Egyptian Journal of Medical Microbiology Volume 25 / No. 1 / January 2016 53-60

ORIGINAL ARTICLE

Phenotypic and Genotypic Characterization of *Pseudomonas Aeruginosa* Isolates from Burn Patients

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Key words:

Burn wound infections, *P. aeruginosa*, , PFGE

ABSTRACT

Background: *Pseudomonas aeruginosa (P. aeruginosa) is an opportunistic pathogen that still develops life threatening infections in patients with immunological system defects like burns. It has been estimated that 75% of all deaths following burns are due to infections.* ***Objectives:*** *This study was designed to evaluate the prevalence and drug susceptibility profile of P. aeruginosa in patients admitted to a burns unit and to investigate the potential sources of infection in burn patients by genotyping using pulsed field gel electrophoresis (PFGE).* ***Methodology:*** *The study was performed in King Fahd Hospital (KFH), Almadinah Almunwarah during a ten month period. A total of 106 burn wound infection (BWI), 40 environmental and 10 health care workers (HCWs) samples were isolated then identified by conventional biochemical methods and API 20E system. Antibiotyping was performed by disc diffusion. Molecular relatedness of P. aeruginosa strains was investigated by pulsed field gel electrophoresis.* ***Results:*** *The most isolated organism was P. aeruginosa (31.6%), followed by Methicillin–Resistant Staphylococcus aureus (MRSA) (21%) and Acinetobacter baumannii (A.baumanii) (17.5). Colistin (100%) was the most effective drug against P. aeruginosa while the least effective drug was ticarcillin (48.1%). Typing of 27 isolates by PFGE revealed four pulso-types A, B, C and D and pulsotype B revealed 3 subtypes, subtypes B_1, B_2 and B_3. The isolates showed that 77.7% of isolates (from patients and environmental specimens) were genetically related. Also, Six patients had the same pulsotypes which were not detected in the environment.* ***Conclusion:*** *P.aeruginosa became one of the major concern in our hospital and implementation of infection control strategies is a major concern to avoid the spread of this threat. Environmental sources may have a significant role in spread of P. aeruginosa among hospitalized patients.*

INTRODUCTION

P. aeruginosa is a Gram-negative, strict aerobic, non-spore forming, non-fermentative, oxidase and catalase positive, straight or slightly curved rods, motile, extracellular bacterium that is able to consume a broad range of organic compounds[1]. It is everywhere in the natural environmental settings as it can be isolated from different living organisms comprising plants, animals, and humans[2], and has a tendency for moist environments, and thus is often cultured in very high numbers from drains and similar semi-aquatic environments[3].

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P. aeruginosa is mostly common in hospital environment having been isolated from soap, disinfectants, respiratory equipment, mattresses, endoscopes, distilled water and suction apparatus[4]. *P. aeruginosa* could be derived from the patient's own endogenous gastrointestinal microbiota. Isolates from hospital environment generally display greater resistance to antimicrobials than environmental samples and are common causes of opportunistic infection in patients[4].

P. aeruginosa is the ideal example of an opportunistic pathogen causes an extremely wide range of disease in both immunocompetent and immunocompromised patients. Infection may vary in severity from a mild, self-limiting illness through to severe and debilitating systemic disease, associated with significant morbidity and mortality[5].

P. aeruginosa is a recognised nosocomial pathogen, responsible for between 10% to 20% of hospital-acquired infections and considered as the most

common cause of burn wound infections in many burn wound centres[6].

P. aeruginosa could produce outbreaks in burn units. In almost all cases the colonized patient is thought to be a major reservoir for the epidemic strain. Other important sources include contaminated hydrotherapy equipment, common treatment areas, and contaminated equipment such as mattresses, which appear to pose unique risks of cross contamination in the burn environment. Risks associated with care of the burn wound, such as hydrotherapy and common treatment rooms, are related to the use of water sources that are frequently contaminated by *P. aeruginosa* intrinsically, and may also be contaminated by organisms from other patients[7]. The other principal modes of transmission in burn units are through the hands of the personnel and contact with inadequately decontaminated equipment or surfaces. The hands and apron area of the person, as the surfaces (e.g., beds, side rails, tables, equipment) are often heavily contaminated with organisms from the patient. Likewise all equipment used on the patient (e.g., blood pressure cuffs, thermometers, wheelchairs, IV pumps) are also heavily contaminated and may be transmitted to other patients if strict barriers are not maintained and appropriate decontamination carried out. In fact, a single cause is uncommon in a burn unit outbreak; in almost all instances, multiple factors contribute to its occurrence and perpetuation[8]. Health care workers (HCWs) acquire microorganisms on gloved hands when touching surfaces near a colonized patient without performing direct patient contact[9]. Also, ungloved hands become contaminated with low levels of microorganisms even from surfaces in rooms that had been terminally cleaned after patient discharge[10].

