

Evaluating Antibiotic Sensitivity Patterns of *Pseudomonas* in Relation to Specimen Type in Jordanian Hospital

Nabil Awni Nimer,¹ Sawsan Ahmad Abdel Dayem,² Gaith Abdul Karim AbouNouar,³ Abdel Naser Husni Dakkah.⁴

Abstract

Objective: To evaluate the sensitivity patterns of different antibiotics of *pseudomonas* in relation to specimen types.

Methods: The quantitative retrospective study was conducted at Princess Iman Research and Laboratory Sciences Centre of Royal Medical Services, Amman, Jordan. The specimens of USS, urine, cerebral spinal fluid, and blood were collected from patients, who visited the hospital from January to September 2015. Drugs analysed included ampicillin, ceftazidime, ciprofloxacin, cefotaxime, ceftazidime, nitrofurantoin and gentamicin.

Results: There were 358 samples collected. Ampicillin was found effective ($p=0.002$). There was a weaker correlation between amikacin and amoxicillin/clavulanic acid ($r=-0.001$). Similarly, nitrofurantoin was also effective ($p=0.001$), and the association between amikacin and ceftazidime was positive ($r=0.998$).

Conclusion: The selected antibiotics were only examined, concerning the sensitivity patterns as data collected from the patients was insufficient for other drugs.

Keywords: Antibiotics, *pseudomonas*, Sensitivity Patterns, Specimen. (JPMA 69: 168; 2019)

Introduction

Pseudomonas (*Ps.*) *aeruginosa* is classified as a gram-negative bacterium, which causes infection to patients, who are compromising the host defence mechanisms. There have been a significant number of nosocomial infections, specifically pneumonia, which are caused by *Ps. aeruginosa*.¹ The consequences of antibiotic resistance have been observed with a gradual increase globally. The bacterial evolution to multi-drug resistance is unavoidable as it provides an uncontrollable bacterial growth with a failure to cease the bacterial reproduction. Achievements of modern organ transplantation, major surgery, cancer chemotherapy, and treatment of preterm babies would not be possible without accessing modern and effective treatment for bacterial infections.² The prescription of empiric antimicrobial drugs to a patient has been considered as the most common cause of antibacterial resistance.³

Ps. aeruginosa causes a broad spectrum of infections and leads to considerable morbidity among immunocompromised patients. Regardless of the therapy, the mortality rate is approximately 70% due to nosocomial *Ps. pneumonia*.⁴ Resistance to several antibiotics is demonstrated by *Ps. aeruginosa*, which has risked the selection of suitable treatment. Antibacterial drugs; such as colistin, are required for the treatment of multi-drug-resistant *Ps. aeruginosa*, but novel anti-pseudomonal antibacterial is probable to be obtainable in future.³ Driscoll et al.⁵ have indicated that the occurrence of multi-drug resistance is related with *Ps. aeruginosa*, which is the leading clinical problem in healthcare. It has been recommended to focus on this problem as a public health concern. Moreover, there is also a need to specify different pathogens, which are responsible for the spread of such infections. The reason for the resistance lays with the synergy with multi-drug efflux system or a type1 AmpC β -lactamase, this provide decreased membrane permeability. To evaluate the sensitivity and resistance, the specimen can be collected from the urine for patients with urinary tract infections (UTIs). Similarly, the

^{1,4}Philadelphia University, Amman, Jordan, ^{2,3}Royal Medical Services, Amman, Jordan.

Correspondence: Nabil Awni Nimer. e-mail: n_nimer@philadelphia.edu.jo

sensitivity of blood sepsis can be evaluated by collecting blood samples, and pus can be collected from any old wound.

