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Investigate the prevalence of multidrug-resistant *Pseudomonas aeruginosa* in patients hospitalized at Qutb al-Din Shirazi with Primer 272

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ABSTRACT

Pseudomonas aeruginosa is a mobile bacterium and cylindrical in shape, which grows easily in many medium. These bacteria can cause serious infections in burn patients due to damages of the skin as the first line of defense so that was a major factor of deaths in about 77% in these patients in the past 25 years. The aim of this study was to investigate the prevalence of multi-drug resistant P. aeruginosa in patients hospitalized at Qutb al-Din Shirazi with Primer 272. This cross-sectional study was conducted experimentally over a period of one year (March 2013 to March 2014) at Burn Research Center, University of Medical Sciences of Shiraz, Iran. In this study, 200 samples were collected by swab from the burn patients admitted to hospital over a period of one year. It should be noted that all samples were taken in the morning before washing the patients and delivered to the microbiology laboratory. All samples were then evaluated using microbiological phenotypic tests for microscopic and macroscopic. Of this number of samples, 50 (25%) cases were confirmed as P. aeruginosa. 83 (41%) samples were from the men's ward; 54 (27%) cases were from the women's ward; 35 (17%) cases were from the department of pediatrics and 28 (14%) samples were from the ICU (117 (58%) males and 83 (42%) females). The most causes of burns were as follows: 49 (25%) gas, 48 (24%) oil and gasoline, 40 (20%) boiling water and hot liquids, 33 (17%) fire and 30 (15%) electricity. Acinetobacter and other bacteria were respectively obtained as 50 (25%); 80 (40%) and 70 (35%). The results showed that resistance to the groups of carbapenem, penicillin, aminoglycoside, quinolone, cephalosporins, macrolides and chloramphenicol were high in different types of genetic patterns of P. aeruginosa and in this case the basic measures should be taken. According to resistance of some samples to antibiotics, this can be a major cause of epidemics of nosocomial infections in burn center of Qutb al-Din Shirazi. The incidence of antibiotic resistance in P. aeruginosa is not new matter and it has been reported upward trend in recent decades.

Keywords: *Pseudomonas aeruginosa*, nosocomial infections, multidrug resistance

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INTRODUCTION

Pseudomonaceae family consists of many species of gram-negative, aerobic and non-fermentation bacteria which live in soil and water. Several species of this genus are pathogenic to animals and plants. Most of isolates of pseudomonas does not infect humans but some of them are important opportunistic pathogen for the hosts with a weakened immune system [1]. This bacterium moves with the help of one or several polar flagella and almost all species are mobile. These bacteria are gram-negative bacilli that are unable to ferment sugars as known Non Fermenting Bacteria [NFB]. In addition to *P. aeruginosa* from Pseudomonaceae family, other genus including Alcaligenes, Acinetobacter, Moraxella, Kingella, Eikenella and Flavbacterium are NFB [2].

Pseudomonas bacteria are classified based on the similarity of rRNA and common cultural characteristics. *P. aeruginosa* is a mobile bacterium and cylindrical in shape [1.5- 3µm×0.5-0.8 µm]. The bacteria are easily grown in many medium, which create sometimes smell like grape. *P. aeruginosa* grows well at 37 to 42 °C. These bacteria are positive oxidize and are the second most common bacteria in nosocomial infections causing 21 percent of these infections. *P. aeruginosa* is a bacterium with a global spread in nature and is completely harmless in most scenarios. It has been isolated from soil, water, and occasionally from normal human skin. It can also be living more than 6% in the nose and throat and lower gastrointestinal system [3].

