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Determination Pattern of Antimicrobial Resistance of Pseudomonas Isolated from Patients in a University Tertiary Hospital

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Abstract

Introduction: Pseudomonas are ubiquitous bacteria widely present in nature. However, they have emerged as an opportunistic pathogen for humans. This bacterium is accountable for many localized and disseminated diseases, especially wounds in burn patients, respiratory infections, septicemia and bacteremia. Among all Pseudomonas species, P. aeruginosa is one of the important and most virulent species in hospital settings, while other pathogenic species include stutzeri, putida and fluorescence. The aim of this study was to assess pattern of antibiotic resistance in these bacteria isolated from a University teaching and treatment center. This cross-sectional study was conducted on 99 Pseudomonas strains (68 strains P. aeruginosa and 31 other Pseudomonas species) isolated from various clinical specimens. Antimicrobial drug susceptibility test was performed using the disc agar diffusion method according to CLSI recommendations. In this study, among various clinical specimens sent to microbiology laboratory, wound (59.59%) was found as a major source of Pseudomonas spp. Among various wards, Pseudomonas spp. was isolated more from patients admitted to burns ward (48.48%). Antibiotic susceptibility assay results revealed non susceptibility pattern towards most of the antibiotics; however, among all antibiotics tested, most common resistance was observed towards ceftazidime (76.76%). The results of this study shows the presence of Pseudomonas infection in the hospital setting and their developed resistance towards many conventional antibiotics, which is a concern at this treatment center. Thus, there exist a need for evaluation of careful and accurate measurement of resistance and assessment of exact drug administration policies. Therefore, to control the infection and prevent from increased prevalence of resistant strains appropriate resolution should be followed.

Keywords: Pseudomonas; Antibiotic Resistance; Nosocomial infection;

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Introduction

Pseudomonas is an opportunistic pathogen involved in many localized and invasive infections envisioning a serious concern in patient care environment (1). Presence of many cellular components as well as virulent genes are some of the factors responsible for fever, motility and adhesion of bacteria to the biological cell membrane (2). In addition, Pseudomonas aeruginosa (P.aeruginosa) produces a variety of exo-enzymes which are one of the factors responsible for augmenting pathogenicity. The attachment of bacteria to medical supplies is accelerated through pilus and fimbriae and secretion of exo-polysaccharide which binds to water and forms a gel assisting bacteria to remain suspended onto the artificial devices (3). Pseudomonas spp. are widely distributed in nature too. Various species of Pseudomonas can survive in different environmental conditions, but they are often found in moist environments of Hospital and considered as opportunistic pathogens for humans (4). The bacterium is also involved in broad spectrum of diseases, including infections especially in burned patients, respiratory infections, septicemia and bacteremia (5). These bacteria are also a primary cause of ventilator-associated pneumonia in the special care unit (ICU) (6). Amongst the various species of Pseudomonas, P.aeruginosa is the most important bacteria in many nosocomial infections (7).

One of the most serious concerns associated with Pseudomonas infections is the emergence of antibiotic resistance towards many traditional therapeutic agents which has doubled their importance particularly in nosocomial infections (8). P.aeruginosa, especially among patients with immunosuppression or long-term hospitalization, leads to irreparable consequences (7).

The treatment of patients with P.aeruginosa infection is problematic because these organisms are innately resistant to several antibiotic classes and, on the other hand, are able to acquire resistance to many other antimicrobial drugs (9, 10). The development of antibiotic resistance, particularly resistance to aminoglycosides which is plasmid mediated, is of interest since such resistance can cause the transfer of resistance genes in sensitive Pseudomonas spp. and other Gram-negative bacteria and turn them non-susceptible to most therapeutic regimens (11). P.aeruginosa is also notorious for being intrinsically resistant to many structurally unrelated antimicrobial agents by exhibiting low permeability of its outer membrane, the constitutive expression of various efflux pumps and the naturally occurring chromosomal AmpC β lactamase, and it can acquire additional resistant gene from other organisms via plasmids, transposons, and bacteriophages. It also shows a very high innate resistance to antimicrobial drugs (jcdr-8-DC30) through biofilm production (12). Horizontal gene transfers via mobile genetic elements coding for Class B Carbapenemase which hydrolysates all beta-lactams except aztreonam is one of the carbapenem resistance mechanisms (13).

