

Carbapenem Resistant Gene of *Pseudomonas Aeruginosa*

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ABSTRACT

Objective: Determining the frequency of MBLs classifying VIM, IMP and NDM in *P. aeruginosa* isolates.

Methods: The isolates of *P. aeruginosa* were isolated from sputum, blood, pus, fluid from patients (non-duplicate samples) in different clinical departments. They had been identified and performed antibiotic susceptibility testing by using Vitek 2 according to CLSI 2017 standards at Cho Ray Hospital from October 2017 to March 2018. The isolates were subcultured, picked colonies, extracted DNA and perform real-time PCR with MDR-MBL kit (VIM, IMP, NDM) of Sacace (Italy) to detect the MBL class VIM, IMP and NDM.

Results: There were 64 isolates of *P. aeruginosa* resistant to carbapenem (either or both Imipenem and Meropenem) were carried out real-time PCR to detect MBL (VIM, IMP, NDM). Among them, 29 (45.3%) isolates had IMP-type gene, 7 (10.9%) isolates had NDM-type gene, 3 (4.7%) isolates had VIM-type gene, 3 (4.7%) isolates had both IMP-type and NDM-type genes, 22 (34.4%) isolates were negative for the above 3 genes. 10 carbapenem-susceptible isolates were performed real-time PCR to detect MBL (IMP-type, NDM-type, VIM-type). Among them, 2 (20%) isolates had IMP-type gene, 8 (80%) isolates were negative for 3 genes.

Conclusion: The results of this study revealed a noticeably high prevalence of MBL-producing *P. aeruginosa* isolates at our hospital. MBL genes among isolates which reported as sensitive to carbapenem was acting as reservoir of such resistance genes with potential risk for the silent spread of these genes in hospitals and community. So, early recognition of MBL-producing isolates, establishing efficacy infection control measures and an appropriate protocol for antimicrobial therapy based on the laboratory data are necessary to prevent the dissemination of these resistance.

Introduction

Background

Antimicrobial resistance is one of the biggest threat to global health especially on developing country as Viet Nam. By 2050, if there is not effective interventions against antimicrobial resistance, 10 million deaths may occur annually [1]. *Paeruginosa* is one of the most frequent and important pathogens in hospital-acquired infections, particularly in critically ill or immunocompromised patients, because they can be resistant to many common used

antimicrobial agents. Carbapenems have been important antibiotics in MDR *P. aeruginosa* treatment. However, resistance to carbapenems has been reported, especially in developing countries [2]. The most common mechanism of resistance to carbapenems is the production of metallo-beta-lactamases (MBLs) [3]. MBLs are encoded either by genes on chromosome or on specific genetic elements so that they can resist to carbapenems and other antimicrobial agents, confer multidrug resistance (MDR) *P. aeruginosa*. In 1991, MBL producing

P. aeruginosa were first reported in Japan, and since then there have been many reports of MBLs among carbapenem-resistant *P. aeruginosa* isolates in the world [4]. Mortality rates are significantly higher in MBL producing *P. aeruginosa* than non-MBL *P. aeruginosa* [5]. Moreover, MBL can be spread from *P. aeruginosa* to members of *Enterobacteriaceae*, increasing the antimicrobial resistant rate and complication in the treatment of infected patients [6]. In addition, new antipseudomonal, such as ceftolozane-tazobactam, ceftazidime-avibactam, have better activity against MDR *P. aeruginosa*, however activity is just only in especially isolates with certain mechanism of resistance. Therefore, understanding the molecular characteristics of MBLs producing *P. aeruginosa* is very important to choose the appropriate treatment, control infection and prevent a possible global health crisis.

There are many different types of acquired MBL depending on epidemiological areas. The most important types that contribute to clinical studies are the IMP-type, VIM-type, NDM-type, SPM-type. However, at present, there is no standard phenotypic confirmatory tests for detection MBLs among *P. aeruginosa* isolates. [3] Several authors show that molecular methods is the proper test for screening the presence of MBLs genes Polymerase chain reaction (PCR) has high sensitivity and reliability for detection of MBL genes among MBL-producing *P. aeruginosa*. At present, there is no systematic evaluation of the molecular epidemiology of MBL-producing *P. aeruginosa* isolates in Viet Nam. Therefore, we carry on the study to detect the features of frequencies of IMP-type, VIM-type, NDM-type genes among *P. aeruginosa* isolates in our hospital.

Objectives

Due to the importance of detection MBLs, in this study we determined the frequency of MBLs classifying VIM, IMP and NDM in *P. aeruginosa* isolates in the patients by the PCR real-time method.

Materials and Methods

Bacterial isolates

This was a cross-sectional study, in which a total of 74 non-duplicated *P. aeruginosa* isolates were collected from various clinical samples received in the microbiology laboratory for bacterial culture and sensitivity from patients admitted in Cho Ray hospital at Ho Chi Minh city, located in the South of Viet Nam, during the period from October 2017 to March 2018. Bacterial species were identified using standard laboratory methods, including Gram staining, oxidase and on automated system such as Vitek 2-compact and MALDITOF-MS (Biomérieux, France). The isolates were stored at - 80oC in trypticase soy broth containing 20% glycerol and subculture twice prior testing.