The bacterium has the capability to show absolute resistance to anti-microbial drugs; therefore, it results in less effective treatment modalities.¹ Tripathi et al.⁶ conducted a study to explain the antibiotic resistance pattern occurring with *Ps. aeruginosa* for UTI. The findings demonstrated that the UTI patients can be best treated with piperacillin (19.6%) and amikacin (AK) (25.3%), which have been observed with a minimum resistance. It has been depicted that AK was proved to be 100% effective while treating UTI. The resistance level with an aminoglycoside, gentamicin (GM) was found to be 35.3% and tobramycin with 69.2%.⁷

The effect of GM has been observed with a raised resistance of *Ps. aeruginosa*, while AK is commonly used to treat the severe degree of disease, due to the higher treatment cost and the avoidance to adopt the invasive procedures. *Ps. aeruginosa* has shown to prove the antimicrobial resistance through acquiring beta-lactamases with the help of extended enzymes like carbapenemases or aminoglycoside-modifying enzymes.⁸ This antimicrobial resistance is achieved by a transfer of plasmid, which carries genes to encode the enzyme. The plasmids are believed to be passed to next generation of bacteria by means of replication, which shows the intense resistance with the antimicrobial agents.⁸

A study⁴ was undertaken to evaluate the antibiotic susceptibility patterns of the pathogenic isolates of *Ps. aeruginosa* from different specimens of the hospital-acquired infections (HAIs). The findings revealed the highest number of *Ps.* infections in urine followed by sputum and pus. Marked resistance was demonstrated by *Ps.* species against monotherapy of cephalosporins, penicillin, tetracyclines, fluoroquinolones, and macrolides. Higher sensitivity to *Ps.* infections was only observed by combining drugs like piperacillin + tazobactam, ticarcillin + clavulanic acid (CA), cefotaxime + sulbactam; however, carbapenems alone showed the maximum sensitivity.

The infections associated with the pseudomonas through the blood have shown a worldwide morbidity and mortality. The data is generally collected from the patients with fever or sepsis. A broad spectrum of

organisms is being engaged in providing blood stream infections. A study⁹ comprised 224 episodes of bloodstream that were examined for infection among patients who were admitted to a surgical intensive care unit (SICU). The results demonstrated that the survival of pathogen was determined by its intensity and severity of bacterial resistance. Generally, mortality has been associated with *Ps. aeruginosa* bloodstream infections, which has been observed in more than 20% for patients receiving antibiotic therapy. A meta-analysis demonstrated that beta-lactam was provided as a monotherapy to treat neutropenic fever and severe sepsis as an empirical treatment for *Ps. aeruginosa*.¹ One study¹⁰ evaluated blood specimens to isolate *Ps. aeruginosa* from the neonates suffering from pneumonia and further evaluated the risk factors associated with it.

Patients of cystic fibrosis (CF) have been largely infected by *Ps. aeruginosa*, especially patients having a long-term chronic infection. These bacteria are largely observed to destroy the highly compartmentalised lungs and the anatomic structure of patients having CF.¹¹ The higher mutation rate has been observed in vivo with higher antibacterial agents' resistance. Being the major cause of morbidity and mortality in patients with CF, the *Ps.* has been observed with a clonal structure along with significant phenotype variations.¹² The use of fluoroquinolone to treat various diseases is being associated with the likelihood of inappropriate treatment.¹ The current study was planned to evaluate antibiotic sensitivity patterns of *Ps.* in relation to specimen types.

Material and Methods

The quantitative retrospective study was conducted at the Princess Iman Research and Laboratory Sciences Centre of the Royal Medical Services, Amman, Jordan. The specimens of USS, urine, cerebral spinal fluid and blood were collected from the patients, who visited the hospital from January to September 2015. *Ps.* detection and testing of antibacterial susceptibility was obtained using the VITEK 2 ID system.¹³ The implementation of VITEK 2 ID was made due to its industrial microbiology-testing environment provided to the researcher for providing increased level of antimicrobial susceptibility testing. The culture requirements of VITEK 2 ID are suitable to evaluate the specimens obtained for pseudomonas isolates. All of the specimens were tested through VITEK 2 ID.

