

Investigation of *Pseudomonas aeruginosa* Resistance Pattern against Antibiotics in Clinical Samples from Iranian Educational Hospital

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Abstract

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the opportunistic pathogens, which is the main cause of prevalent hospital infections worldwide. The aim of this study was to determine the prevalence of antibiotic resistance pattern against *P. aeruginosa* from clinical samples in our population. This study was performed during March 2009 to September 2011. During this period 233 clinical isolated samples from hospital patients were examined. In these studies, different strains of *P. aeruginosa* were isolated from samples, then microbiologically tested. Bacterial susceptibility was performed by the disc-diffusion tests with Kirby Baur disc diffusion tests in Muller-Hinto environment. Our results showed maximum antibiotic resistance (99.5%) of *P. aeruginosa* against Trimetoprim Sulfametoxazole and Ciprofloxacin (55.33%), Amikacin (61%), Imipenem (33%), which were identified as the most effective antibiotics in this study. In conclusion, indeed most *Pseudomonas aeruginosa* strains infections are treated as soon as possible due to their severe resistance against antibiotics. So, we have to apply an accurate antibiotic treatment discipline, according to the finding, based on antibiogram, in order to prevent its spread and also, monitoring and optimization of antimicrobial use should be considered carefully.

Keywords

Pseudomonas aeruginosa, Resistance, Antibiotics

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1. Introduction

P. aeruginosa is a gram-negative, aerobic, rod shaped bacterium with unipolar Motility, which is the main cause of prevalent hospital infections [1] [2]. It is recognized as a serious opportunistic pathogen that causes infections in hospitalized patients [3] [4]. The symptoms of these infections are generalized inflammation and sepsis. Particularly in burns patients where the skin host defenses are destroyed [5] [6], orthopedic related infection, respiratory disease [7], corneal infections caused by contact lens user [8], corneal ulceration [8], patient with impaired immune defense [9] [10] including HIV infection [11] [12] and, also is the most frequent colonizer in medical devices [13]. *P. aeruginosa* causes clinical problem as a result of its high resistance to a large rang of antibiotics [14] and may demonstrate additional resistance after unsuccessful treatment. Indeed treatment of *P. aeruginosa* infections in most cases should be conflicted, due to the resistance of these bacterial species and further acquired resistance to extensive range of antibiotics [3]. *P. aeruginosa* frequently displays resistance to multiple antimicrobial agents [15] [16]. Gentamycine is the only antibacterial agent through which pseudomonas strains are remains sensitive against it. There are different ranges of cross resistance between Carbencilin, Polymaxin, Colistin, Streptomycin, Quinolones, Cephalosporin against it reported [14] [17]. Therefore, antimicrobial resistance is a growing problem worldwide, especially in hospitals. Serious infections due to *P. aeruginosa* strains that exhibit resistance patterns to most common antibacterial agents are increasingly serious problems [16]. In this regard, the *Pseudomonas areuginosa* resistance pattern against antibiotics has not been studied in Urmia, Iran. We aimed to determine sensitivity and resistance patterns of *P. aeruginosa* against various antibiotics among clinical samples of educational Hospitals of Iran, Urmia University of medical science in western of Iran.

2. Material and Methods

2.1. Study Setting

A cross-sectional study during March 2009 to September 2011 was performed. This study was conducted in the Internal, surgery, burns and transplantation ward of educational hospitals of Urmia University of medical science, Iran. Indeed we examined 233 clinical samples were collected from different clinical sampling from wound swab, urine samples, blood, trachea samples, eye swabs, ear swabs and pus from their patients then examined. All procedures of this investigation performed with the approval of the scientific and ethical board of this college, and patient consent form signed with each subjects for permission to use of their samples as case of study.

Isolation and identification of *Pseudomonas aeruginosa*

For these sample processing, in sterill condition, Nutrient agar, Mac Conkey agar and blood agar used for each samples. All plates incubated at 37°C for 24 h. After incubation period to obtain pure cultures according to Chessborough (Cheesborough 2002 based protocol), single pure colonies were subcultured. Then pure samples used To isolation and Different identification tests of *Pseudomonas aeruginosa* strains using macroscopic and microscopic biochemical methods Based on Bergey's manual of determinative bacteriology that describes *Pseudomonas aeruginosa* Oxidative and non-fermentative strain, with positive oxidase reaction, citrate utilization test, production of green pigment (Pyocyanin) on Mueller-Hinton agar medium.

