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The frequency of genes encoding exotoxin A and exoenzyme S in *Pseudomonas aeruginosa* strains isolated from burn patients

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ABSTRACT

Background: *Pseudomonas aeruginosa* infections have emerged as a major infectious disease threat in recent decades with infection particularly in immunocompromised hosts. *P. aeruginosa* possesses several virulence factors with involvement in pathogenesis. The aim of this study was to examine the prevalence of virulence genes of *toxA* and *toxS* and to analyze their relation to antimicrobial resistance of the isolates.

Methods: In total 185 clinical isolates of *P. aeruginosa* were collected from burn patients. Antimicrobial susceptibility testing was done by disk diffusion method. PCR amplification was performed on extracted DNA from the isolates and the presence of encoding genes for exotoxin A (*toxA*) and exoenzyme S (*toxS*) were investigated by using specific primers.

Results: In disk diffusion method, the isolates showed high sensitivity to colistin sulfate (100%) followed by imipenem (41.9%). The most prevalent resistance was seen against ceftazidime (90.5%) and gentamicin (88.5%). Multidrug resistance (MDR) demonstrated in 113 isolates (76.35%). According to PCR amplification, 133 (89.8%) and 127 (85.8%) isolates possessed *toxA* and *toxS* genes respectively. The frequencies of genes among MDR strains were 102 (76.6%) for *toxA* and 98 (77.1%) for *toxS*. Eighty five MDR isolates possessed both genes (73.9%). The non-MDR strains (23.65%), harbored lower prevalence of simultaneous *toxA* and *toxS* genes (26%) compared to MDR strains.

Conclusion: The present study established a higher frequency of MDR among *P. aeruginosa* isolates from burn patients. It was found that the frequency of both *toxA* &*S* genes were significantly higher in MDR strains *P. aeruginosa* strains.

1. Introduction

Pseudomonas aeruginosa is a gram negative opportunistic pathogen causing hospital-acquired infections. It accounts for 11–13.8% of nosocomial infections due to its ubiquitous presence in the hospital environment [1,2]. *P. aeruginosa* infections have emerged as a major infectious disease threat in recent decades with infection particularly in immunocompromised patients and those with predisposing factors,

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including cystic fibrosis, cancer [3,4], and patients with severe buns with a high mortality rate [5,6]. The bacterium has the capability to infecting all tissues and is known for its resistance to antibiotics. The emergence of multidrug resistance (MDR) *P. aeruginosa* in burn wounds is becoming a challenging problem worldwide [7].

The pathogenesis of *P. aeruginosa* infection is multifactorial, comprises a wide array of virulence determinants expressed by the bacterium and also rapid development of antibiotic resistance. *P. aeruginosa* possesses several cell-associated and extracellular virulence factors including exotoxin A (ETA), exoenzyme S (ETS), alginate, elastase, and phospholipase C. In burn infection, the tissue damage is contributed to the role of these factors, although exotoxin A and exoenzyme S which is secreted by type III secretion system (TTSS), are the most important contributors to this pathologic condition [8]. ETA which is produced by the majority of *P. aeruginosa* strains, encoded by *toxA* gene and is the most toxic virulence factor of this bacterium with ADP-ribosylation activity affecting the protein synthesis of the host cells [9]. Exoenzyme S encoded by *toxS* gene is also an ADP-ribosyltransferase which inhibits protein synthesis [10]. The aim of this study was to examine the prevalence of virulence genes of *toxA* and *exoS* and to analyze their relation to antimicrobial resistance of the isolates. For this purpose the PCR amplification was performed for rapid detection of related genes in *P. aeruginosa* strains isolated from burn samples.

2. Materials and methods

2.1. Sampling

A total of 185 non-duplicated isolates of *P. aeruginosa* from different clinical samples including infected wound discharge, blood, biopsy and urine of burn patients in the Taleghani burn Hospital, Ahvaz, Iran, were collected between March and August 2014. The study was approved by Institutional Ethics Committee after submission of preliminary proposal of the study. The isolates were identified as *P. aeruginosa* by application of culture and standard biochemical tests including SIM, MRVP, Oxidation Fermentation (OF) tests and pigment production in Mueller Hinton agar (Merck, Germany) [11].

