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Vancomycin-Resistant *Pseudomonas Aeruginosa* in the Cases of Trauma

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ABSTRACT

Background: One of the main problems in the treatment of cases of *P. aeruginosa* especially in the orthopedic infections is the occurrence of high antibiotic resistance. The present study was carried out in order to investigate the prevalence of vancomycin-resistant *P. aeruginosa* in the cases of trauma in Iran. **Methods:** Two hundred and fifty swab samples were collected from the site of trauma from the patients who referred to the orthopedic wards of the Iranian health centers. Samples were cultured immediately and those that were *P. aeruginosa*-positive were analyzed by the disk diffusion method. **Results:** Of 250 swab samples collected, 43 were positive for *P. aeruginosa* (17.2%). The results of the culture technique were also confirmed by the PCR reaction. Of 43 *P. aeruginosa* isolates, 32 strains (74.41%) were resistant to vancomycin. Total prevalence of bacteria in Tehran and Mashhad hospitals were 18.46% and 15.83%, respectively. Statistically significant difference was seen for the prevalence of vancomycin-resistant *P. aeruginosa* between the samples collected from Tehran and Mashhad ($P=0.027$). More than 55 years old and less than 10 years old patients had the highest prevalence of *P. aeruginosa*. *P. aeruginosa* strains of male and more than 55 years old patients harbored the highest levels of resistance against vancomycin. **Conclusions:** It is logical to primary identification of type of bacteria causing infection in the site of trauma and then using from the disk diffusion method to choose the best antimicrobial agent. Highest levels of health care should be performed for the patients less than 10 years and more than 55 years old patients.

Key words: *Pseudomonas aeruginosa*, Trauma, orthopedic wards, Vancomycin resistance, sex.

1. INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a ubiquitous organism present in many diverse environmental settings and is a prominent cause of nosocomial infections all around the world (1, 2). The ability of this bacterium to survive on minimal nutritional requirements and to endure against variety of physical conditions has allowed it to persevere in both community and hospital settings. Hospital reservoirs of *P. aeruginosa* include respiratory equipment, soap, antiseptic, mop, sink, artificial fingernails, hot tubes, physiotherapy and hydrotherapy pools, gastrointestinal tract of patients on anticancer therapy, muco-

sa and skin of patients treated with broad-spectrum antibiotics, lower respiratory tract of mechanically ventilated patients, and finally the hands of hospital personnel, caused to the constant persistence of *P. aeruginosa*-infection in the hospitals (3, 4). In hospitals, *P. aeruginosa* can be isolated from a variety of sources, including respiratory tract, urinary tract, burn and wound, soft tissue and superficial and deep infections and also the cases of food-poisoning, orthopedic operation and trauma (5). *P. aeruginosa* may be presented into the bone or joint via direct inoculation during trauma, the surgical procedure, hematogenous spread, or spread from a contiguous infection.

Pseudomonas infection often has a delayed presentation and may become a chronic infection following fracture repair (6, 7). According to the destruction of the keratin layer of the skin close exposure of the substrate layers with the polluted environment, the possibility for the occurrence of severe infection and hardening treatment is not unbelievable (8). *P. aeruginosa* has the capacity to carry plasmids containing genes that regulate antimicrobial resistance, and this feature has led to the appearance of some strains that are resistant to normally reliable antibiotics (9). In the other hand, infections caused by *P. aeruginosa* are complicated by the fact that antibiotic resistance is both widespread and rapidly acquired. Documented researches revealed an increase in the levels of *P. aeruginosa* resistance against several classes of antibiotic including aminoglycosides, penicillins, carbapenems, cephalosporins and fluoroquinolones (10, 11).

Vancomycin is an antibiotic used to treat variety of nosocomial infections (12, 13). Vancomycin is not a primary choice for treatment of the cases of *P. aeruginosa*, but its high prescription caused to occurrence of excessive levels of resistance (14). Based on the uncertain epidemiology of *P. aeruginosa* in the cases of trauma, the present investigation was carried out in order to determine the prevalence of *P. aeruginosa* in the Iranian cases of trauma as well as study the levels of *P. aeruginosa* isolates against vancomycin antibiotic.