Typing of *P. aeruginosa* is very important step. The main role of the typing is to assess the relationships between microbial isolates. Understanding clonal relatedness between the microbial strains is essential to determine the source and routes of infections, confirm or rule out outbreaks, trace cross-transmission of healthcare-associated pathogens, recognize particularly virulent strains and evaluate the effectiveness of control measures[11]. There are several typing methods used in laboratories today. These range from methods based on simple phenotypic features to DNA sequencing[12].Phenotyping methods are not discriminatory enough to identify strains belonging to the same genotype. Instead, more reliable molecular typing methods are required[13].

DNA typing methods are known as the most suitable approaches for epidemiological studies[14]. A variety of molecular methods have been used to type *P. aeruginosa* strains, each varying in their discriminatory potentials these methods include pulsed-field gel electrophoresis (PFGE), amplified fragment-length polymorphism (AFLP) analysis, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) and exotoxin *A* or pilin gene probing of enzyme-digested DNA fragments, multilocus sequence typing (MLST), restriction fragment length polymorphism (RFLP) and Repetitive-sequence-based PCR (Rep-PCR)[15]. PFGE remains the "gold standard" and prevalent genotypic method for fingerprinting and comparing isolates because it utilizes more than 80% of the chromosomal DNA to produce the molecular profiles of bacteria[16].

Since effective management of nosocomial infections, especially in burn units, needs to inform about infection transmission routes and drug susceptibility of pathogens, this study will be conducted to evaluate the incidence of *P. aeruginosa* in burn patients and to trace the source of infections and investigate relationship of strains by studying the antibiotic susceptibility of *P. aeruginosa* and characterization the clinical and environmental isolates of *P. aeruginosa* by PFGE analysis.

METHDOLOGY

Microbiological analysis

The present study was conducted in the burn unit at king Fahd Hospital (K.F.H), Al-Madinah Al-Munawarah, Saudi Arabia, during ten month period from March 2013 to December 2013. Swabs were taken from wound infections from burn ward using sterile cotton wool swabs by introducing them deeply into the depth of the infected wounds. Swabs were taken also from hospital environmental samples (from floors, doors, sinks, dressing trolleys, beds, water tap, environmental surfaces and other equipments). Environmental samples were collected before the regular daily cleaning by rolling sterile cotton swabs moistened with sterile saline several times over a surface area of approximately 5×5 cm^2 and then inoculated into 5% SBA plates. Swabs were taken from hands of health care workers over the same period. The right and left hand specimens of HCWs were obtained by placing their hands into 2 separate SBA plates directly without washing their hands before culture[17]. *P. aeruginosa* were finally identified and tested to the species level using API20E (Bio-Merieux, France).

Antibiotypying

The isolated strains were tested for their susceptibilities to 11 antibiotics; Ceftazidime, Cefepime, Piperacillin, Aztreonam, Imipenem, Gentamicin, Amikacin, Ciprofloxacin, Ticarcillin, Colistin and Tobramycin. The inhibition zones were measured and results of disk diffusion method were then reported according the guidelines of the Clinical and Laboratory Standards Institute[18].

Molecular Typing

Genomic DNA in agarose gel plugs was prepared according to the manufacturer's instructions of the reagents used (Bio-Rad Laboratories, USA). The

chromosomal DNA in the plug was digested with *SpeI* (Promega, USA) overnight at 37ºC[19]. Electrophoresis was performed in a 1% Sea Kem agarose gel (BMA, Rockland, USA) prepared and run in 0.5 × Tris-borate-EDTA buffer on a CHEF-DR III apparatus (Bio- Rad Laboratories, USA). The initial switch time was 5 s, the final switch time was 20 s, and the run time was 22 h at 6 V/cm. Gels were stained in ethidium bromide, destained in distilled water, and photographed under ultraviolet (UV) light. PFGE DNA patterns were compared and interpreted. Isolates with ≥ 80% similarity were considered to belong to the same pulsotype and subtypes were assigned to isolates having ≤ 3 DNA band differences within the same pulsotype[20].

