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ORIGINAL ARTICLE

Detection of *bla*$_{PER-1}$ & *bla*$_{Oxa10}$ among imipenem resistant isolates of *Pseudomonas aeruginosa* isolated from burn patients hospitalized in Shiraz Burn Hospital

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ABSTRACT

Background and Objectives: *Pseudomonas aeruginosa* is one of the most important Gram negative opportunistic bacteria which causes infection among burn patients. Resistance to the antibiotics in this group of bacteria is increased due to the activity of extended spectrum β-lactamase (ESBLs) genes. In the current study, we investigated the prevalence of two genes (*bla*$_{PER-1}$ & *bla*$_{Oxa10}$) related β-lactamase genes among imipenem resistance clinical isolates of *P. aeruginosa* in hospitalized patients.

Materials and Methods: From May 2010 to March 2011, 270 *P. aeruginosa* isolated from hospitalized burned patients' wounds in Shiraz Burn Hospital**,** were tested for Imipenem resistance by disk diffusion method. Presence of ESBLs exo-enzyme, *bla*$_{PER-1}$ and *bla*$_{Oxa10}$ genes were also evaluated in the resistant isolate.

Results: 210 (77.7%) of 270 *P. aeruginosa* isolates were resistant to imipenem. *bla*$_{PER-1}$ and *bla*$_{Oxa10}$ were detected among 168 (80.0%) of imipenem resistant isolates. Furthermore, 160 (76.2%) of them had *bla*$_{Oxa10}$ gene and 84 (40.0%) of them had *bla*$_{PER-1}$ while 63 (30.0%) resistant isolates contained both genes simultaneously.

Conclusion: This study showed a high prevalence of *bla*$_{PER-1}$ and *bla*$_{Oxa10}$ genes in hospitalized burn patients in south west of Iran. Therefore, it's highly recommended to perform such tests routinely to evaluate the resistance pattern in order to better antibiotic selection in the burned patients.

Keywords: *bla*$_{Oxa10}$, *bla*$_{PER-1}$, Burn, ESBLs, Resistance, *Pseudomonas aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa is a Gram negative opportunistic human pathogen, which causes various acute and chronic nosocomial infections such as pneumonia, urinary tract and wound infections in immunocompromised hosts, particularly in burn hospitalized patients (1).

These infections are responsible for significant human mortality, morbidity, prolonged hospital stays and increased health care costs (2,3). This organism possesses different factors that promote adherence to host cells and mucosal tissues, damage host tissue, elicit inflammation and disrupt defense

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Table 1. Primer seq. target genes, product size and PCR protocol

Bacteria Gene Primer	Seq. (5/-3/ direction)	cycle profile (35X)	Product size
P. aeruginosa			
bla_{OXA-10}F	TAT CGC GTG TCT TTC GAG TA	610C/1min- 720/2min- 950/1min	760 bp
bla_{OXA-10}R	TTA GCC ACC AAT GAT GCC C		
bla_{PER-1} F	ATG AAT GTC ATT ATA AAA GCT	520C/1min- 720/2min- 950/1min	927 bp
bla_{PER-1} R	TA ATT TGG GCT TAG G		

mechanisms. This conditions aggravate in burn patients due to impairment of the skin barrier in burn patients and frequent scrubbing, debridement and manipulation of the burn site (1). Resistance of *P. aeruginosa* to a wide spectrum of antibiotics has become a major clinical concern worldwide (1, 2). The extended spectrum β-lactamases (ESBLs) , may lead *P. aeruginosa* to be resistant to β -lactam antibiotics, including penicillins, cephalosporins and monobactams (4,5).

OXA and PER can be mentioned as the two of important β-lactamase enzymes, in *P. aeruginosa,* (3, 4). bal_{PER-1} was the first group of this gene reported from France in 1991 in a single *P. aeruginosa* isolate from a Turkish patient (3). *P. aeruginosa* strains contacting bal_{PER-1} are highly resistant to β-lactamase, and have strong hydrolytic activity against cephalosporins but can not hydrolyze carbapenems and cephamycins (5). Another group of such enzymes which has high incidence in Enterobacteriaceae especially in *P. aeruginosa* is OXA. Owing to hydrolytic activity OXA-10 (a class D β-lactamase) is responsible for a high resistance to amino-group antibiotics, carboxy-penicillins, ureido-penicillins and cephalosporins in *P. aeruginosa* isolates (6).

Due to importance of the carbapenems in resistance infections management, and increasing of the imipenem resistance ESBL *P. aeruginosa* strains, finding the true frequency of such enzymes is mandatory. The purpose of the present study was to investigate the prevalence of these two β-lactamase genes (bla_{PER-1} and bla_{Oxa-10}) in imipenem resistant clinical isolates of *P. aeruginosa* in hospitalized patients in a main burn center of southwest of Iran.