Fecal transmission of these bacteria is low in human beings [about 3%], but with long-term stay in the hospital after three weeks comes to about 30-50%, which can be a risk for internal infection [4]. *P. aeruginosa* can be seen in two forms on solid medium: Large colonies and smooth with flat edges and protruding center [like scrambled eggs] as well as rough and convex small colonies. Medical facilities are good sources of large colonies, while the small colonies usually are obtained from natural sources. The transformation of large colonies to small ones is possible, but the reverse mode is extremely rare. The third type is the mucoid colony that is obtained most from the urinary tract and respiratory secretions. The bacteria use carbohydrates through the aerobic metabolism and among sugars is only able to oxidize glucose. Oxygen is the final electron acceptor; although, the *P. aeruginosa* is obligatory aerobic bacterium, but can anaerobically grow using arginine or nitrate as subsidiary electron acceptor [5].Considering that *P. aeruginosa* is an important cause of nosocomial infections particularly in burn patients, so knowing how to release bacterial strains have particular epidemiological importance in order to find the sources of infection, assess the propagation of pathogenic isolates and quick control. Since it seems certain genetic pattern of bacteria appears in different infections, the aim of this study was to determine the prevalence of *P. aeruginosa* in burns patients admitted to hospital Qutb al-Din Shirazi during the period of 2013 to 2014.

MATERIALS AND METHODS

This cross-sectional study was conducted experimentally over a period of one year (March 2013 to March 2014) at Burn Research Center, University of Medical Sciences of Shiraz, Iran. 200 samples were directly taken by swab from the burn patients referred to hospital over a period of one year. It should be noted that all samples were collected in the morning before washing the patients and the experiments were performed at the microbiology laboratory. All samples were then examined be applying microbiological phenotypic tests for microscopic and macroscopic.

Determination of antibiotic susceptibility:

Evaluation of drug-resistant strains and different antibiotic sensitivity of each sample were measured using the Kirby-Bauer method. In this way, the numbers of 2-3 colony of any monoculture were inoculated in 2 ml nutrient broth and incubated for 2 h at 37 ° C. The turbidity of cultures was compared with 0.5 McFarland (6).

RESULTS

83 (41%) samples were obtained from the men's ward; 54 (27%) cases were from the women's ward; 35 (17%) cases were from the children's ward and 28 (14%) samples were from the ICU (overall 117 (58%) males and 83 (42%) females). The most causes of burns were as follows: 49 (25%) gas, 48 (24%) oil and gasoline, 40 (20%) boiling water and hot liquids, 33 (17%) fire and 30 (15%) electricity.

Of the total 200 samples taken from patients with burns, those that results of microbiological tests were positive for citrate, oxidase, catalase, indole, with pigment production and no sugar fermentation in TSI as well as growth was at a temperature of 42 °C have been identified as *P. aeruginosa* and stored in specific medium at -20 °C. Acinetobacter and other bacteria were respectively obtained as 50 (25%); 80 (40%) and 70 (35%).

Resistance pattern of different wards of hospital with carbapenem group:

- Children's Ward: this ward had no specific genetic pattern and contamination of this ward was as a result of genetic pattern contamination of other wards of the hospital. On the other hand, isolates of *P. aeruginosa* causing infection in children were resistant to imipenem and meropenem drugs. So, the children's ward needs correct planning and proper use of medications to reduce drug-resistant strains.

- Men's Ward: The genetic pattern 13 was dedicated to the men, as semi-sensitive to imipenem and meropenem antibiotics. The rest of the genetic pattern was as resistant to carbapenems group. So, drug-resistant infection is high in this ward. Therefore, men's ward needs correct planning and proper use of medications to reduce the drug-resistant strains.

- Women's Ward: The genetic pattern 5 was shown as semi-sensitive to imipenem and meropenem antibiotics. Although, this genetic pattern was common to other wards of the hospital, but it was more dispersed in the women's ward. The specific genetic patterns of women's ward were as resistance to various antibiotics, particularly carbapenems group. As a result, anti-susceptibility testing should be done with proper planning to prevent the bacteria resistance before prescribing antibiotics.

- ICU: isolates of *P. aeruginosa* from the ICU have been placed in the genetic pattern, which are all resistant to imipenem and meropenem antibiotics. The prevalence of drug-resistant strains is high in this ward, like other wards of the hospital, and so stronger actions are needed to control of infections and treatments.