RESULTS

Description of specimens from burn wound infection

A total of 122 patients were admitted in burn unit during the study period**.** The total number of burn wound swabs was 106. The overall percentage of positive cultures from burn wound swabs was 57 (53.7%). The negative cultures accounted for 49 (46.2%).

Isolation and identification of the microbial isolates

Bacterial isolates from BWI were subjected after purification to identification. Distribution of positive bacterial isolates among all cases is shown in table 1. They constituted (57) isolates, the most prevalent isolate was *P. aeruginosa* (31.6%), followed by methicillin resistant *Staphylococcus aureus* (MRSA) (21%)**.** No fungi ere detected in the culture media. Most wound infection 49/57 (86%) were monomicrobial, while the rest 8 (14%) were polymicrobial.

Table 1. Distribution of bacterial isolates from burn wound infection

Organism	Number (No=57)	Percent %
P. aeruginosa	18	31.6
MRSA	12	21
Acinetobacter baumanii	10	17.5
Klebsiella pneumoniae.	6	10.5
Staphylococcus aureus	4	7
Enterobacter spp.	3	5.3
Escherichia. Coli	3	5.3
Serratia spp.	1	1.8
Total isolates	57	100.0%

Description of specimens from environmental & HCWs samples

Forty different environmental samples were investigated. Eighteen samples (45%) were negative and 22 samples (55%) were positive. The percentage of *P. aeruginosa* among positive isolates was 40.9 (9 samples).

Ten samples from hands of HCWs (nurses) were collected in these study, two isolates (20%) were positive (Coagulase- negative *Staphylococcus*) and eight isolates was negative (80%). All cultures from the hands of the HCWs were negative for *P. aeruginosa.*

Clinical data of patients with *P. aeruginosa* infection

Clinical data of the patients with *P. aeruginosa* burn wound infection are listed in table 2. As regard the age, The main findings of this study were patients below 15 years (66.6%). The burn wound infection caused by *P. aeruginosa* was common in males 88.8% more than females 11.2%. Flame was the predominant type of burn in patients and accounted for 61.1% of the burn cases. The total body surface area on patients that has been burned was estimated by using rule of nines**.** The category (31-40%) showed the highest percentage of patients, while (33.3%), and (>41%) categories showed the lowest percentage of patients (11.1%).

Table 2. Clinical data of patients with *P. aeruginosa* infection

Patients	Age (Y)/sex	Causes of burn	TBSA
P1	10Y/ M	Flam	19%
P2	65Y/ M	Scald	4%
P3	72Y/ F	Flam	35%
P4	45Y/ M	Scald	19%
P5	39Y/ M	Flam	40%
P6	45Y/ M	Electrical	40%
P7	65Y/ M	Scald	8%
P8	32Y/ M	Flam	30%
P9	27Yl M	Flam	70%
P10	24Y/ M	Flam	36%
P11	75Y/M	Flam	40%
P12	30Y/ M	Flam	36%
P13	4Y/ M	Flam	5%
P14	27Y/ M	Scald	1%
P15	41Y/ M	Flam	65%
P16	12Y/M	Flam	25%
P17	8Y/ M	Scald	16%
P18	1Y/ F	Scald	18%

P= patient, M= male, F= female

Comparison of antimicrobial sensitivity pattern of *P. aeruginosa* strains

Data in tables 3 indicated the antimicrobial susceptibility pattern of 11 antibiotics against 27 *P. aeruginosa* strains (from the patients and the environmental samples)**.** The most effective drugs against *P. aeruginosa* were colistin (100%), followed by imipenem and amikacin (77.8%). The other commonly used drugs; piperacillin, ceftazidime and gentamycin showed moderate senstivity in the range of (50–60%). The least effective drug was ticarcillin that showed high level of resistance (51.9%).