MATERIALS AND METHODS

Bacterial Isolation. The study included 270 *P. aeruginosa* isolates that were recovered consecutively from clinical sites of separate patients' wounds hospitalized in Shiraz Burn Hospital (the main burn center in southwest of Iran) from May 2010 to March 2011. Collected strains were assessed with routine microbiology methods like Gram stain, pigment production on Muller-Hinton agar media, Oxidase test and non-fermentative result in TSI media. Then, PCR based assay was performed by specific primers for 16s rDNA (7) to confirm presence of *P. aeruginosa*. Confirmed strains were stored at -200C in Trypticase Soy Broth containing 10% glycerol.

Antimicrobial susceptibility testing. Isolated strains were tested for their resistance to imipenem using the disc diffusion method (DD), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (8). Resistance to imipenem, were also evaluated for ESBL production using the combination disc diffusion method (CDD), recommended by the CLSI guidelines (9). In this step, antibiotic disks (aztreonam (/30µg), ceftazidime (/30µg) cephotaxime (/30µg)) were placed around a clavulanic acid (15µg) disk on 10 cm Mueller-Hinton agar plates inoculated with 0.5 McFarland suspensions of the isolates, with 30 mm distance between each disk. After the incubation time (18 hours/37˚C) inhibition zone diameters were measured to the minimum distance. Difference of 5mm ≥ in the zone between each disk with clavulanic acid disk compare to another side of the disk showed that the strain is ESBL positive. For ESBL negative control, *P. aeruginosa (*ATCC 27853) was used.

Detection of bla_{OXA-10} and bla_{PER-1} PCR. Imipenem-Resistant *P. aeruginosa* strains were refreshed in Muller-Hinton broth for about 4 hours and their DNA were extracted with Accuprep® Genomic DNA Extraction Kit (Bioneer-USA) according to the manufacture protocol. To evaluate the presence of bla_{OXA-10}, bla_{PER-1} genes in these strains, the PCR was performed with the specific primers for these regions according to the Table-1.

RESULTS

In this cross-sectional of 270 isolates of *P. aeruginosa*, 210 (77.7%) were resistant to the imipenem. According to the results of CDD screen, 168(80.0%) isolates were ESBL producing strains. 160 (76.2%) of them contain bla_{OXA-10} and 84 (40%) of resistant isolates contain bla_{PER-1} related genes, and 63(30.0%) of them contain both genes. 148(92.5%) of 160 bla_{OXA-10} containing isolates, and all of bla_{PER-1} containing isolates produced ESBL (Figs. 1- 2).

DISCUSSION

Burn injury is a major public health problem in many countries, and requires immediate specialized care in order to minimize mortality and morbidity (10,11). It is estimated that 75% of all deaths following burn injury are related to infection (12). The infection in such patients is difficult to control due to the presence of dead burn eschar, and moist environment, that act as a good growth medium for microbes. Prolonged hospital stay and invasive diagnostic and therapeutic procedures (13, 14).

P. aeruginosa, known as major colonizer of the burn wound, is able to accumulate different resistance and virulence factors, thrives on moist burn wound surface and survives well in the hospital environment, once it is established (14). Burn hospitals often harbor multidrug-resistant *P. aeruginosa* that can serve as the source of infection (2). Previous studies in Iran confirmed resistance to many antibiotics used routinely for treatment of burn wounds infected by *P. aeruginosa*. Hadadi *et al*. showed that *P. aeruginosa* isolates were resistance to ceftizoxime (99%), ceftazidime (59.6%), ticarcilin (50%), ceftriaxone (44.3%), and cefoperazone (37.5%) (15). According to a study conducted in Shiraz Burn hospital in 2006 by Japoni A *et al.* almost all *P. aeruginosa* isolated from burn patients were resistant to all tested anti-Pseudomonal antibiotics except carbapenems (meropenem and imipenem) (16).

Carbapenems are useful in treatment of some cases of multi-drug resistant strains of *P. aeruginosa* (16). The resistance of *P. aeruginosa* was 48% against imipenem in a study conducted by Singh *et al*. in Korea in 2001 (17). In another study in Iran in 2009 Shahcheraghi *et al.* reported 75% resistance for imipenem in *P. aeruginosa* isolated from nosocomial

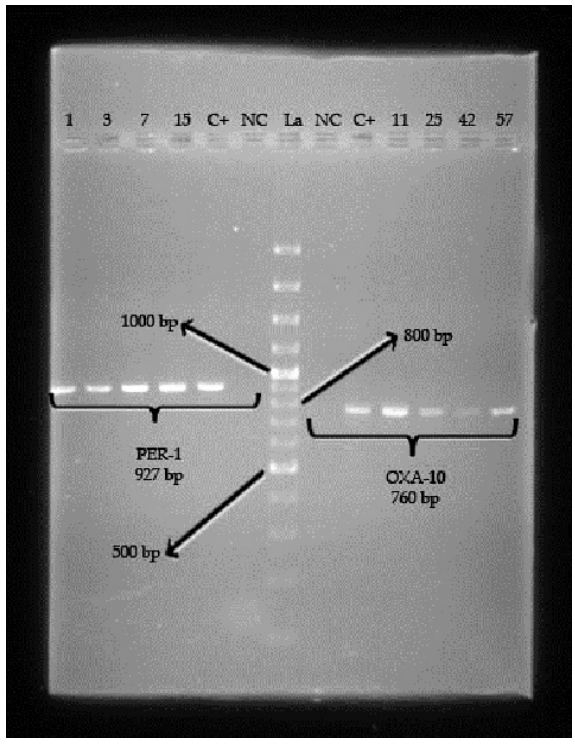

Fig. 1. PCR amplification of bla_{PER-1} *and* bla_{OXA-10} genes**.**1, 3, 7, 15: No. of positive samples for *per-1* (927bp) - 11, 25, 42, 57: No. of positive samples for bla_{OXA-10} (760 bp)
La: DNA ladder, **NC:** negative control, **C+:** Positive control.

sources (12). In the current study 210 (77.7%) of 270 isolated *P. aeruginosa*, were resistant to imipenem.

