Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 6:248-254

Correlation of Frequency of Pseudomonas Aeruginosa and Exos & Exou Genes and Their Antibiotic Sensitivity Pattern in Specimen Isolated from ICU Ward

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ABSTRACT

Pseudomonas aeruginosa is a cause of nosocomial infections that can be destroyer by antibiotic-resistant strains. This study conducted to determine the antibiotic susceptibility pattern and distribution of exoU and exoS among clinical isolates of P. aeruginosa. Fifty three specimens of tracheal tube were collected from patients who were hospitalized in ICU wards and P. aeruginosa were isolated and identified by phenotypic and molecular methods. Antibiotic resistance performs by disk diffusion and analyzed their virulence factors genes by PCR method. Susceptibility pattern of 53 isolates of P. aeruginosa showed that majority and minority of resistance belong to cefepime (55.4%),and Meropenem (50%) Respectively. Twenty four (45.2%) isolates were not susceptible to three or more different groups of antibiotics. Forty (71.4%) of isolated have had exoSand1(1.8%) exoU, 8(15%)both of exoS and exoU and the rest being negative for exoS or exoU. Distribution of MDR(resistance to three or more group of antibiotics) exoenzymes were shown: exoU(7.5%)and exoS(90.5%). According to statistically analysis there were not significant relationship between presence of exo SandexoU and antibiotic resistance.

Key words: *Pseudomonas aeruginosa*, antibiotic susceptibility, *exoU*, *exoS*

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, aerobic and non-fermenting bacteria that was first remote from green pus [1].*Pseudomonas aeruginosa* is an important cause of the bacteria most of tenliable for 20%Ventilator Associated Pneumonia(VAP) and nosocomial infections [2]. VAP has one of the highest mortality rates ranging from 20 to 50% and increases length of hospital stay and hospital costs [3].

Lower respiratory tract infection causes 3.9 million deaths per year worldwide, of which 1.8 million are shown in children under the age of five years [4].VAP caused by *P. aeruginosa* is the most difficult to be treated thus mortality due to this bacteria has been presented as high as70% and directly mortality rates are almost 40% [5]. Patients in intensive care unit (ICU) have a high risk to give VAP because; their immune system is suppressed by increased use of multiple antibiotics. In recent, Gram- negative bacteria more isolated than Gram-positives in this ward.

The ICUs are considerable for special conditions because micro organism in this area often resistance to multiple classes of antibiotics. Their prevalence and rates of resistance are different in every location geographic region [6, 7].

P. aeruginosa able to cause acute and chronic infections in respiratory system. It has multi virulence factor such as: hemolysins , lipopolysaccharide, pili, alkaline proteases, pyocyanin, phospholipases, elastase, and type 3 of secretion system(TTSS) that contain exotoxins including: *ExoS, ExoU, ExoY,* and *ExoT*[7]. *TTSS* is a main virulence factor for pathogenesis of P. aeruginosa that uses the *TTSS*to carry effector toxins (*ExoS, ExoU, ExoY,* and*ExoT*) into host cells. After the enter once of the exotoxins, cell necrosis and cytoskeleton will be damaged therefore pathogen able to invade host cells [8].

*ExoS*and *exoT*are bio functional enzymes and have a parallel activity. They are same in 75% amino acid and encode Gtpase-activating protein (GAP) and ADP-ribosyltransferas (ADP-RT) activities [9].*ExoY* is an enzyme by adenylate cyclase activity. The activity of *exoU* is attributable to phospholipase A2. *ExoU* is a potent cytotoxin with phospholipase activity, capable of killing a variety group of cells of eukaryotic cells in *in vitro*. Additionally, *exoU* has a greater effect than other (TTSS) effectors on the virulence of the bacteria [9].Upon inoculation into host cells, *exoU* is activated and targeted to the plasma membrane and cleaves membrane phospholipids thus the cell is going to lyses [10,11]. Most strains have either *exoS*or *exoU*, but strains which having both of genes are rare [10, 11].

Recently research shows that exoU of *P. aeruginosa* noticeable cytotoxic

Capabilities to quick cytotoxic effects in many cell types. Omission of *exoU* sternly limits the harm of this organism in lung; this enzyme has been implicated as an agent associated with septic shock and increased disease harshness and rate of mortality in pneumonia[12].

This study was carried out to determine the prevalence of *P. aeruginosa* isolated, antimicrobial susceptibility profile, and their *exoS* and *exoU* in patients who were hospitalized in ICU ward.