2. MATERIALS AND METHODS

From January 2015 to August 2015, a total of 250 swab samples were collected from the patients suffered from the various types of trauma. Samples were collected from the site of trauma using the sterile cotton swabs under aseptic precautions. Sex and age of all patients were also recorded. Swab samples were immediately transferred to the laboratory in a cooler with ice-packs.

Swab samples were inoculated on to blood, MacConkey (Merck, Germany) and Nutrient agar (Merck, Germany) and incubated at 37°C for 18–24 h; colonies that produce pyoverdin, pyocyanin and pyorubin pigments were transferred to nutrient agar and subcultured more than one time to obtain pure cultures. The isolates were identified using conventional biochemical tests.

Vancomycin-resistant strains of *P. aeruginosa* was determined using the simple disk diffusion technique. The Mueller–Hinton agar (Merck, Germany) medium was used for this purpose. Resistance of *P. aeruginosa* strains against vancomycin disk (5 µg/disk, Oxoid, UK) was determined using the instruction of Clinical and Laboratory Standards Institute (15). All of the inoculated plates were aerobically incubated at 37 °C for 18-24 h in an aerobic atmosphere. Results were interpreted based on the instruction provided by CLSI (2012) (15). In all reactions, the *P. aeruginosa* (ATCC 27853) was used as quality control organisms.

Total genomic DNA was extracted from the bacterial colonies. A single colony was inoculated on 5ml of brain heart infusion broth and incubated over night at 37°C. Then 1.5 ml of a saturated culture was harvested with centrifugation for 5 min. at 14,000 rpm. The cell pellet

was resuspended and lysed in 200µl of lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1% SDS) by vigorous pipetting. To remove most proteins and cell debris, 66 µl of 5M NaCl solution was added and mixed well, and then the viscous mixture was centrifuged at 12,000 rpm for 10min. at 4°C. After transferring the clear supernatant into a new eppendorf tube, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Following centrifugation at 14,000 rpm for 5min., the supernatant is then removed to another eppendorf tube and double volume of 100% ethanol was added. The tubes were inverted 5 to 6 times gently, then centrifuged at 10,000rpm for 5minutes. The supernatant was discarded and 1ml of ethanol (70%) was added to the pellet, and tubes centrifuged at 10,000 rpm for 5 minutes. Finally the supernatant discarded and the pellet was dried for 10 min at room temperature, the pellet was resuspended by 100µl H₂O. The stock was kept at -20°C until use. The DNA concentration has been determined by measuring absorbancy of the sample at 260 nm using spectrophotometer (16). The bacteria were confirmed using the PCR method for *16S rRNA* gene of the *P. aeruginosa* (17). PCR was carried out with 2 µL template DNA, 0.25 µM of each primer (F: 5'- GGGG-GATCTTCGGACCTCA-3' and R: 5'- TCCTTAGAGT-GCCCACCCG-3') (956 bp size of product), 0.2 mM deoxyribonucleoside triphosphates, 1X reaction buffer, 2 mM MgCl₂ and 1.5 U Taq DNA polymerase (Fermentas) in a total volume of 25 µL. The DNA was amplified using the following protocol: initial denaturation (95 °C for 2 min), followed by 25 cycles of denaturation (94°C for 20 s), annealing (58°C for 20 s) and extension (72°C for 40 s), with a single final extension of 1 min at 72°C. In all PCR reaction, *P. aeruginosa* (ATCC 27853) was used as positive control and sterile distilled water was used as a negative control. Fifteen microliters of PCR products were resolved on a 1.5% agarose gel containing 0.5 mg/ml of ethidium bromide in Tris–borate–EDTA buffer at 90 V for 1 h, also using suitable molecular weight markers. The products were examined under ultraviolet illumination.

Statistical analysis: The chi-square test and Fisher's exact 2-tailed test analysis were performed in this study. Statistical significance was regarded at a *P* value < 0.05.

