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DETECTION METALLO-BETA-LACTAMASE GENE IMP-1 AND IMP-2 OF *Pseudomonas aeruginosa* CLINICAL ISOLATES IN SANGLAH HOSPITAL BALI

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ABSTRACT

Pseudomonas aeruginosa is a pathogen frequently found as an agent of Hospital Acquired infections. This bacterium is very easy to be resistant to several types of antibiotics through various mechanisms. Carbapenem such as Imipenem and Meropenem is a potential option for the therapy of this bacterium, but unfortunately *P. aeruginosa* has ability in hydrolyzing these antibiotics through enzyme metallo-β-lactamases (MBLs). Recently, IMP and VIM, MBLs enzyme group are reported common from various countries, but no data is reported for these enzymes in Indonesia especially in Bali. In fact, the resistant data of *P. aeruginosa* against carbapenem group antibiotics such as meropenem and imipenem is quite high in Sanglah General Hospital in 2014 was 35% and 45% respectively. Therefore, the aim of this study was to detect IMP-1 and IMP-2 genes of MDR *P. aeruginosa,* which are phenotypically resistant to the antibiotic Imipenem and Meropenem disks based on CLSI standards in Clinical Microbiology Laboratory, Sanglah General Hospital, Denpasar, Bali. Eighty-six isolates were isolated from sputum (25 / 29.1%), wound (25 / 29.1%), urine (15 / 17.4%), endotracheal Tube (11 / 12.8), pus (6/7%), blood (3 / 3.5%) and tissue (1 / 1.1%). In this study, all isolates were subjected to PCR for detection of IMP-1 and IMP-2. The result showed that 9 isolates were positive IMP-1 gene (10.5%), but there was no isolate positive for IMP-2 gene. The result was similar with that of the other countries, especially for the gene IMP-1. Detection and molecular characterization of MBL-producing *P. aeruginosa* strains are very important for infection control purposes. Currently, this study is still continued for detection of another MBL genes.

Keyword : *Pseudomonas aeruginosa,* MBLs gene, PCR, Positive Control

INTRODUCTION

Bacterial Multidrug Resistance (MDR) is an indicator of the changes in the strains of bacteria that are often associated with the administration of antibiotic unwisely, such as unnecessary empirical therapy, the selection of antibiotic regimens improperly, and prolonged use of antibiotics. One of bacteria found in hospital setting that easy to become an MDR bacteria is *Pseudomonas aeruginosa*. It is a pathogen frequently found as an agent of Hospital Acquired infections. This bacterium is very easy to be resistant to several types of antibiotics through various mechanisms. Prolonged use of antibiotics causing this bacteria to become resistant immediately against several groups of antibiotics such as Beta-lactam, Aminoglycosides, Chloram-phenicol, Quinolone, Tetracycline and Sulphonamides. Thereby making the infections caused by these bacteria very difficult to treat (Arunagiri K *et al*.,2012). Carbapenem, such as Imipenem and Meropenem, is a potential option for the therapy of this bacterium, but unfortunately *P. aeruginosa* has an ability in hydrolyzing these antibiotics through enzyme metallo-β-lactamases (MBLs). (Zhao WH *et al*., 2009). Prevalence rates of *P. aeruginosa* produce MBLs vary widely. Study in India in 2012 reported MDR *P. aeruginosa* isolate produced MBLs in 70.1% of isolates, which was higher than that in 2002 that is only

