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The Antibiotic Resistance Patterns of *Pseudomonas Aeruginosa* Strains Isolated from Microbiological Specimens

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Research Article

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ABSTRACT

To determine the antibiotic resistance rates of *Pseudomonas aeruginosa* strains isolated from samples collected by the microbiology laboratory of a single center for four years. The samples of wound, blood, tracheal aspirate, abscess, vagina, cerebrospinal fluid, sputum, and urine culture isolated from 789 patients who were hospitalized in the service, intensive care and outpatient services between 2017-2020 were evaluated retrospectively for *Pseudomonas* species. Most of culture was urine (42.7%) and sputum cultures (20.4%). Most patients applied to the service were from department of chest diseases (38.6%) or from department of urology (14.3%) or palliative care unit (12.5%). The highest resistances were against cefuroxime, levofloxacin and netilmicin; lowest resistance was against amikacin. The aztreonam, cefepime and gentamicin resistances were significantly reduced by years (P=0.0321, 0.0038 and 0.0004, respectively) while colistin and levofloxacin resistance considerably increased (P<0.0001 and P=0.0407, respectively). Significant decreases were observed in resistance against cefepime, ceftazidime and ciprofloxacin over years (P=0.0321, 0.0038 and 0.0004, respectively). A significant decrease in resistance of strains isolated from urine culture was only observed for cefepime over years (P=0.0003). The resistance of strains isolated from cultures of sputum, urine and respiratory secretions against levofloxacin significantly increased in 2019 while those of wound culture increased in 2020 (P=0.0145). Alterations in the antibiotic resistance profile were detected in patients over years due to frequently varied use of antimicrobials.

INRODUCTION

Pseudomonas aeruginosa is one of the most common gram negative bacteria causing the hospital and other healthcare associated infections in hospitalized patients [1]. It is of particular importance due to being one of the main causes of morbidity and mortality in immunocompromised patients, the leading nosocomial pathogens affecting the hospitalized patients, and is intrinsically resistant to a wide variety of antibiotics [2-4].

P. aeruginosa is an organism naturally found in many different environments, however, it can be isolated from a variety of living sources such as animals, plants and human beings. The survival of *P. aeruginosa* with the minimal nutritional requirements and its ability to tolerate a variety of physical conditions has been the reasons for its viability in both population and hospital settings. In hospital settings, *P. aeruginosa* can be isolated from various sources such as respiratory therapy equipment, antiseptics, soap, washbasins, mats, medicines, etc [5]. Due to its adaptive nature and high survivability, it can survive from 6 hours to 6 months on dry inanimate surfaces in a hospital setting [6]. In last decade, the antibiotic resistance rates have been increasing due to the unconscious use of inappropriate and broad spectrum antibiotics and the lack of appropriate disinfection methods. More than 2.8 million antibiotic resistant infections occur in the U.S. each year, and more than 35,000 people die as a result [7]. In a recent study from Turkey, the overall mortality of *P. aeruginosa* infections in a hospital was found as 46% and it was 68% for carbapenem

resistant colistin susceptible *P. aeruginosa* infections among patients hospitalized the Intensive Care Unit (ICU) [8]. Although common prevention and infection control efforts reduced deaths from antibiotic resistant infections in hospitals, there is limited amount of data on the magnitude of this issue for Turkey [9]. Therefore, in this study, we aimed to determine the antibiotic resistance rates of *P. Aeruginosa* strains isolated from the samples collected by the microbiology laboratory of a single center for four years.

MATERIALS AND METHODS

This retrospective study involving the data of human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval was granted by non-interventional research ethics committee of Bandirma Onyedi Eylul university health sciences.

The samples of wound, blood, tracheal aspirate, abscess, vagina, cerebrospinal fluid, sputum, and urine culture isolated from 789 patients who were hospitalized in the service, ICU and received outpatient services in our hospital between 2017-2020 were evaluated retrospectively for *Pseudomonas* species. Urine samples from different clinical specimens collected from patients were inoculated on 5% sheep blood agar, and Eosin-Methylene Blue (EMB) agar media (RTA Laboratories, Gebze, Turkey). Other clinical specimens were inoculated on 5% sheep blood agar, EMB agar and chocolate (CHOC) agar plates and incubated at 37°C for 24 hours. At the end of incubation, the bacterial growths were evaluated by the presence and type of colony and the culture samples considered appropriate to be included in the antibiogram were selected for the study for the bacterial identification. Samples taken from the patients who were contaminated, the patients whose culture findings could not be reached, and who were younger than 18 years of age were not included in the study.