3. RESULTS

A total of 250 swab samples of traumatic patients of orthopedic wards of the educational Hospitals, Tehran and Mashhad, Iran were tested for presence of vancomycin-resistant *P. aeruginosa*. Table 1 shows the total distribution of vancomycin-resistant *P. aeruginosa* in various studied groups of patients. Of 250 swab samples collected, 43 samples were positive for *P. aeruginosa* (17.2%). Of 43 *P. aeruginosa* isolates, 32 strains (74.41%) were resistant to vancomycin. Total prevalence of vancomycin-resistant *P. aeruginosa* in Tehran and Mashhad hospitals were 62.5% and 89.47%, respectively. Statistically significant difference was seen for the prevalence of vancomycin-resistant *P. aeruginosa* between the samples collect-

ed from Tehran and Mashhad ($P = 0.027$). Statistically significant difference was also seen for the prevalence of *P. aeruginosa* between male and female ($P = 0.029$). More than 55 years old and less than 10 years old patients were the most commonly infected age groups of our study. Statistically significant difference were seen for the prevalence of *P. aeruginosa* between more than 55 years old and 25-40 years old patients ($P = 0.034$) and also between less than 10 years old patients and 10-25 years old patients ($P = 0.041$). *P. aeruginosa* strains of male and more than 55 years old patients harbored the highest levels of resistance against vancomycin. The results of the culture technique were also confirmed by the PCR reaction. In the other hand, all of the 43 *P. aeruginosa* strains isolated from the swab samples using the culture method, have been approved for presence of the *16S rRNA* gene using PCR (Figure 1).

4. DISCUSSION

The results of the present study revealed that 17.2% of the swab samples taken from the site of trauma in patients hospitalized in Iranian hospitals and health centers were infected with *P. aeruginosa*. Besides, 74.41% of all isolates were resistant to vancomycin which was considerably high. As far as we know, this is the first and most interesting research about the high prevalence of vancomycin-resistant *P. aeruginosa* in the swab samples taken from the site of trauma in patients hospitalized in Iranian hospitals and health centers. Higher prevalence of bacterium was seen in the groups of more than 55 years old and less than 10 years old patients. Females had the higher prevalence of *P. aeruginosa*, while male had the higher prevalence of resistance against vancomycin. *P. aeruginosa* strains recovered from the samples of Tehran had the higher frequency, while those that were recovered from the samples of Mashhad had the higher prevalence of resistance against vancomycin.

There are some logical explanation for above findings. Conceivable clarifications for the high distribution of *P. aeruginosa* in our study is due to the low levels of health care and lack of sanitary conditions in the orthopedic parts of hospitals, indecorous prescription of operative drugs and existence of antibiotic resistance in bacterial strains. A possible reason for the lower prevalence of antibiotic resistance in less than 10 years old patients is that these patients were not usually used from antibiotics. It is because of the number of diseases in these patients is much less than those of elderly. Therefore, the frequency of using antibiotics is less. The cases of trauma in males are more than those of female. It is because of males are more at a risk of accident which cause trauma. In addition, working in difficult conditions that predispose to accident and trauma is more prevalent among males. A possible reason for the higher prevalence of bacteria in females is that they are usually weaker and more sensitive. The higher levels of fat under the skin of women is another risk factor. A possible reason for the high prevalence of resistance in males than females is the fact that they are more resistant to get sick and usually antibiotic-resistant strains of bacteria causes diseases in males. There were few studies

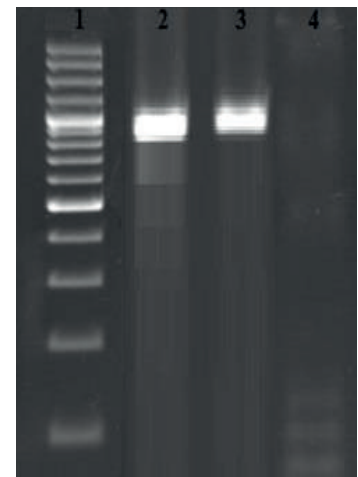


Figure 1. Electrophoresis of PCR products for 16S rRNA gene of the *P. aeruginosa* strains in the swab samples taken from traumatic patients. 1: 100 bp ladder, 2: Positive sample (956 bp), 3: Positive control and 4: Negative control.