Table 3. Comparison of antimicrobial sensitivity pattern against *P. aeruginosa* strains

Antibiotic disc	Disc potency	Sensitivity patterns					
		Sensitive		Intermediate		Resistant	
		No	%	No	%	No	%
Tobramycin	10 µg	19	70.4	2	7.4	6	22.2
Piperacillin	100 µg	16	59.3	1	3.7	10	37
Ticarcillin	75 µg	13	48.1	0	0	14	51.9
Cefepime	30 µg	18	66.7	0	0	9	33.3
Ceftazidime	30 µg	17	63	1	3.7	9	33.3
Gentamycin	10 µg	15	55.6	1	3.7	11	40.7
Amikacin	30 µg	21	77.8	1	3.7	5	18.5
Imipenem	10 µg	21	77.8	0	0	6	22.2
Aztereonam	30 µg	18	66.7	1	3.7	8	29.6
Ciprofloxacin	5 µg	19	70.4	1	3.7	7	25.9
Colistin	10 µg	27	100	0	0	0	0

PFGE Analysis of *P. aeruginosa* isolates

The total *P. aeruginosa* isolates from the patients and the environment were subjected to molecular typing by PFGE. PFGE pattern of *P. aeruginosa* is presented in table 4. A total of 4 PFGE profiles; pulsotypes A, B, C and D were identified among 27 *P. aeruginosa*. Pulsotype B isolates were further separated into 3 subtypes; subtypes B_1, B_2 and B_3 (Fig.1). Most of the subtypes among pulsotype B were subtype B_3.

Table 4. PFGE pattern of *P. aeruginosa* isolates

Isolate No	Sample	PFGE typing
E1	Tap water	A
E2	Suction tube	B1
E3	Suction bottle	B1
E4	Bed $_1$	C
E5	Bed $_2$	D
E6	Bed rails	B2
E7	Unit floor	C
E8	Bathroom floor	A
E9	Nurse gloves	B2
P1	Wound swab	A
P2	Wound swab	D
P3	Wound swab	B3
P4	Wound swab	A
P5	Wound swab	B3
P6	Wound swab	B3
P7	Wound swab	A
P8	Wound swab	B3
P9	Wound swab	A
P10	Wound swab	B3
P11	Wound swab	D
P12	Wound swab	B2
P13	Wound swab	A
P14	Wound swab	B3
P15	Wound swab	B1
P16	Wound swab	C
P17	Wound swab	B2
P18	Wound swab	A

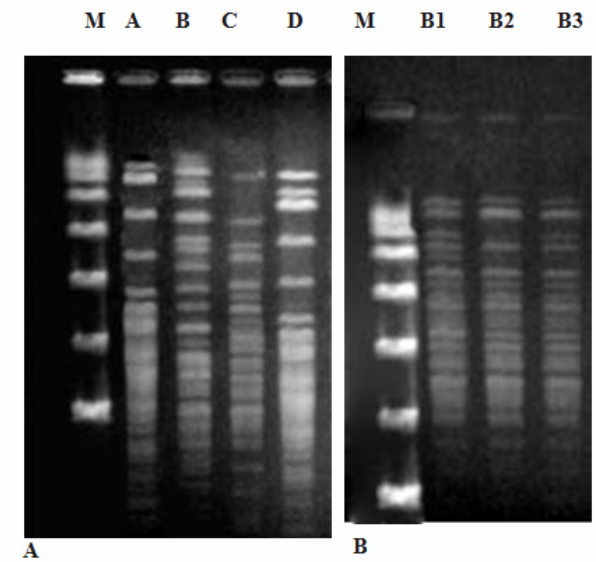

Fig. 1. *P. aeruginosa* strains obtained by PFGE after digestion with *Spe I*. **A:** four pulsotypes; in lanes A to D and Lane M; lambda marker. **B:** The three B subtypes; B1 to B3

DISCUSSION

Nosocomial Infection is an important cause of mortality in burns. It has been estimated that 75% of all deaths following thermal injuries are related to infections. The rate of nosocomial infections are higher in burn patients due to various factors like nature of burn injury itself, immunocompromised status of the patient, invasive diagnostic, and therapeutic procedures and prolonged ICU stay. Moreover, cross-infection results among different burn patients due to overcrowding in burn wards. Complicating this high

rate of infection is the fact that the spectrum of bacterial isolates varies with time and geographical area[21].

