Hormozgan University of Medical Sciences

ORIGINAL ARTICLE

Health Institute

Hormozgan University of Medical Sciences

REP-PCR Typing, Antibiogram Pattern and Distribution of Clinical Isolates of *Pseudomonas aeruginosa* in a Teaching Hospital in South of Iran

Foroogh Faridi[1] , Sedigheh Javadpour [2]

[1] Infectious and Tropical Diseases Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

[2] Molecular Medicine Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Accepted 20 December 2015; Published Online 12 January 2016

ABSTRACT

Introduction: *P. aeruginosa* is an important multi-drug resistant (MDR) opportunistic pathogen in hospital-acquired infections. Several methods based on DNA analysis have been used for investigating genetic diversity of *P. aeruginosa* isolates. Among them, REP-PCR typing has been introduced as a rapid, low cost method with high discriminatory power. The aims of this study were to investigate genotypic relatedness and antibiotic resistance in clinical isolates of *P. aeruginosa*.

Methods: This cross - sectional study was carried out on 67 *P.aeruginosa* at Shahid Mohammadi hospital, Bandar Abbas, Iran, between May 2012 and June 2013. Identification and antimicrobial susceptibility of the isolates were determined by conventional biochemical reactions and Kirby-Bauer method. The genetic similarity was investigated by REP-PCR technique and data analysis was performed with NTSYS PC software, and Jacquard's method. The correlation between the molecular patterns and the other parameters was determined by Pearson's chi-square test using SPSS software, version 19.

Results: REP-PCR results, created 7 genotypic clusters with 76% similarity. Clusters I and III were the most prevalent clones, identified in 16 (23.88%) of isolates, distinctly. ICU had the most cluster diversity. The majority of isolates were obtained from sputum (29.85 %). The isolates were most resistant to cotrimoxazole (80.6%) and most susceptible to ciprofloxacin (85.1%).

Conclusion: REP-PCR type A and C were the predominant strains in our hospital. Because of serious infections and high drug resistance, it is necessary to implement the guidelines of infection control committee to reduce Pseudomonas infections.

Key words: *Pseudomonas aeruginosa*, Distribution, Microbial Sensitivity Tests , Nucleic Acid

*Corresponding author: Tel: +98-761-333668478

E-mail addres: sjavadpour@hums.ac.ir (S. Javadpour)

Introduction:

Pseudomonas aeruginosa is a major opportunistic pathogen that causes severe nosocomial infections, particularly in immune-compromised patients or those with underlying diseases (1). It is also responsible for the majority of morbidities and mortalities in patients with severe burn wounds, AIDS, lung cancer and other serious pulmonary diseases (2, 3).

P. aeruginosa has many virulence factors, including cell-associated determinants such as lipopolysaccharides, pili, flagella and numerous secreted factors like elastases, proteases, exotoxins, pyocyanin and extracellular polysaccharides (2, 4).

P. aeruginosa is inherently resistant to most currently used antibiotics due to a variety of resistance mechanisms. Treatment is further limited by the ability of the organism to rapidly develop additional resistance during treatment (5).

P. aeruginosa retains a wide set of accessory genes conferring functional uniqueness to individual strains. Its genome complexity is presumed to be the main reason for its skills for adaptation to various environmental niches and its ability to cause a wide range of infections. Because of large phenotypic diversity and high genomic polymorphism of *P. aeruginosa*, precise identification of this bacterium by conventional microbiology techniques is not accessible (6). Currently, several molecular typing methods are available that provide information about the genetic similarities between isolates within the same species (7). One of these commonly used methods is repetitive extragenic palindromic-PCR (REP-PCR). It is an extragenic typing technique that amplifies many uniquely sized amplicons, demonstrating regions between target-non-coding, repetitive sequences in the genome (7, 8).

The aims of this study were to investigate genotypic relatedness, antibiotic resistance and distribution of clinical isolates of *P.aeruginosa*, at Shahid Mohammadi hospital, Bandar Abbas, Iran..

