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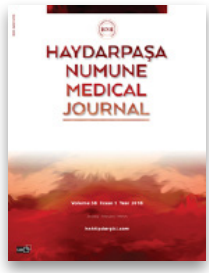

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ORIGINAL ARTICLE

Frequencies and Antibiotic Susceptibilities of *Pseudomonas spp.* and *Acinetobacter spp.* Isolated from Blood Culture: A 7-year Trend Analysis of *Pseudomonas spp.* and *Acinetobacter spp.* Bacteremias

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Abstract

Introduction: In this study, we aimed to investigate the changes in the frequencies and antibiotic susceptibilities of Pseudomonas spp and Acinetobacter spp isolated from blood cultures in our hospital.

Methods: Results of blood cultures, which were obtained from inpatients of our hospital and accepted by our microbiology laboratory between January 1st, 2013 and November 1st, 2019, were retrospectively searched. Automated blood culture system BACTEC FX (Becton–Dickinson, USA) was used. Identification and susceptibility tests of the strains were made using VITEK MS MALDI-TOF (bioMérieux, USA) and VITEK® 2 Compac automated system; (Biomerieux, French).

Results: Bacterial growth was detected in 20.5% of the total 21,367 blood cultures. Of the positive cultures, 263 (5.9%) were Pseudomonas spp. and 254 (5.7%) were Acinetobacter spp. The frequency of Pseudomonas spp. in the blood cultures over the years did not change (p=0.2), whereas the frequency of Acinetobacter spp decreased (p=0.004). Tigecycline resistance of Acinetobacter spp increased over the years (p=0.0005). However, ceftazidime and amikacin resistance of Pseudomonas spp decreased over the years (p=0.01 and p=0.04, respectively).

Discussion and Conclusion: Multidrug resistance among Gram-negative bacteria is an increasing problem. Estimating the probable resistance pattern of especially Acinetobacter and Pseudomonas infections is difficult. Thus, in our opinion, empirical treatment strategies should be defined by local and national studies.

Keywords: Acinetobacter; blood culture; pseudomonas.

Bloodstream infections [1] are major causes of mortality and morbidity despite effective antimicrobial therapy. Both healthcare-associated and community-acquired Gram-negative bacteremias are critical health problems because of increasing multi-resistance[2,3]. Gram-negative bacteremias have increased worldwide parallel to the increase in invasive interventions in intensive care units recently[4]. This increase is particularly present in catheter-related bloodstream infections that arise from Gram-negative bacteria[5]. Gram-negative bloodstream infections can be troublesome due to high resistance against antibiotics in empiric treatment. Regional surveillance data may be helpful in conditions in which prompt administration of antibiotics is necessary, such as bloodstream infections.

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Especially in bacteremias caused by non-fermentative Gram-negative rods, such as *Pseudomonas spp*. and *Acinetobacter spp*., in which multidrug resistance is a big challenge, available surveillance data affects the treatment success[6]. In this study, we aimed to investigate the trends in frequencies and antibiotic susceptibilities of *Pseudomonas spp*. and *Acinetobacter spp*., which were isolated from the blood cultures of our inpatients.

Materials and Methods

We investigated the blood cultures of our inpatients between January 1st, 2013, and November 1st, 2019, retrospectively. First blood culture was collected in patients who had multiple blood cultures. We used an automated blood culture system BACTEC FX (Becton–Dickinson, USA). Specimens that signaled reproduction were inoculated into four quadrants of the medium using sterile standard loops using the dilution method on the surface of 5% sheep blood agar (Becton Dickinson, USA) and MacConkey agar (Standard media, Turkey) and chocolate agar (Becton Dickinson, USA). The inoculated medium plates were incubated aerobically for 24-48 hours in a CO_2 incubator at a temperature of 35±2 °C. Colonies with bacterial growth were processed for further identification. Bacterial identification and antibiotic susceptibility of the strains were performed using VITEK MS MALDI-TOF (bioMérieux, USA) and VITEK® 2 Compac automated system (Biomerieux, France). A strain which was found to be moderately sensitive to an antibiotic as a result of the antimicrobial susceptibility test was accepted as resistant to that antibiotic. Antimicrobial susceptibility tests were evaluated according to the "Clinical and Laboratory Standards Institute" (CLSI) criteria before 2015 and the "European Committee on Antimicrobial Susceptibility Testing" (EUCAST) criteria after 2015. Colistin susceptibility test was performed with colistin sulfate (Sigma Aldrich, Germany) in microplates, which were prepared in double-fold dilutions between 128 and 0.125 mg/L, using ISO standard liquid microdilution method according to EUCAST and CLSI recommendations (4 mg/L for Pseudomonas aeruginosa and ≥2 mg/L for other Gram-negative bacteria).

