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Isolation, Identification and Antimicrobial Susceptibility of *Pseudomonas* spp. Isolated from Hospital Environment in Tonekabon, North of Iran

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Abstract A wide variety of opportunistic pathogens has been detected in hospital surfaces. Medical center surfaces can serve as reservoirs of pathogenic bacteria. Among this pathogens, *Pseudomonas* species are one of the leading causes of nosocomial infections, frequently found in hospital environments. In this research, we studied the presence of *Pseudomonas* spp. in a hospital wards surfaces. 460 samples from a hospital sections were collected in the city of Tonekabon, in North of Iran, between December 2012 and June 2013. The identification of strains was performed by using biochemical tests and API20NE (Biomerieux). Finally, the identification of some strains was verified by 16S rRNA gene sequencing. In general, 61 strains of *Pseudomonas* were isolated from all the sources. The highest isolation rate of *Pseudomonas* spp. was recorded in Surgery section (19/71%), followed by ICU (19/23%), Labor (17/46%), CCU (14/10%), Pediatric (11/76%), Internal (9/87%) and while lowest isolation was recorded in Dialysis section (1/56%). out of 61 isolates 52 (85/25%) were belonged to *Pseudomonas aeruginosa*, 6 (9/83%) to *Pseudomonas stutzeri*, 2 (3/28%) to *Pseudomonas putida*and 1 (1/64%) to *Pseudomonas fluorescens.* In addition, all *pseudomonas* species isolates were resistant to penicillin, cephalexin and vancomycin, while they showed different levels of susceptibility to other antibiotics. Environments in hospital are vehicle of *Pseudomonas* spp. and therefore the patients and people working in this area must attention to their personal hygiene in order to avoid *Pseudomonas* infection.

Keywords: *isolation, Pseudomonas, hospital surfaces, nosocomial infections, antibiotic susceptibility, Iran*

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1. Introduction

Nosocomial infection is one of the most important problems in the worldwide. It is an old problem and it starts when a patient is admitted in a hospital for reason other than that infection. It is called nosocomial infection if it develops 72 h after the admission to the hospital. These infections are more dangerous than other infections because they are caused by bacteria have a high resistance to antibiotics. These infections are an important cause of increased morbidity, mortality and health care costs worldwide [1]. Nowadays although modern antibiotics have improved; still sometimes the treatment is difficult and causes morbidity and mortality to patients. Many outbreaks of nosocomial infections have raised from reservoirs of pathogens in the inanimate hospital surfaces. The contribution of the environment surfaces is so important. It has been reported that 10% hospitals acquire this infection while staying hospital [2]. Based on statistics and the result of the researches, the rate of infection varies in different part of the world. The etiological agent of Hospital-Acquired Infections varies from hospital to hospital and in different geographical areas [3,4].

*P.aeruginosa*is one of the most important factors of nosocomial infection which threatens the lives of many patients annually. *Pseudomonas* spp. are considered opportunistic pathogens that causes nosocomial infection, very commonly found in nature (soils, water, plants and animals) and water treatment systems, thus demonstrating their adaptation to environments with low nutrient concentration, and over a large temperature range, between 4 and 42°C [5].

In sampling from hospital sentiment, *Pseudomonas*is taken from different surfaces of the hospital like, taps water, sinks and other places. The researches have shown that these bacteria grow better in wet surfaces [6]**.** The typical *Pseudomonas* bacterium in nature might be found in biofilm, and is one of the most vigorous, fast swimming bacteria seen in infusions and water samples [7]. Because these bacteria have simple growth requirements, they spread extensively. *Pseudomonas*spp. may be present as a part of the normal flora of humans, although the prevalence of colonization of healthy individuals outside the hospital is rather low [8].

P.aeruginosa is considered as a major hospital problem, as they cause opportunistic infections in humans, particularly among immunocompromised patients or in those with faulty homeostasis mechanisms [9,10]. It causes urinary tract, respiratory tract and soft tissue infections, dermatitis, bacteremia and great variety of systemic infections [11]. It is the second most common bacteria cause of nosocomial infections, accounting for 21% of cases. Incidences reported 16% of nosocomial pneumonia, 12% of urinary tract infections, 17-26% of wound infections and 10% of septicemia are due to *P.aeruginosa* [12,13]. Also it causes significant morbidity and mortality in immunocompromised subjects showing burns, cystic fibrosis, chronic bronchitis and cancer [14].