It seems that most of this multidrug resistance reflects the accumulation of multiple mutations and acquirement of many resistance genes (2). Different studies evaluated the *P. aeruginosa* resistant strains in different world centers specially in ICU & Burn wards so far (5,16,18), which showed different pattern for the resistance of this bacteria to the different antibiotics and the frequency of the important β-lactamases (such as bla_{PER-1} *and* bla_{OXA-10}) among resistant isolates. According to the results of CDD screening in our study, 168 (80.0%) isolates from 210 Imipenem resistant isolates were ESBL producing strains. Mirsalehian *et al*. highlighted that 39.41% of the *P. aeruginosa* strains isolated from hospitalized burn patients in Tehran were ESBL producers (16).

For many years, PER β-lactamases were thought to be significant only in Turkey (*PER-1*) and Argentina (*PER-2*) (5, 18, 19). Since 1995, PER-1 producing organisms have been disseminating in Italy (20, 21), France (21), Spain (22), Romania (23), Korea (24), Japan (26), and China (27). Bacteria with the OXA (a class D β-lactamase) have evolved to destroy β-lactam

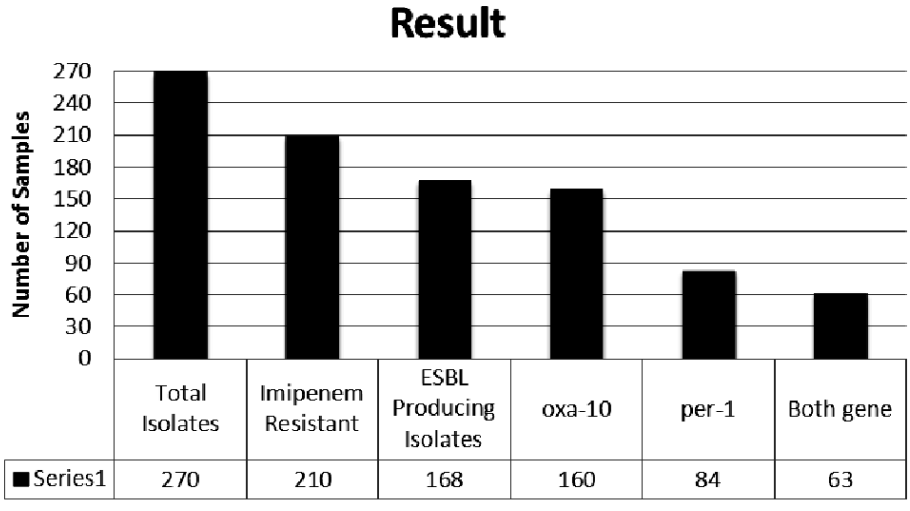

Fig. 2. Number of Imipenem resistant isolates, ESBL producing isolates in Imipenem Resistance's, number of bla_{PER-1} *and* bla_{OXA-10} related genes.

antibiotics, and presented high levels of resistance to a broad spectrum of β-lactam antibiotics (28, 29).

Mirsalehian *et al.* in 2010 stated that 74.62% and 49.25% of the isolated *P. aeruginosa* strains from hospitalized burn patients in Tehran contain bla_{PER-1} and bla_{OXA-10} gene, respectively (17). In addition, Vahaboglu *et al.* detected PER-1-type b-lactamases in 11% (40/367) of *P. aeruginosa* strains (30). While in our study, 160 (76.2%) of the resistant isolates contain bla_{OXA-10} and 84 (40%) of resistant isolates contain bla_{PER-1} related genes, and 63 (30.0%) of them contains both related genes simultaneously. 148 (92.5%) of 160 bla_{OXA-10} containing isolates, and all of bla_{PER-1} containing isolates were ESBL producing.

The results of our study showed a high prevalence of the imipenem resistant strains in burn patients, which is an alarming sign and should be taken into the consideration, because increasing of the antimicrobial resistant bacteria isolated from burn patients is an important issue (13).

The accumulation of multiple mutations and acquirement of resistance genes is believed as one of the most important reasons for the multidrug resistance (2). Hence, it is very important to set up a strict and logical infection control program to detect the source of infection, bacteriological profile, antibiogram of burn wound isolates and find the responsible genes for the antimicrobial resistant in order to decrease the incidence of nosocomial infections in hospitalized burn patients and help the clinicians to better drug selection for this kind of patients.

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