2.2. Antibiotic Susceptibility Test

Antibiotics MIC with Agar dilution methods was determined. In this way isolates based on disk diffusion method (using Kirby-baur method) with common and valid Antibiotics on Mueller-Hinton agar (Mueller-Hinton agar Formula/Liter: Beef Extract: 2 g, Acid Hydrolysate of Casein: 17.5 g, Starch: 1.5 g, Agar: 17 g, Final pH 7.3 ± 0.1 at 25°C) medium, antibiotic susceptibility pattern according to Clinical Laboratory Standard Institute (CLSI) principals (NCCLS 2011) was determined [18]. Paper disk were smeary with antibiotics: Amikacin (30 ug), Gentomycin (10 µg), Imipenem (10 µg), Sparfloxacin (5 µg), Ceftazidime (30 µg), Piperacilin (100µg), Nalidik acid (30 ug), Ceftizoxime (30 µg), Ceftriaxone (30µg), Carpencilin (100 µg) and Trimtoprine (5 µg) Sol-fametoxazole (23.75 µg) and incubated at 37°C for 18 hours. We determined the diameter of zone of inhibition as sensitive and semi-sensitive and resistance patterns of *P. aeruginosa* against different antibiotics based on CLSI guidelines (NCCLS 2011).

Statistical analysis

Statistical analysis performed with SPSS (ver.18) software, data were reported as mean ± SD for continuous

variables.

3. Results

Our study takes almost two years during March 2009 to September 2011. 233 samples were isolated from wound swab, urine, trachea, eye swabs, ear swabs and pus of patients.

From Imam Khomeini Hospital, Urmia, Iran, Isolation rates of *Pseudomonas aeruginosa* according to departments presented in **Table 1**.

The mean age of the patients was 35.4 ± 14.5 years old and most of the patients were more than 34 years old. 81% were female and the remaining (18.2%) were male. 20.9% patients were illiterate, 20% had studied until the elementary level, 11% attained guidance school, 12% attained high school, 35% had diplomas, and 1.1% patients had an academic degree. Data shown sixty samples were positive to *Pseudomonas aeruginosa*. Also 25.75% of samples were resistant to all antibiotic, which the majority of them found in Transplantation ward. The resistance pattern against Cefotaxime (94.34%), Ceftazidim (95.68%), Nalidixic acid (95.32%), Gentamycine (79.23%), Carpenicilin (81.54%) and Piperacilin (94.37%) was also observed in this study. The maximum antibiotic resistance 99.5% was observed against Trimtoprine Solfametoxole. The most effective antibiotics (sensitive pattern) for *P. aeruginosa* were Ciprofloxacin (55.33%), Amikacin (61%) and Imipenem (33%). Resistance pattern rate (%) of *Pseudomonas aeruginosa* against antibiotics presented in **Figure 1**.

4. Discussion

P. aeruginosa emerged as an important pathogen and was reasonable for nosocomial infections. So, that is one of the important causes of morbidity and mortality among Hospital patients [3] [4] [19]. The current study showed that *P. aeruginosa* was highly prevalent in clinical samples of urine and pus, was also observed in Arshi, et al. and Murase et al. study [20] [21]. This study shows that *P. aeruginosa* resistant rate against Gentamycin was 79.23%, while reports of the susceptibility of *P. aeruginosa* against Gentamycin in different studies have ranged from 49.8%, in Greek to as high as 96.6% in the United Kingdom [22] and in another study, in 1988, from Turkey, it was reported that 54% of gram negative Bacilius were resistant to Gentamycin [23]. This survey shown

Table 1. Isolation rates of *Pseudomonas aeruginosa* according to departments.