Resistance pattern of different wards of hospital with penicillin group:

- Children's Ward: there was no specific genetic pattern and contamination of this ward was due to other wards. Also, isolates of *P. aeruginosa* causing infection in children were resistant to *piperacillin*. So, the children's ward needs appropriate actions to use of suitable medications for decreasing drug-resistant strains.

- Men's Ward: The genetic patterns dedicated for men's ward were observed as resistant to piperacillin antibiotic. Hence, high drug-resistant infections were observed, so that men's ward needs correct planning to proper use of medications for decreasing the drug-resistant strains.

- Women's Ward: The genetic pattern 5 was indicated as semi-sensitive to imipenem resistant to piperacillin antibiotic. This common genetic pattern was more dispersed in the women's ward. The specific genetic patterns of women's ward were as resistance to various antibiotics, particularly penicillin group. So, proper tests require preventing the resistance of bacteria.

- ICU: isolates of *P. aeruginosa* from the ICU have been placed in the genetic pattern, which are all resistant to piperacillin antibiotics. The prevalence of drug-resistant strains is high in this ward, like other wards of the hospital, and so serious measures are required to control of infections and treatments. Semi-sensitive genetic patterns 17 and 12 to piperacillin antibiotic have the same diffusion rate among different units of hospitals.

Resistance pattern of different wards of hospital with aminoglycosides group:

- Children's Ward: no specific genetic pattern was found and contamination observed is due to release from other sectors. On the other hand, isolates of *P. aeruginosa* causing infection in children were resistant to imipenem and meropenem drugs. So, more attention and care must be taken in the children's ward to reduce drug-resistant strains.

- Men's Ward: The genetic pattern 2 was special to the men, as semi-sensitive to gentamicin and amikacin antibiotics. The rest of the genetic pattern was as resistant to aminoglycosides group. So, there is great drug-resistant infection in the ward; this means that special care is required for lowering the drug-resistant strains.

- Women's Ward: The genetic pattern 5 was shown as semi-sensitive to gentamicin and amikacin antibiotics. However, this similar pattern was high in the women's ward. The specific genetic patterns of women's ward were as resistance to various antibiotics, particularly aminoglycosides group. Therefore, anti-susceptibility test and effective antibiotics can be suitable to fight against resistant bacteria.

- ICU: isolates of *P. aeruginosa* from the ICU have been placed in the genetic pattern, which are all resistant to gentamicin and amikacin antibiotics. The serious measures are able to destroy high prevalence of drug-resistant strains in this ward.

Resistance pattern of different wards of hospital with quinolones group:
- Children's Ward: genetic pattern was not specific and other parts of the hospital have caused contamination in this ward. On the other hand, isolates of *P. aeruginosa* causing infection in children were resistant to imipenem and meropenem drugs. So, the children's ward needs correct medications to decrease the drug-resistant strains.

- Men's Ward: The genetic pattern 2 was special to the men, as semi-sensitive to quinolones antibiotics. The rest of the genetic pattern was as resistant to aminoglycosides group. So, extremely drug-resistant infection is seen in this ward. Therefore, men's ward requires particular measure to fight the drug-resistant strains.

- Women's Ward: The genetic pattern 5 was shown as semi-sensitive to ciprofloxacin and nalidixic acid antibiotics. The ratio of the release of this genetic pattern was the greatest in the women's ward. The specific genetic patterns of women's ward were as resistance to various antibiotics, particularly quinolones group. Then, it should be noted that repeated tests to determine the appropriate pattern decrease the resistance.

- ICU: isolates of *P. aeruginosa* from the ICU have been placed in the genetic pattern, which are all resistant to ciprofloxacin and nalidixic acid antibiotics. By applying correct methods and effective antibiotics, we can fight against drug-resistant strains.