Identifying common microorganisms and their antibiotic resistance pattern assists physicians with selecting the appropriate antibiotic and preventing the unnecessary prescription of antibiotics and spread of antibiotic resistance. This study aimed to study prevalence and antibiotic resistance pattern in Pseudomonas species isolated from different clinical specimens taken from in-patient hospitalized patients admitted to various wards of Sina Hospital, one of University Teaching Hospitals of Tabriz University of Medical Sciences, Iran. Sina Hospital is a tertiary

hospital which receives patients from North West Iran.

Materials and Methods

In this study, 99 clinical isolates including P.aeruginosa and other Pseudomonas species were collected from various wards of the hospital including, burn, infectious, surgery, internal and emergency wards and all ICUs from July 2015 until June 2016. These bacteria specimens were isolated from blood, urine, sputum, ulcers, abscesses and tracheal tubes. The samples were processed for the isolation and identification of Pseudomonas spp. along with other pathogenic bacteria according to phenotypic methods. Wound and urine specimens yielding >105 colony forming units (cfu/ml) were included in this study. Duplicate isolates from the same patient were not enrolled. Antibiotic susceptibility testing was performed on all isolates using the Kirby-Bauer method. The tests were performed according to the guidelines of clinical and laboratory standards institute (CLSI) (14), with a panel of following antibiotics: Ofloxacin (10µg), Ciprofloxacin (5µg), Gentamicin (10µg), Amikacin (30µg) Imipenem (10µg), Ceftriaxone (30µg), Ceftazidime (30µg), Levofloxacin (5µg), Tobramycin (10 µg), and Carbenicillin (25 µg). Statistical analysis: Categorical variables were compared by means of either $\chi 2$ analysis or Fisher's exact test when needed. A 2-tailed P value of <0.5 was considered significant. All statistical calculations were done using standard programs in SPSS 16 software.

Results

A total of 99 Pseudomonas species were isolated from diverse clinical specimens. Of these, 68 (68.7%) were Pseudomonas aeruginosa and 31 (31.3%) other species of Pseudomonas. P.aeruginosa was isolated from 18 (26.5%) ICU patients (burn and infectious), 35 (51.45%) burn unit, 6 (8.8%) internal ward, and 9 (13.2%) emergency unit patients. Other species of Pseudomonas were isolated from 11(35.5%) ICU (burn and infectious), 13 (41.9%) burn, 4 (12.9%) internal ward and 3 (9.7%) emergency unit cases. Source of majority of Pseudomonas isolates was wound (59.9%). Of these, 46 (63.23%) isolates were P.aeruginosa and 16 (51.61%) other Pseudomonas species (Table 1).

Regarding antibiotic susceptibility of isolates, our results revealed P.aeruginosa to be non- susceptible to cephalosporins, while majority of other Pseudomonas species were found resistant to ceftazidime (Table 2).

Discussion

It is noticeable that Pseudomonas play significant role in causing severe infections, especially in immunocompromised patients. Unfortunately, despite the progress in the field of antibiotics against Pseudomonas, mortality is still a concern (15). Pseudomonas are opportunistic bacteria which have become one of the most important pathogens in the second half of the last century, considering their resistance towards traditional therapeutic agents (16). In our study, majority of Pseudomonas spp. were isolated from wound specimen, which is in concordance with other published reports. A study conducted in Saudi Arabia disclosed majority of Pseudomonas to be isolated from wounds, followed by respiratory tract specimens and wound from out-patients (17). Another study performed in Turkey reported Pseudomonas from respiratory specimens of patients admitted in ICU to be common (18). Having substantially increased in recent years, acquired resistance to ceftazidime has been estimated to be 10 to 40% (19). In our study, among the 99 isolates of Pseudomonas, 76 isolates were resistant to ceftazidime. Majority of P.aeruginosa isolates were non-susceptible to ceftriaxone (80.88%); among other Pseudomonas species, non-susceptibility was related to ceftazidime (83.87%). In the present study, all of the resistance among