Methods:

This cross – sectional study was carried out on 67 non-repetitive *P.aeruginosa* strains collected from different clinical specimen at Shahid Mohammadi hospital, Bandar Abbas, Iran, between May, 2012 and June, 2013. Isolation of the bacteria was performed by culturing the specimens on appropriate bacteriological media, including Blood agar and EMB agar media. Identification of the isolates was performed by routine microbiological methods. The strains were stored in Tryptic Soy Broth medium containing 30% glycerol at – 20°C until further testing (9, 10).

Antimicrobial susceptibility: In vitro antibiotic susceptibility of each isolate was assessed by the Kirby-Bauer disc diffusion method, and interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (11, 12). Antibiogram discs were purchased from PadTanTeb, Iran, and contained the following antibiotics, at the specific concentrations indicated in parentheses cotrimoxazole 25 µg, ceftriaxone 30 µg, ceftazidime 30 µg, meropenem 10 µg, imipenem10 µg, ciprofloxacin 5 µg, amikacin 30 µg.

Extraction of DNA for REP-PCR: DNA was extracted from isolates with the modified TE boiling method (13). Each sample was suspended in 200 µL TE buffer [10 mmol/L Tris-HCl, 1 mmol/L EDTA (pH 7.5)]. In order to wash the pellets, each preparation was centrifuged at 8000 g for 4 min at 4°C by TE buffer for 3 times . The pellet was resuspended in 200 µL TE buffer, mixed on a vortex mixer and subjected to boiling at 100°C in a water bath for 1 min, 3 freeze-thaw cycles alternating between -70°C for 3 min and 100°C for 2 min, and then centrifuged at 10000 g for 5 min. Supernatant was transferred to a sterile tube and stored at 20°C (14, 15).

REP-PCR DNA fingerprinting: The REP-PCR oligonucleotide primers used in this study were REP1 5′-IIIICGICGICATCIGGC-3′, REP2 5′ICGICTTATCIGGCCTAC-3′. REP amplification was performed according to the following program: initial denaturation of 94°C for 7 min; 35 cycles of denaturation at 94°C for 45sec; annealing at 40°C for 60 sec; extension at 72°C for 8 min; and a final extension step at 70°C for 16 min. Total PCR reaction volume was 25 µl, containing 2 µl of purified genomic DNA, 2.5 µl MgCl2, 1.25 µl of dNTP, 2 µl of each primer and 0.4 µl of Taq DNA polymerase. The DNA fingerprint patterns were presented as virtual electrophorese analysis in 1.2% agarose gel in TAE buffer (0.1 M EDTA pH 8.0, 0.04 M TRIS pH 8.0, and 0.02 M acetic acid) with the race of 80V for 3hours. The amplification of the

products was analyzed under UV transilluminator (8, 11).

Analysis of DNA Fingerprints: The amplification products were transformed into binary matrixes where 1(one) was attributed to the presence of the band and 0 (zero) for absence. The NTSYS PC software coefficient was employed to determine genetic similarities between P. aeruginosa strains and create dendrograms. Samples were classified into the same REP-PCR group if the similarity was at least 97% (1, 12).

Analysis of genetic diversity: In order to evaluate the genetic relationships between strains a matrix of genetic distances, was constructed using the complement of the Jaccard Similarity Coefficient (CSJ), this matrix does not consider negative similarities and the absence of the product. From estimates of the dissimilarities, the strains were grouped using hierarchical UPGMA method (Unweighted Pair-Group Mean Average) with the test of bootstrap (1000 times) to evaluate the consistency of the group (1).

Statistical analysis: The correlation between molecular typing and antimicrobial susceptibility patterns was determined by Pearson's chi-square test using SPSS software, version 19.0 for Windows. A P value of < 0.05 was considered for statistically significant (12).

Results:

A total of 67 non repetitive isolates of *P.aeruginosa*, was recovered from sixty seven patients. 45 patients were males and 22 were females. Average age of the patients was 48 years. There was greater incidence of *P. aeruginosa* infections in the 21 to 40 years age group.

The majority of isolates were obtained from sputum (29.85%), wound swabs and tracheal tube, each one (25.37%), urine (14.29%) and blood (4.47%).