Resistance pattern of different wards of hospital with cephalosporins group:
- Children's Ward: there was no particular genetic pattern and contamination of this ward was as a result of genetic pattern contamination of other wards of the hospital. On the other hand, isolates of *P. aeruginosa* causing infection in children were resistant to ceftazidime and cefotaxime antibiotics. So, the ward requires appropriate antibiotics to fight the drug-resistant strains.

- Men's Ward: The genetic pattern 13 was special to the men, as semi-sensitive to ceftazidime and cefotaxime antibiotics. The rest of the genetic pattern was as resistant to aminoglycosides group. So, special actions require for lowering the high drug-resistant.

- Women's Ward: The genetic pattern 5 was shown as semi-sensitive to ceftazidime and cefotaxime antibiotics. The women's ward had the highest genetic pattern. The specific genetic patterns of women's ward were as resistance to various antibiotics, particularly cephalosporins group. Thus, prescribed antibiotics based on correct anti-susceptibility test can prevent the resistance in bacteria.

- ICU: isolates of *P. aeruginosa* from the ICU have been placed in the genetic pattern, which are all resistant to ceftazidime and cefotaxime antibiotics. Control of antibiotic-resistant bacteria in this section is possible with right medications.

Resistance pattern of different wards of hospital with macrolides group:
- Children's Ward: genetic pattern of this ward was not special and genetic pattern contamination of other units contaminated this ward. On the other hand, isolates of *P. aeruginosa* causing infection in children were resistant to erythromycin antibiotic. So, drug-resistant strains should be reduced with intensive care and appropriate medications.

- Men's Ward: The genetic pattern 13 was special to the men, as semi-sensitive to macrolides groups. So, serious measures must be taken to combat the high incidence of drug-resistant infection.

- Women's Ward: The genetic pattern 5 was shown as semi-sensitive to erythromycin antibiotic. The specific genetic patterns of women's ward were as resistance to various antibiotics, particularly macrolides group. By administrating right antibiotics, we can reduce the high genetic pattern in the women's ward.

- ICU: isolates of *P. aeruginosa* from the ICU have been placed in the genetic pattern, which are all resistant to erythromycin antibiotic. As mentioned in other parts, the basic measures should be done for the control and treatment of resistant infectious agents.

Resistance pattern of different wards of hospital with chloramphenicol group:
- Children's Ward: genetic pattern in this ward was not particular. The contamination was due to genetic pattern contamination of other units of hospital. On the other hand, isolates of *P. aeruginosa* causing infection in children were resistant to chloramphenicol antibiotic. Hence, reduced resistance must be done with basic health measures and proper medications.

- Men's Ward: The genetic pattern 13 was special to the men, as semi-sensitive to chloramphenicol groups. So, effective drugs, using the correct tests, should be used to prevent the spread of drug-resistant infections.

- Women's Ward: The genetic pattern 5 was shown as semi-sensitive to chloramphenicol antibiotic. The specific genetic patterns of women's ward were as resistance to various antibiotics, particularly chloramphenicol group. It would be better to use specific tests to cope with the genetic pattern in this ward. As a result, anti-susceptibility testing should be done with proper planning to prevent the bacteria resistance before prescribing antibiotics.

- ICU: isolates of *P. aeruginosa* from the ICU have been placed in the genetic pattern, which are all resistant to chloramphenicol antibiotic. The high incidence of drug-resistant strains in this ward is a serious threat for patients; so, it is essential to find effective treatments to control of infections.

DISCUSSION AND CONCLUSION

Considering that *P. aeruginosa* is an important cause of nosocomial infections particularly in burn patients, so knowing how to release of bacterial strains has special epidemiological importance to find the source of infection, assess the propagation of pathogenic isolates and quick control of pathogens. In this study, 200 samples were collected from patients, 117 (58%) was related to men and 83 (42%) was belonged to women.

