Iranian Journal of Microbiology
Volume 7 Number 5 (Octobr 2015) 247-250

ORIGINAL ARTICLE

IS*Ppu22*, a novel insertion sequence in the *oprD* porin gene of a carbapenem-resistant *Pseudomonas aeruginosa* isolate from a burn patient in Tehran, Iran

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Received: March 2015**, Accepted:** September 2015

ABSTRACT

Background and Objectives: The *oprD* mutation and AmpC overproduction are the main mechanisms of intrinsic resistance to carbapenems such as imipenem and meropenem in *Pseudomonas aeruginosa*.
Materials and Methods: In this study, we investigated intrinsic resistance to carbapenems including mutation of *oprD* and AmpC overproduction in a carbapenem-resistant *P. aeruginosa* isolated from a burn patient by phenotypic and molecular methods.
Results: In our study, the carbapenem-resistant *P. aeruginosa* isolate was resistant to imipenem, meropenem, cefepime, gentamicin, ceftriaxone, carbenicillin, aztreonam and ciprofloxacin but was susceptible to ceftazidime and polymyxin B. The minimum inhibitory concentrations (MICs) against imipenem, meropenem and ceftazidime were 64 µg/ml, 16 µg/ml and 2µg/ml, respectively. The isolate was ESBLs and AmpC overproducer. No carbapenemase activity was detected by Modified Hodge test (MHT). This isolate was carrying only bla_{OXA-10}. PCR amplification and sequencing of *oprD* performed on isolate resulted in PCR product of 2647bp. Sequence analysis of the 2647bp product revealed insertion of a sequence of 1232 bp at position 8 in coding region of *oprD*.
Conclusion: According to the results of this study, *oprD* mutation and AmpC overproduction can cause the main mechanism of resistance of *P. aeruginosa* to carbapenems.

Keywords: ISPpu22, *oprD*, AmpC, Carbapenem-resistant *P. aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous organism present in many diverse environmental settings and it is an important cause of bacterial infections associated with hospitalization (1). It presents a serious therapeutic challenge for treatment of related infections because the majority of isolates show a high level of intrinsic resistance to antimicrobial drugs and has potential to acquire resistance (2,3). Carbapenems such as imipenem and meropenem are the major antibiotics used for treatment of *P. aeruginosa* infections (2,3). In recent years, carbapenem resistance has developed among *P. aeruginosa* worldwide (2-4). Several mechanism including loss of the outer

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membrane OprD porin, efflux-pumps over-expression and production of β-lactamases are involved in resistance to carbapenems. However, *oprD* mutation and AmpC overproduction are the main mechanisms of intrinsic resistance to carbapenem. Loss of the outer membrane OprD porin causes blocking of entrance of carbapenems particularly imipenem into the cell (5,6). One of the factors that causes loss of the OprD is the disruption of *oprD* by insertion sequence (IS) elements (7). Also, the mutation of *oprD* usually couples with AmpC over-expression and thus leads to increased resistance to imipenem (8,9). In this study, we report a novel insertion sequence in the *oprD* porin gene of a carbapenem-resistant *P. aeruginosa* isolate from a burn patient in Tehran, Iran.

MATERIALS AND METHODS

The carbapenem-resistant *P. aeruginosa* strain was isolated in 2011 from a wound sample of a female burn patient who was admitted to Shahid Motahari Hospital of Tehran University of Medical Sciences. The identification of isolate was performed by standard biochemical tests (10). The antibiotic resistance pattern of isolate was determined using disk diffusion method according to the CLSI guidelines (11). The following antibiotics (MAST, UK) were tested: imipenem (10 µg), meropenem (10 µg), polymyxin B (300 Unit), ceftazidime (30 µg), cefepime (30 µg), gentamicin (10 µg), amikacin (10 µg) ceftriaxone (30 µg), carbenicillin (30 µg), aztreonam (30 µg) and ciprofloxacin (5 µg). The isolate was also screened for production of carbapenemase using the Modified Hodge test (11). The isolate screened for extended spectrum β-lactamases (ESBLs) production using combined double-disk test, double-disk synergy and β-lactamase disk test (9,11). The β-lactamase disk test is based on use of Tris-EDTA (TE) buffer to permeabilize a bacterial cell and release of β-lactamases into the external environment. In brief, the surface of a Mueller-Hinton agar plate was inoculated with a lawn of the cefotaxime (CTX) susceptible (*P. aeruginosa* ATCC 27853) according to the standard disk diffusion method. A CTX (30µg) disk was placed on the bacterial lawn on the surface of the Mueller-Hinton agar and flanked by two blank disks, each containing 20 µl of a 1:1 mixture of saline and 100X Tris-EDTA solution. Colonies of the test strain and control strains were applied to blank disks (Fig.1). Flattening or indentation of the growth inhibition zone of the CTX disk at the side of blank disks containing the test strain indicated the release of β-lactamase. The minimum inhibitory concentrations (MICs) of imipenem (IMI), meropenem (MEM) and ceftazidime (CAZ) were determined in micro broth dilution assay. Susceptibility breakpoints were defined according to CLSI recommendations (11). AmpC overproduction was confirmed according to the method of Rodrı´guez-Martı´nez et al. (12). Furthermore, the presence of carbapenemase genes (bla_{VIM}, bla_{IMP}, bla_{GIM}, bla_{AIM}, bla_{SPM}, bla_{NDM}, bla_{SIM}, bla_{OXA-48}, bla_{KPC} and bla_{GES}) and ESBLs genes (bla_{TEM}, bla_{SHV}, bla_{PER}, bla_{PSE}, bla_{CTX-M} and bla_{OXA-10}) were investigated by PCR method as previously described (4). PCR amplification and sequencing of *oprD* and the IS*Ppu*22 was performed using primers *oprD*-F-1-5´-CGCCGACAAGAAGAACTAGC-3´ and *oprD*-R-5´-GTCGATTACAGGATCGACAG-3´(9) and with additional primer designed in this study (IS*Ppu*22-F-5´-GGTAGCCGTATACACCTCCG-3´, *oprD*-F-2-5´-TCGATGCCTTCGGCTACCT-3´)

