Available online at www.sciencedirect.com

ScienceDirect

Journal of the Chinese Medical Association xx (2017) 1−6

www.jcma-online.com

Original Article

Investigating of four main carbapenem-resistance mechanisms in high-level carbapenem resistant *Pseudomonas aeruginosa* isolated from burn patients

Soodabeh Rostami [a,*], Ahmad Farajzadeh Sheikh [b], Saeed Shoja [c], Abbas Farahani [b], Mohammad Amin Tabatabaiefar [d], Abbas Jolodar [e], Raheleh Sheikhi [f]

[a] *Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
[b] *Department of Microbiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[c] *Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
[d] *Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
[e] *Department of Biomolecular and Biochemistry, School of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran*
[f] *Department of Microbiology, Guilan University of Medical Sciences, Rasht, Iran*

Received November 1, 2016; accepted August 1, 2017

Abstract

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen involved in many infections. Carbapenem-resistant *P. aeruginosa* has emerged as an important cause of infection in different hospitals worldwide. We aimed to determine frequencies of the four main resistance mechanisms [metallo-beta lactamase (MBL) production (bla_{IMP}, bla_{VIM}, bla_{SPM} and bla_{NDM}), overproduction of the MexAB−OprM and MexXY efflux pumps, overproduction of chromosome-encoded AmpC β−lactamase, and reduced *OprD* expression] in high-level carbapenem-resistant *P. aeruginosa* isolated from patients with burns.

Methods: In a descriptive study, 107 *P. aeruginosa* isolates were collected from patients with burn injuries and tested for antibiotic susceptibility, by an E-test for carbapenems, an E-test for metallo-β-lactamase producer isolates, and PCR to detect MBL genes. Furthermore, high-level carbapenem-resistant isolates were tested by real-time PCR for the expression levels of the *mexB*, *mexY*, *ampC*, and *oprD* genes.

Results: Amongst all *P. aeruginosa* isolates, 78.5%, 46.7%, and 15% were imipenem-, meropenem-, and doripenem-resistant, respectively; 72% of isolates were multidrug-resistant. The bla_{IMP} and bla_{VIM} genes were detected in 17.9% and 1.2% of isolates; respectively. The bla_{SPM} and bla_{NDM} genes were not observed. Among the resistant isolates, *mexB* overexpression (63.2%) was the most frequent mechanism, followed by *mexY* overexpression (52.6%), *ampC* overexpression (36.8%), and reduced *oprD* expression (21.1%).

Conclusion: Emerging antimicrobial resistance in burn wound bacterial pathogens is a serious therapeutic challenge for clinicians. In the present study, most of the isolates were MDR. This finding indicated an alarming spread of resistant isolates and suggested that infection control strategies should be considered. Resistance to carbapenems is influenced by several factors, not all of which were evaluated in our study; however, the results showed that production of MBLs and overexpression of the *mexB* gene were the most frequent mechanisms in carbapenem-resistant isolates.

Keywords: Burns; Carbapenems; Efflux pumps; *Pseudomonas aeruginosa*

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Soodabeh Rostami, Isfahan University of Medical Sciences, School of Medicine, Infectious Diseases and Tropical Medicine Research Center, Hezar Jarib Street, Postal code: 8174673461 Isfahan, Iran.
E-mail address: srostami1876@gmail.com (S. Rostami).

https://doi.org/10.1016/j.jcma.2017.08.016

1. Introduction

Pseudomonas aeruginosa is an opportunistic pathogen involved in many infections, such as respiratory infections, urinary tract infections, wound and soft tissue infections, and bacteremia in immunocompromised patients, including patients with thermal injuries.[1] Despite advances in medicine, *P. aeruginosa* is considered an important infectious threat to patients with burns.[2]

P. aeruginosa displays a primary resistance to many antimicrobial agents because of the outer-membrane barriers, presence of multidrug efflux pumps, and endogenous antimicrobial inactivation.[1] Selection of a suitable antibiotic is complicated by the ability of *P. aeruginosa* to develop resistance to multiple classes of antibacterial agents, and the appropriate choice of antibiotic to begin treatment with is essential to optimizing the clinical outcome.[3]

Carbapenems (e.g., imipenem, meropenem, and doripenem) are members of the β-lactam antibiotic class, and are used commonly to treat infections caused by *P. aeruginosa.*[1] Although carbapenems are the most effective antibiotics for therapy of multidrug-resistant *P. aeruginosa* infections, increased emergence of high-carbapenem resistant isolates has been observed worldwide.[4] Multiple mechanisms are involved in resistance to carbapenems. One of the mechanisms is the acquisition of resistance genes on mobile genetic elements, and another way is through mutations in genes that change the expression and/or function of chromosomally encoded proteins.[3]