Blood samples were inoculated into the blood culture bottles (Render C/Horacio Lengo N 18, Malaga, Spain) and incubated in an automated system (RENDER BC128, automated blood culture systems, Jinan, Shandong, China). Samples that gave a growth signal within five days were pre-identified by a gram staining. After pre identification, the samples were inoculated on 5% sheep blood agar, chocolate agar and EMB agar media and incubated between 35.5°C -37°C for 18-24 hours.

At the end of 24 hours of incubation of all specimens belonging to 801 patients, the conventional methods (gram staining, oxidase test, fermentation feature) were used to identify the Pseudomonas isolates at species level in the growths detected, and the antibiotic resistance rates were determined by using of Phoenix 100 (Becton Dickinson, Sparks, Md, BD) automated system. Drug susceptibilities were categorized by the automated device after 24 hours. The results were evaluated according to the recommendations of the "European Committee on Antimicrobial Susceptibility Testing (EUCAST)" [10].

Statistical method

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for the statistical analysis. The study data were evaluated with the descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum), and the Pearson *chi-square* test was used to compare the qualitative data. The significance was determined at $p<0.05$ level.

RESULTS

Pseudomonas aeruginosa strains isolated from 789 patients who applied to our hospital between 2017-2020 were included in the study. 63.5% of the participants were male (n=501) and 36.5% were female (n=288). The ages of the participants ranged from 18 to 103, with a mean of 68.90 ± 15.12 years. The distribution of units sending the culture samples showed that 35.5% of patients were hospitalized in the service (n=280), 22.7% applied to the polyclinic units (n=179), and 41.8% were hospitalized in the intensive care units (n=330).

The distribution of types of microbiological cultures showed that most of the culture was the urine cultures (42.7%) and sputum cultures (20.4%). The least frequent types of cultures were the catheter tip culture (0.4%), peritoneal fluid culture (0.4%) and Cerebro Spinal Fluid (CSF) culture (0.3%). Only one vaginal secretion and only one pleura fluid were cultured for the microbiological examination (Tables 1 and 2).

Table 1. The distribution of types of microbiological cultures.

Type of culture	n (%)
Abscess culture	10 (1.3)
Sputum culture	161 (20.4)
Urine culture	337 (42.7)
Blood culture	35 (4.4)

Catheter tip culture	3 (0.4)
Ear culture	15 (1.9)
Peritoneal fluid culture	3 (0.4)
Pleural fluid culture	1 (0.1)
CSF culture	2 (0.3)
Quantitative cultures of respiratory secretions	121 (15.3)
Vaginal secretion culture	1 (0.1)
Wound culture	100 (12.7)
Total	789 (100.0)
CSF: Cerebro Spinal Fluid	

Table 2. The detailed distribution of units according to the medical departments.

	Service (n=280)		Polyclinic (n=179)		Intensive care unit (n=330)	
Department	**n**	**%**	**n**	**%**	**n**	**%**
General surgery	18	6.4	15	8.4	0	0
Urology	40	14.3	76	42.5	0	0
Chest diseases	108	38.6	25	14	0	0
Intensive care unit	0	0	0	0	330	100
Home health care	0	0	21	11.7	0	0
Internal diseases	25	8.9	4	2.2	0	0
Orthopedics	11	3.9	13	7.2	0	0
Palliative care	35	12.5	0	0	0	0
Otorhinolaryngology	1	0.4	12	6.7	0	0
Infectious diseases	5	1.8	7	3.9	0	0
Neurology	11	3.9	0	0	0	0
Cardiology	5	1.8	1	0.6	0	0
Emergency	5	1.8	0	0	0	0
Gynecological diseases	0	0	4	2.2	0	0
Cardiovascular surgery	5	1.8	0	0	0	0
Neurosurgery	5	1.8	1	0.6	0	0
Hemodialysis	3	1.1	0	0	0	0
Physiotherapy	2	0.7	0	0	0	0
Psychiatry	1	0.4	0	0	0	0

Table 2 represents the units where microbiological samples were collected in detail. The majority of the patients applied to the service were from the department of chest diseases (38.6%) or from the department of urology (14.3%) or palliative care unit (12.5%). The majority of patients who applied to the polyclinics were from the department of urology (42.5%), or the department of chest diseases (14%) or the home care unit (11.7%). The percentage of intensive care patients whose microbiological samples were collected was 41.8% of all patients.