MATERIALS AND METHODS

The specimens were collected in the period from Octobr 2014 to Octobr 2015, of tracheal tube from ICU patients of teaching Hospitals (Emam Khomainy, Golestan, Abuzar, Razi,Taleghani and Sina) in Ahvaz south of the Iran.

The samples transferred to Department of microbiology, Faculty of Medicine, in Judishapur University of Ahvaz, Iran.

Microbiology processing

The specimens were cultured on MacConkey agar , blood agar(Merk ,Germany) and cetrimide agar(Merk ,Germany) and incubated at 37°C for 24 hours. All isolates were confirmed as *P. aeruginosa* according to colony morphology, oxidase reaction, growth on Muler –Hinton agar (Merk, Germany) at 42°C and create piocyanin pigment. The isolated were inoculated in 15% glycerol plus TSB broth and stored at −80°C [13].

Antibiotic susceptibility test:

Disk diffusion method for antibiotic susceptibility patternwas performed on Mueller-Hinton agar medium according to Clinical Laboratory Standard Institute (CLSI). The disks were impregnated with antibiotics included:

imipenem (10 µg), Meropenem(10 µg),Gentamicin(10 µg), Ceftasidim (30 µg),Ciprofloxacin (5 µg), cefipime(30 µg)(Padtanteb, Iran)and piperacillin / tazobactam (100/10 µg)(Mast ,England) and Incubated at 37°C for18 hours. After defined incubation period the inhibition diameter zone was measured and explanation of result based on CLSI guidelines [14].

Identification of *exoU* and *exoS* by molecular method:

Nucleotide sequences of Primers used in PCR for amplification and detection for *exoU*′ (428bp) and *exoS*′ (504bp) were(15): *exoU*-F .5′-GGG AAT ACT TTC CGG GAA GTT- 3′ , *exoU*-R 5′-CGA TCT CGC TGC TAA TGT GTT-3′ and *exoS*-F 5′-CTT GAA GGG ACT CGA CAA GG-3′ , 3′-TTC AGG TCC GCG TAG TGA AT-3.′

The genomic DNA extraction was performed by boiling method [16].

PCR was carried out in a 25 µl reaction volume using a Eppendorf thermal cycler (Eppendrof,Germany Com)**.** The reaction mixture contained 1.5 µl of template DNA, 0.4µM of each primer, 0.2 mM dNTP, 1X reaction buffer, 1.5 mM $MgCl_2$ and 0.2 U/µl Taq DNA polymerase. PCR protocol was done as an initial denaturation 94°C (5min) followed by denaturation 94°C (40s), Annealing60°C for *exoS* and 59°C for exo,Extension72°C (1:30s), and final extension72°C(7min) was followed by 30 cycles.

Figure1and Figure2:Identification of *exoU*(428)bp and *exoS*(504)bp by PCR method.

Gel Electrophoresis

Gel Electrophoresis performed by inoculated 10 µl of PCR product on to 1% agarose gel in the TBE buffer plus ethidium bromide (0.5µg/ML) and visualized under ultraviolet illumination(Proteinsimp Company,USA). Products size was analyzed in evaluation to a M100-1000 bp marker (Sinnagen, Iran). Present of exoU and exos with amplification size 428, and504 respectively were shown in figure 1and 2.

RESULTS

Fifty three isolates of *P. aeruginosa* obtained from tracheal tube of patients were submitted in ICU. The ages of patients ranged from 1 month to 90 years old. The gender of patients was 33(58.9%) male and 20(35.7%) Female. The specimen's site of isolation just involved respiratory system that exactly referred to secretion obtained from tracheal tubes. Total of 90 specimens which collected from tracheal tube, 53 isolated confirmed as *P. aeruginosa* by culture and biochemical tests.

Also 24(45.2%) of the *P. aeruginosa* were multi drug resistant (MDR).

Antibacterial sensitivity result of 53 isolated of *P. aeruginosa* with seven antibiotics is shown in(table1).

Table 1: Antimicrobial susceptibility patterns of *P. aeruginosa* isolated from tracheal tubs

Susceptibility / Antibiotic	No (%) Sensitivity	No (%) Intermediate	No (%) Resistant
Cefepime	18(32.1%)	4(7.1%)	31(55.4%)
Ceftazidime	28(50%)	3(5.4%)	22(39.3%)
Gentamicin	31(55.4%)	3(5.4%)	19(33.9%)
Imipenem	26(46.4%)	7(12.5%)	20(35.7%)
Meropenem	21(37.5%)	4(7.1%)	28(50%)
Ciprofloxacin	27(48.2%)	8(14.3%)	18(32.1%)
Piperacillin-tazobactam	28(50%)	8(14.3%)	17(30.4%)

Result of PCR for identification frequency of *exoU* and *exoS*in 53 isolated of *P. aeruginosa* were shown that 8(15%) and 40(71.4%) have had *exoU*and *exoS* respectively, and the rest were negative for both of gens (table 2).