Carbapenems are relatively stable to hydrolysis by most β-lactamases[5,6]; however, metallo-β-lactamases (MBL) are able to hydrolyze them efficiently. Genes encoding MBLs are transferred by mobile genetic elements.[7−9]

The three most studied, chromosomally encoded resistance mechanisms against carbapenems in *P. aeruginosa* are: i) inactivation of the outer membrane protein *OprD*; ii) overexpression of chromosome-encoded *ampC* (β−lactamase); and iii) overproduction of multidrug efflux pumps, such as MexAB−OprM and MexXY−OprM.[3]

In our country, studies have been carried out on enzymatic carbapenem resistance mechanisms in *P. aeruginosa* collected from patients with burns[10−12]; however, there is a little information about contribution of different mechanisms to carbapenem resistance in these isolates.[13]

In this study, four main resistance mechanisms (MBLs production, overproduction of the MexAB−OprM and MexXY efflux pumps, overproduction chromosome-encoded AmpC β−lactamase and reducing the *OprD* expression) were examined in high-level carbapenem-resistant *P. aeruginosa* (CRPA) isolated from patients with thermal injury.

2. Methods

2.1. Bacterial isolates

This descriptive study was approved by the ethics committee of the Ahvaz Jundishapur University of Medical Sciences (Grant No: CMRC-55). During the period from June 2011 through May 2012, a total of 107 non-duplicate *Pseudomonas* spp. were collected from the microbiology laboratory of a burns teaching hospital (Taleghani hospital) in Ahvaz, in the South west of Iran. Identification of *P. aeruginosa* was performed using previously described standard phenotypic tests, and verified by polymerase chain reaction (PCR), using specific primers for the *P. aeruginosa gyrB* gene.[14]

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for the bacterial isolates was carried out using the Kirby−Bauer method, as recommended by the Clinical and Laboratory Standards Institute (CLSI).[15] The following antibiotics were tested: imipenem (10 μg), meropenem (10 μg), doripenem (10 μg), ceftazidime (30 μg), cefepime (30 μg), piperacillin (100 μg), piperacillin/tazobactam (100/10 μg), gentamicin (10 μg), amikacin (30 μg), tobramycin (10 μg), ciprofloxacin (5 μg), aztreonam (30 μg), polymyxin B (300 units), and colistin (10 μg) (Mast Group Ltd, UK). The minimum inhibitory concentrations (MICs) of carbapenems (imipenem [IMI], meropenem [MRP], and doripenem [DOR]) were obtained by an E-test (Liofilchem, Italy), as described in the manufacturer's instructions. Carbapenem resistance was determined based on the MIC breakpoints. When an isolate was resistant to three carbapenems (imipenem, meropenem, and doripenem), that isolate was considered high-level carbapenem resistant. If an isolate was resistant to three or more classes of antimicrobial agents (i.e., penicillins/cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones), that isolate was considered multidrug resistant (MDR). In accordance with the CLSI guidelines, *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as control strains in all susceptibility assays.

2.3. Phenotypic and molecular detection of MBLs

For the phenotypic detection of metallo-β-lactamase producer isolates, an E-test MBL strip containing a double-sided seven-dilution range of imipenem (4−256 μg/ml; IMI) and imipenem in combination with a fixed concentration of EDTA (1−64 μg/ml; IMD) (Liofilchem, Italy) was used. The test was considered positive when the IMI/IMD ratio was ≥8 μg/ml.

All imipenem-resistant *P. aeruginosa* isolates were screened by PCR for the bla_{IMP}, bla_{VIM}, bla_{SPM}, and bla_{NDM} genes. For the bla_{SPM} and bla_{NDM} genes, the primers used were as reported by Poirel et al.[16] and primers newly designed in this study were used for the bla_{IMP} and bla_{VIM} genes (Table 1). Primers were prepared by TAG Copenhagen A/S, Denmark, and all chemical materials were from SinaClon (Iran). DNA was extracted by the boiling method. Briefly, frozen bacteria were sub-cultured on a Mueller−Hinton's agar plate (Merck, Germany) and incubated at 35 °C overnight. After growth, one to five colonies were suspended in 500 μl of 1 × Tris-EDTA buffer, heated at 95 °C for 10 min, and placed at room

Please cite this article in press as: Rostami S, et al., Investigating of four main carbapenem-resistance mechanisms in high-level carbapenem resistant *Pseudomonas aeruginosa* isolated from burn patients, Journal of the Chinese Medical Association (2017), https://doi.org/10.1016/j.jcma.2017.08.016