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Detection and Determination of the Antibiotic Resistance Patterns in *Pseudomonas aeruginosa* Strains Isolated From Clinical Specimens in Hospitals of Isfahan, Iran, 2012

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Background: *Pseudomonas aeruginosa* is an opportunistic bacterial pathogen commonly found in the environment mainly in soil and water, but is also regularly found on animals, including humans. It is particularly harmful to patients who already have a compromised immune system, particularly those suffering from the immunodeficiency diseases. Over the years, this particular bacterium has been known to be highly resistant to current antibiotics due to both intrinsic and acquired resistance mechanisms within the bacteria.
Objectives: This study aimed to detect and determine drug resistance patterns in different *P. aeruginosa* strains isolated from clinical specimens in hospitals of Isfahan, Iran.
Materials and Methods: In this descriptive cross-sectional study, approximately 255 different bacterial isolates were gathered from samples of blood, sputum, urine, wounds, burns, respiratory systems, catheter, eye and peritoneum from the major hospitals of Isfahan, Iran, in 2012. Biochemical tests revealed that 106 of these isolates were *P. aeruginosa*. The resistance rate of these bacterial strains to different antibiotics was then assessed by antibiogram (kirby-bauer method). Data were analyzed using SPSS (version 16, SPSS Inc., Chicago, IL, USA).
Results: Resistance rates of the isolates to various antibiotics were obtained. It was found that cefepime and cefotaxime had the highest resistance rates (100%). However, the resistance rates were also high for the drugs imipenem (58.5%), meropenem (58.5%), ceftazidime (89.6%), aztreonm (96.2%), ciprofloxacin (77.4%) and gentamicin (66%). Moreover, the lowest resistance rate was observed for amikacin (43.4%).
Conclusions: The prevalence of *P. aeruginosa* strains with multiple-drug resistance was very high amongst the clinical samples in the major hospitals of Isfahan. This is quite a worrisome problem and makes the controlling of the strains more difficult.

Keywords:Pseudomonas aeruginosa; Multiple Resistance; Efflux Pumps; Beta-Lactamase

1. Background

Pseudomonas aeruginosa is an opportunistic pathogen and the most abundant organism on earth. It is a Gram-negative, aerobic and rod-shaped bacterium. It can vary in size and range from 1-5 µm long to 0.5-1.0 µm wide. It is an extremely ubiquitous organism and abundantly found in soil, water, plants, humans, animals and in hospital settings. There are usually low numbers of the bacteria in humans, found mainly in the gastrointestinal tract and in some instances, on the skin.

This bacterium has the ability to colonize healthy subjects and act opportunistically but it is particularly harmful in hospital and poses quite a significant threat. It has been known to be isolated from patients, hospital personnel as well as medical equipment (1, 2). It is most harmful to immune compromised people, such as those suffering from cancer, cystic fibrosis, burns, neutropenia and AIDS. It can also lead to several hospital-acquired infections such as wound infections, burns, meningitis, urinary tract infections, necrotizing pneumonia and outer ear and eye infections. The bacterium can also cause fatal septicemia and invasive infections of the blood in patients suffering from disabilities as well as in infants (3, 4).

The treatment of infections that arise from the bacterium is proving problematic as it has high resistance to antibiotics (5, 6). The antibiotic resistance is caused due to the synergistic phenomenon between the bacterial efflux pumps, which contain chromosomally encoded antibiotic resistance genes, and the low permeability of the outer membrane. The beta-lactamase enzyme contributes to the development of resistance within the bacteria. It is in particular noticeable in nosocomial infection agents, which can lead to severe health problems when it comes to their treatment (7). Beta-lactamase could be classified in many strata according to Ambler or Bush-Jacoby classification that many of them are extended-spectrum beta-lactamase lead to multidrug-resistance in drugs. Other class is metallo-beta-lactamases (MBLs) that constitute the molecular class B of Ambler as well as the group 3 in the functional classification of Bush, Jacoby and Medeiros. These enzymes that is able to hydrolyze and inactivate class of beta-lactam antibiotics such as imipenem family of antibiotics. Since the early 90s, new genes coding for distinct MBLs have been described in clinical important pathogens like *Pseudomonas* spp, *Acinetobacter* spp. and even among members of the family *Enterobacteriaceae*. These genes are usually inserted into mobile elements facilitating the exchange of these resistance genes among several bacterial species. An important point is that genes producing these enzymes are on transferable genetic elements (integrin and transposon) and could be transferred to another Gram-negative species by different ways including conjugation, transformation and transduction that are normal protocols in bacteria (8-11).

2. Objectives

This study aimed to detect and determine drug resistant patterns in different strains of *P. aeruginosa* isolated from clinical samples in hospitals of Isfahan, Iran.