In the present study, the most commonly isolated organisms from burned patients were *P. aeruginosa* (31.6%). These finding is similar to several other studies from several countries; Turkey[22], Korea[23], and Egypt[24]. The prevalence of *P. aeruginosa* in the burn wards may be due to the fact that *P. aeruginosa* is known to thrive well in moist and humid habitats in which the organism can multiply to large numbers, even in the presence of minimal amounts of nutrients[12].

The second most common isolate was MRSA. Some reports from Lithuania, showed that the rate of MRSA infection (23.4%) was marginally higher than that of *Pseudomonas* spp. (17.6%)[25]. However, multi-drug resistant *A. baumannii* is rapidly emerging as an important pathogen in our hospital, they accounted for (17.5%), of all the organisms isolated in our study. This organism was also reported by some studies in India, U.S.A and China as the most common organism in burn patients followed by *P.aeruginosa*[26-28]. *K. pneumoniae* in this study accounted 10.5% of the total isolates. Our results were similar with other results[29]. In contrast, other researchers in Egypt have observed that *K. pneumoniae* was among one of the most common organisms isolated from burn patients[30]. In our study *S. aureus* accounted 7% of the total isolates. This low incidence *of S. aureus* infection was different with many of the studies in which *S. aureus* was the most predominant organism as in Yemen[31]. We also found *E.coli* accounted for 5.3% of the total isolates. This low incidence of *E.coli* was in agreement with other studies in which the frequency of the organism does not exceed 5%[32]. Most of the isolates in our research were monomicrobial 86%, and this was similar to another study[32], while on other study polymicrobial infection was the predominant[33].

As regard the age of paients with *P. aeruginosa* BWI, The main findings of this study were patients below 15 years (66.6%), this may be due to the fact that children have more mobility inside houses and have less sense and awareness of dangers[34].

The present study demonstrated an overall male 88.8% to female 11.2%. This was in agreement with several studies[35]. This reflects the preponderance of males in risk-taking activities at work and leisure. However, some studies reported the opposite[32].

As regard the cause of burn, flame was the predominant type of burn in patients and accounted for 61.1% of the burn cases. This finding was consistent with other studies[32]. Another study from Egypt reported that scald was the most common cause of burns followed by Flame[36].

The present study found that by using Rule of nines; the category (31-40%) included the highest percentage of patients, while (33.3%), and (>41%) category showed the lowest percentage of patients (11.1%). Another result observed that majority of the patients had less than 20% TBSA, while another study observed that TBSA was between 10 to 30%[37].

As regard the environment, positive environmental samples were 55%. It means that environment may play an important role in transmission of the infection to burn patients. This may be an indicator of the cleanliness in units and the use of unsuitable disinfectants. This result was similar to the results of Ikpeme et al [35]. The percentage of *P. aeruginosa* among positive isolates was 40.9% (9 samples), and this was similar to results by other investigators[33]**.** This finding made attention to *P. aeruginosa* ability to develop resistance to multiple types of disinfectants. this finding supported by some researchers where they found that *P. aeruginosa* exhibited moderate susceptibility to all disinfectants used[36].

Regarding the antimicrobial sensitivity pattern of *P. aeruginosa* strains, we found that the most effective drugs against *P. aeruginosa* were colistin. This result agreed with Mansour et al[38]. In this study, imipenem, amikacin, ciprofloxacine and tobramycin though not ideal but were comparatively better than other drugs, and this was in accordance to another study which reported that amikacin and imipenem had resistance rates of 24.6 and 22.6%, respectively[28]. We also found that 51.9% of *P. aeruginosa* isolates were resistant to ticarcillin, which was the highest percentage of resistance among the other antimicrobials tested. Our finding was in agreement with other studies[38]. *P. aeruginosa* isolates in the present study showed low sensitivity against gentamicin. Gentamicin is a cheap and easily available drug that is used extensively in general and hospital practice in clinically suspected Gram-negative infections. This may be the main reason for the development of resistance in bacteria against this drug[39]**.**

Some of our isolates have shown to be resistant to many antimicrobials, and this indicates the high contamination of burn wounds in our hospitals. Several reports confirmed an increasing multi-drug resistance among *P. aeruginosa* strains isolated from burn wound infections[34].