12% (Arunagiri K *et al.,* 2012). Several types of MBLs reported from *Enterobacteriaceae* and Gram-negative non-fermenter group bacteria such as IMP, VIM, SPM, SIM and GIM isolated from clinical specimens. Enzymes such as IMP and VIM are two groups of enzymes were predominantly found in the previous studies (Giske CG *et al.,* 2006). Moreover, both of enzymes are also found predominantly in Asia. Recently, IMP and VIM, MBLs enzyme group are reported commonly found from various countries. Study in India, 2012, the MDR *P. aeruginosa* isolates showed that both of MBLs gene such as *bla*VIM genes and *bla*IMP gene were 87.2% and 4.3%, respectively. However, it has been different with study conducted in Iran in the same year, which reported *bla*VIM gene and *bla*IPM gene were18.2% and 9,6%, respectively. (Sepehriseresht S *et al.,* 2012). No data is reported for MBLs enzymes in Indonesia especially in Bali. In fact, the resistant data of *P. aeruginosa* against Carbapenem group antibiotics such as Meropenem and Imipenem is quite high in Sanglah General Hospital in 2014 was 35% and 45% respectively. Based on this condition, therefore, the aim of this study was to detect IMP-1 and IMP-2 genes of MDR *P. aeruginosa,* which are phenotypically resistant to the antibiotic Imipenem and Meropenem disks based on CLSI standards in Clinical Microbiology Laboratory, Sanglah General Hospital, Denpasar, Bali using PCR.

MATERIALS AND METHODS

Bacterial isolates and identification

Eighty-six glycerol stock isolates of *P. aeruginosa* were cultured on MacConkey Agar and incubated aerobically at 35±2°C, 18-24h. The isolates were isolated from sputum (25 / 29.1%), wound (25 / 29.1%), urine (15 / 17.4%), Endotracheal Tube (11 / 12.8), pus (6/7%), blood (3 / 3.5%) and tissue (1 / 1.1%) during 2013-2015. Identification of *P. aeruginosa* and drug susceptibility test by VITEK-2 based on CLSI Standard.

Bacterial Genomic DNA Isolation

Isolates of *P. aeruginosa* that have grown on MacConkey agar plates were harvested around 5-10 colonies and suspended in 200 µl PBS (Phosphate Buffered Saline) pH 7.3. Bacterial genomic DNA was isolated by using Roche High Pure PCR Template Isolation Kit (Roche Life Science, Indianapolis, USA) based on manufacturer's instruction from bacterial suspension. DNA was eluted with 50 µl of elution buffer.

PCR for *bla*IMP1 and *bla*IMP2 gene

PCR was conducted using Go Taq® Green Master Mix (Promega, Madison, USA). Uniplex PCR to detect 16S rRNA gene was performed. It was followed by Uniplex PCR to detect IMP-1 and IMP-2 gene used primer Listed in Table 1. The final concentration of primers were 0.4 µM each. Protocols of PCR were described in previous study (Sepehriseresht et al, 2012). PCR cycle was initiated with pre denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 1 min, annealing primer at 55°C (16sRNA and IMP-1) and 48°C (IMP-2) for 1 min and extension at 72°C for 1 min; and final extension at 72°C for 7 min (iCycler, Biorad thermal cycler).

Table 1. Primer used for Gene VIM and IPM Detection (Shibata et al.,2003)

No.	Nama Primer	Sekuens Primer (5'→ 3')	bp
1.	*bla*IMP1	Forward: ACC GCA GCA GAG TCT TTG CC	587
		Reverse: ACA ACC AGT TTT GCC TTA CC	
2.	*bla*IMP2	Forward: GTT TTA TGT GTA TGC TTC C	678
		Reverse: AGC CTG TTC CCA TGT AC	

Amplicons were electrophoresed on 1.5% agarose gel in TBE buffer at 60 volt, and for 35 min. DNA was visualized with GelRed™ Nucleic Acid Gel Stain (Biotium, Hayward, CA 94545) and

RESULTS AND DISCUSSION

A total of 86 glycerol stock isolates of *P. aeruginosa* from clinical samples, which were phenotypicall resistant to antibiotics imipenem and meropenem using VITEK-2 based on CLSI Standard, were successfully cultured on MacConkey agar media plates. The colonies showed pale yellow colonies. All clinical isolates were positive tested for the presence of 16sRNA gene (Fig.1), confirmed that all isolates were *P. aeruginosa*. All isolates were subjected to PCR for detection of IMP-1 and IMP-2. The result showed that 9 (10.5%) isolates were positive IMP-1 gene (Fig.2), but none of isolate was positive for IMP-2 gene. The study to detect MBLs gene have been conducted in the other countries. Study in Iran showed that 6.6% and 3.3% of the *P. aeruginosa* had IMP-1 and IMP-2 genes, respectively (Sepehriseresht S et al,2012). In Asian Countries and regions, two MBLs gene are commonly prevalent. Study in Japan showed IMP-1 gene was 0.5% and IMP-2 was 64.5% (Shibata et al, 2003), whereas in China reported that IMP-1 type of MBLs was 89.7% (Arunagiri K, 2012)