Table 2: Distribution of *exoU* and *exo S*among 53 clinical isolates of *P. aeruginosa* in thrachial tube:

Exoenzyme	*Frequency*	*Percent*
exos	*40*	*71.4*
exou	*1*	*1.8*
exo s + exo u	*8*	*15*
exo s or exo u Negative	*5*	*8.9*

The antibiotic resistance of *P. aeruginosa* and relationship to *exoS* and *exoU* genes were shown that the majority of antibiotics resistant belong to *P. aeruginosa* which had these two genes together (table 3).

Table 3: Relationship of exoS *and exoU* genes of *P. aeruginosa* and antibiotics resistance

Exogens / *Antibiotics*	*exoU* No (%)	*exoS* No (%)	*exoS&exoU* No (%)	*Negative exoS&exoU* No (%)
Cefepime	4(7%)	30(56%)	34(64%)	2(3%)
Ceftazidime	3(5%)	21(39%)	24(45%)	2(3%)
Gentamicin	3(5%)	21(39%)	24(45%)	2(3%)
Imipenem	3(5%)	19(35%)	22(41%)	2(3%)
Meropenem	4(7%)	29(54%)	33(62%)	2(3%)
Ciprofloxacin	3(5%)	17(32%)	20(37%)	2(3%)
Piperacillin-tazobactam	2(3%)	20(37%)	22(41%)	1(1%)

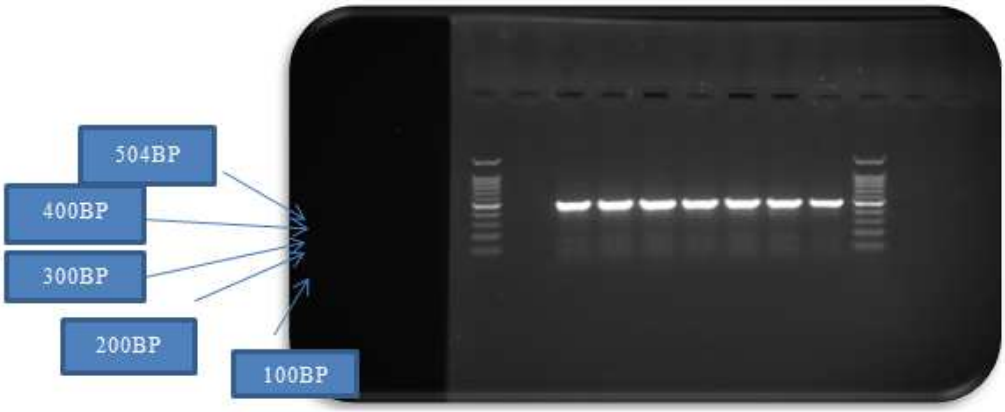

Figure1: Electrophoresis of *exoS*(504)bp PCR products on agarose

gel. Line 1 shows100–1000 bp ladder. Lines 2shows the negative control, Line 3 shows the positive control,4-9 shows *P. aeruginosa* strains and Line 5 shows100–1000 bp ladder.

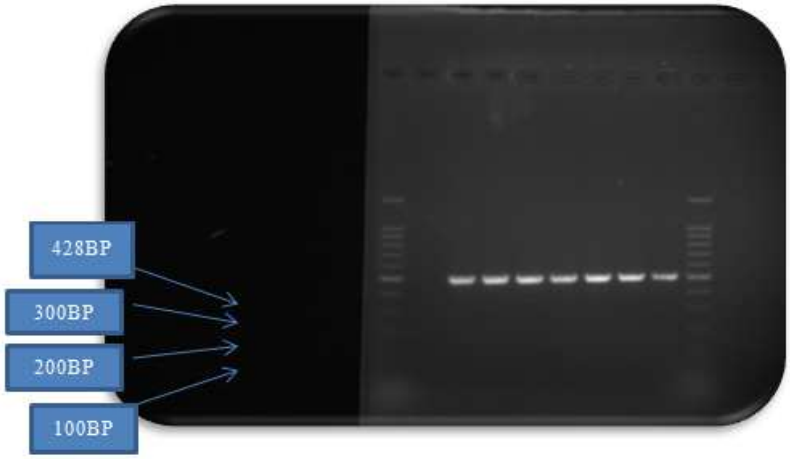

Figure 2: Electrophoresis of *exoU*(428)bp PCR products on agarose

gel. Line 1 shows100–1000 bp ladder. Lines 2shows the negative control, Line 3 shows the positive control,4-9 shows *P. aeruginosa* strains and Line 5 shows100–1000 bpladde.

