Annals of Medical Research
DOI: 10.5455/annalsmedres.2018.09.186 2019;26(2):213-6

Original Article

Assessment of antibiotic resistance rates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated in intensive care units between 2014 and 2017

Mustafa Kerem Calgin, Yeliz Cetinkol

Ordu University Faculty of Medicine, Department of Medical Microbiology, Ordu, Turkey

Abstract

Aim: Non-fermenter microorganisms are commonly found in hospital environments and the treatment of infections caused by these pathogens is becoming difficult due to increasing resistance developing against antimicrobials. This study aimed to identify the resistance status of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates against a variety of antimicrobial agents. Clinical samples taken from patients admitted to the adult intensive care units of Ordu University Education and Research Hospital.

Material and Methods: Antibiogram results for 162 *P.aeruginosa* and 380 *A.baumannii* isolates obtained from a variety of clinical samples taken from the intensive care units from January 2014 to December 2017 were retrospectively assessed. The definition of the isolates and antimicrobial susceptibility tests were studied with a VITEK 2 Compact system.

Results: At our hospital, the two bacteria were most commonly isolated in respiratory tract samples. The second-highest incidence for *P.aeruginosa* was in urine samples and for *A.baumannii* strains was in blood samples. The resistance rates of *P.aeruginosa* strains were observed to reduce in the last two years and this change was statistically significant for cefepime, imipenem, meropenem and ciprofloxacin. There was no change in the resistance of *A.baumannii* strains to antibiotics used routinely during the years.

Conclusion: *P.aeruginosa* and *A.baumannii* have high antibiotic resistance and are microorganisms that rapidly develop resistance during treatment. As resistance development may vary in each hospital, determination of resistance phenotype in each center will be an indicator in terms of being able to administer effective and appropriate treatment.

Keywords: *Pseudomonas aeruginosa; Acinetobacter baumannii*; Intensive Care Unit; Antibiotic Resistance.

INTRODUCTION

Intensive care units are units where infections are most commonly observed in hospitals and antibiotics are most commonly used. The largest share in the occurrence and spread of antibiotic resistance among bacteria belongs to this environment (1). Gram-negative bacteria are responsible for the majority of intensive-care-sourced infections and multiple resistance is increasing around the world at worrying levels (2,3). *Pseudomonas aeruginosa* is an infectious agent that is very difficult to treat due to intrinsic resistance to many antibiotics (4).

Generally, described as a commensal bacterium, *Acinetobacter baumannii* has become a clinically dangerous bacterium in recent years due to infections caused especially in critical patients (5). Both microorganisms are commonly found in hospital environments. The treatment of infections caused by these pathogens is very difficult due to increasingly developing resistance against antimicrobials.

Currently the most commonly accepted strategy in combating hospital infections is to regularly complete patient and laboratory based prospective active surveillance studies and to organize treatment according to the data obtained (6). As a result, culture results should be noted and the regional resistance phenotype should be considered when deciding on empirical antibiotic treatment. This study aimed to identify the resistance status of *P. aeruginosa* and *A. baumannii* isolates from clinical samples taken from patients admitted to the adult intensive care units of Ordu University Education and Research Hospital against a variety of antimicrobial agents.

Received: 14.09.2018 **Accepted:** 23.11.2018 **Available online:** 29.11.2018
Corresponding Author: Mustafa Kerem Calgin, Ordu University Faculty of Medicine, Department of Medical Microbiology, Ordu, Turkey
E-mail: mkcalgin@gmail.com

MATERIAL and METHODS

Antibiogram results of *P. aeruginosa* and A. *baumannii* isolates obtained from a variety of clinical samples sent to Ordu University, Education and Research Hospital microbiology laboratory from the surgical and anesthesia intensive care units between January 2014 and December 2017 were retrospectively assessed.

Samples taken from patients under appropriate conditions were inoculated on the surface of 5% sheep's blood agar (Salubris, İstanbul, Turkey) and eosin methylene blue (EMB) (Salubris, İstanbul, Turkey) agar media. The media plates were incubated in an aerobic environment in a 37 °C incubator for 18-24 hours and cultures with proliferation were investigated. The definition and antimicrobial susceptibility tests for the obtained isolates were studied in line with the Clinical and Laboratory Standards Institute (CLSI) recommendations until January 2017 and then according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations with a VITEK 2 Compact (Biomerieux, Marcy l'Etoile, France) system. Ceftazidime, piperacillin/tazobactam, gentamicin, amikacin, imipenem, meropenem, cefepime, ciprofloxacin, trimethoprim sulfamethoxazole (SXT) and tigecycline resistance rates were analyzed. Strains with intermediate resistance identified were accepted as resistant. Repeated proliferation in the same type of samples from patients only had one case included in the study.