RESULTS

The carbapenem-resistant *P. aeruginosa* isolate was resistant to imipenem, meropenem, cefepime, gentamicin, ceftriaxone, carbenicillin, aztreonam and ciprofloxacin but was susceptible to ceftazidime and polymyxin B using disk diffusion method. MICs revealed a high resistance to imipenem (MICs=64 µg/ml). MICs against meropenem and ceftazidime were 16 and 2µg/ml, respectively. This strain was AmpC overproducer. Addition of cloxacillin reduced the MICs from 64 to 16µg/ml for imipenem. No carbapenemase activity was detected in MHT. Diameter of inhibition zone around the antibiotics in combined disk test and double-disk synergy was zero without detection of ESBLs by these tests but β-lactamase disk test was positive for this isolate (Fig. 1). PCR results were negative for bla_{TEM}, bla_{SHV}, bla_{PER}, bla_{PSE}, bla_{CTX-M}, bla_{OXA-48}, bla_{KPC}, bla_{GES}, bla_{VIM}, bla_{IMP}, bla_{GIM}, bla_{AIM}, bla_{SPM}, bla_{NDM} and bla_{SIM} genes. This isolate was positive only for bla_{OXA-10}. PCR amplification and sequencing of *oprD* performed on strain resulted in PCR product of 2647bp instead of 1413 bp by *oprD*-F-1 and *oprD*-R primers (Fig. 2). Sequence analysis of the 2647bp product revealed insertion of a sequence of 1232 bp at position 8 in coding region

of *oprD*. This IS that had 97% identity with IS3 family transposed from *P. aeruginosa* PA96 (GenBank accession no. CP007224). This IS named IS*Ppu*22, belonging to the IS3 family according to the IS finder database nomenclature *(https://www-is.biotoul.fr)*. The full sequence of the *oprD* gene interrupted by this novel IS element has been deposited in the GenBank database with accession no. KJ825703.

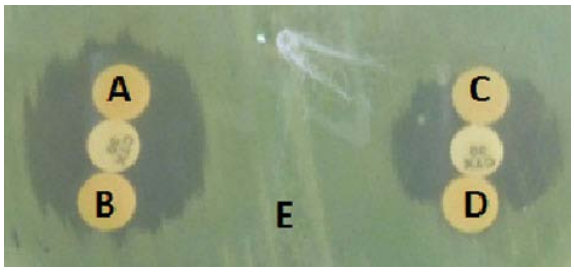

Fig.1. β-lactamase Disk Test. A, B; Negative controls (*P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922), C; Positive control, D; Clinical isolate and E; Lawn culture (*P. aeruginosa* ATCC 27853).

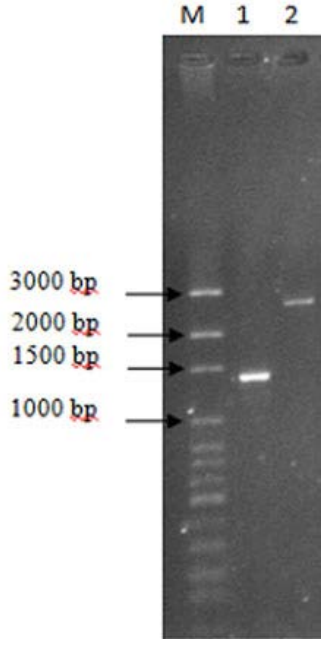

Fig.2. PCR product of *oprD*, Lane M: DNA size marker, Lane 1:PAO1, Lane 2: Clinical isolate

DISCUSSION

In this study, the isolate was positive for β-lactamase by β-lactamase disk test method. This result shows a probable activation of one of the efflux pumps system and impermeability, or both, against beta-lactam antibiotics which causes falsely negative results in β-lactamase detections in combined disk method and double-disk synergy (4,8). This investigation showed that, in the absence of carbapenemases, the *oprD* mutation and AmpC over expression can cause resistance to carbapenems, however, *oprD* mutation can cause decreased meropenem susceptibility and AmpC over expression not effective on meropenem (5,6,13). Literature revealed various routes, including deletion or insertion of sequences, can cause of *oprD* inactivation. Recently, IS elements disrupting of *oprD* has been reported in France, USA, China, Korea and Spain among resistant carbapenem *P. aeruginosa* isolates (7,12). In this study AmpC-β-lactamase caused a reduction in susceptibility to imipenem but not to meropenem. These data suggest that AmpC may play an important role in the level of intrinsic susceptibility of *P. aeruginosa* to imipenem. Quale, Rodríguez and Lee et al, reported that AmpC-β-lactamase causes an increase of MIC to carbapenems in clinical *P. aeroginosa* isolates (3,12-15). Therefore, mutation of *oprD* and AmpC overproduction are the main mechanisms of carbapenem resistance in the absence of acquired carbapenemases such as metallo-beta-lactamases.

CONCLUSION

According to the results in our study, intrinsic resistance mechanism such as *oprD* mutation and AmpC overproduction are the main mechanism of resistance of *P. aeruginosa* to carbapenems.

ACKNOWLEDGMENT

This research has been supported by Tehran University of Medical Sciences & Health Services grant no19204//91-04-30.

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