One of the most significant problems which is to be raised in connection with *pseudomonas* is their antibiotic resistance problem. *P.aeruginosa* is naturally resistant to many antibiotics. Their antimicrobial susceptibility is limited to only a few drugs, and the emergence of resistance during therapy against initially susceptible strains occurs at relatively high frequency. Antimicrobial resistance in hospital-acquired pathogens is associated with adverse clinical outcomes and increased healthcare expenditures. Carbapenem antibiotics are often reserved for the therapy of serious nosocomial infections due to *P.aeruginosa*, because these nosocomial pathogens are frequently resistance to other classes of antibiotics [15,16,17,18]. The detection of *Pseudomonas* spp. contamination and antimicrobial susceptibility of this bacterium from North of Iran has not been investigated. The purpose of this study was to determine the rate of existence and antibiotic susceptibility of this bacterium from ShahidRajaii hospital wards, Tonekabon, North of Iran.

2. Materials and Methods

2.1. Sample Collection

In total, 460 sample were collected from every environmental surfaces including moist habitats (sinks, tubs) and dry surfaces (ground, walls, beds, blankets, doors, doors handle, nurse tables, chairs, electronic equipment's, medicine cabinet, windows) of 7 sections (Surgery, Labor, Internal, Intensive Care Unit (ICU), Coronary Care Unit (CCU), Pediatric, Dialysis) in ShahidRajaii general hospital, Tonekabon, Mazandaran, Iran from December 2012 to June 2013 were rolled in this cross-sectional study. Environmental samples were taken with wet sterile cotton swabs. During sampling put swabs in 5 ml of brain heart infusion broth (Pronadisa, Spain). After collection BHI tubes with the swabs arrival in the laboratory.

2.2. Sample Processing and Isolation

After transported to the laboratory tubes shacked and swabs emitted. The BHI tubes were incubated at 37°C for 24 h. then a loopful of BHI was withdrawn and spread on the *pseudomonas* agar base (Himedia, India), and incubated at 37°C for 24-48 h.

2.3. Identification of *Pseudomonas* spp. By Biochemical Test

Pseudomonas spp. identification was performed by gram stain, oxidase and catalase test, sugar fermentation in Triple Sugar Iron agar (TSI) and Oxidation-Fermentation (OF) tubes, and all *Pseudomonas* collected were tested by conventional biochemical tests using API20NE (Biomerieux, France).

2.4. Authentication of *Pseudomonas* spp. Isolates by PCR Method

Some of *Pseudomonas* strains were selected randomly and subjected to gene sequencing. Identification of these strains was verified by gene sequence of 16S rRNA according to the following method. DNA was extracted from some *Pseudomonas* spp. was subjected to gene sequencing using phenol-chloroform method [19]. Universal primers produced by TAG kopenhagen (Denmark) were used to amplify 16S rRNA gene. The sequence of forward and reverse primers was 5'-CAACGAGCGCAACCCT-3' and 5'-GGTTACCTTGTTACGACTT-3', respectively [20]. Each reaction was performed in totalvolume of 25 µl, which contained 13 µl DNase free water (Cinagen-Iran), 2.5 µl of1X PCR buffer (Isogene-Russian), 1 µl of 10 pmol from each one of the primers, 1 µl of 10 mMdNTPs (Cinagen-Iran), 1 µl of 50 mM $MgCl_2$ (Cinagen-Iran), 0.5 µl Taqpolymeras enzyme (Cinagen-Iran) and 5 µl of the sample DNA. PCR amplification conditions on thermo cycler (Eppendrof-Germany) were as follows: 95°C for 5 min, followed by 30 cycles of 95°C for 45 s, 56°C for 40 s, 72°C for 45s with final extension at 72°C for 3 min and storage at 4°C. All PCR products were run on 1.5% (w/v) agarose gel with a 100 bp DNA ladder (Fermntas-Russia). Aliquots of PCR products were electrophoresed at 75 V for 40 min; DNA was visualized using ethidium bromide and photographed after UV transillumination with Uvidoc (England).Observation of band in the 375 bp region specified the amplification of 16S rRNA gene. PCR products were sent to Macrogen Company in South Korea (http://macrogen.com/) for DNA sequencing.All sequence data were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) to definitively each respective 16S rRNA gene amplicon.

2.5. Antimicrobial Resistance by Disc Diffusion Method

In this study, the antimicrobial resistance of *Pseudomonas* spp. Isolates was determined by disc diffusion test [21]. To perform the disc diffusion test, each culture was grown in 5 ml of Muller-Hinton broth until the turbidity corresponded to 0.5 McFarland standard tubes (1.5×10^8 cells ml^{-1}). This microbial suspension was spread out on the surface of Muller-Hinton agar by sterile swap and various antibiotic discs were placed on it, then incubating the plates at 37°C for 48 h.