The most numbers of *P. aeruginosa* strains were obtained from Internal and ICU wards, 19 (28.35%) distinctly, followed by emergency ward 14 (20.88%), surgery (8.95%), burn (7.46%), orthopedic and neurosurgery wards (2.98%).

Antimicrobial susceptibility: The susceptibility rates of isolates to tested antibiotics are presented in Table 1.

Table 1. Antibiogram pattern of *Pseudomonas aeruginosa* isolates (expressed as percentage)

Antibiotic	Sensitive	Intermediate	Resistant
Ciprofloxacin	82.09	2.98	14.93
Imipenem	74.63	0	25.37
Amikacin	74.63	4.47	20.90
Meropenem	73.13	1.49	25.37
Ceftazidime	65.67	2.98	31.34
Ceftriaxone	25.37	11.94	62.69
Cotrimoxazole	19.40	0	80.60

As it is considered, ciprofloxacin with 82.09% susceptibility rate was the most active agent, followed by imipenem and amikacin with 74.63% efficacy. The highest resistance rate was seen to cotrimoxazole (80.60%).

Analysis of DNA Fingerprints: Using NTSYS PC and Jacquard's method, REP-PCR results created 7 genotypic clusters (A- G) with 76% or more similarity. REP- PCR created 1-10 fragments per strain, with a total of 20 distinct fragments with the size ranging between 150 to 3000 bp (Figure 1). Strains belonging to the same cluster showed identical or highly similar profiles (up to two bands different). Clusters A and C were the most prevalent clones, identified in 16 bacterial isolates (23.88%).

REP-PCR dendrogram based on the rate of similarity is illustrated in figure 2.

Isolates P2 and P11 of group (A), P6, P8 and P40 of group (B), P25, P29, P33, P34, P37, P45, P48, P53, P55, P57 and P66 of group (C), P56 and P58 also in group (C), P35, P52 and P59, P62 in group (E) showed the smallest genetic distance (0%) within

their group, whereas strains P36 and P24 with about 67% had the highest distance.

Table 2. Frequency of *Pseudomonas aeruginosa* isolates in different REP type

REP type	Frequency	Percent
A	16	23.88
B	8	11.94
C	16	23.88
D	7	10.44
E	13	19.40
F	3	4.47
G	4	5.97
Totall	**67**	**100**

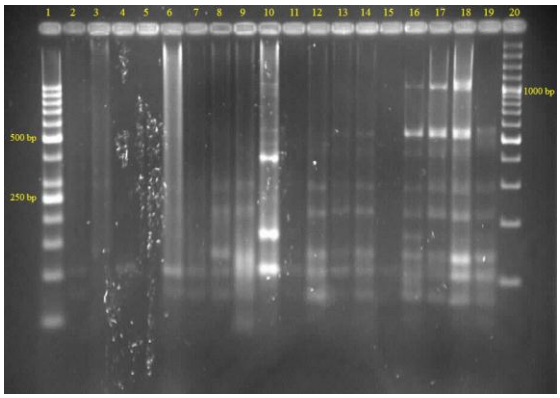

Figure 1. Repetitive-PCR fragments of *Pseudomonas aeruginosa* isolates

Lane 1. Molecular weight marker (50 bp), 2 – 19 Clinical isolates, 20. Molecular weight marker (100 bp)

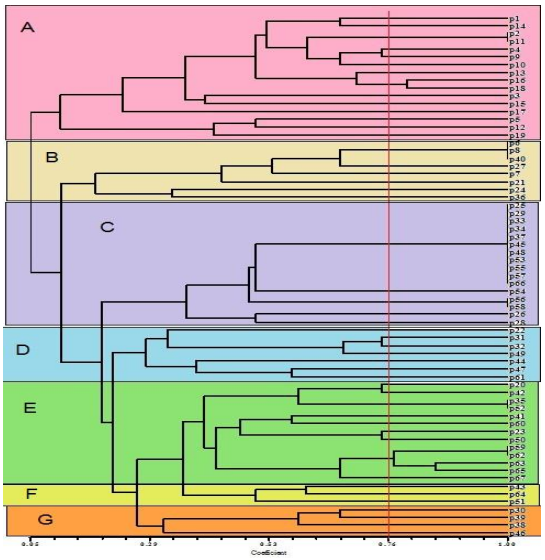

Figure.2. Dendrogram showing similar REP-PCR type

Table 3. Clinical Sources of *Pseudomonas aeruginosa* isolates in different Rep type