Of the total samples collected from the burn center of Qutb al-Din Shirazi (Shiraz) hospital, 83 (41%) cases were from men's ward; 54 (27%) cases were from women's ward; 35 (17%) cases were from children's ward; 28 (14%) samples were from the intensive care unit, all of the samples had been taken from the ulcers of patients. In this research, standard microbiological tests were carried out on 200 samples taken from patients to identify isolates of *P. aeruginosa*, which 50 samples were identified as *P. aeruginosa* isolates. The results show that resistance to carbapenem and penicillin groups in the genetic patterns of *P. aeruginosa* is high. As well as resistance to aminoglycosides is more in the genetic patterns of *P. aeruginosa*. Semi-sensitive genetic pattern 17 was related to gentamicin and amikacin antibiotics and had same propagation speed among different wards. Similarly, resistance to quinolone in the genetic patterns of *P. aeruginosa* is also high. Semi-sensitive genetic pattern 5 to ciprofloxacin antibiotic was discussed. Semi-sensitive genetic patterns 17 and 12 were associated to ciprofloxacin and nalidixic acid antibiotics and had same propagation speed among different wards. So we can prevent the spread of common genetic patterns among wards using the right medicines.

It should be noted that resistance to cephalosporins, macrolides and chloramphenicol is high in a variety of genetic patterns of *P. aeruginosa*. Semi-sensitive genetic pattern 11 was linked to erythromycin antibiotic with the same propagation speed among different wards. The common genetic pattern 11 that was semi-sensitive to chloramphenicol had the same propagation speed in different wards of the hospital.

A study was conducted by Polavarapu et al., in 2008 for six months on all patients hospitalized in the burn ward. The findings of this study showed that 72% were infected with *Pseudomonas aeruginosa* and others, respectively, with *E. coli*, *Staphylococcus aureus*, Candida and Proteus (7). The results of this study were not consistent with the present study and infections with P. aeruginosa isolates were much more from this study, it could be evidence of improper sterilization and lack of hygiene and other factors in the study o Polavarapu. In this study, other organisms listed have not been studied. A research was performed by Gürtler et al., in 2007; in this study the most common causative organism of burn wound infections were, respectively, Staphylococcus and Klebsiella as well as *P. aeruginosa* were in third place (8). The *Pseudomonas aeruginosa* isolates in this study with 25% frequency were similar to the present study; it was shown that the incidence of these bacteria has decreased in burn patients in contrast to previous years. Bertrand et al., in 2009 in America investigated the prevalence of *P. aeruginosa* isolates among 200 burn patients over a period of 8 months. The frequency of these bacteria was 40 (80%) cases, more than the present study (9).

A study was conducted by Mahenthiralingam et al., in 2006 in the burn hospital of Netherlands. The prevalence of bacteria involved in infections over a period of two years was as follows:

Staphylococcus aureus (70%), *Pseudomonas aeruginosa* (15%), Klebsiella (9%), Candida (5%) and Proteus (1%) (10). The prevalence of *Pseudomonas aeruginosa* isolates in this study was lower than the present study, which represents the difference of bacteria contributes to the development of infections in various parts of the world. A study was done by Renders and et al, on 59 patients with cystic fibrosis to isolate the strains of *Pseudomonas aeruginosa*. 113 samples were also collected from hospital environments (devices, hallways, faucets, bathrooms and hand washing liquids). 21 patients with cystic fibrosis (35.5%) and 25 patients with urinary tract infection (92.5 percent) were infected with *P. aeruginosa* and 11 isolates of *P. aeruginosa* were obtained from 113 samples of hospital environment. Of 21 strains of *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis, 19 isolates (42.9 percent) had mucoid colonies and the antibiotic resistance included: 9.5% gentamicin and amikacin, 14.2% ciprofloxacin, 19% piperacillin and 86% and ceftazidime. In patients with urinary tract infections, 4 isolates (16%) had mucoid colonies and 21 isolates (84%) had non-mucoid colonies. Also 50% of isolates of *Pseudomonas aeruginosa* isolated from hospital environments formed mucoid colonies. 96% of strains isolated from patients with urinary tract infections and 91% of strains isolated from hospital environments were resistant to ceftazidime and the least resistance was related to amikacin. Mucoid colonies of *Pseudomonas aeruginosa* was mostly observed in patients with cystic fibrosis compared to strains isolated from patients with urinary tract infection at hospital environments. The most antibiotic resistance in all groups was belonged to ceftazidime and the most antibiotic susceptibility was observed to amikacin (11). The ratio of resistance of isolates obtained from this study to antibiotics was lower than the present study. But isolates resistant to ceftazidime antibiotic in the present study was lower than this study.