PFGE was used as a gold standard for determining similarities between *P. aeruginosa* isolates obtained from patients and environmental samples in burn ward. *SpeI* digestions of the 18 clinical and 9 environmental *P. aeruginosa* isolates were performed, resulting in four patterns (designated as pulsotypes, A, B, C and D), Pulsotype B were separated into 3 subtypes, subtypes B_1, B_2 and B_3.

In this setting, among patients with isolates that were not found in the environmental samples, six patients hospitalized during the same period in the same unit had the same pulsotype (B3), suggesting a possible patient-to-patient transmission. Medical staff can be a vehicle in the spread of *P. aeruginosa* within a hospital, as direct person-to-person contact contributes to the transmission of *P. aeruginosa*. Transmission between

patients from different rooms could be explained by their survival via hospital staff, several hospital devices or from the environment[37].

In this study, we found six patients had isolates identical to those found in the tap water (pulsotype A). This finding suggests that the water system was the reservoir. Several studies identified tap water as source of *P. aeruginosa* infection[40]. It was noted that taps could play a part in patients infections. In some cases, taps had become colonized with *Pseudomonas* from a patient already colonized on arrival on the ward. Also, pseudomonad bacteria were most likely to travel from tap water to the patients during facial washing, or via gauze dressings that had become wet during washing[40].

Also, we observed that pulsotype (B_1) was detected in one patient (p15), who was on mechanical ventilator from which the same pulsotype was isolated from (Endotracheal tube (E2), Suction bottle (E3), this implies that patient was probably colonized from an endogenous source or from ventilator equipment. In agreement with our study, Mansour et al in Egypt and Saudi Arabia, found a relationship between suction tubes, drainage containers, ventilator tubes and patients[38]. On the other hand, Berthelot et al found that the origin of lung colonization by *P. aeruginosa* was endogenous in 80% of the cases[41].

In the remaining cases, the environmental isolates showed the same pulso-types which were isolated from patients, there were two patients (p12 and p17) who had infection with the same subtype (B2) as that isolated from a bed rails. In addition, pulso-type (C) was isolated from both bed, unit floor and patient (p16), indicating that the source was probably hospital surfaces or derived from the patients themselves.

Environmental contamination may contribute to transmission of healthcare pathogens when HCWs contaminate their hands or gloves by touching contaminated surfaces, or when patients come into direct contact with contaminated surfaces. Environment may serve as a reservoir for *P. aeruginosa*. *P. aeruginosa* can survive at least 3-6 months on dried blood or cotton and as long as four weeks on other surfaces, and it is particularly well adapted to wet or damp conditions[4].

So, lapses in infection-control measures, or the non-restrictive antibiotic use at the field hospitals could result in an increase in cross-transmission between patients. So, future research on prevention of *P. aeruginosa* must focus on ways to improve compliance with hand hygiene, and improved methods of disinfecting the hospital environment are needed.

Ethical approval:

Ethical Committee of King Fahd Hospital in Al- Madinah Al-Monawarah and Scientific Research Deanship of Taibah University approved the study.