REP type	A		B		C		D		E		F		G	
Sample	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**
Sputum	18.75	3	37.50	0	25	4	0	0	64.54	8	33.34	1	25	1
Wound	25	4	37.50	1	31.25	5	28.58	2	15.39	2	0	0	25	1
Trachea tube	43.75	7	25	4	6.25	1	42.86	3	7.70	1	33.34	1	50	0
Urine	6.25	1	0	2	31.25	5	28.58	2	7.70	1	33.34	1	0	0
Blood	6.25	1	0	0	6.25	1	0	0	7.70	1	0	0	0	0
Total	100	16	100	8	100	16	100	7	100	13	100	3	100	4

Table 4. Frequency of REP type of *Pseudomonas aeruginosa* isolates in different wards

REP type	A		B		C		D		E		F		G	
Wards	%	N	%	N	%	N	%	N	%	N	%	N	%	N
Neurosurgery	0	0	0	0	0	0	14.28	1	7.69	1	0	0	0	0
Surgery	12.5	2	12.5	1	12.5	2	0	0	7.69	1	0	0	0	0
Emergency	6.25	1	50	4	25	4	14.28	1	15.38	2	66.66	2	0	0
ICU	56.25	9	25	2	6.25	1	28.57	2	15.38	2	33.33	1	50	2
Orthoppedic	6.25	1	0	0	0	0	14.28	1	0	0	0	0	0	0
Burn	0	0	0	0	25	4	0	0	15.38	1	0	0	0	0
Internal	18.75	3	12.5	1	31.25	5	28.57	2	46.15	6	0	0	50	2
ToTal	100	16	100	8	100	16	100	7	100	13	100	3	100	4

Table 5. Antibiotic resistance of different REP types (expressed as percentage)

REP type Antibiotics	A	B	C	D	E	F	G
Cotrimoxazolee	68.75	87.50	75	100	84.62	100	75
Imipenem	18.75	50	25	14.29	15.39	100	0
Amikacin	18.75	33.34	6.25	57.15	23.08	33.34	0
Ceftriaxone	75	62.50	50	71.43	53.85	100	50
Ciprofloxacin	18.75	12.50	25	14.29	0	33.34	0
Meropenem	12.50	37.50	25	14.29	30.77	100	0
Ceftazidime	50	37.50	25	14.29	23.08	66.67	0
Mean	**37.50**	**45.84**	**33.04**	**40.82**	**32.97**	**76.20**	**17.86**

Relationship of REP types and source of infection: Most of the group E isolates were obtained from sputum specimens (61.54%), whereas group A were mostly isolated from tracheal tubes (43.75%). Most of the wound and urine isolates belonged to group C (31.25%).

Relationship of REP-PCR types and infection dispersion in wards: Table 4 demonstrates the distribution of REP types in different wards of hospital. The most REP type diversity was observed in ICU and internal wards. In each ward different REP type was dominant. The most isolates of internal, burn and ICU wards belonged to REP type C, E and A, respectively.

Antimicrobial susceptibility among different REP-PCR types: The relationships of REP types and antimicrobial susceptibility patterns are shown in Table 5. Eleven isolates (16.42%) were resistant to 3 antibiotics tested, 7 (10.44%) to 4 antibiotics, 4(5.97%) to 5 antibiotics, and seven isolates (10.44%) were resistant to six agents. Only two isolates (2.98%) were resistant to all seven tested antibiotics. These two isolates belonged to different REP-PCR types (A and B). In addition, REP type F members had the highest drug-resistance (mean = 76.20%).