Samples	Wound samples	Urine samples	Trachea samples	Ear swabs	Eye swabs	Pus	Blood	Total
Frequency	20	102	12	25	6	48	20	233
Percent (%)	8.58%	43.78%	5.15%	10.73%	2.57%	20.7%	8.25%	100%

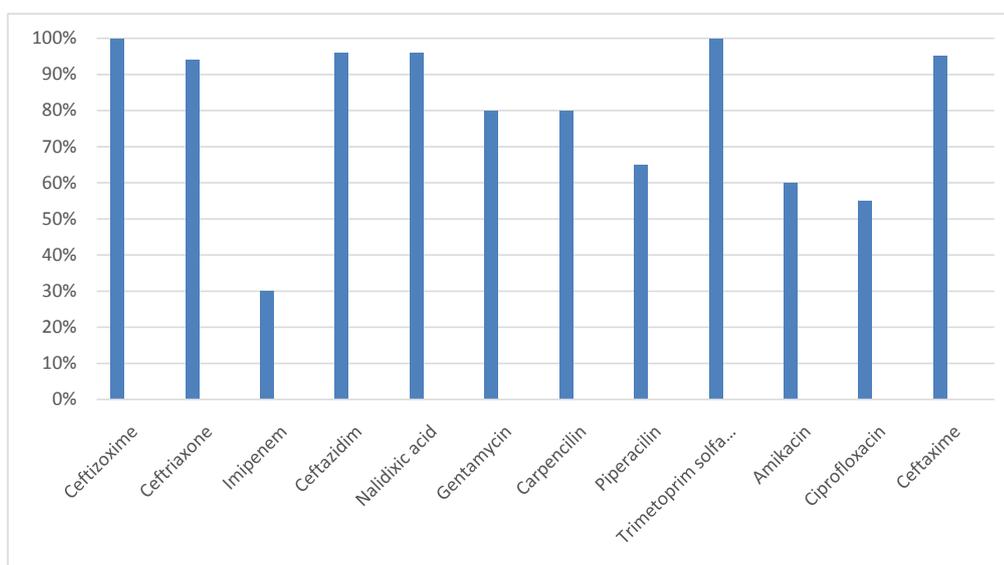


Figure 1. Resistance rate (%) of *Pseudomonas aeruginosa* against Antibiotics.

in our population study samples analysis revealed resistant patterns to Ceftazidime (95.67%), while, according to different reports, resistance to Ceftazidime was 15% - 22% in the world, also Resistance to Ceftriaxime, Ceftriaxon and Nalidixic acid are significant in our study (99.37, 94.37, 95.32 respectively). Previous studies suggested that selective pressure from the use of anti-microbial agents is a major determinant for the emergence of resistant strains [24]-[26] that could be considerable. Our results indicated that the rate of ciprofloxacin resistance pattern against *P. aeruginosa* was found to be relatively high percent in our population. Other studies show that ciprofloxacin resistance pattern was 23% in Spain [27], 31.9% in Italy [28] and 26.8% in Latin America [29]. Overall results show that the rate of *P. aeruginosa* antibiotic resistance was observed in highest rate in case of Trimetoprim Sulfametaxazole, while, Ciprofloxacin, Imipenem and Amikacin were identified as the most effective antibiotics in this study against *P. aeruginosa*. In previous studies it was reported that resistance to Imipenem was 14% in Spain [27], 19.3% in Italy [28] and 68% in Saudi Arabia [30] that approved with our results. Previous studies show that antibacterial effects of Amikacin against pseudomonas were greater than Gentamycin [23] [31] and only 0.9% resistant reported to Amikacin in Turkey [23]. So, Amikacin is effective antibiotic against *P. aeruginosa* in their study. In current study, resistance pattern to Piperacillin was also higher, while Piperacillin resistance rate was 10% in Spain [27], 12% in Italy [28], 14% in Latin American population [29]. Indeed *P. aeruginosa* strains Infections, must be treated as soon as possible due to their severe resistance against antibiotics. We have to apply an accurate antibiotic treatment discipline, according to the findings based on antibiogram method in order to prevent its spread. Data showed that this bacterium has developed its resistance pattern against amikacin and Ciprofloxacin in comparison to previous years. So the use of effective infection control practices can go a long way to limiting the development and spread of antimicrobial resistance ensuring that these agents continue to find a place in treatment of *P. aeruginosa* infections and also, hospital infection monitoring committee needs to force the tasks of more severe monitoring on hospital pathogens and bacterial resistance pattern development studies as important objectives to finding effective antibiotics against *P. aeruginosa*.

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