regarding the presence of *P. aeruginosa* in the cases of trauma and orthopedic operations. In a study which was conducted by Al-Mulhim et al. (2014) (18) the most commonly detected pathogens in the sites of orthopedic operations were Methicillin Resistant *S. aureus* (MRSA) (29.11%), *Acinetobacter* species (21.5%), *P. aeruginosa* (18.9%) and *Enterococcus* species (17.7%). In the United Kingdom (UK) *P. aeruginosa* is one of the most prevalent causes of infections in the orthopedic operations (19). A study which was conducted in Germany (20) showed that the *P. aeruginosa* is responsible for 28% of the cases of wound infections in hospitalized patients. Total prevalence of *P. aeruginosa* in the cases orthopedic surgical site infections in Egypt was 10.6% (21). Differences in the type of orthopedic diseases, method of sampling, number of samples collected, method of experiment, sex and age of patients and geographical area which the samples were collected are the main fac-

Samples and criteria		No. samples collected	No. <i>P. aeruginosa</i> (%)	No. vancomycin-resistant (%)	
Tehran	Sex	Male	80	11 (13.75)	8 (72.72)
		Female	50	13 (26)	7 (53.84)
	Age	<10	30	6 (20)	1 (16.66)
		10-25	25	3 (12)	1 (33.33)
		25-40	20	3 (15)	2 (66.66)
		40-55	20	3 (15)	3 (100)
		>55	35	9 (25.7)	8 (88.88)
Total	130	24 (18.46)	15 (62.5)		
Mashhad	Sex	Male	70	10 (14.28)	9 (90)
		Female	50	9 (18)	8 (88.88)
	Age	<10	25	4 (16)	2 (50)
		10-25	22	3 (13.63)	3 (100)
		25-40	20	3 (15)	3 (100)
		40-55	23	3 (13.04)	3 (100)
		>55	30	6 (20)	6 (100)
Total	120	19 (15.83)	17 (89.47)		
Total	250	43 (17.2)	32 (74.41)		

Table 1. Total prevalence of vancomycin-resistant *P. aeruginosa* in the swab samples taken from traumatic patients.

tors for differences in the prevalence of *P. aeruginosa* in various investigations. The results of our investigation revealed that 74.41% of all *P. aeruginosa* isolates were resistant to vancomycin. Another Iranian investigation (22) showed that *P. aeruginosa* strains of clinical infections exhibited the highest level of resistance to penicillin (100%), followed by tetracycline (90.19%), streptomycin (64.70%), and erythromycin (43.13%). Another study which was conducted on the samples recovered from the orthopedic wards (23) showed that the highest levels of resistance was observed against ampicillin (> or = 98.4%), ampicillin/sulbactam (85.3%), co-amoxiclave (83.8%) and ofloxacin (68.4%) and least resistance was observed against amikacin (24%). In a study which was conducted in Iraq (24), *P. aeruginosa* strains showed sensitivity to amikacin, erythromycin and penicillin, while showed resistance to penicillin, erythromycin, and norfloxacin, amoxicillin, amoxicillin + clavulanic acid and azithromycin. Irregular, unusual and excessive prescription of vancomycin in the orthopedic wards of the Iranian Hospitals caused to increase in the levels of resistance of *P. aeruginosa* isolates to this antibiotic. Medical practitioners of Tehran. Therefore, the prevalence of resistance against this antibiotic in Tehran was entirely lower than Mashhad. In fact, differences in the idea of medical practitioners in antibiotic prescription and availability of antibiotics causes variations in the levels of antibiotic resistance in each city and/or even each hospital. Unfortunately, selection of the most suitable antibiotic is complicated by the ability of *P. aeruginosa* to develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection. Studies have shown that infections caused by drug-resistant *P. aeruginosa* are associated with significant increases in morbidity, mortality, need for surgical intervention, length of hospital stay and chronic care, and overall cost of treating the infection (25). This bacterium can develop resistance to antibiotic agents either through the attainment of resistance genes on mobile genetic elements (i.e., plasmids) or through mutational procedures that modify the expression and/or function of chromosomally encoded mechanisms.

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