Conclusion:

P.aeruginosa is a major cause of nosocomial infections, particularly in ICU patients. Its intrinsically resistant to multiple classes of antimicrobial agents, limits choice of drugs, and complicates the therapeutic management of patients. Due to large phenotypic diversity and genomic complexity, precise identification of *P.aeruginosa* isolates is important for epidemiologic studies to recognize identical or closely related strains, sources of infection, and for detecting cross-transmissions in the hospital environments (6).

In the present study, we acknowledged the spread of *P.aeruginosa* infections in Shahid Mohammadi Hospital. In addition, we applied the REP- PCR method to determine the relationship of the strains and to dominant clonal groups, present in different wards.

Sixty seven patients were entry in this study; when factors such as age and sex of the patients were considered, we found that occurrence of *P. aeruginosa* was higher in males 45 (67.16%) compared to females 22 (32.83%). Our results were in consistence with similar studies carried out by Jamshaid et al, and Ranjan, in which *P. aeruginosa* infections were more common in males, 61.78 % and 58%, respectively (16, 17).

We categorized our patients into five groups, according to their age. There was a greater incidence of *P. aeruginosa* infections in the age group 21 - 40 years, and patients with more than 40 years old, but we didn't recognize significant association between age and incidence of *P.aeruginosa* infections (p= 0.23). Ranjan and colleagues found that *P. aeruginosa* was more commonly isolated from patients in the age group 21–40 years (17). In a study performed by Ghorbanalizadegan on 155 cases (69% male 31% female), the average age was 52 years, and *P. aeruginosa* infection was mostly observed in patients with more than 50 years old (18). Similar to these findings have been reported in other studies (19). We therefore report it as a significant finding, which is in agreement with that obtained in other studies.

In our study *P. aeruginosa* strains were most frequently isolated from sputum samples (29.85%), followed by wounds and tracheal tube, both with the same rate (25.37%). Correspondingly in Ghorbanalizadegan study most of the isolates were obtained from sputum (16.9%) (18). In Khalili study urine with 41% and tracheal tube with 29% rates were the main sources of *P. aeruginosa* isolates. However, *P. aeruginosa* strains were typically isolated from Blood 33%, sputum 24% and urine 22% in Rahimi's report. But in our study 14.92% and 4.47% of isolates were obtained from urine and blood samples, respectively, which are quite less than their findings (20, 21).

In the present study, the majority of *P. aeruginosa* strains were obtained from ICU (28.35%), internal ward (28.35%) and emergency ward (20.88%). This is in agreement with other investigations carried out in other hospitals (18, 21).

Regarding antibiogram pattern, our result showed that ciprofloxacin with 82.09% susceptibility rate was the most active agent, followed by imipenem and amikacin, both with 74.63% activity. In Razavi study, Imipenem (99.10%) ceftazidim (86.4%), and Ciprofloxacin (75.5%) were the three most effective agents (22). In Rajabpour study imipenem (90.40%), amikacin (80%) and ofloxacin (60%) were the three most effective antibiotics (23). Amikacin, imipenem and ciprofloxacin has been also ranked as one of the three most effective agents against *P. aeruginosa* in other studies (24). Surprisingly, amikacin has been found as the most resistant agent in Biswal study, which is in contrast with our results and studies mentioned above. He has reported colistin (100%), meropenem (79.3%) and ciprofloxacin (79.31%) as the three most effective agents and imipenem ranked the fourth with 72.4% susceptibility rate (25). In Sabir study in Karachi, imipenem (100%), piperacillin/tazobactam(90.91%) and sulbactam/cefoperazon (88.43%) were the three most effective agents against *P. aeruginosa* infections, followed by amikacin, aztreonam and ciprofloxacin with 80.99% , 78.51% and 65.29% susceptibility rates, respectively (26). Unfortunately, we did not evaluate piperacillin/tazobactam and sulbactam/cefoperazon activity in our study to compare the results.