Different specimen samples of urine, pus, and blood and cerebrospinal fluid (CSF) were taken from the patients to test the drugs on the organisms, obtained from the test. Ampicillin (AM; class=penicillin), Ceftazidime (CAZ; class=cephalosporin), Ciprofloxacin (CIP; class=fluoroquinolone), Cefotaxime (CTX; class=cephalosporin), Cefoxitin (FOX; class=cephalosporin), Nitrofurantoin (FT; class=nitrofurantoin), Gentamicin (GM; class=aminoglycoside) were the drugs particularly observed as the data, collected from the patients, was incomplete for other drugs. The selection of antimicrobial agents was based on the Clinical & Laboratory Standards Institute (CLSI) Guidelines¹⁴. These guidelines were followed to ensure testing conditions as recommended by CLSI. Similarly, clinical efficacy, clinical indications, and prevalence of resistance were also focused in the CLSI guidelines. Cotton-based paper discs of 6.5mm

were used in the laboratory settings and silica-gel was used for maintaining dryness. The inclusion of these suitable features of antibiotic susceptibility discs allowed us to ensure the regulatory environments and quality. SPSS version 20 was used to predict effective antibiotics. Cross-tabulation technique was applied to measure the specimen type collected with respect to the organisms. Pearson correlation test was applied to assess the association between antibiotics. Regression analysis was also performed in order to examine the effectiveness of the drugs on the organism obtained. Specimens were collected from those patients only, who signed the consent form.

Results

There were 358 samples. Mostly, *Ps. aeruginosa* was detected by observing different specimen (Table 1). A

Table-1: Cross-Tabulation of Specimen type and Organism.

Organism Name * Specimen Type Crosstabulation		SpecimenType				Total
Count		Urine	Pus	CSF	Blood	
Organism Name	Ps.aeruginosa	35 (74%)	115 (40.49%)	3 (50%)	11 (52.38%)	164 (45.8%)
	Pseudomonas spp	9 (19.1%)	139 (48.94%)	1 (16.67%)	1 (4.76%)	150 (41.8%)
	Ps.stutzeri	1 (2%)	3 (1%)	0 (0%)	2 (9.52%)	6 (1.675%)
	Ps.luteola	0 (0%)	13 (4%)	1 (16.67%)	3 (14.28%)	17 (4.74%)
	Ps.fluorescens	1 (2%)	5 (1.7%)	0 (0%)	3 (14.28%)	9 (2.5%)
	Ps.alcaligenes	0 (0%)	1 (0.35%)	0 (0%)	0 (0%)	1 (0.279%)
	Ps.putida	1 (2%)	8 (2.81%)	1 (16.67%)	1 (4.76%)	11 (3.072%)
Total		47 (100%)	284 (100%)	6 (100%)	21 (100%)	358 (100%)

CSF: Cerebrospinal fluid.

Table-2: Pearson Correlation Test.

Correlations		Amoxicillin/Clavulanic Acid	Amikacin	Ceftazidime	Ciprofloxacin	Cefoxitin	Nitrofurantoin
Amoxicillin/Clavulanic Acid	Pearson Correlation	1	-.001	-.080	.043	.165**	.228**
	Sig. (2-tailed)		.987	.141	.430	.002	.000
	N	341	340	340	340	341	341
Amikacin	Pearson Correlation	-.001	1	.000	.246**	.017	.081
	Sig. (2-tailed)	.987		.998	.000	.760	.136
	N	340	340	339	339	340	340
Ceftazidime	Pearson Correlation	-.080	.000	1	-.096	.025	.028
	Sig. (2-tailed)	.141	.998		.076	.642	.612
	N	340	339	340	339	340	340
Ciprofloxacin	Pearson Correlation	.043	.246**	-.096	1	-.017	.014
	Sig. (2-tailed)	.430	.000	.076		.756	.793
	N	340	339	339	340	340	340
Cefoxitin	Pearson Correlation	.165**	.017	.025	-.017	1	.168**
	Sig. (2-tailed)	.002	.760	.642	.756		.002
	N	341	340	340	340	341	341
Nitrofurantoin	Pearson Correlation	.228**	.081	.028	.014	.168**	1
	Sig. (2-tailed)	.000	.136	.612	.793	.002	
	N	341	340	340	340	341	341

** . Correlation is significant at the 0.01 level (2-tailed).

Table-3: Regression Analysis.