The distribution of findings of antibiotics resistance tests performed for the *Pseudomonas aeruginosa* compared by years is shown in Table 3. Totally, the highest resistances of the organism to antibiotics evaluated were against the cefuroxime, levofloxacin and netilmicin. The lowest resistance was against the amikacin. The amikacin resistance of the organism was highest in 2019 and lowest in 2020 (P=0.0107). The aztreonam, cefepime and gentamicin resistances of the organism were significantly reduced by years (P=0.0321, 0.0038 and 0.0004, respectively) while the colistin and levofloxacin resistance considerably increased (P<0.0001 and P=0.0407, respectively). The piperacillin/tazobactam resistance was highest in 2018 and lowest in 2020 (P=0.0148). The resistances to the other antibiotics, namely ciprofloxacin, imipenem, meropenem and netilmicin did not change significantly in years (Table 3).

Table 3. The distribution of antibiotics resistance findings of the *Pseudomonas aeruginosa* organism presented by years.

	2017		2018		2019		2020		Total		
Antibiotic type	n/Total	%	n/Total	%	n/Total	%	n/Total	%	n/Total	%	P value
Amikacin	9/177	5.1	13/215	6	29/242	12	8/169	4.7	59	7.3	0.0107
Aztreonam	63/145	43.4	83/183	45.4	6/30	20	0/0	0	152	42.5	0.0321
Cefepime	69/174	39.7	85/212	40.1	35/136	25.7	24/96	25	213	34.5	0.0038
Cefuroxime	89/89	100	98/98	100	101/101	100	81/81	100	369	100	-
Ciprofloxacin	75/175	42.9	107/214	50	108/243	44.4	75/169	44.4	365	45.6	0.5819
Colistin	10/150	6.7	24/71	33.8	11/11	100	3/3	100	48	20.4	<0.0001
Gentamicin	57/181	31.5	62/214	29	57/242	23.6	23/170	13.5	199	24.7	0.0004
Imipenem	44/171	25.7	48/209	23	47/241	19.5	42/166	25.3	181	23	0.4102
Levofloxacin	2/12	16.7	0/0	0	106/205	51.7	92/168	54.8	200	51.9	0.0407
Meropenem	40/170	23.5	45/210	21.4	48/240	20	30/167	18	163	20.7	0.6304
Netilmicin	51/97	52.6	44/101	43.6	7/20	35	0/0	0	102	46.8	0.2413
Piperacillin/ Tazobactam	43/180	23.9	58/215	27	45/245	18.4	28/168	16.7	174	21.5	0.0148
Chi-squared test for independence											

The distribution of antibiotic resistance of *Pseudomonas aeruginosa* organism in different culture types presented by years is presented in Table 4. Considering the antimicrobial resistance of *Pseudomonas aeruginosa* strains isolated from the cultures of sputum, wound and respiratory secretions, significant decreases were observed in the resistance profile against the cefepime, ceftazidime and ciprofloxacin over the years (P=0.0003, 0.0422 and 0.0335, respectively). A significant decrease in the antimicrobial resistance of strains isolated from the urine culture was only observed in the resistance profile against the cefepime over the years (P=0.0003). The resistance profile of strains isolated from the cultures of sputum, urine and respiratory secretions against the levofloxacin significantly increased in 2019 while those of wound culture significantly increased in 2020 (P=0.0145). The distribution of antibiotic resistance of organism against amikacin, aztreonam, colistin, gentamicin, imipenem and piperacillin/tazobactam did not altered according to the different culture types by years.

Table 4. The distribution of antibiotic resistance of *Pseudomonas aeruginosa* organism in different culture types presented by years.

Cultures	Years	Sputum culture		Urine culture		Quantitative culture of respiratory secretions		Wound culture		P value
		n	%	n	%	n	%	n	%	
Amikacin	2017	4	22.2	2	9.5	2	20	1	20	0.8406
	2018	5	27.8	4	19	1	10	2	40	
	2019	7	38.9	11	52.4	6	60	2	40	
	2020	2	11.1	4	19	1	10	0	0	
Aztreonam	2017	18	46.2	18	33.3	11	40.7	7	41.2	0.7177
	2018	19	48.7	35	64.8	14	51.9	9	52.9	
	2019	2	5.1	1	1.9	2	7.4	1	5.9	
Cefepime	2017	18	35.3	20	32.8	15	34.9	8	25	0.0003
	2018	15	29.4	39	63.9	12	27.9	12	37.5	
	2019	12	23.5	1	1.6	11	25.6	6	18.8	
	2020	6	11.8	1	1.6	5	11.6	6	18.8	
Ceftazidime	2017	21	38.2	15	15	15	31.3	9	33.3	0.0422
	2018	17	30.9	29	29	15	31.3	8	29.6	
	2019	13	23.6	34	34	13	27.1	5	18.5	
	2020	4	7.3	22	22	5	10.4	5	18.5	
Ciprofloxacin	2017	23	34.8	18	12.9	15	22.7	8	17	0.0335
	2018	19	28.8	47	33.6	16	24.2	13	27.7	
	2019	18	27.3	42	30	20	30.3	15	31.9	
	2020	6	9.1	33	23.6	15	22.7	11	23.4	
Colistin	2017	2	14.3	4	36.4	2	18.2	2	20	0.3074
	2018	7	50	7	63.6	7	63.6	3	30	
	2019	4	28.6	0	0	2	18.2	3	30	
	2020	1	7.1	0	0	0	0	2	20	
Gentamicin	2017	18	36	12	17.4	13	37.1	6	27.3	0.3649
	2018	15	30	27	39.1	7	20	8	36.4	
	2019	12	24	24	34.8	11	31.4	5	22.7	