Although Bonfiglio and his colleagues, summarized that meropenem was the most active drug against *P. aeruginosa* isolates (24), in our study susceptibility rate to meropenem was (73.13%) ranking the fourth active agent, comparable to Gamal's study with 71% (27). We observed the highest antibiotic resistance rate to sulfamethoxazol (80.60%), which is consistent with Imani's findings (96.4%) (28). In his analysis, Sabir found highest resistant rate to cefurexim (91.7%) and sulfamethoxazol (84.3%) (26). Resistance to third generation of cephalosporins has been observed in

several studies, ranging from 78.9% to 97.1 % (29, 30). We also found a 62.69% resistance rate to ceftriaxone.

Those strains resistant to three or more antibiotics were considered as MDR making 46.27% of our isolates, which is lower than Bayani's finding (60%) and more than Biswal and Khan with 36.2% and 30% rates, respectively (25, 31, 32). Dissimilar antibiogram patterns of *P. aeruginosa* strains has been reported in different area, which can be due to diversity of strains, quality of antibiogram discs and also precise of practice. Any way it can be concluded that constant antibiotic screening should be performed, before prescribing any drug.

The average length of hospitalization in our study was 17.75 days which is close to Hadji Bagheri's report (15.38 days) (33). Period of hospitalization, especially in ICU patients is an important risk factor for *P. aeruginosa* infection (5).

In this study, we detected seven REP-PCR types within the clinical isolates of *P. aeruginosa*.

The strain similarities within type A isolates (16 isolates) was less than that of Rep-type F (3 isolates) and G (4 isolates). Although REP-PCR type G (4 isolates) was more likely to be associated with antibiotic-susceptible strains, and REP-PCR type F (3 isolates), were highly correlated with antibiotic-resistance, because of so low number of isolates in each group, no statistically acceptable conclusion can be make. Concerning the source of infections, those *P. aeruginosa* strains recovered from burn ward patients showed higher similarity, most of them belonging to cluster C. On the other hand, isolates obtained from other wards showed higher genetic diversity in their group. The highly heterogeneous strains were isolated from tracheal tube. While the group of isolates of blood samples, showed the least genetic variation.

Due to the high genetic variety of *P. aeruginosa* strains, precise typing of clinical isolates is necessary for epidemiological surveillance, and finding clonal relationships between individual isolates in hospital settings.

There were some limitations to our study, including the small sample size. To know much more about the relation between different genotypes and their antibiogram patterns, additional prospective clinical studies with large sample sizes are needed.

Conclusions:

In conclusion, clinical isolates of *P. aeruginosa* with type A and C clones were the predominant strains from December 2012 to January 2013 in our hospital. To our knowledge, there are not many articles on REP-PCR genotyping of *P. aeruginosa* and this is the first report of REP-PCR typing of *P. aeruginosa* in Iran.

Acknowledgement:

This study was conducted in Molecular Medicine Research Center and supported by Vice-chancellor of Research and Technology, Infectious and Tropical Diseases Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Conflicts of Interests

The authors declare no conflict of interest.

References:

1. MILENA G. RIKALOVIĆ GG-C, MIROSLAV M. VRVIĆ, IVANKA KARADŽIĆ. Production and characterization of rhamnolipids from *Pseudomonas aeruginosa* san-ai. Journal of the Serbian Chemical Society. 77(1):27-42.
2. H. Arabian Hossein Abadi HR, G. Amoabediny and M. Mazaheri Asadi. Purification of rhamnolipid using colloidal magnetic Nanoparticles. African Journal Biotechnology. 2009;8(13):3097-106..
3. Witek-Krowiak. A. WJ, Gruszczyn´ska. A., G. Szafran., Koz´lecki. T., Modelski. S. . Ultrafiltrative separation of rhamnolipid from culture medium. World Journal Microbiol Biotechnol. 2011..
4. patrick R. Murray KSR, Michael A.Pfaller. medical microbiology. seventh ed2013. 288 p.
5. Mudau M, Jacobson R, Minenza N, Kuonza L, Morris V, Engelbrecht H, et al. Outbreak of multi-drug resistant *Pseudomonas aeruginosa* bloodstream infection in the haematology unit of a South African Academic Hospital. PloS one. 2013;8(3):e55985..
6. Le Gall F, Le Berre R, Rosec S, Hardy J, Gouriou S, Boisrame-Gastrin S, et al. Proposal of a quantitative PCR-based protocol for an optimal *Pseudomonas aeruginosa* detection in patients with cystic fibrosis. BMC microbiology. 2013;13:143..
7. Marco Ligozzi RF, Marco Aldegheri, Giovanna Scalet,Giuliana Lo Cascio. Comparative Evaluation of an Automated Repetitive-Sequence-Based PCR Instrument versus Pulsed-Field Gel Electrophoresis in the Setting of a *Serratia marcescens* Nosocomial Infection Outbreak. Journal of Clinical Microbiology. 2010;48(5):1690–5.
8. Cheon K, Moser SA, Whiddon J, Osgood RC, Momeni S, Ruby JD, et al. Genetic diversity of plaque mutans *streptococci* with rep-PCR. Journal Dental Research. 2011;90(3):331-5
9. Rhame F. The Ecology and Epidemiology of *Pseudomonas aeruginosa*. In: Sabath, L. D. (ed.) *Pseudomonas aeruginosa*, Hans Huber, Berne 2000; pp. 31-51.
10. Rosa Daza JGr, Gonzalo Pie´drola. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. International Journal of Antimicrobial Agents. 2001;18:211-5.
11. Cheryl K. Shutt JIP, 1 Sam R. Page,1 Barbara J. Schaecher,2 and Gail L. Woods, . Clinical Evaluation of the DiversiLab Microbial Typing System Using Repetitive-Sequence-Based PCR for Characterization of *Staphylococcus aureus* Strains. Journal of Clinical Microbiology. 2005;mar 1187-92
12. Yu-Chung Chuang J-TW, Mei-Ling Chen,Yee-Chun Chen. Comparison of an Automated Repetitive-Sequence-Based PCR Microbial Typing System with Pulsed-Field Gel Electrophoresis for Molecular Typing of Vancomycin-Resistant *Enterococcus faecium*. Journal of Clinical Microbiology. 2010;48(8):2897–901
13. Hossein Goudarzi1 FK, Fahimeh Asadi Amoli, Zohreh Abedinyfar. Farahnoosh Doustdar,Faramarz Mehrnejad. Genetic fingerprinting and antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* isolates from eye infections. Iranian journal of clinical infectious diseases. 2011;6(1):41-6.
14. Maria Isabel Queipo-Ortun˜o JDDC, 2 Manuel Macias,3, Maria Jose Bravo aPM. Preparation of Bacterial DNA Template by Boiling and Effect of Immunoglobulin G as an Inhibitor in Real-Time PCR for Serum Samples from Patients with Brucellosis. Clinical and Vaccine Immunology. 2008;15(2):293-6
15. Paul G. Higgins KJ, Maximilian M. Fresen, Hilmar Wisplinghoff, and Harald Seifert. Molecular Epidemiology of *Acinetobacter baumannii* Bloodstream Isolates Obtained in the United States from 1995 to 2004 Using rep-PCR and Multilocus Sequence Typing. Journal of Clinical Microbiology. 2012:3493–500.
16. Jamshid Alikhan zi, Saeed Ur Rahman, Kalsoom Farzana and Abbaskhan. Prevalence ans Resistance Pattern of *Pseudomonas aeruginosa* Against Various Antibiotics. Pakistan Journal of Pharmaceutical Sciences. 2008;21(3):311-5.
17. Ranjan KP, Ranjan N, Bansal SK, Arora DR. Prevalence of *Pseudomonas aeruginosa* in post-operative wound infection in a referral hospital in Haryana, India. Journal of Laboratory Physicians. 2010;2(2):74-7
18. Jamali SH. Bahar M.A.Houshmand S. M. Detection of bla VIM Gene among Imipenem – Resistant *Pseudomonas aeruginosa* Isolated from Burn Wounds from Tehran Shahid Motahari Hospital. Journal of medical microbiology 2009;1(1)
19. Rahimi B, Shojapour M, Sadeghi A, Pourbabayi AA. The study of the antibiotic resistance pattern of *Pseudomonas aeruginosa* strains isolated from hospitalized patients in Arak. Arak Medical University Journal. 2012;15(3):8-14.
20. Yunes Khalili MTA, Reza Gotasloo, Mohammad Aghazadeh, Behrooz Naghili, Majid Pornoor, . Prevalence of Antibiotic Resistance and VEB-1