3. Materials and Methods

This descriptive cross-sectional study was performed on 255 patients referred to the major hospitals of Isfahan, Iran. From these patients, bacterial isolates were gathered from samples of blood, sputum, urine, wounds, burns, respiratory systems, catheter, eye and peritoneum using conventional sampling method from October 2012 to April 2013. Biochemical tests performed for the identification of the isolates were as follows: growth of the bacteria on cetrimide agar, gram staining, reaction in TSI condition, oxidase test, catalase, indole production and motion study in sulfide indole motility (SIM), growth at 42°C, and green and blue pigment production (8, 12). The bacteria were then maintained in liquid culture with 15% glycerol at -70°C (13). The biochemical tests revealed that 106 of the isolates were strains of *P. aeruginosa* and then the resistance rates of the bacterial strains to specific antibiotics were assessed.

3.1. Sensitivity Tests

The antibiotic resistance pattern of the different strains of *P. aeruginosa* was determined using CLSI guideline with the Kirby-Bauer disk diffusion method in Mueller hinton agar (14). During the use of this method, microbial suspension equal to half of the McFarland scale was prepared and then was planted on Mueller hinton agar culture via the grass method (similar to the planting of grass). Related antibiotic discs were then pasted in culture, one centimeter apart from each other. The diameter of the non-growing areolae of antibiogram was measured after 18-24 hours of incubation. Antibiotic discs, which were used, were imipenem, meropenem, cefotaxime, amikacin, ceftazidime, cefepime, aztreonam, ciprofloxacin and gentamicin. These discs were all purchased from MAST (Merseyside, UK) (13). For quality control of tests such as the antibiogram, standard strains of *P. aeruginosa* (ATCC 27853) were used (5). Ethical approval was obtained from the Research Ethics Committee of Isfahan University of Medical Sciences. A written consent was obtained from the medical director and manager of the hospital to conduct the study. Descriptive statistics were used in this study with help of SPSS for Windows (version 16, SPSS Inc., Chicago, IL, USA).

4. Results

There were 255 patients who participated in this study; from this cohort 106 strains of *P. aeruginosa* were found. Sixty-eight percent of the *P. aeruginosa* isolates were

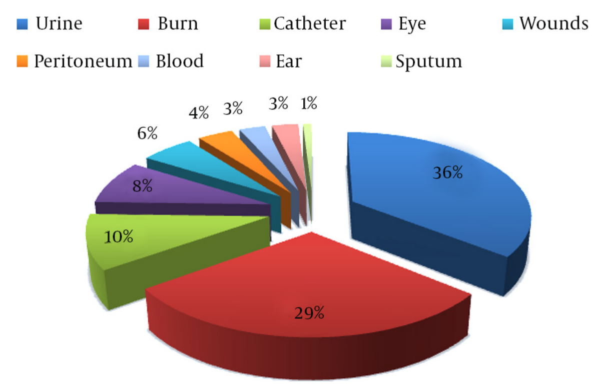

Figure 1. Frequency Percent of *Pseudomonas aeruginosa* Isolates Collected From Clinical Samples

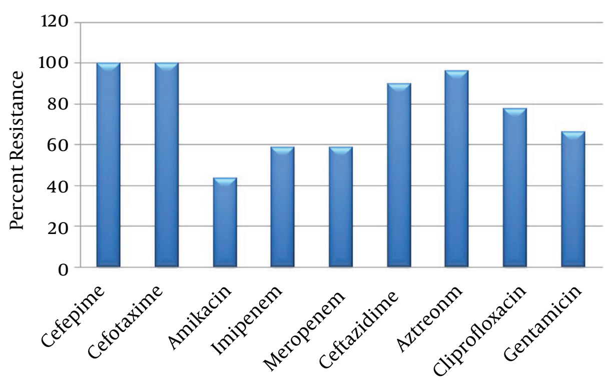

Figure 2. Antimicrobial Susceptibility of *Pseudomonas aeruginosa* Strains Collected From Clinical Samples

found in males and the mean age was 43 in the whole cohort. The isolates were most commonly found in urine (n = 31) and thereafter burns (n = 31) and catheters (n = 11). However, isolates were also found in the eye (n = 9), wounds (n = 6), peritoneum (n = 4), blood (n = 3), ear (n = 3) and sputum (n = 1) (Figure 1).

The results of the different types of antibiotics revealed that amikacin showed the lowest resistance (43.5%), imipenem and meropenem had equal resistance rates (58.5%) whilst cefipime and cefotaxime had the highest antibiotic resistance rates (100%) (Figure 2).