	2020	5	10	6	8.7	4	11.4	3	13.6	
Imipenem	2017	13	31.7	10	17.9	11	28.2	5	25	0.3763
	2018	10	24.4	19	33.9	12	30.8	3	15	
	2019	12	29.3	12	21.4	11	28.2	5	25	
	2020	6	14.6	15	26.8	5	12.8	7	35	
Levofloxacin	2017	2	8	0	0	0	0	0	0	0.0145
	2019	16	64	44	54.3	21	56.8	11	40.7	
	2020	7	28	37	45.7	16	43.2	16	59.3	
Piperacillin/ Tazobactam	2017	13	29.5	13	19.7	5	19.2	6	33.3	0.1577
	2018	13	29.5	22	33.3	10	38.5	9	50	
	2019	15	34.1	15	22.7	6	23.1	2	11.1	
	2020	3	6.8	16	24.2	5	19.2	1	5.6	
Chi-squared test for independence										

DISCUSSION

Pseudomonas aeruginosa represents a notorious pathogen of nosocomial infections often characterized by Multi Drug Resistant (MDR), especially among the hospitalized patients [11]. The success of treatment is seriously challenged by the intrinsic and adaptive resistance of organisms to nearly all available antipseudomonal antibiotics. Therefore, understanding the antibiotic resistance profile of *Pseudomonas aeruginosa* in a population is crucial and the combination therapy is suggested in the treatment of infections caused by *P. aeruginosa* to prevent the development of resistance [12]. In the present study, we investigated the bacterial isolates that were repetitively derived from 789 patients treated or hospitalized in our clinic to study changes to antimicrobial susceptibility in response to antibiotic therapy in years. For all *P. aeruginosa* isolates analyzed for four years, we found very high levels of drug resistance to the cefuroxime, levofloxacin and netilmicin and lowest resistance was against the amikacin. In addition, the aztreonam, cefepime and gentamicin resistances of the organism were significantly reduced by years while the colistin and levofloxacin resistance considerably increased.

In a very recent study by Gysin, et al., antimicrobial susceptibility patterns of respiratory gram negative bacterial isolates from COVID-19 patients in Switzerland were investigated and a high proportion of P. aeruginosa isolates was found to be resistant to the standard of care antibiotics cefepime (56.3%), ceftazidime (46.9%), and meropenem (50.0%). The resistance to piperacillin/tazobactam (65.6%) was the highest for any of the relevant drugs tested. Resistance to ciprofloxacin was comparatively low in P. aeruginosa isolates (15.6%). We found lower ratios of resistance to cefepime (34.5%), meropenem (20.7%) and piperacillin/tazobactam (21.5%) which decreased in time dependent manner while resistance to ciprofloxacin (45.6%) was higher than the above-mentioned study but did not change significantly by years [13].

Aminoglycosides are a group of antibiotics primarily used to treat a wide spectrum of bacterial infections. Of the numerous aminoglycosides known to date, amikacin, gentamicin, neomycin, streptomycin, kanamycin, paromomycin, and tobramycin are approved by the US Food and Drug Administration (FDA) for clinical use [14]. Amikacin is a semi-synthetic, widely sued aminoglycoside antibiotic which is active against a broad spectrum of gram negative organisms, including pseudomonas and some gram positive organisms [15]. Since amikacin is affected by a small number of aminoglycoside modifying enzymes, it is more effective in *P. aeruginosa* infections than other aminoglycosides [16]. In studies, amikacin resistance was found to be between 1-30% [17]. In a study by Tozlu Keten, et al., amikacin resistance (30%) was found to be less than ciprofloxacin (48%) and piperacillin tazobactam (32%) resistance [18]. In our study, the total amikacin resistance of *Pseudomonas aeruginosa* was measured as 7.3%, and the ratio was highest in 2019 (12%) One main reason for the low antibiotic resistance against amikacin may be that it is not