Statistical Analyses

Statistical Package for the Social Sciences (SPSS)-17 (SPSS Inc.; Chicago, IL, USA) was used for calculations. Categorical variables were presented as percentages and numbers. Non-normally distributed ages of the patients were presented as median (IQR, minimum-maximum). Trends in microorganisms and their antibiotic susceptibilities were analyzed by chi-square Mantel-Haenszel linear-by-linear trend analysis and Fisher exact correction was made when needed. A p-value <0.05 was considered statistically significant.

Results

A total of 21,367 blood cultures were accepted to our microbiology laboratory between January 1st, 2013, and November 1st, 2019. Of those, 4,386 (20.5%) were positive. In positive cultures, growing bacteria were Pseudomonas spp. in 263 (5.9%) samples and *Acinetobacter spp*. in 254 (5.7%) samples (Table 1). Of these cultures, 319 (61.7%) were from intensive care unit, 75 (14.5%) were from internal medicine clinics (except Hematology-Oncology clinic), 51 (9.9%) were from pediatrics, 35 (6.8%) were from surgery clinics, 21 (4.1%) were from Hematology-Oncology clinic, and 16 (3.1%) were from the emergency department. Pseudomonas spp. frequency was significantly higher in blood cultures from surgical clinics (p=0.0001).

Pseudomonas spp. frequency in blood cultures did not change in years (p=0.02). However, a significant decrease in the frequency of *Acinetobacter spp*. was present in years (p=0.004). In addition, the total antibiotic resistance of *Acinetobacter spp*. decreased (p=0.04) in contrast to increasing resistance to tigecycline in years (p=0.0005). Co-trimoxazole resistance decreased between 2013 and 2019 (p=0.006) (Table 2). The total antibiotic resistance of Pseudomonas strains did not change significantly in years (p=1). Resistance to ceftazidime and amikacin decreased in years (p=0.01 and p=0.04, respectively) (Table 3).

Table 1. Between 2013-2019, *Pseudomonas spp.* and *Acinetobacter spp.* trend analysis of frequency

	2013 n (%)	2014 n (%)	2015 n (%)	2016 n (%)	2017 n (%)	2018 n (%)	2019 n (%)	Total n (%)	p	Trend
Pseudomonas spp.	45 (17.1)	36 (13.7)	43 (16.4)	33 (12.5)	19 (7.2)	47 (17.9)	40 (15.2)	263 (5.9)	0.2	-
Acinetobacter spp.	49 (19.3)	60 (23.6)	31 (12.2)	25 (9.9)	28 (11)	34 (13.4)	27 (10.6)	254 (5.7)	0.004*	⬇
Positive blood culture	696	699	637	576	471	622	685	4.386 (20.5)		
Total blood culture	2.513	3.102	3.075	3.046	2.793	3.082	3.756	21.367		

Table 2. Between 2013 and 2019, *Acinetobacter spp*. trend analysis of antibiotic resistance rates

	2013	2014	2015	2016	2017	2018	2019	p	Trend
Ceftazidime	87.7	96.6	83.8	83.3	96.4	88.2	85.1	0.19	-
Piperacillin-tazobactam	89.1	91.1	80.6	84	96.4	91.1	81.4	0.41	-
Imipenem	89.8	86.6	77.4	80	96.4	85.2	88	0.37	-
Meropenem	89.8	86.6	77.4	80	96.4	85.2	88	0.37	-
Ciprofloxacin	91.8	86.6	77.4	76	96.4	79.4	81.4	0.13	-
Levofloxacin	91.8	86.6	77.4	76	96.4	82.3	88	0.17	-
Amikacin	65.3	63.3	38.7	52	46.4	61.7	55.5	0.2	-
Colistin	0	1.6	3.3	0	7.6	3.1	4.1	0.42	-
Tigecycline	27	11.6	9.6	47.8	29.6	44	40	0.0005*	⬆
Co-trimaxazole	56.2	83.3	67.7	58.3	42.8	62.5	66.6	0.006*	⬇
Total	69	69	59.5	64.3	71.1	69.4	68.3	0.04*	⬆

Table 3. Between 2013-2019, *Pseudomonas spp*. trend analysis of antibiotic resistance rates

