$ |
| DIM-R | CGTTCGGCTGGATTGATTTG | 699 | |
| KPC-Fm | CGTCTAGTTCTGCTGTCTTG | 798 | $bla_{KPC}$ |
| KPC-Rm | CTTGTCATCCTTGTTAGGCG | 232 | |

**Amplicon detection**

DNA fragments were analyzed by electrophoresis in 0.5×Tris-borate-EDTA on a 1% agarose gel stained with ethidium bromide and then visualized under UV transillumination. The PCR product was sent for sequencing and data analysis (BLAST).

**Statistical analysis**

Information on the patients and isolates were collected from clinical records (microbiological data; the source of the isolate, antibiotic treatment, type of infection) of all patients enrolled in the study. Data were presented as percentages unless otherwise stated

# RESULTS

**Prevalence of *P. aeruginosa* isolates and antibiotics susceptibility profile**

In total, 209 of *P. aeruginosa* isolates were detected in the period of August 2015 to December 2016 from tertiaries care hospitals in southern of Thailand. The specimens were positive about carbapenem-resistant that was shown in Figure 1A. Out of 32 samples, we found the highest rate of *P. aeruginosa* infection that was on April 2016 (15.3%). The maximum rate of *P. aeruginosa* infection was found in sputum (111, 53.1%), followed by urine (17, 23.9%), and secretions (17, 8.1%) (Figure 1B). Out of 209 isolates, 76 isolates (36.4%) were from the medical ward, 42 isolates (22.5%) were from ICU, and 27 isolates (9.9%) were from the surgical wards that were presented in Figure 1C.

Of the 209 isolates, 101 (48.3%), 88 (42.1%), and 77 (36.8%) were susceptible to amikacin, piperacillin/tazobactam, and gentamicin, respectively. All the isolates were susceptible to colistin (polymycin). The percentage of resistance showed by *P. aeruginosa* strains was 184 (88.0%) for imipenem, 164 (78.5%) meropenem, 127 (60.8%) gentamicin, and 124 (59.3%) ciprofloxacin that were represented in Figure 1D. The multidrug resistance of *P. aeruginosa* infection has 55.0% (115/209) promoted to the problem (data not shown). The study was not found both XDR and PDR isolates.

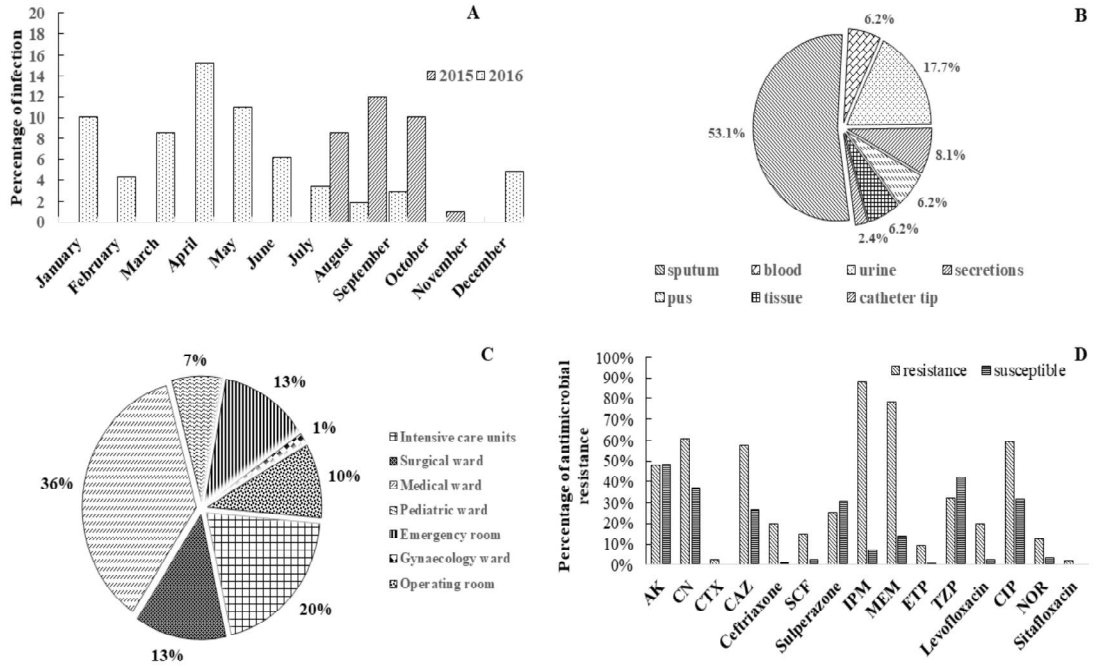


**Figure 1.** The prevalence of MBLs-producing *P. aeruginosa* isolates from hospitalized patients between August 2015 to December 2016 from two tertiaries care hospitals of Songkhla Province : incidence rate of MBLs-producing *P. aeruginosa* infection (A), isolation rates of MBLs- producing *P. aeruginosa* infection from various clinical specimens (B), source of MBLs-producing *P. aeruginosa* isolates among patients (C), and percentage of antibiotic resistance of MBLs-producing *P. aeruginosa* isolates to various antibiotics (D).