Model Summary						
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate		
1	.303 ^a	.092	.073	1.257		
a. Predictors: (Constant), Gentamicin, Cefoxitin, Ceftazidime, Cefotaxime, Nitrofurantoin, Ampicillin, Ciprofloxacin						
ANOVA ^a						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	52.345	7	7.478	4.731	.000 ^b
	Residual	516.879	327	1.581		
	Total	569.224	334			
a. Dependent Variable: Organism Name						
b. Predictors: (Constant), Gentamicin, Cefoxitin, Ceftazidime, Cefotaxime, Nitrofurantoin, Ampicillin, Ciprofloxacin						
Coefficients ^a						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.004	.240		8.357	.000
	Ampicillin	.087	.027	.179	3.188	.002
	Ceftazidime	-.002	.012	-.009	-.171	.864
	Ciprofloxacin	-.125	.138	-.052	-.908	.365
	Cefotaxime	-.011	.006	-.105	-1.877	.061
	Cefoxitin	.004	.015	.015	.268	.789
	Nitrofurantoin	.003	.001	.179	3.265	.001
	Gentamicin	-.042	.048	-.049	-.873	.383
a. Dependent Variable: OrganismName						

ANOVA: Analysis of variance.

weak correlation was found between AK and AMC/CA ($r=0.001$). The association between CAZ and AK was very strong and positive ($r=0.998$). The association between AK and CAZ was positive and strong ($r=0.998$). The association between FT and FOX was also positive ($r=0.168$) (Table 2). In regression analysis, AM was statistically significant on different organisms on different specimen types ($p=0.002$). CAZ was statistically insignificant on different organisms on different specimen types ($p=0.864$). CIP was statistically insignificant on different organisms on different specimen types ($p=0.365$). CTX was statistically insignificant on different organisms and different specimen types ($p=0.061$). FOX was also statistically insignificant ($p=0.789$). GM was statistically insignificant on different organisms and different specimen types ($p=0.383$). FT was statistically significant ($p=0.001$). AM ($p=0.002$) and FT ($p=0.001$) were effective on the organisms (Table 3).

Discussion

The study aimed at providing clinical investigations for the use of several drugs to determine the antimicrobial resistance for the species of *Ps.*, particularly *Ps. aeruginosa*.

Therefore, there is a need to minimise the production of drugs, which are proved to be resistant to a greater number of populations, and raise the demand of drugs, which are tested to provide efficacious parameters with diseases. It has been demonstrated that the improper initial antimicrobial treatment of *Ps. aeruginosa* for blood infection, sepsis, fever, and UTI is generally associated with antimicrobial resistance. The *Ps.* is needed to be considered for differential diagnosis for the gram-negative bacteria.³ An effective and comprehensive treatment is required as it is a HAI for patients, who are immune-compromised. For treating the immune-compromised patients, a combination of anti-pseudomonal drug therapy is provided. This aspect prevents the selection of resistant mutants to the resistant strains. Carbapenems and aztreonam, which is a monobactam antibiotic, are generally prescribed for serious and intense infections. These are provided for the antibacterial resistance, which is usually provided by the beta-lactam antibiotic.^{15,16}

Drug resistance has been observed to facilitate the counter-productivity of pathogens, proving them as having the strength to resist extermination. According to A study⁶, ofloxacin showed a greater extent of activity in

quinolones, being 51% sensitive with the resistant bacteria. The effects of ciprofloxacin were observed to be 50% sensitive; however, some studies showed 25% results with it. The effects of ceftazidime have been proved to be around 90%.⁶

A study determined the susceptibility of antimicrobials and found piperacillin-tazobactam as having 91% susceptibility.¹⁷ The most common cause of sepsis, pneumonia, UTI and infections after surgeries are deemed as the most common cause of gram-negative bacilli (GNB). In the intensive care units, GNB resistance problem is of specific concern. Therefore, it has been believed that effective antimicrobial therapy plays a major role in the successful management of patients with infections in the ICU.¹⁸ *Pseudomonas* species can survive in double distilled water and in both high and low nutrition environment.