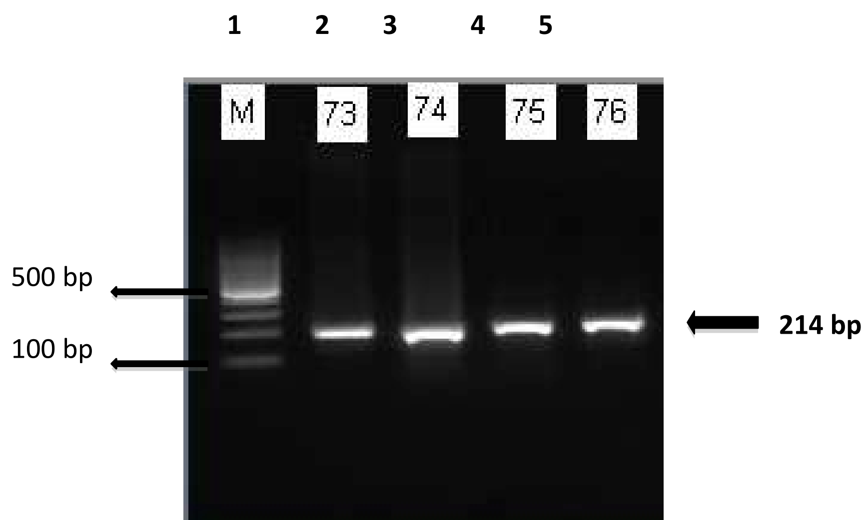

Fig. 1. PCR for 16sRNA gene detection. The expected band was 214 bp. Amplicon was electrophoresed on 1.5% agarose gel. (M = Marker 100 bp; Lane 2 - 5 = 16sRNA positive band = 214 bp)

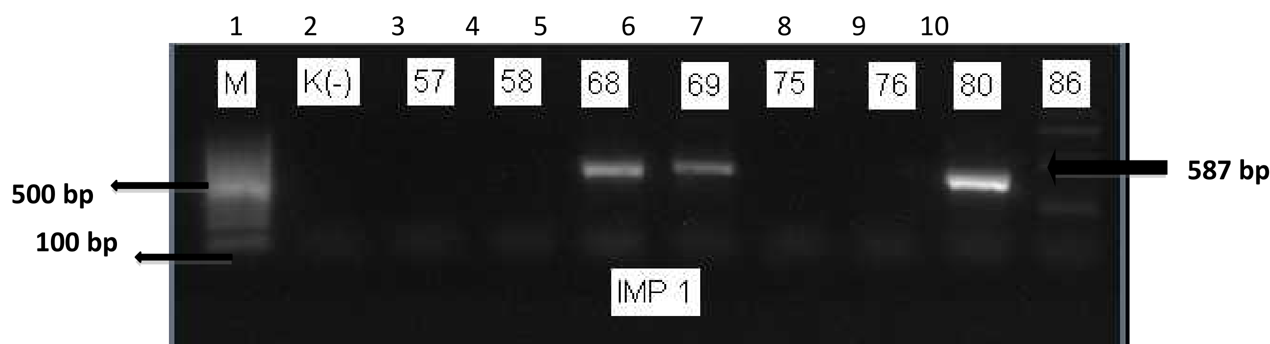

Fig. 2. PCR for IMP-1 gene detection. Lanes 5,6 and 9 were positif for IMP-1 gene (587 bp). Amplicon was electrophoresed on 1.5% agarose gel. (lane 1 = marker, lane 2 = negative control)

This study showed the similar condition with the MBL gene research studies from the other countries, especially for the gene IMP-1. However, it was slightly different for IMP-2 gene. Detection and molecular characterization of MBL-producing *P. aeruginosa* strains very important for infection control purposes. Currently, this study is still continued for detection of another MBL genes.

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