For disc diffusion test, Penicillin (10 µg), Ampicillin (10 µg), Cephalexin (30 µg), Vancomycin (30 µg), Nalidixic Acid (30 µg), Chloramphenicol (30 µg), Amoxicillin (25 µg), Piperacilin (10 µg), Ceftriaxone (30 µg), Erithromycin (15 µg), Imipenem (10 µg), Amikacin

(30 µg), Tetracycline (30 µg), Ticarcillin (75 µg), Ciprofloxacin (5 µg) and Gentamicin (10 µg) (PADTAN TEB - Iran) were used. The disc strengths and the zone size interpretation were done in accordance with National Committee for Clinical Laboratory Standards.

2.6. Statistical Analysis

Chi square test was used to determine whether there was any significant difference between the frequency of Pseudomonas spp. isolated from hospital environment and antimicrobial susceptibility (SPSS software 16).

3. Results

From the 460 environmental samples 61 isolates were taken from the surfaces in a hospital sections. The overall rate of *Pseudomonas* isolates in hospital was about 13/26%. Evidence showed that contamination in sections were variable. Among wards most prevalent was related to surgery section (19/71%), followed by ICU (19/23%), labor (17/46%), CCU (14/10%), Pediatric (11/76%), Internal (9/87%) and while lowest isolation rates were recorded from Dialysis (1/56%).

Table 1. frequency of occurrence of *Pseudomonas* spp. isolates in different section

sector	No. of samples collected	*P. aeruginosa*	*P. stutzeri*	*P. putida*	*P. fluorescens*	Total of Isolates
Surgery	71	13 (18.30%)	1 (1.40%)	0 (0%)	0 (0%)	14 (19.71%)
Labor	63	11 (17.46%)	0 (0%)	0 (0%)	0 (0%)	11 (17.46%)
CCU	78	9 (11.53%)	1 (1.28%)	1 (1.28%)	0 (0%)	11 (14.10%)
ICU	52	10 (19.23%)	0 (0%)	0 (0%)	0 (0%)	10 (19.23%)
Internal	81	3 (3.70%)	3 (3.70%)	1 (1.23%)	1 (1.23%)	8 (9.87%)
Pediatric	51	6 (11.76%)	0 (0%)	0 (0%)	0 (0%)	6 (11.76%)
Dialysis	64	0 (0%)	1 (1.56%)	0 (0%)	0 (0%)	1 (1.56%)
Total	460	52 (11.30%)	6 (1.30%)	2 (0.43%)	1 (0.21%)	61 (13.26%)

Available research results have shown the frequency of isolation of *Pseudomonas* spp. in hospital was different. The most prevalent of *Pseudomonas* spp. found were related to *P.aeruginosa* (11/30%), followed by *P.stutzeri* (1/30%), *P.putida* (0/43%) and *P.fluorescens* (0/21%) (Table 1).The Result obtain from Statistical analyses of data showed significant correlation (P<0.05) between the isolation rates of *P.aeruginosa* and hospital sectors.

In present research, most isolates were obtained from sinks 17 (27/86%) and then taps water 9 (14/75%), doors handle 7 (11/47%), ground 6 (9/83%), medicine cabinet 4 (6/57%), bed 4 (6/57%), wall 3 (4/91%), window 2 (3/28%), door 2 (3/28%), Nurse Table 2 (3/28%), Ashcan surface 2 (3/28%), chair 1 (1/64%), Electric switch 1 (1/64%), Refrigerator handle 1 (1/64%).

The result obtained for antibiotic susceptibility of *Pseudomonas* isolates from hospital environment samples by disc diffusion method indicated that all strains were resistant to Penicillin, Cephalexin and Vancomycin, while they showed different levels of resistance to other antibiotic, among 61 spp. 47 (77%) of them showed resistance to Nalidixic acid and Ampicillin, 44 (72%) to Tetracycline, 41 (67%) to Chloramphenicol, 37 (61%) to Amoxicillin, 32 (52%) to Erythromycin, 17 (28%) to Ceftriaxone and piperacillin, 14 (23%) to Imipenem, 10 (16%) to Amikacin, 3 (5%) to Ciprofloxacin, Gentamicin and Ticarcillin (Table 2).