Statistical analyses were completed using the SPSS statistical program (SPSS, Chicago, IL, USA, version 20). The chi-square and Fisher's exact tests were used to analyze categorical variables. For the tests, $p<0.05$ was accepted as statistically significant.

RESULTS

Strains of 162 *P. aeruginosa* and 380 A. *baumannii* isolates obtained from a variety of clinical samples were tested in the microbiology lab. When the distribution of *P. aeruginosa* strains are evaluated according to the organs from which samples are taken; 92 were isolated from respiratory tract samples, 45 from urine samples, 16 from blood samples, 4 from wound samples, 4 from IV catheters and 1 from a drain. For the 380 *A. baumannii* strains, 237 were isolated from respiratory tract samples, 82 from blood samples, 34 from urine samples, 12 from wound samples, 9 from IV catheter, 3 from drains, 1 from peritoneal fluid and 2 from pleura fluid samples (Table 1). With the aim of observing the variation in resistance rates through the years, the resistance rates for strains from 2014 to 2015 were compared with the resistance rates for strains from 2016 to 2017. The resistance rates of *P. aeruginosa* strains were observed to reduce in the last two years and this change was found to be statistically significant for cefepime, imipenem, meropenem and ciprofloxacin (Table 2). Apart from SXT, variation in resistance among *A. baumannii* strains was not observed. In the last two years SXT resistance increased and this situation was identified to be statistically significant (Table 3).

Table 1. Distribution of strains according to clinical samples

	P.aeruginosa		*A.baumannii*	
Sample type	n	%	n	%
Respiratory tract*	92	56.7	237	62.4
Urine	45	27.8	34	8.9
Blood	16	9.9	82	21.6
Wound	4	2.5	12	3.1
IV catheter	4	2.5	9	2.4
Others**	1	0.6	6	1.6
Total	162	100	380	100

n: Number, %: Percent
*Sputum, Endotracheal aspirate, Bronchoalveolar lavage
** *P.aeruginosa*: Drain (n=1); *A.baumannii:* Drain (n=3), peritoneal fluid (n=1), pleura fluid (n=2)

Table 2. Resistance rates of *P. aeruginosa* strains between 2014-2015 and 2016-2017

	2014-2015			2016-2017			
	Number of strains	Resistant		Number of strains	Resistant		p
Antibiyotics	n	n	(%)	n	n	(%)	
Piperacillin/ tazobactam	68	44	(64.7)	84	46	(54.8)	0.215
Ceftazidime	73	28	(38.4)	87	29	(33.3)	0.509
Cefepime	72	33	(45.8)	88	27	(30.7)	0.049
İmipenem	70	35	(50)	79	19	(24.1)	0.001
Meropenem	73	35	(47.9)	87	25	(28.7)	0.012
Gentamicin	75	21	(28)	87	21	(24.1)	0.576
Amikacin	72	22	(30.6)	87	16	(18.4)	0.073
Ciprofloxacin	74	34	(45.9)	88	21	(23.9)	0.003

n: Number, %: Percent

Table 3. Resistance rates of *A. baumannii* strains between 2014-2015 and 2016-2017

	2014-2015			2016-2017			
	Number of strains	Resistant		Number of strains	Resistant		p
	n	n	(%)	n	n	(%)	
Piperacillin/ tazobactam	154	154	(100)	151	148	(98)	0.120
Ceftazidime	170	170	(100)	172	168	(97.7)	0.123
Cefepime	167	167	(100)	151	150	(99.3)	0.475
İmipenem	147	145	(98.6)	177	174	(98.3)	1.000
Meropenem	162	161	(99.4)	208	204	(98.1)	0.391
Gentamicin	170	158	(92.9)	209	201	(96.2)	0.162
Amikacin	161	147	(91.3)	208	190	(91.3)	0.989
Ciprofloxacin	171	171	(100)	209	205	(98.1)	0.131
SXT	169	35	(20.7)	209	118	(56.5)	<0.001
Tigecycline	125	77	(61.6)	167	101	(60.5)	0.846

n: Number, %: Percent, SXT: Trimethoprim sulfamethoxazole

DISCUSSION

Hospital infections developing in high-risk departments like intensive care units cause treatment difficulties (7). *P. aeruginosa* and *A. baumannii* are non-fermenter gram-negative bacteria with minimal nutritional requirements able to live on a variety of surfaces and fluid environments. These bacteria are the greatest source of worry about serious invasive infections that may develop in hospitals, especially in intensive care units where patients are generally critical and have suppressed immune systems (8). These species are also resistant to many antibiotics. Additionally, the resistance rates might be reduced by the effective antibiotic use policies and infection control procedures applied in hospitals.