5. Discussion

The results of this study indicated that antibiotic resistance amongst the population of *P. aeruginosa* in the Isfahan hospitals was very high. The results of this study also showed that the resistance rate to cefepime and cefotaxime was 100%, which is quite worrisome. Furthermore, the results revealed that antibiotic resistance rate was increasing to other commonly prescribed antibiotics. Resistance rates to aztreonam and ceftazidime were also high at 96.2% and 89.6%, respectively. This rising trend in antibiotic resistance is supported by several other studies. In 2006, Khosravi et al. study found that imipenem and ceftazidime resistance rates were 41% and 83%, respectively (15). While, in 2007 Saderi et al. found that the resistance rates for imipenem, ceftazidime and ciprofloxacin were 38.2%, 74.2% and 49.2%, respectively (16). Furthermore, in 2008 Mirsalehian et al. reported the resistance rates to imipenem, ceftazidime, ciprofloxacin and cefipime was 63%, 85%, 83%, and 88%, respectively (17). In another study has been done by Salimi et al. in 2009, resistance rates to imipenem and ceftazidime were reported 30% and 75%, respectively (18). In addition, in a study has been done by Shahcheraghi et al. in 2010, resistance rates to imipenem, ciprofloxacin, and ceftazidime were 9%, 41%, and 42%, respectively. In addition, resistance rates to gentamicin and cefotaxime were 42% and 70%, respectively (19). Fazeli et al. in 2009 found the highest rates of resistance in their study to imipenem (94%), ciprofloxacin (98.7%) and ceftazidime (100%) (20). In a study has been done by Norouzi et al. in 2010, the isolates were 100% resistant to cefotaxime and ceftazidime (21). According to a study has been done by Doosti et al. in 2012, resistance rates to imipenem, ciprofloxacin, ceftazidime were reported 64%, 41%, and 79%, respectively. In addition, resistance to gentamicin and cefotaxime were 55% and 92%, respectively (22). Tirodimos et al. in 2010 also found that, resistance rate to imipenem, meropenem, and aztreonam was 2.2%. In addition, resistance to ciprofloxacin, ceftazidime, gentamicin, and cefipime were not reported (23). In a study has been done by Franco et al. in 2010, resistance rate to imipenem was reported 34% (24). A study has been done by Mohanasoundaram et al. in 2011, resistance to carbapenem, ceftazidime, gentamicin were 58%, 62%, and 64%, respectively. In addition, resistance to cefipime and amikacin were 68% and 37%, respectively (25). Chander et al. in 2013 found that resistance to ciprofloxacin, amikacin, and ceftriaxone were 52%, 17%, and 52%, respectively (26). Furthermore, in 2013 Moazami-Goudarzi et al. found that resistance to ciprofloxacin, cefepime, amikacin, aztreonam and imipenem were 96%, 92%, 89%, 93% and 95%, respectively (27). In a study has been done by Alikhani et al. in 2013, resistance rates to amikacin, ceftazidime, aztreonam and cefipime were reported 48.3%, 66.7%, 41.7%, and 96.7%, respectively. They also found that, resistance to cefotaxime, ciprofloxacin, gentamicin, imipenem, and meropenem were 68.3%, 8.3%, 53.3%, 11.7%, and 21.7%, respectively (28). Golshani et al. in 2013 also found that, resistance to ciprofloxacin (56%), cefotaxime (62%), ceftazidime (57%), cefipime (55%), amikacin (65%), gentamicin (59%), and imipenem (55%) were considerable (29). Finally, in a study has been done by Ghamgosha et al. in 2014, resistance to ceftazidime, ciprofloxacin, and imipenem were found 62.8%, 47.1%, and 5.7%, respectively (30). The results of all these studies lead to the same conclusion; that antibiotic resistance rate is increasing amongst strains of *P. aeruginosa*. Resistance has been found particularly high in the family of Carbapenem. This has been proved to be a growing and very concerning problem for physicians' worldwide, and making the control and treatment of infections caused by difficult (31). To tackle this growing problem and minimize the chance of these resistant strains of bacteria spreading in hospitals and to the greater global population there is an urgent need for the establishment of antibiotic use prevention programs to teach patients how to take antibiotics properly as well as raising awareness amongst clinicians themselves on how to best prescribe these types of drugs knowing their risk and to minimize the chance of antibiotic resistance spreading. Finally, it can be concluded that immediate detection of the resistant strains to drugs and gene creating it, and awareness about the antibiotic resistance rate could be led to better control of spreading these strains and genes creating it to other bacteria. It is also to be noted that awareness about the resistant strains could be helpful in treating the infections with appropriate drugs. Finally, it seems necessary for physicians and health-related personnel to be aware of the status of the drug resistance, and prevent from the uncontrolled prescriptions of drugs.

5.1. Limitations of the Study

Although resistance rates of *P. aeroginosa* isolates to a wide range of antibiotics were assessed using the phenotypic method, lack of the molecular and genetic assessments can be considered as one of the limitations of the study. False positive results may be increased when phenotypic methods are used, which can be removed in molecular and genetic methods.

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