**Molecular characterization of MBLs-producing *P. aeruginosa* isolates**

A total of 209 isolates were positive for 16S rRNA genes by PCR (Figure 2A). One hundred and twelve (53.6%) isolates were $bla_{\mathrm{DIM}}$ positive and 61 (29.2%) were $bla_{\mathrm{VIM}}$ positive, and $bla_{\mathrm{IMP}}$ and $bla_{\mathrm{AIM}}$ showed in 26 (12.4%) (Table 2). Four possible MBL-producing *P. aeruginosa* ($bla_{\mathrm{oxa48}}$, $bla_{\mathrm{NDM}}$, $bla_{\mathrm{KPC}}$, and $bla_{\mathrm{SIM}}$) are shown in this study using multiplex-PCR were illustrated on an agarose gel according to their amplicon size (Figure 2B-2D). The $bla_{\mathrm{SPM}}$, $bla_{\mathrm{BIC}}$ and $bla_{\mathrm{GIM}}$ were not detected among *P. aeruginosa* isolates.

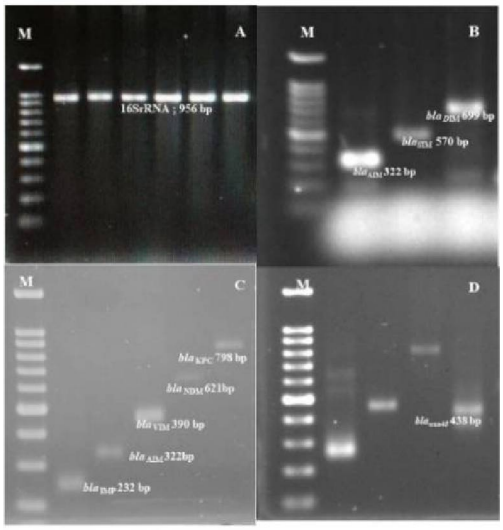


**Figure 2.** Identification of *P. aeruginosa* isolates by 16S rRNA gene (A) and multiplex PCR based genotypic characterization of MBLs-producing *P. aeruginosa* isolates recovered from clinical (B, C, and D) lane M: DNA ladder 100 bp. The size of each amplicon is indicated on the right.

**Table 2.** Percentage of detection MBL-producing *P. aeruginosa* isolates by multiplex polymerase chain reaction**.**

| Gene | No. of isolates | Percentage (%) |
|---|---|---|
| *bla*$_{DIM}$ | 112 | 53.6 |
| *bla*$_{VIM}$ | 61 | 29.2 |
| *bla*$_{IMP}$ | 26 | 12.4 |
| *bla*$_{AIM}$ | 26 | 12.4 |
| *bla*$_{oxa48}$ | 20 | 9.6 |
| *bla*$_{NDM}$ | 5 | 2.4 |
| *bla*$_{KPC}$ | 3 | 1.4 |
| *bla*$_{SIM}$ | 2 | 1.0 |

## DISCUSSION

This study showed that the majority of the clinical isolates were recovered from sputum (53.1%) which is less than report from Kosovo that showed 65% of *P. aeruginosa* infection (Lila et al., 2018). In our study, the highest number of isolates were from patients hospitalized in the medical ward (36.4%). Differences in the frequency of *P. aeruginosa* infection have also been reported in other studies. Harris and Dou reported that *P. aeruginosa* infection was in 82.0% from ICU and burn wards from China (Dou et al., 2017; Harris et al., 2016). The regional difference observed in this study support the understanding that infection control of resistant pathogens needs to be based on local epidemiology.

Our study showed that 88.0% of *P. aeruginosa* isolates with reduced susceptibility to imipenem were MBL-producer. This finding was higher than those reports in the previous study from Nepal (35.7%) (Acharya et al., 2017), Taiwan (55.1%) (Lee et al., 2008), Egypt (68.7%) (Zafer et al., 2015), indicating that MBL-producing *P. aeruginosa* isolates is increasing. From the results, we found the high rate of MDR *P. aeruginosa* isolates that could be related to misuse, or overuse of antibiotics (Nguyen et al., 2018).

Surprisingly, our present study showed that the detection of the $bla_{\text{DIM}}$ among carbapenem-resistant *P. aeruginosa* isolates (60, 52.2%) using multiplex-PCR was first reported in tertiaries care hospitals of Songkhla Province, Thailand. Its detection in our hospital setting may be carried within transposons which help the bacteria to transfer antibiotic resistance gene from one to another (Zhao and Hu, 2015). The $bla_{\text{VIM}}$ was high prevalence represent 32 (38.6%) among 83 carbapenem-resistant multidrug-resistant *P. aeruginosa* isolates (CR-MDR PA), the $bla_{\text{IMP}}$ represents 27 (23.9%) among 115 carbapenem-resistant *P. aeruginosa* isolates (CR-PA) and the $bla_{\text{SIM}}$ was the least detected in 1.0%. This result is consistent with the finding of other investigators who reported the prevalence of MBL genes in carbapenem-resistant *P. aeruginosa* isolates was 31.9% in Sudan (Adam and Elhag, 2018), 24.0% in India (Garg et al., 2019). The difference may be due to the variation of circulating strains.

Our examination demonstrated that multiplex-PCR can be used for detecting antimicrobial resistance genes among *P. aeruginosa* isolates that play a role in the dissemination, persistence of drug resistance genes of pathogenesis and extending it into important epidemiological data for *P. aeruginosa* infection control.

## CONCLUSION

The results of this study reveal a noticeability high prevalence of MBLs producing *P. aeruginosa* isolates in our tertiaries care center hospital. MBL-encoding genes are often carried by motile genetic elements that can rapidly spread between strains through horizontal gene transfer. Therefore, the multiplex-PCR technique is rapid for the detection of the most prevalent carbapenemase genes in tertiaries care hospitals of Songkhla Province. Furthermore, our detection of $bla_{DIM}$ using multiplex-PCR in tertiaries cares hospitals of Songkhla Province was first reported in Thailand.

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