frequently preferred in the empirical treatment.
The resistance of *P. aeruginosa* to gentamicin owing to enzymatic N-acetylation has been known for a long time [19]. The gentamicin resistance from various hospitals is examined, a resistance profile varying between 4 and 51% was observed in the literature [20]. In a study by Varisli, et al., gentamicin resistance of *P. aeruginosa* isolates increased from 13.2% to 21% in the wound samples of patients who applied to outpatient clinics. In our study, the gentamicin resistance of *P. aeruginosa* significantly decreased in four years from 31.5% to 13.5%, probably due to the less frequent usage in the clinic.
Fluoroquinolones including ciprofloxacin and levofloxacin are frequently used in the empirical treatment of *P. aeruginosa* infections. In a study by Varisli, et al., the antibiotic resistance rates of *Pseudomonas aeruginosa* strains isolated from clinical specimens were also examined by years and no increase was observed in the ciprofloxacin resistance of abscess cultures in outpatients between 2011 and 2015, while the resistance increased in urine cultures from 14.2% to 20.3% in the same years. They also found that ciprofloxacin resistance of *P. aeruginosa* was between 22-25% in abscess culture and between 22-25.4% in wound culture. In a study by Eyigor, et al., the highest resistance among antibiotics was found against ciprofloxacin (16%). Another study by Ozturk, et al. found the ratio as 15% [21]. Other studies performed in Turkish population showed that ciprofloxacin resistance was in the range of 7.2-47% [22,23]. In our study, the total antimicrobial resistance of *P aeruginosa* to ciprofloxacin was 45.6% and our data are compatible with the literature. The resistance of strains isolated from the cultures of sputum significantly decreased by years, but total resistance profile did not change over the years. On the other hand, the resistance profile of strains isolated from the cultures of sputum, urine and respiratory secretions against the levofloxacin significantly increased in 2019 while those of wound culture significantly increased in 2020. The reason for this situation may be the frequent use of quinolone group antibiotics in the empirical treatment of urinary tract infections of outpatients since the most frequent microbiological samples were collected from urine cultures. The relatively high ciprofloxacin resistance suggests that it would be appropriate to apply these fluoroquinolones in combination with a beta-lactam [24,25].

CONCLUSION

Carbapenems are broad spectrum beta-lactam antibiotics and are not affected by many hydrolyzing enzymes. The overall mortality of *P. aeruginosa* infections in the hospital was found to increase for carbapenem resistant colistin susceptible *P. aeruginosa* infections. Considering t the studies conducted in our country, it is observed that the well-known carbapenem, imipenem resistance of *P. aeruginosa* isolates varies by between 3-53% Varisli, et al. reported that the resistance rate of *P. aeruginosa* strains against the imipenem and meropenem determined by EUCAST criteria in the hospitalized patients decreased significantly from 31% to 29% in years. Tozlu Keten, et al. found imipenem resistance determined by EUCAST criteria as 31%, and meropenem resistance as 27%. Determined by EUCAST criteria, we found the imipenem resistance of all *P. aeruginosa* isolates as 23%, and the meropenem resistance as 20.7% both of which did not change significantly in years. Out data were in agreement with the literature.
Piperacillin-tazobactam is frequently used to treat *Pseudomonas aeruginosa* infections in critically ill patients. In a study, the percentage of piperacillin-tazobactam resistance of *P. aeruginosa* isolates was found to be 39% according to EUCAST criteria. In our study, the total resistance against piperacillin-tazobactam was 21.5% which was lower than before mentioned study, suggesting the difference in the patient population and type of diseases.
Continuous changes in antimicrobial drug resistance in patients isolated from *P. aeruginosa* complicate the empirical treatment options. Therefore, in order to determine effective treatment protocols, it is necessary to regularly examine the microorganisms and their antibiotic resistance for each hospital. As a result of the evaluations, an alteration in the antibiotic resistance profile was detected in patients over the years due to the frequently varied use of antimicrobials. The combination of aminoglycosides with antipseudomonal beta-lactams or quinolones is considered appropriate in outpatients for the empirical treatment of *P. aeruginosa* infections. As a result, timely and accurate interpretation of antibiotic susceptibility tests has been found to be extremely crucial for the success of antimicrobial treatments.

STATEMENTS AND DECLARATIONS

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

Author contributions
The author contributed to the study conception and design. The data collection and analysis were performed by DKT. The first draft of the manuscript was written by DKT and DKT commented on previous versions of the manuscript. DKT read and approved the final manuscript.

Ethics approval and consent to participate

This retrospective study involving the data of human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval was granted by non-interventional research ethics committee of Bandirma Onyedi Eylul university health sciences.

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