Samples	*P.aeruginosa* N (%)	*Pseudomonas.spp* N (%)	Total
Wound	43(63.23)	16(51.61)	59(59.59)
Urine	15(22.05)	11(35.48)	26(26.26)
Blood	8(11.76)	3(9.67)	11(11.11)
Tracheal tube	1(1.47)	1(3.22)	2(2.02)
Abscess	1(1.47)	0	1(1.01)
Total	68(68.68)	31(31.31)	99(100)

Table1: *Pseudomonas* isolates from different specimen

isolates of P.aeruginosa and other Pseudomonas species were related to ceftazidime (76.76%). The sensitivity of the bacteria to at least 3 of the antibiotics namely, amikacin, gentamicin, ciprofloxacin, ceftazidime and imipenem was increased from 13% in 1997 to 21% in 2000 in the US. (20). In a study conducted by Mihani and colleagues, it was reported that 71% of P. aeruginosa isolates obtained from the patients in Ahvaz Taleghani Hospital were resistant to ceftazidime, 41% to imipenem and 67% towards ciprofloxacin (21) which is similar to our findings. However, non-susceptibility varies from ward to ward, even among diverse hospitals. The published literature from Tehran, Kashan, Iran and Turkey reported Pseudomonas resistance to ceftazidime of 72.9% (16), 86% (22) and 84%, respectively (18); these are consistent with our study.

Most sensitivity of P.aeruginosa isolates was related to carbenicillin (33.8%), and among the other Pseudomonas isolates were related to Imipenem (32.2%). Among all isolates of P.aeruginosa, the sensitivity to imipenem was much more than other antibiotics in this study. In a study conducted by Shojapour et al., 48% of P.aeruginosa isolates were resistant to imipenem (23). Rate of resistance to imipenem was reported (26%) in Latvia, 20.5% in South Korea (24) and 14%-18% in Spain (25). In Turkey also, the sensitivity of the isolates decreased and the resistance of Pseudomonas to imipenem increased in recent years (26). In another study conducted by Munir and colleagues, cases of sepsis in newborns were investigated; later, the resistance of Pseudomonas isolates to imipenem reported to be 83.3% (23). In this study, the amount of imipenem resistance among isolates of Pseudomonas (aeruginosa and other species) was 61.61%, which indicates the high resistance to these antibiotics.

About 20.5% of P.aeruginosa isolates had intermediate resistant to ciprofloxacin and other Pseudomonas isolates had the most intermediate resistant to ceftriaxone. Generally, among all isolates of Pseudomonas the highest intermediate resistance was related to ciprofloxacin (16.1%). Based on the results presented in this article, antibiotics such as ceftazidime, ceftriaxone, ofloxacin, gentamicin and tobramycin, have lost their effectiveness against Pseudomonas to some extent, and probably this class of drugs will be useless in the foreseeable future. Although the use of antibiotics such as ceftazidime and ceftriaxone for treatment of infections caused by gram-negative bacteria are not recommended since long time ago, resistance to them is impressive. Imipenem, carbenicillin and ciprofloxacin can be more effective than other antibiotics. Nevertheless, according to the recent studies, it can be predicted that resistant bacteria will be replaced by the partly sensitive strains soon and treatment will be more difficult.