	2013	2014	2015	2016	2017	2018	2019	p	Trend
Ceftazidime	35.5	47.2	20.9	28.1	52.6	51.1	27.5	0.01*	⬇
Piperacillin-tazobactam	42.2	61.1	41.8	40.6	63.1	54.5	37.5	0.19	-
Imipenem	31.1	47.2	37.2	40.6	57.8	48.8	47.5	0.39	-
Meropenem	31.1	45.9	37.2	40.6	57.8	50	45	0.4	-
Ciprofloxacin	31.1	30.5	30.2	21.2	47.3	47.8	30	0.19	-
Levofloxacin	31.1	30.5	30.2	24.2	50	47.8	30	0.23	-
Amikacin	28.8	22.2	9.3	12.1	21	8.8	7.5	0.04*	⬇
Colistin	0	6.06	2.3	6.06	0	0	0	0.14	-
Total	28.8	36.4	26.2	26.5	44	38.8	28.2	1	-

Discussion

We investigated *Pseudomonas spp*. and *Acinetobacter spp*. isolated from blood cultures in our Microbiology laboratory and their resistance trends in the last seven years.

Isolation of Pseudomonas in blood cultures is of critical importance because it causes nosocomial infections in especially immunocompromised patients. It is generally resistant to antibiotics; therefore, selecting the appropriate treatment regimen is difficult and the disease has a high mortality. In a study from the USA, it is reported that *Pseudomonas spp*. are responsible for 64% of 24.179 nosocomial bacteremias, and they are the third frequent type among the Gram-negative bacteria[7]. In our study, *Pseudomonas spp*. were 5.9% of the total blood culture isolates, but the proportion of community-acquired ones to healthcare-associated ones was not available. The ratio of *Pseudomonas spp*. in blood culture isolates did not change in years. Albrecht et al. isolated *Pseudomonas spp*. in 14.8% of blood cultures and the ratio did not change between 1996 and 2003. All positive blood cultures were healthcare-associated in this study[5]. Diekema et al.[6] demonstrated no change in frequency of *Pseudomonas spp*. bacteremias in years in 20-year data of the "SENTRY" antimicrobial surveillance program. Gandra et al.[8] from India reported no change in the frequency of *Pseudomonas spp*. among positive blood cultures between 2008 and 2014 in their countrywide study. However, Babaei et al.[9] showed an increase in *Pseudomonas spp*. caused bacteremias between 2010 and 2016. A study from our country reported an increase in *P. aeruginosa* bacteremias between 2013 and 2017[10].

An increase in antibiotic resistance was observed over the years. Especially in Pseudomonas and *Acinetobacter spp*., multidrug resistance became a severe problem. We did not find out an increase in total antibiotic resistance among *Pseudomonas spp*., but we observed a decrease in resistance to ceftazidime and amikacin. In the SCOPE study between 1995 and 2002, an increase in resistance to ceftazidime in *P. aeruginosa* strains was reported[7]. However, decreasing resistance to ceftazidime was reported among *P. aeruginosa* in a study from our country between 2004 and 2011[11]. Gandra et al.[8] showed a decreasing resistance to ceftazidime among *P. aeruginosa* strains in their study

between 2008 and 2014. Shortridge et al. demonstrated a higher than 20% resistance rate to ceftazidime among *P. aeruginosa* strains in all years in their study investigating antibiotic microbial surveillance data between 1997 and 2016[6]. In our study, nearly half of *Pseudomonas spp*. was resistant to carbapenems. Therefore, carbapenems should not be a good option in the empirical treatment of the *Pseudomonas spp*. infections in our hospital. In a study from -a neighboring country- Iran, a decrease in meropenem resistance between 2010 and 2016 was reported[8]. In a study from our country, a two-fold increase of up to 50% in carbapenem resistance was demonstrated[11]. An increasing carbapenem resistance between 2012 and 2016 was shown in a meta-analysis performed by Acar et al.[12]. In addition, an increase in amikacin resistance was reported in the same study. "SENTRY Program", in which more than 200 centers worldwide and centers from Turkey are included, is an ongoing surveillance program since 1997. In the study by Diekema et al.[6] investigating "SENTRY Program" surveillance data, amikacin resistance is reported in 10% of 14.559 *P. aeruginosa* strains between 1997 and 2016. In our study, a downward trend in amikacin resistance of Pseudomonas strains was seen and amikacin resistance decreased below 10% in years. Additionally, we did not detect colistin resistance in the last three years. Diekema et al.[6] reported a colistin resistance lower than 1% among *P. aeruginosa* strains in 20 years. In a meta-analysis from our country, the authors advocated that colistin was an effective alternative against multidrug-resistant *P. aeruginosa* strains in the last years, despite several reports indicating a substantial increase in colistin resistance between 2012 and 2016[12]. They reported that the use of colistin might be restricted in the future because of increasing resistance. Antibiotic Resistance Threats in the USA, 2013 by CDC demonstrated a multidrug resistance rate of 13% among *Pseudomonas spp*.[13]. However, the 2019 report indicated a substantial decrease in multidrug resistance among *Pseudomonas spp*.[14]. We think this decrease might be a result of rational antibiotic use.