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of isolates were performed by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [12], using following antibiotic disks: piperacillin (100 μg), piperacillin-tazobactam (100/10 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), imipenem (10 μg), meropenem (100 μg), amikacin (30 μg), gentamicin (10 μg) and colistin sulfate (10 μg) (MAST Co., UK). The test were performed by preparation of standard bacterial suspension equal to 0.5 McFarland and inoculated in Muller Hinton agar and the antibiotic disks were placed in the plate at identical distances. Plates were incubated for 16–24 h at 35 °C. The diameter of zone of growth inhibition were measured and compared with standard values.

2.3. PCR amplification

DNA extracted from colonies of all isolates by simple boiling method as described elsewhere [13]. In brief, a few colonies were dissolved in TE (Tris-EDTA) buffer and boiled at 100 °C for 10 min with subsequent precipitation in a 14,000×*g* refrigerated centrifuge at 4 °C for 3 min. The supernatant containing DNA was used as template for PCR amplification. The concentration of extracted DNA was measured by biophotometer (Eppendorf, Germany) at 260–280 nanometer and were kept at −20 °C until use.

PCR amplification was performed on extracted DNA using previously described primers of Exo A and *toxS*, as: *toxA*: 5′-CTGCGCGGGTCTATGTGCC and 5′-GATGCTGGACGGGTCGAG; and *toxS*: 5′-CGTCGTGTTCAAGCAGATGGTGCTG and 5′-CCGA-ACCGCTTCACCAGGC. These primers are designed to amplify a 270 bp and 444 bp fragments of *toxA* and *toxS* genes sequence respectively [10].

PCR mixture were prepared in final volume of 25 μl and consisted: 10X PCR Buffer, 50 mM $MgCl_2$, 10 mM dNTPs, 10 μM of each primer, 5 Unit Taq DNA Polymerase, and 5 μl of template DNA. The amplification program was consisted of initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 s, annealing for toxA, 58 °C and for toxS, 55 °C for 60 s, extension at 72 °C for 90 s and a final extension at 72 °C for 5 min. *P. aeruginosa* ATCC 27853 was used as positive control.

The PCR products were loaded on a 1.5% agarose gel containing 0.5 μg/ml ethidium bromide in electrophoresis, with a 100 bp DNA marker used for size determination, and photographed by using the gel documentation system (Proteinsimp, USA). SPSS software (SPSS Inc., No. 13) was used for data analysis.

3. Results

The *P. aeruginosa* strains in this study were originated from samples of burn wound discharge (117, 79%), blood (16, 10.8%), biopsy (13, 8.78%) and urine (2, 1.35%). The samples were belonged to 82 (54.5%) female and 66 male (45.5%) patients.

According to the results from disk diffusion, high sensitivity of the isolates to colistin sulfate (100%) followed by imipenem (41.9%) were demonstrated. The most prevalent resistance was seen against ceftazidime (90.5%) and gentamicin (88.5%). MDR according to the previously determined criteria of simultaneous resistance to 3 classes or more of antibiotics including carbapenems, aminoglycosides and fluoroquinolones [14], were noticed in 113 isolates (76.35%). The MDR strains were majority isolated from blood samples (15, 93.75%). The frequencies of these strains among other samples were as: wound discharge (87, 74.3%), biopsy (10, 76.92%) and urine (1, 50%). The overall results of disk diffusion method is presented in Table 1.

PCR amplification for the presence of *toxA* and S genes (Fig. 1), showed that 133 (89.8%) isolates comprised *toxA* gene, 127 (85.8%) isolates possessed *toxS* gene and simultaneous presence of both *toxA* and *toxS* genes were noticed in 115 isolates (77.7%). In two isolates none of the genes were presented.