A study in 2012 by Rosen in patients hospitalized in the intensive care unit was conducted in four categories of antibiotics including cephalosporins, fluoroquinolones, aminoglycosides and carbapenems. 75% of *Pseudomonas aeruginosa* isolates were MDR (resistant to more than 3 groups of antibiotics) and 50% of them were resistant to all antibiotic groups (12). One of the findings of the present study was to find samples with multiple antibiotic resistances, which the results of this study were consistent with the present research. According to the resistance of some samples to antibiotics, this resistance can be a major cause of epidemic of nosocomial infection in burn center of Qutb al-Din Shirazi hospital (Shiraz). The incidence of antibiotic resistance in *P. aeruginosa* is not new matter and it has been reported upward trend in recent decades. A study performed by Rastegar- Lari et al., in 2012, 89 strains of *P. aeruginosa* were isolated from samples, which the highest prevalence in urine samples was 51.5%. The results showed that these organisms were resistant to ceftazidime and imipenem, respectively, 56% and 24.7% and the ratio of resistance in isolates of present research than the study shows the annual increase (13). The present study showed the increased resistance to imipenem and ceftazidime antibiotics, which the results are in line with different studies done in the other regions and neighboring countries. However, the study shows clear difference with industrialized countries, this suggests the importance of the proper use of these antibiotics in developed countries. Behzadiyan-Nejad et al., in 2006 in Uzbekistan carried out a study on antimicrobial resistance of 170 isolates of *P. aeruginosa* isolated from burn patients, which included 87%, 74%, 81.7%, 88.2% and 84.7% for imipenem, carbenicillin, amikacin, tobramycin, Ticarcilin, respectively (14). The results showed an increase in antibiotic resistance in *P. aeruginosa* isolates compared to similar studies, and the results were consistent with the findings of the present study. Ranjbar et al in 2007 in Italy studied on 104 samples for *P. aeruginosa*; isolates resistance was evaluated by disk diffusion for 11 antibiotics and broth microdilution for 4 antibiotics. In this study, the highest rate of resistance was to antibiotics of nalidixic acid (86.54%), ceftriaxone (82.2%) and ofloxacin (81.78%). There was also the least resistance to the antibiotic of imipenem (40.19%), piperacillin (44.9%) and tetracycline (48.03%). MIC results showed the greatest sensitivity was related to tetracycline and the most resistant was to ceftriaxone (15). The results of this study were in line with findings of present research.

A research was performed by Ahangarzadeh-Rezaee et al in 2009, on 85 isolates of *P. aeruginosa* to determine their antibiotic susceptibility with different disks. *P. aeruginosa* isolates were resistant to ceftazidime (50%), cefotaxime (38.9%) and imipenem (15.5%) (16). Resistance of *P. aeruginosa* isolates in this study to ceftazidime, cefotaxime and imipenem discs was, respectively, 90, 78 and 84%, which was much more than our study and the results of these two studies were not consistent. Since *P. aeruginosa* is an important factor for nosocomial infection, especially in burn patients, so awareness of how to release strains of bacteria has epidemiological importance to find the source of infection, assess the propagation of pathogenic isolates and quick control. And apparently certain genetic patterns come from different bacterial infections; so, it is necessary to determine the genetic patterns of resistant and susceptible strains of *Pseudomonas aeruginosa* isolated from burn patients seems.

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