Type Extended-Spectrum Beta-Lactamase in *Pseudomonas aeruginosa* Isolates from Intensive Care Unit Patients. Tabriz University of Medical Sciences. 2011;33(4)

21. Razavi M, Mansouri S, Norouzi F. Antibiotic resistance pattern among nonfermenting gram-negative bacteria isolated from clinical specimens during 2007-2008 in Kerman, IRAN. Indian Journal of Medical Microbiology. 2011;4(4):7-13.
22. . Rajabpour M, Arabestani mR, Yousefi mashof R, Alikhani MY. MIC determination of *Pseudomonas aeruginosa* strains were isolated from clinical specimens of patients admitted to educational hospitals in Hamedan (90-91). Indian Journal of Medical Microbiology. 2013;7(3):18-25.
23. Bonfiglio G CV, Russo G, Stefani S, Schito GC, Debbia E, Nicoletti G. Antibiotic resistance in *Pseudomonas aeruginosa*: an Italian survey, Journal of Antimicrobial Chemotherapy. 1998; 41(2):307-10.
24. Biswal I, Arora BS, Kasana D, Neetushree. Incidence of multidrug resistant *pseudomonas aeruginosa* isolated from burn patients and environment of teaching institution. Journal of Clinical and Diagnostic Research 2014;8(5):Dc26-9.
25. Sabir R, Alvi SF, Fawwad A, Basit A. Antibiogram of Pseudomonas aeruginosa and Methicillin-resistant *Staphylococcus aureus* in patients with diabetes. Pakistan Journal of Medical Sciences 2014;30(4):814-8.
26. Imani Foolad AA; Rostami Z; Shapouri. Antimicrobial resistance and ESBL prevalence in *Pseudomonas aeruginosa* strains isolated from clinical specimen by phenotypic and genotypic methods. Journal of Ardabil University of Medical Sciences 2010;10(3):189-98. [In Persian]
27. Gamal F. Gad RAE-D, Sahar Zaki and Hossam M. Ashour. Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. Journal of Antimicrobial Chemotherapy. 2007;60:1010-7.
28. Imani Foolad AA; Rostami Z; Shapouri. Antimicrobial resistance and ESBL prevalence in *Pseudomonas aeruginosa* strains isolated from clinical specimen by phenotypic and genotypic methods. Journal of Ardabil University of Medical Sciences 2010;10(3):189-98. [In Persian].
29. Massumeh Doosti, Mehdi Haj Ojagh Faghihi , Ali Ramazani ,Mohammad Reza Saini. Comparison of Conventional Culture Methods and Polymerase Chain Reaction (PCR) for Specific Detection of *Pseudomonas Aeruginosa*, Journal of Isfahan Medical School 2012;30(192). [In Persian]
30. Mitra Salehi MH, Farzaneh Hosseini. Quinolone resistance associated with efflux pumps mexAB-oprM in clinical isolates of *Pseudomonas aeruginosa*, The Journal of Microbial World 2014;6(4). [In Persian]
31. Bayani M, Siadati S, Rajabnia R, Taher AA. Drug Resistance of *Pseudomonas aeruginosa* and *Enterobacter cloacae* Isolated from ICU, Babol, Northern Iran. International Journal of Molecular and Cellular Medicine. 2013;2(4):204-9.
32. Khan F, Khan A, Kazmi SU. Prevalence and Susceptibility Pattern of Multi Drug Resistant Clinical Isolates of *Pseudomonas aeruginosa* in Karachi. Pakistan Journal of Medical Sciences. 2014;30(5):951-4.
33. K hajibagheri SA. An epidemiologic study of nosocomial infections and its related factors at the intensive care unit of Tohid Hospital, in Sanandaj during 2003-2004, Scientific Journal of Kurdistan University of Medical Sciences 2006;10(4):44-50. [In Persian]