REFERENCES

1. Forbes, B., Sahm, D., Weissfeld, A. Bailey & Scott's. Diagnostic Microbiology, 12th Ed. Elsevier. 2007; pp. 340-350.
2. Lister, P. D., Wolter, D. J., Hansoni, N.D. Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin. Microbiol. Rev. 2009, 22 (4): 582-610.
3. Brisse, S., Milatovic, D., Fluit, A. C., Kusters, K., Toelstra, A., Verhoef, J., Schmitz, F. J. Molecular surveillance of European quinolone-resistant clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter spp*. using automated ribotyping. J. Clin. Microbiol. 2000, 38 (10): 3636-3644.
4. Catano, J. C., Echeverri, L. M., Szela, C. Bacterial contamination of clothes and environmental items in a third-level hospital in Colombia. Interdiscip. Perspect. Infect. Dis., Article ID 507640, 5 pages, 2012.
5. Bradbury, R., Champion, A., Reid, D.W Poor clinical outcomes associated with a multi-drug resistant clonal strain of *Pseudomonas aeruginosa* in the Tasmanian cystic fibrosis population. Respirology 2008, 13 (6): 886-892.
6. Matar, G. M., Chaar, M. H., Araj, G., Srour, Z., Jamaleddine, G., Hadi, U. Detection of a highly prevalent and potentially virulent strain of *Pseudomonas aeruginosa* from nosocomial infections in a medical center. BMC Microbiol. 2005, 5: 29-35.
7. Rafla, K., Tredget, E. Infection control in the burn unit. Burns 2011, 37 (1): 5-15.
8. Sharma, B., Singh, V., Bangar, S., Gupta, N. Septicemia: the principal killer of burns patients. Am. J. Infect. Dis. 2005, 1 (3): 132-138.
9. Ray, J., Hoyen, K., Das, M. Nosocomial transmission of vancomycin-resistant enterococci from surfaces. JAMA 2002, 287 (11): 1400-1401.
10. Bhalla, A., Pultz, J., Gries, M. Acquisition of nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. Infect. Control. Hosp. Epidemiol. 2004, 25 (2): 164-167.
11. Pereyre, S., Touati, A., Petitjean-Lecherbonnier, J., Charron, A., Vabret, A., Bébéar, C. The increased incidence of *Mycoplasma pneumoniae* in France in 2011 was polyclonal, mainly involving *M. pneumoniae* type 1 strains. Clin. Microbiol. Infect. 2013, 19: 212-217.
12. Belkum, A., Tassios, P. T., Dijkshoorn, L., Haeggmanl, S., Cookson, B., Fry, N. K., Fussing, V., Green, J., Feil, E., Gerner-Smidt, P., Brisse, S., Struelens, M. Guidelines for the validation and application of typing methods for use in bacterial