Antibiotics	*P.aeruginosa* isolated N (%)			*Pseudomonas.spp* isolated N (%)			Total *Pseudomonas* antibiotics Resistance N (%)		
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Ofloxacin	47(69.11)	3(4.41)	18(26.47)	25(80.64)	0	6(19.35)	72(73.72)	3(3.03)	24(24.24)
Ciprofloxacin	37(54.41)	14(20.58)	17(25)	25(80.64)	2(6.45)	4(12.90)	62(62.62)	16(16.16)	21(21.21)
Gentamicin	50(73.52)	2(2.94)	16(23.52)	23(74.19)	1(3.22)	7(22.58)	73(73.73)	3(3.03)	23(23.23)
Amikacin	43(63.23)	5(7.35)	20(29.41)	22(70.96)	2(6.45)	7(22.58)	65(65.65)	7(7.07)	27(27.27)
Imipenem	41(60.29)	5(7.35)	22(32.35)	20(64.5)	1(3.22)	10(32.25)	61(61.61)	6(6.06)	32(32.32)
Ceftriaxone	55(80.88)	1(1.47)	12(17.64)	18(58.06)	5(16.12)	8(25.80)	73(73.73)	6(6.06)	20(20.20)
Ceftazidime	50(73.52)	2(2.94)	16(32.52)	26(83.87)	2(6.45)	3(9.67)	76(76.76)	4(4.04)	19(19.19)
Levofloxacin	46(67.64)	4(5.88)	18(26.47)	21(67.74)	2(6.45)	8(25.80)	67(67.67)	6(6.06)	26(26.26)
Tobramycin	48(70.58)	0	20(29.41)	24(77.41)	0	7(22.58)	72(72.72)	0	27(27.27)
Carbenicillin	42(61.76)	3(4.41)	23(33.82)	21(67.74)	2(6.45)	8(25.80)	63(63.63)	5(5.05)	31(31.31)

Table2: Antibiotic resistance profile among *Pseudomonas* isolated from patients

Antibiotic resistance of Pseudomonas to different antibiotics is distinct around the world and even this resistance is also different between various species of Pseudomonas. As a result, the correct treatment against this type of infection requires careful consideration of drug resistance of infectious agent and then prescription of the useful medicine. On the other hand, emergence of resistant strains can be partly avoided by completing the treatment duration and not using antibiotics inappropriately as much as possible.

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References

1. El-Rehewy MS, Saboor EA, Afifi NA, Ibrahim MA, Qayed SS. Detection and Characterization of Nosocomial Carbapenem-Resistant Gram Negative Bacilli from Assuit University Hospitals. The Egyptian Journal of Medical Microbiology (EJMM). 2016 Jul 19;25(3).
2. Leylabadlo HE, Kafil HS, Yousefi M, Aghazadeh M, Asgharzadeh M. Persistent infection with metallo-beta-lactamase and extended spectrum β-lactamase producer Morganella morganii in a patient with urinary tract infection after kidney transplantation. Journal of natural science, biology, and medicine. 2016;7(2):179.
3. Qiu D, Eisinger VM, Rowen DW, Hongwei DY. Regulated proteolysis controls mucoid conversion in Pseudomonas aeruginosa. Proceedings of the National Academy of Sciences. 2007;104(19):8107-12.