According to statistically analysis there were not significant relationship between presence of *exoS* and *exoU* and antibiotic resistance .

DISCUSSION

Resistance to antibiotic is a main concern of modern medicine. *P. aeruginosa* is a mainly dangerous bacteria .Its natural resistance to vast range of antibiotics and its ability to acquire new resistance mechanisms. The heightened level of drug resistance is a result of the denovo emergence of resistance in a specific organism after exposure to antimicrobials as well as of patient-to-patient spread of resistant organisms. This organism liable for morbidity and mortality among hospitalized patients. Prevalence of antibiotical- resistant *P. aeruginosa* is increasing among ICU patients [10,16,17].

Patients in ICU have a high risk to give VAP. ICUs are considerable for special conditions because micro organism in this area often resistance to multiple classes of antibiotics [6, 7].

The case-fatality rate of bacteremia due to P. aeruginosa is severe, ranging from 32% to 73%, with attributable mortality estimated to be 30% [6]. Kurahashi K and et al was reported that if the exoU deletion gene, the infection significant reduction in lung pathogenesis [18].

According to antimicrobial susceptibility test our study indicated that the majority of *P. aeruginosa* resistance to *Cefepime*(55.4%) and the minority of them (30.4%) were resist to *Piperacillin-tazobactam*(Table 1). Also 24(45.2%) of the *P. aeruginosa* were multi drug resistant (MDR). This resistance show the force major for choosing the co-selected antibiotics for treatment the resistant strains of *P.* aeruginosa. The antibiotics were used in our study generally used in treating infections caused by *P. aeruginosa*[19].

The result of antibiogram of our study is agreement with SimaTokajian [19] in Lebanon and Narges Noritalab[20] in Iran. Difference in reports ranges about antimicrobial susceptibility pattern have relation with geographical regions. SitiNurAtiqah Idris and et al [15] indicated that frequency of *P.aeruginosa* exoenzymes in tracheal tubes, were *exoU*(61%)and *exoS*(41%) that this data were deferent with our finding. Also Philips Bertholet worked on the same issue and showed the frequency of *exoU*(28.3%)and *exoS* (52.2%) in *P.aeruginosa* bacteremia [6]. Other researcher with deferent ranges reported the*exoU* and *exoS* frequency in clinical specimens. Fortunately the finding of our study about *exoU* much less than reported of other investigator [10, 15, 23].

The data of Vajiheh Sadat NikBin and et al [21] in Iran, perfectly accordance to our finding, she's reported that the prevalence of *exoS* were 47.4% in secretion of tracheal tube. Difference in reports ranges about exoenzymes have relation with geographical regions.

According to statistically analysis and correlation between presence of *exoS* and *exoU genes* in *P. aeruginosa* and antibiotic resistance were not shown satisfy relationship between them (p>0/05) as shown in table 4. Therefore it suppose that present of *exoS* and *exoU* genes together or alone cannot be play the important role in antibiotics resistance in *P. aeruginosa*.. Makaoui Maatallah and Melisa Agnello, worked on multi drug resistant (MDR) in *P.aeruginosa* and reported that there were significant relationship between *exoU* and MDR[22,24], this deferent probably due to specimen site of our study and site of collection in study of Makaoui Maatallah and Melisa Agnello.

CONCLUSION

Its concluded that in this study frequency of exoS *is* more than *exoU*. In other hand presence of *exoS* and *exoU* gens of *P.aeruginosa* have no effect on pattern of antimicrobial resistance. Its satisfaction that prevalence of *exoU* and multidrug resistance in our region is little and its good prognoses for patients who were admitted in ICU in this region.

Acknowledgements
This study was supported by a grant (93143) from Health Research Institute, Infectious and Tropical Disease Research Canter, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran. We would like to thank from (Emamkhomeiny, Golestan, Razi, Abuzar, Sina and Taleghani) Hospitals for giving the permission to conduct this study, specifically from the Microbiology personals for providing the isolates. We also like to thank to Department of Medical Microbiology, Faculty of Medicine, Jundishapur University for providing research materials and facilities.

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