epidemiology. Clin. Microbiol. Infect. 2007, 13: 1-46.
13. Hafiane, A., Ravaoarinoro, M. Various typing methods of *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients. Med. Mal. Infect. 2008, 38 (5): 238-247.
14. Bertrand, X., Thouverez, M., Patry, C., Balvay, P., Talon, D. *Pseudomonas aeruginosa*: Antibiotic susceptibility and genotypic characterization of strains isolated in the intensive care unit. Clin. Microbiol. Infect. 2001, 7 (12): 706-708.
15. Wolska, K., Barbara Kot, B., Jakubczak, A. Phenotypic and genotypic diversity of *Pseudomonas aeruginosa* strains isolated from hospitals in Siedlce (Poland). Braz. J. Microbio. 2012, 43(1): 274-282.
16. Curran, B., Jonas, D., Grundmann, H., Pitt, T., Dowson, C.G. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J. Clin. Microbiol. 2004, 42 (12): 5644-5649.
17. Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., Winn, W.C. Color atlas and textbook of diagnostic microbiology; 5th ed. Williams and Wilkins, 1997.
18. National Committe For Clinical Laboratory Standards. Methods for antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard; 5th ed. Wayne: NCCLS M7-A5, 2000.
19. Salimi, H., Yakhchali, B., Owlia, P., Lari, A. Molecular epidemiology and drug susceptibility of *Pseudomonas aeruginosa* strains isolated from burn patients. Lab. Medicine 2010, 41 (9): 540-544.
20. Tenover, F. C., Arbiet, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H., Swaminathan, B. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 1995, 33: 2233-2239.
21. Mehta, M., Dutta, P., Gupta, V. Bacterial isolates from burn wound infections and their antibiograms: A eight-year study. Indian J .Plast., Surg. 2007, 40 (1): 25-28.
22. Oncul, O., Ulkur, E., Acar, A., Turhan, V., Yeniz, E., Karacaer, Z., Yildiz, F. Prospective analysis of nosocomial infections in a burn care unit, Turkey. Indian J. Med. Res. 2009, 130 (6): 758-764.
23. Song, W., Lee, k., Kang, H., Shin, D., Kim, D. Microbiologic aspects of predominant bacteria isolated from the burn patients in Korea. Burns 2001, 27 (2): 136-139.
24. Badr, R. I., El- Nagdy, M., El. Sabagh, A., Bahaa El. Din, A. *Pseudomonas aeruginosa* exotoxin *A* as a virulence factor in burn wound infections. Egyp. J. Med. Microbiol. 2008, 17 (1): 125-133.
25. Bagdonas, R., Algimantas, T., Rytis, R., Mindaugas, K. Analysis of burn patients and the isolated pathogens. Lithuanian Surg. 2004, 2 (3): 190-193.
26. Bhat, A., VinodKumar, C. Prevalence of burn wound infections with aerobic and anaerobic bacteria and their antimicrobial susceptibility pattern at tertiary care hospital. Int. J.Sci. Res. 2013, 2(11): 410-413.
27. Keen, F., Robinson, B.. Hospenthal, D., Aldous, W., Wolf, S., Chung, K., Murray, C. Incidence and bacteriology of burn infections at a military burn center. Burns 2009, 36 (4): 461-468.
28. Wang, L., Li, J., Ma, W., Li, J. Drug resistance analysis of 1914 bacterial strains isolated from burn patients. Genet. Mol. Res. 2014, 13 (4): 9727-9734.
29. Shahzad, M., Ahmed, N., Khan, I., Mirza, A., waheed, F. Bacterial profile of burn wound infections in burn patients. Ann. Pak. Inst. Med. Sci. 2012, 8 (1): 54-57.
30. Ibrahim, N, Amer, T. (2008): Frequency of bacterial and fungal infections of burn wounds at Cairo University Burn Center. Egyp. J. Med. Microbiol., 17 (4): 573-583.
31. Alghalibi, S., Humaid, A., Alshaibani, E., Alhamzy, E. Microorganisms associated with burn wound infection in Sana'a, Yemen. Egypt. Acad. J. biolog. Sci. 2011, 3 (1): 19-25.
32. Rajeshwar, S. R., Jaya, L. L., Pavani, S., Kawle, V., Jaya, J. P. Bacteriological profile, antibiogram of burn wound isolates and MRSA and ESBL production at tertiary care hospital, Hyderabad. WJPPS 2014, 3 (10): 1691-1698.
33. Magnet, M., Khan, A. , Arongozeb, M., Ahmed, Z. Isolation and identification of different bacteria from different types of burn wound infections and study their antimicrobial sensitivity pattern. IJRANSS 2013, 1(3): 125-132.
34. Sabzghabaee, A., Abedi, D., Fazeli, H., Javadi, A., Jalali, M., Maracy, M., Soltani, R., Karamyafti, M. Antimicrobial resistance pattern of bacterial isolates from burn wounds in an Iranian University Hospital. J. Pharm. Pract. Res. 2012, 1(1): 30-33.
35. Ikpeme, E. M., Enyi-Idoh, K. H., Nfongeh, J. F., Etim, L. B., Akubuenyi, F. C. Prevalence, antibiogram profile and cross transmission of *Pseudomonas aeruginosa* in a tertiary burn unit. Mal. J. Microbiol. 2013, 9(1): 116-119.
36. Nasser, S., Mabrouk, A., Maher, A. Colonization of burn wounds in Ain Shams University Burn Unit. Burns 2003, 29 (3): 229-233.
37. Ekrami, A., Hemadi, A., Kalantar, E., Latifi, M., Kayedani, A. Epidemiology of hospitalized burn patients during 5 years in Khuzestan province, Iran. Iran. J. Clin. Infect. Dis. 2010, 5 (1): 40-44.
38. Mansour, S. A., Eldaly, O., Jiman-Fatani, A., Mohamed, M. L., Ibrahim, E.M. Epidemiological characterization of *P. aeruginosa* isolates of

intensive care units in Egypt and Saudi Arabia. East Mediterr. Health 2013, 19(1): 71-80.
39. Moazami-Goudarzi, S., Eftekhar, F. Assessment of carbapenem susceptibility and multidrug-resistance in *Pseudomonas aeruginosa* burn isolates in Tehran. Jundishapur J. Microbiol. 2013, 6 (2): 162-165.
40. Pearson, S. A practical approach to ensuring safe water. Health Estate. J. 2012, 66 (7): 41-47.
41. Berthelot, P., Grattard, F., Mahul, P., Pain, P., Jospé, R., Venet, C., Carricajo, A., Aubert, G., Ros, A., Dumont, A., Lucht, F., Zéni, F., Auboyer, C., Bertrand, J. C., Pozzetto, B. Prospective study of nosocomial colonization and infection due to *Pseudomonas aeruginosa* in mechanically ventilated patients. Intensive Care Med. 2001, 27 (3): 503-512.