4. Cross A, Allen JR, Burke J, Ducel G, Harris A, John J, et al. Nosocomial infections due to Pseudomonas aeruginosa: review of recent trends. Review of Infectious Diseases. 1983;5(Supplement 5):S837-S45.
5. Leylabadlo HE, Kafil HS, Aghazadeh M, Hazratian T. Nosocomial oral myiasis in ICU patients: occurrence of three sequential cases. GMS hygiene and infection control. 2015;10.
6. Moolenaar RL, Crutcher JM, San Joaquin VH, Sewell LV, Hutwagner LC, Carson LA, et al. A Prolonged Outbreak of Pseudomonas Aeruginosa in a Neonatal Intensive Care Unit Did Staff Fingernails Play a Role in Disease Transmission? Infection Control & Hospital Epidemiology. 2000;21(02):80-5.
7. Zaborina O, Kohler JE, Wang Y, Bethel C, Shevchenko O, Wu L, et al. Identification of multi-drug resistant Pseudomonas aeruginosa clinical isolates that are highly disruptive to the intestinal epithelial barrier. Annals of clinical microbiology and antimicrobials. 2006;5(1):1.
8. Eriksen H, Iversen B, Aavitsland P. Prevalence of nosocomial infections in hospitals in Norway, 2002 and 2003. Journal of Hospital Infection. 2005;60(1):40-5.
9. Taylor G, Kibsey P, Kirkland T, Burroughs E, Tredget E. Predominance of staphylococcal organisms in infections occurring in a burns intensive care unit. Burns. 1992;18(4):332-5.
10. Leylabadlo HE, Asgharzadeh M, Aghazadeh M. Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. Iranian journal of microbiology. 2015;7(5):226.
11. Nicas TI, Iglewski BH. Isolation and characterization of transposon-induced mutants of Pseudomonas aeruginosa deficient in production of exoenzyme S. Infection and immunity. 1984;45(2):470-4.
12. Kitao T, Tada T, Tanaka M, Narahara K, Shimojima M, Shimada K, et al. Emergence of a novel multidrug-resistant Pseudomonas aeruginosa strain producing IMP-type metallo-β-lactamases and AAC (6')-Iae in Japan. International journal of antimicrobial agents. 2012;39(6):518-21.
13. Hota S, Hirji Z, Stockton K, Lemieux C, Dedier H, Wolfaardt G, et al. Outbreak of multidrug-resistant Pseudomonas aeruginosa colonization and infection secondary to imperfect intensive care unit room design. Infection Control & Hospital Epidemiology. 2009;30(01):25-33.
14. Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods. Manual of Clinical Microbiology, Eleventh Edition: American Society of Microbiology; 2015. p. 1253-73.
15. Mauldin PD, Salgado CD, Hansen IS, Durup DT, Bosso JA. Attributable hospital cost and length of stay associated with health care-associated infections caused by antibiotic-resistant gram-negative bacteria. Antimicrobial agents and chemotherapy. 2010;54(1):109-15.
16. Driscoll JA, Brody SL, Kollef MH. The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections. Drugs. 2007;67(3):351-68.
17. Babay HAH. Antimicrobial resistance among clinical isolates of Pseudomonas aeruginosa from patients in a teaching hospital, Riyadh, Saudi Arabia, 2001-2005. Japanese journal of infectious diseases. 2007;60(2/3):123.
18. Yüce A, Yapar N, Eren KO. [Evaluation of antibiotic resistance patterns of Pseudomonas aeruginosa and Acinetobacter spp. strains isolated from intensive care patients between 2000-2002 and 2003-2006 periods in Dokuz Eylul University Hospital, Izmir]. Mikrobiyoloji bulteni. 2009;43(2):195-202.
19. Nordmann P, Guibert M. Extended-spectrum β-lactamases in Pseudomonas aeruginosa. Journal of Antimicrobial Chemotherapy. 1998;42(2):128-31.
20. Rossolini G, Mantengoli E. Treatment and control of severe infections caused by multiresistant Pseudomonas aeruginosa. Clinical Microbiology and Infection. 2005;11(s4):17-32.
21. Khosravi AD, Mihani F. Detection of metallo-β-lactamase–producing Pseudomonas aeruginosa strains isolated from burn patients in Ahwaz, Iran. Diagnostic microbiology and infectious disease. 2008;60(1):125-8.
22. Moniri R, Mosayebi Z, Movahedian A. Increasing trend of antimicrobial drug-resistance in Pseudomonas aeruginosa causing septicemia. Iranian Journal of Public Health. 2006;35(1):58-62.
23. Ekrami A, Kalantar E. Bacterial infections in burn patients at a burn hospital in Iran. Indian Journal of Medical Research. 2007;126(6):541.
24. Nehme D, Poole K. Assembly of the MexAB-OprM multidrug pump of Pseudomonas aeruginosa: component interactions defined by the study of pump mutant suppressors. Journal of bacteriology. 2007;189(17):6118-27.
25. Dambrauskiene A, Adukauskiene D, Jeroch J, Vitkauskiene A. [Pseudomonas aeruginosa bacteremia: associations with a source of infection and antibiotic resistance]. Medicina (Kaunas, Lithuania). 2008;45(1):1-7.
26. Alişkan H, Colakoğlu S, Turunç T, Demiroğlu Y, Erdoğan F, Akin S, et al. [Four years of monitoring of antibiotic sensitivity rates of Pseudomonas aeruginosa and Acinetobacter baumannii strains isolated from patients in intensive care unit and inpatient clinics]. Mikrobiyoloji bulteni. 2008;42(2):321-9.