A study¹⁹ investigated the multi-drug resistance and discovered that the GNB and multi-drug resistance has increased from 1% to 16% for *Ps. aeruginosa* and 4% to 13% for enterobacter species. It is concluded that these pathogens can be included for limiting the widespread of antimicrobial resistance. Therefore, *Ps.* known as the starting point of many important carbapenemases categories. According to a research, the gram-negative resistance (GNR) is linked to the prolonged stay at hospital that eventually increases the rate of mortality. Consequently, it is said that GNR independently estimates the mortality. Moreover, it is more effective to implement altering infection control practices for limiting the spread of some bacterial species.²⁰ There is a highly significant difference in the cost among the infected and non-infected patients and certainly it has been found that cost of patients with anti-microbial resistance was higher.²¹

Antibiotic resistance is more frequently observed in a country where the higher frequency of drugs is prescribed to patients with an improper drug controlling process. The easy access of over-the-counter drugs is associated with frequent increase in antibacterial resistance. Despite the major research work performed in this field, a lot of parameters further require to be accomplished with clinical findings. A study²⁰ evaluated the effects of bioelectric current on 11 different antimicrobial agents, which represented different classes opposing *Ps. aeruginosa*. It concluded that enhancements in the antibacterial agents by the use of electric current against biofilms was not considered a generalised phenomenon across other micro-organisms.²³ It has been observed that the 12 micro-

bacterial agents versus GNB were obtained from the ICU setting in US hospitals. It was discovered that occurrence of multi-drug resistance increases considerably between the patients of ICU isolates of *acinetobacter* spp., and *E. Cloacae*. The study also indicated that the drug resistance has turned into a severe problem, which consisted of antibiotics, specifically ciprofloxacin.²² Moreover, the study examined the antimicrobial susceptibility and explored that each antimicrobial tested in the study was susceptible compared to the nonmucoid with the mucoid isolates, but mucoid phenotype results in the increased resistance. Data analysed in another study²³ indicated higher resistance among gram-negative bacteria for aminoglycoside and β -lactam antibiotics. Whereas the antibiotics, which are commonly used, like ceftriaxone and ampicillin, revealed poor activity against most of the organisms.²⁴

Regular antimicrobial susceptibility surveillance is necessary for field-wise monitoring of the resistance patterns. Certain efficient state and national level strategies and guidelines must be presented to preserve the efficiency of the antibiotics and effective treatment of patients. Moreover, the study recommends testing specimen type frequently as the efficiency of the antibiotic varies with different specimen type.

Conclusion

Among different antibiotics considered to measure the sensitivity patterns; AM and FT were found effective. The association between the AK and CAZ was also positive. Sensitivity patterns of each antibiotic varied in different specimen type.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Burns JL, Saiman L, Whittier S, Larone D, Krzewinski J, Liu Z, et al. Comparison of agar diffusion methodologies for antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *J Clin Microbiol* 2000; 38: 1818-22.
2. Chastre J, Trouillet JL. Problem pathogens (*Pseudomonas aeruginosa* and *Acinetobacter*). *Semin Respir Infect* 2000; 15: 287-98.
3. Chaudhari V, Gunjal S, Mehta M. Antibiotic resistance patterns of *Pseudomonas aeruginosa* in a tertiary care hospital in Central India. *Int J Med Sci Public Health* 2013; 2: 386-9
4. Christensen LD, van Gennip M, Jakobsen TH, Alhede M, Hougen HP, Høiby N, et al. Synergistic antibacterial efficacy of early combination treatment with tobramycin and quorum-sensing