Table 2. Antibiotic resistance of *Pseudomonas* isolated from hospital environments by disc diffusion method

		Numberof *Pseudomonas* spp. Isolates resistance to															
	No.of Isolated	P	CN	V	NA	AM	T	C	AMX	E	PIP	CRO	IPM	AN	CP	GM	TIC
P.aeruginos	52	52	52	52	42	42	40	34	32	29	16	15	13	9	3	3	2
P. stutzeri	6	6	6	6	4	2	2	5	2	1	0	0	0	1	0	0	0
P. putida	2	2	2	2	0	2	1	1	2	1	1	2	1	0	0	0	1
P.fluoresces	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0

P, Penicillin,. CN, Cephalexin,. V, Vancomycin,. NA, Nalidixic acid,. AM, Ampicillin,. T, Tetracycline,. C, Chloramphenicol,. AMX,Amoxicillin,. E, Erythromycin,. PIP, piperacillin,. CRO, Ceftriaxone,. IPM, Imipenem,. AN, Amikacin,. CP, Ciprofloxacin,. GM, Gentamicin,. TIC, Ticarcillin.

4. Discussion

Pseudomonas spp. are the main causes of nosocomial infections causing morbidity and mortality as these infections are difficult to eradicate. There is a global emergence of multidrug resistant strains of *Pseudomonas*. The transmission of infection during patient remedy in hospital can occur by direct contact with surfaces [22]. The goal of our study was to demonstrate the importance of the evaluation of the presence of *Pseudomonas* spp. in hospital sections environment. The results showed that different species of *Pseudomonas* exist in hospital environment. Among them, the most prevalent was related to *P.aeruginosa*.

P.aeruginosa often has been isolated from sinks and other moist sites in hospitals. The possible transmission of *P.aeruginosa* from sinks to patients has been reported. Recent report showed extensive bacteriological screening of the inanimate hospital environment has identified *pseudomonas* spp. in the majority of moist sites of hospital. The important and the most environmental sources of *Pseudomonas* spp. are humid places. The role of contamination of the environment with *Pseudomonas* in hospital has been the subject of several studies [23,24]. Intensive environmental contamination was found in some studies, especially in moist sites, and transmission of *P.aeruginosa* from sinks to hands during hand washing has been shown. Also in other research showed that when the sink was used for hand washing, drain contents splashed at least 1 meter from the sink and pollute water infect patients [25].

However, even in a situation of intense environmental contamination, direct transmission from the environmental to patients has only been demonstrated occasionally. Hence, based on foregoing evidence, environments in hospital are vehicle of *Pseudomonas* spp. and therefore the patients and people working in this area must attention to their personal hygiene in order to avoid *Pseudomonas* infection. Decrease frequency of *Pseudomonas* spp. in hospital is probably due to improvements in the methods

of dispensing and use of disinfectants, to the elimination of the main sources of the organism, and improvements in aseptic techniques. In hospital, even the least important inanimate surfaces could contaminate the hands of nurses, patients and wards staff [26].

One of the most significant problems which are to be raised in connection with *pseudomonas* is their antibiotic resistance, particularly in the Intensive care unit of the hospitals. High consumption of the antibiotics has led to the increase of vulnerability of the hospitalized patients toward the opportunistic infections [27]. Because of the antibiotic resistance, especially in form of the multidrug, *P.aeruginosa* has created a lot of problems in order to treat the infections resulting from this bacterium. The especial resistance of this bacterium to various antibiotics has increased the significance of controlling this bacterium in the hospital environments [28]. Of total of 61 strains of the *pseudomonas* isolated from the hospital environment, all were resistance toward the Penicillin, Cephalexin and vancomycin. Instead, the isolated strains showed a relative good sensitivity toward the Amino glycosides. In the researches implemented by loueriaet al, Pietro et al, Oliveriaet al, Van Elder and Ahani Azariet al, the high resistance to drugs of the β-Lactam group and sensitivity toward the Amino glycosides and have been observed [29,30,31,32,33]. The information obtained from the current research and other researches show that the β-Lactam drugs, although have been introduced as one of the anti-*pseudomonas* drugs, today they have become ineffectual by developing the resistance. Anyway, the rate of the resistance of the *pseudomonas* toward antibiotics is different in the various parts of the world and even, this resistance among the various species of *pseudomonas* and diverse strains of a species is different. As well as a result, in order to treat correctly the infections originating from it, examination of the drugs resistance model is first required and then, the prescription of drug.

5. Conclusion

In addition, environments in hospital are vehicle of *Pseudomonas* spp. and therefore the patients and people working in this area must attention to their personal hygiene in order to avoid *Pseudomonas* infection. Furthermore, due to high occurrence frequency of penicillin, cephalexin and vancomycin resistant *Pseudomonas* spp., it is suggested that these antibiotics do not be considered as drugs of choice for treatment of infection. In contrast, ciprofloxacin, gentamicin and ticarcillin could be used as drugs of choice, piperacillin, ceftriaxone, imipenem and amikacin as alternatives for treatment of nosocomial infection due to *pseudomonas* spp. in this geographical area.

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