Studies in Turkey have reported *P. aeruginosa* strains are most commonly isolated from respiratory tract samples (sputum, bronchoalveolar lavage, tracheal aspirate), followed by wound and urine samples (9-11). In our study, *P. aeruginosa* strains were most commonly isolated in respiratory tract samples followed by urine, blood and wound samples, similar to the study by Demiral et al. (9). Similar to this study, the elevation in isolation rates from blood cultures was linked to the monitoring of patients in the intensive care unit and generally among patients with suppressed immune systems. In our study *A. baumannii* was most commonly isolated in respiratory tract samples, with second highest frequency in blood samples. These were followed by urinary tract samples. A variety of studies in Turkey have identified that *A. baumannii* strains were most commonly isolated from respiratory tract samples (12).

For infections developing with *P. aeruginosa*, antipseudomonal penicillin, cephalosporins, fluoroquinolones, aminoglycosides and carbapenems are the most commonly used antimicrobial medications (13). *P. aeruginosa* strains with high resistance rates to aminoglycosides, ceftazidime, quinolones, piperacillin-tazobactam and carbapenems are common in south and east Europe (14). Additionally, *P. aeruginosa* clinical isolates showing multiple resistance patterns from 2008 to 2011 in French hospitals showed a reducing trend in resistance patterns and this situation was observed to continue until 2015 (14,15). Similarly, surveillance data from China showed the resistance levels of *P. aeruginosa* to fourteen antimicrobial agents tested over ten years reduced (16). Uzun et al. in a study in Izmir showed that the resistance of *P. aeruginosa* strains reduced from 2010 to 2011 (17). According to our data, there was a reduction in resistance in recent years in our hospital. This situation was observed to be statistically significant for cefepime, imipenem, meropenem and ciprofloxacin and this is considered to be due to the fact that these medications are used less often in routine *P. aeruginosa* treatment. Additionally, while antibiotic susceptibility tests were interpreted according to CLSI standards until 2015 in Turkey, from 2015 the transition to EUCAST standards began. Studies related to this transition by Hombach et al. identified higher resistance rates according to CLSI standards for *P. aeruginosa* to a variety of antibiotics compared to EUCAST interpretations (18). Our laboratory transitioned to the EUCAST standards in January 2017 and it is considered the increase in susceptibility may be due to this situation.

Acinetobacter infections are a common problem globally due to showing intrinsic resistance to many antibiotics and the potential to rapidly develop resistance to a variety of antibiotic classes. Nearly half of the invasive blood isolates reported in the EARS-Net in 30 countries in Europe were determined to have combined resistance against fluoroquinolones, aminoglycosides and carbapenems (14). Carbapenems are known as the broadest spectrum beta-lactam antibiotics with bacterial resistance developed, with the increase in production of carbapenemase by Pseudomonas and Acinetobacter isolates in recent periods allowing resistance to these antibiotics (19). Xia et al. (20) in a study of resistance rates of microorganisms isolated from respiratory tract samples from 2006-2010 reported a statistically significant increase in resistance of Acinetobacter isolates against cephalosporins and carbapenems reaching 86-99%. In our study, the resistance of up to 98% for Acinetobacter spp. strains in both periods against beta-lactam/beta-lactamase inhibitor combinations, cephalosporins, carbapenems and quinolone groups' antibiotics are noteworthy. It appears infection developing with Acinetobacter strains is a situation requiring attention in our hospital. Many studies have reported resistance rates of 5-66% for *A. baumannii* isolates against tigecycline (12). In our hospital, these rates were 61.6% for 2014-2015 and 60.5% for 2016-2017 which is close to the upper limit. Additionally, the most susceptible antibiotic compared to other choices is tigecycline which shows that there may be limitations on the use of this medication in the future linked to increases in frequency of use. In the future, there is a need for new antibiotics to treat infections caused by Acinetobacter species. The increase in new treatment choices is limited. After empirical treatment, treatment should be reorganized according to culture results.

CONCLUSION

The most significant bacteria in terms of hospital infections of *P. aeruginosa* and *A. baumannii* have high antibiotic resistance and are microorganisms that rapidly develop resistance during treatment. It is necessary to regularly monitor the resistance phenotypes of these isolates. Personnel should be trained, care should be taken about the choice and doses of antibiotics to be used and infection control should be completed according to the rules. Additionally, as the resistance development may change in each hospital, determination of the resistance phenotype in each center will be an indicator in terms of administering effective and appropriate treatment.

Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: There are no financial supports

Mustafa Kerem Calgin ORCID:0000-0003-4236-6177
Yeliz Cetinkol ORCID:0000-0003-4940-4498

REFERENCES

1. Nafziger DA, Lundstrom T, Chandra S, et al. Infection control in ambulatory care. Infect Dis Clin North Am 1997;11:279-96.
2. Brusselaers N, Vogelaers D, Blot S. The rising problem of antimicrobial resistance in the intensive care unit. Ann Intensive Care 2011;1:47.
3. Mehrad B, Clark NM, Zhanel GG, Lynch JP, 3rd. Antimicrobial resistance in hospital-acquired gram-negative bacterial infections. Chest 2015;147:1413-21.
4. Livermore DM. Of Pseudomonas, porins, pumps and carbapenems. J Antimicrob Chemother 2001;47:247-50.
5. Richet H, Fournier PE. Nosocomial infections caused by *Acinetobacter baumannii*: a major threat worldwide. Infect Control Hosp Epidemiol 2006;27:645-6.
6. Alp E. Surveillance methods. Turk J ICU 2007;5:35-6.
7. Ding JG, Sun QF, Li KC, et al. Retrospective analysis of nosocomial infections in the intensive care unit of a tertiary hospital in China during 2003 and 2007. BMC Infect Dis 2009;9:115.
8. Karlowsky JA, Draghi DC, Jones ME, et al. Surveillance for antimicrobial susceptibility among clinical isolates of P*seudomonas aeruginosa* and A*cinetobacter baumannii* from hospitalized patients in the united states, 1998 to 2001. Antimicrob Agents Chemother 2003;47:1681-8.
9. Demirdal T, Şen P, Yula E, ve ark. Yoğun bakım ünitelerinden izole edilen *Pseudomonas aeruginosa* suşlarının direnç profilleri: Beş yıllık değerlendirme. Ortadoğu Tıp Dergisi. 2017;9:108-12.
10. Dündar D, Tamer GS. Çeşitli klinik örneklerden izole edilen *Pseudomonas aeruginosa* suşlarının antimikrobiyal direnci: Üç yıllık değerlendirme. Ankem Derg 2009;23:17-21.
11. Şafak B, Kılınç O, Tunç N, ve ark. Türkiye'de bir devlet hastanesinde 2010-2016 yılları arasında *Pseudomonas aeruginosa* antimikrobiyal duyarlılık sonuçları. ANKEM Derg 2018;31:31-6.
12. Eroğlu C, Ünal N, Karadağ A, et al. Acinetobacter species isolated from various clinical specimens between 2006-2011 years and their susceptibilities against antibiotics. Turk Hij Den Biyol Derg 2016;73:25-32.
13. İnce N, Geyik MF, Özdemir D, et al. A Comparison of the Antibiotic Susceptibility Rates of *Pseudomonas aeruginosa* Strains Causing Hospital Acquired Infections According to Years. ANKEM Derg 2014;28:94-9.
14. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2015. Stockholm: ECDC 2017.
15. Slekovec C, Robert J, Trystram D, et al. *Pseudomonas aeruginosa* in French hospitals between 2001 and 2011: back to susceptibility. Eur J Clin Microbiol Infect Dis 2014;33:1713-7.
16. Hu FP, Guo Y, Zhu DM, et al. Resistance trends among clinical isolates in China reported from CHINET surveillance of bacterial resistance, 2005-2014. Clin Microbiol Infect 2016;22:9-14.
17. Uzun B, Güngör S, Sezak N, ve ark. Yoğun bakım hastalarının kan kültürlerinden izole edilen *Pseudomonas aeruginosa* ve *Acinetobacter baumannii* izolatlarının antibiyotik direnç yüzdelerindeki değişim. Turk Hij Den Biyol Derg 2014;71:1-8.
18. Hombach M, Bloemberg GV, Bottger EC. Effects of clinical breakpoint changes in CLSI guidelines 2010/2011 and EUCAST guidelines 2011 on antibiotic susceptibility test reporting of Gram-negative bacilli. J Antimicrob Chemother 2012;67:622-32.
19. Yuce A, Yapar N, Kutsoylu OE. 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. Mikrobiyol Bul 2009;43:195-202.
20. Xia WY, Chen Y, Mei YN, et al. Changing trend of antimicrobial resistance among pathogens isolated from lower respiratory tract at a university-affiliated hospital of China, 2006-2010. J Thorac Dis 2012;4:284-91.