- inhibitors against *Pseudomonas aeruginosa* in an intraperitoneal foreign-body infection mouse model. *J Antimicrob Chemother* 2012; 67: 1198-206.
5. CLSI. About CLSI's Subcommittee on Antimicrobial Susceptibility Testing (AST). [Onlien]. [Cited 2018 May 31]. Available from: URL: <https://clsi.org/education/microbiology/ast/>
 6. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. *Clin Infect Dis* 2006; 42 (Suppl 2): S82-9.
 7. D'Agata EM. Rapidly rising prevalence of nosocomial multidrug-resistant, gram-negative bacilli: a 97-year surveillance study. *Infect Control Hosp Epidemiol* 2004; 25: 842-6.
 8. Del Pozo JL, Rouse MS, Mandrekar JN, Sampedro MF, Steckelberg JM, Patel R. Effect of electrical current on the activities of antimicrobial agents against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* biofilms. *Antimicrob Agents Chemother* 2009; 53: 35-40.
 9. Driscoll JA, Brody SL, Kollef MH. The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs* 2007; 67: 351-68.
 10. Frank DW. Research topic on *Pseudomonas aeruginosa*, biology, genetics, and host-pathogen interactions. *Pseudomonas Aeruginosa, Biology, Genetics, and Host-pathogen Interactions*. *Front Microbiol* 2012; 3: 20.
 11. Harbarth S, Ferrière K, Hugonnet S, Ricou B, Suter P, Pittet D. Epidemiology and prognostic determinants of bloodstream infections in surgical intensive care. *Arch Surg* 2002; 137: 1353-9.
 12. Khare A, Kothari S, Misra V. Incidence and sensitivity pattern of *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* in a tertiary care hospital. *Int J Basic Clin Pharmacol* 2017; 6: 329-33.
 13. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, et al. Antibiotic resistance-the need for global solutions. *Lancet Infect Dis* 2013; 13: 1057-98.
 14. Zawacki A, O'Rourke E, Potter B, Bynoe G, Macone A, Harbarth S, Goldmann D. An outbreak of *Pseudomonas aeruginosa* pneumonia and bloodstream infection associated with intermittent otitis externa in a healthcare worker. *Infect Control Hosp Epidemiol* 2004; 25: 1083-9.
 15. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare?. *Clin Infect Dis* 2002; 34: 634-40.
 16. Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. *Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment. *Antimicrob Agents Chemother* 2005; 49: 1306-11.
 17. Mulcahy LR, Burns JL, Lory S, Lewis K. Emergence of *Pseudomonas aeruginosa* strains producing high levels of persister cells in patients with cystic fibrosis. *J Bacteriol* 2010; 192: 6191-9.
 18. Nimer NA, Al-Saa'da RJ, Abuelaish O. Accuracy of the VITEK® 2 system for a rapid and direct identification and susceptibility testing of Gram-negative rods and Gram-positive cocci in blood samples. *East Mediterr Health J* 2016; 22: 193-200.
 19. Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 2000; 288: 1251-4.
 20. Pathmanathan SG, Samat NA, Mohamed R. Antimicrobial susceptibility of clinical isolates of *Pseudomonas aeruginosa* from a Malaysian Hospital. *Malays J Med Sci* 2009; 16: 27-32.
 21. Raymond DP, Pelletier SJ, Crabtree TD, Evans HL, Pruett TL, Sawyer RG. Impact of antibiotic-resistant Gram-negative bacilli infections on outcome in hospitalized patients. *Crit Care Med* 2003; 31: 1035-41.
 22. Tripathi P, Banerjee G, Saxena S, Gupta MK, Ramteke PW. Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from patients of lower respiratory tract infection. *Afr J Microbiol Res* 2011; 5: 2955-9.
 23. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *PT* 2015; 40: 277-83.
 24. Verma P, Berwal PK, Nagaraj N, Swami S, Jivaji P, Narayan S. Neonatal sepsis: epidemiology, clinical spectrum, recent antimicrobial agents and their antibiotic susceptibility pattern. *Int J Contemp Pediatr* 2017; 2: 176-80.