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing for all the isolates was done by automatic system Vitek2-compact to detect the minimum inhibitory concentrations (MICs). The following antibiotics were

tested Piperacillin, Piperacillin/tazobactam, Ceftazidime, Cefepime, Gentamicin, Amikacin, Ciprofloxacin, Levofloxacin, Colistin, Meropenem, Imipenem. Susceptibility and MICs breakpoints were defined according to Clinical and Laboratory Standards Institute (CLSI; 2017). Carbapenem sensitivity was determined based on the MIC breakpoint. *P. aeruginosa* was defined non-susceptible to carbapenem when it's indeterminate or resistant to imipenem and/or meropenem. *P. aeruginosa* ATCC 27853, E.coli ATCC 25922, E.coli ATCC 35218 were used as control isolates in all susceptibility assays.

Detection of MBLs by PCR

Carbapenem -non susceptible and carbapenem - susceptible isolates were tested by Sacace MDR MBL (VIM, IMP, NDM) Real-TM PCR kit. DNA was extracted by the SaMag™ Bacterial DNA Extraction Kit. Frozen bacteria were sub-cultured onto Mac-conkey agar (BD, American) before DNA extraction. International reference strain *P. aeruginosa* ATCC 27853 was used as the blaMBL gene's negative control strain.

Results

74 isolates of *P. aeruginosa* from different clinical samples were collected. In total, 64 isolates were non-susceptible to these carbapenem, of which 42 (65.6%) isolates were found to be MBL producers. Twenty-nine (45.3%) isolates encoded IMP-type, which was the most commonly encoded gene among those investigated, followed by the NDM-type 7 (10.9%) and VIM-type 3 (4.7%), both IMP-type and NDM-type 3 (4.7%), and 22 (34.4%) isolates were negative for the above 3 genes. Ten carbapenem-susceptible isolates were performed real-time PCR to detect MBL, of which, 2 (20%) isolates had IMP-type gene, 8 (80%) isolates were negative for 3 genes IMP-type, VIM-type, NDM-type (Table 1).

Table 1: Distribution of MBL-type in *P. aeruginosa*.

	Isolates non-susceptible to Carbapenem n (%)	Isolates susceptible to Carbapenem n (%)
IMP-type	29 (45.3%)	2 (20%)
NDM-type	7 (10.9%)	-
VIM-type	3 (4.7%)	-
NDM-type + VIM-type	3 (4.7%)	-
No MBLs genes (IMP-type, VIM-type, NDM-type)	22 (34.4%)	8 (80%)
TOTAL	64 (100%)	10 (100%)

Discussion

This discussion illustrated screening by molecular approach. In current study, 64 non-susceptible carbapenem isolates were collected. Among them, 42 (65.6%) isolates were MBL producers based on the PCR. This frequency was higher than in other studies at Asia or another region. In one report from Iran- 2017, prevalence of MBL-producing carbapenem-resistant *P. aeruginosa* was 43.7%.

[7] In Thailand- 2019, of 153 carbapenem-resistant *P. aeruginosa*, this prevalence was 20.3% (31 isolates), divided into VIM-type (14 isolates) and IMP-type (17 isolates) [3]. This difference may be due to various research populations: Iran's report was focus on the burn patients, and the Thailand's report was based on the isolates from 5 regions of Thailand, our study was examined at our general hospital. This study found 29 (45.3%) isolates carried IMP-type, which was the most common MBL genes, followed by the genes NDM-type (10.9%) and VIM-type (4.7%). In 1988, transferable IMP-1 was isolated from *P. aeruginosa* in Japan and was found in a class 1 integron located on a conjugational plasmid. Thereafter, it was identified in many other species suggesting horizontal gene transfer between unrelated Gram-negative species [4]. Therefore, in most studies, IMP type-producing isolates has a high prevalence. However, our result was different from a study in Iran and Thailand, that had equivalent frequency between VIM-type and IMP-type (17.5% and 15.6%) and (14 isolates and 17 isolates), respectively [8,9]. The difference may be due to the variation of geographical circulating strain.

In our present study, the NDM-type and VIM-type genes coexisted in 3 (4.7%) isolates. The genes encoding these enzymes are often located on transferable genetic platforms and can potentially spread to other species through horizontal gene transfer. Therefore, appropriate infection control guidelines and treatment protocols are needed to prevent the further dissemination of these resistant genes to other bacterial agents in hospital.

Unfortunately, we also detected 2 (20%) MBL-producing isolates (IMP-type) in carbapenem-susceptible *P. aeruginosa*. This result is similar to study in Sudan, from 2015 to 2016, prevalence of MBL genes by multiplex PCR among 100 carbapenems-susceptible gram-negative clinical isolates (including *P. aeruginosa*) was 27 (27%) [1]. It shows that, in some cases, MBL – producing isolates express low resistance, thus we cannot use the phenotypic test to detect them. This result has the important meaning, because MBL detection by PCR assays has not been routinely performed in most of clinical microbiology laboratories. Consequently, the occurrence of MBL production in *P. aeruginosa* that remains susceptible to carbapenem might be unrecognized by the clinical laboratory, they can transfer the genes to other gram negative because MBL-encoding genes are often carried by mobile genetic elements,

increasing the antibiotic resistance rate. In addition, failing in detection MBL production among carbapenem-susceptible isolates may lead to inadequate treatment, especially in serious ill patient.

Conclusion

The results of this study revealed a noticeably high prevalence of MBL- producing *P. aeruginosa* isolates at our hospital, especially in some carbapenem-susceptible isolates. MBL genes among isolates which reported as sensitive to carbapenem was acting as reservoir of such resistance genes with potential risk for the silent spread of these genes in hospitals and community. Thus, early recognition of MBL-producing isolates, establishing efficacy infection control measures and an appropriate protocol for antimicrobial therapy based on the laboratory data are necessary to prevent the dissemination of these resistance.

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