Some risk factors for bacteremias caused by *Acinetobacter spp*. are intensive care unit stay, mechanical ventilation support, previous surgery, use of wide-spectrum antibiotics, immunosuppression, trauma, burns, malignancies, indwelling catheters, invasive interventions, and prolonged hospital stay[15,16]. The incidence could be changed seasonally, and especially it could increase in summer with high temperatures and humidity[15].

Of isolated microorganisms from blood cultures, 5.7% were *Acinetobacter spp*. in our study. Akcay et al.[11] demonstrated a decrease in *Acinetobacter spp*. among isolates from blood cultures between 2004 and 2011. In another study from our country, 11.2% of isolates from blood cultures were Acinetobacter baumannii and the ratio was increased recently, but it did not show an upward trend in years[10]. Similarly, Gandra et al.[8] did not report an increase in A. baumannii isolation from blood cultures between 2008 and 2014. In our study, we found a decrease in A. baumannii isolation from blood cultures in years.

In the "Central Asian and European Surveillance of Antimicrobial Resistance" 2017 report, which was published by the WHO Regional Office for Europe and included the Republic of Turkey Ministry of Health national surveillance data, carbapenem resistance of *Acinetobacter spp*. isolated from cerebrospinal fluid and blood cultures was 93%[17]. Carbapenem resistance of *Acinetobacter spp*. decreased between 2012 and 2017 in CDC 2019 report[14]. Muderris et al.[10] showed a decreasing carbapenem-resistance among *Acinetobacter spp*. between 2013 and 2017. However, Akcay et al.[11] demonstrated an increase in carbapenem and quinolone-resistance between 2004 and 2011. Babaet et al.[8] showed that meropenem-resistance among Acinetobacter strains was above 85% and they reported colistin-resistant strains between 2014 and 2016. In the same study, amikacin-resistant strains were above 80%, especially with an increase between 2014 and 2016. Colistin-resistance among *Acinetobacter spp*. was 7.6% in a study analyzing SENTRY surveillance data between 2014 and 2018. It is indicated as one of the most effective antibiotics in this study[18]. In another study investigating SENTRY surveillance data, susceptibility to colistin was 96.9% among Acinetobacter strains isolated from blood cultures between 1996 and 2016. However, increased resistance against colistin was reported in SENTRY data in 2018[6].

In the SCOPE study, bacteremias caused by A. baumannii were more frequent in intensive care units than those in wards[7]. However, Wisplinghoff et al.[7] did not find a difference in frequencies of bacteremias caused by A. baumannii between intensive care units and in wards. In our study, frequencies of bacteremias caused by Acinetobacter were not different between intensive care units and wards, but bacteremias caused by Pseudomonas were more common in surgery clinics. Muderris et al.[10] reported that bacteremias caused by Acinetobacter and Pseudomonas were more common in intensive care units.

In conclusion, *Acinetobacter spp*. and *Pseudomonas spp*. are the major causes of healthcare-associated bacteremias. Because of the high likelihood of multidrug resistance in

both agents, the selection of an empirical treatment regimen might be challenging. Therefore, empirical treatment strategies should be determined by local and national studies. In the presence of risk factors for *Pseudomonas spp*. bacteremia, empirical antimicrobial treatment should include effective antibiotics against these bacteria. In this geographic location encompassing our country, multidrug resistance among Gram-negative bacteria is an emerging issue. National and international strategies for rational antibiotic use should be implemented to manage this problem, and all healthcare providers should comply with the regulations.

Ethics Committee Approval: Ethical approval was received from the Bezmialem Vakif University Ethics Committee. No: 24/459, date: 17.12.2019.

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Authorship Contributions: Concept: S.B.; Design: S.B.; Data Collection or Processing: S.B., G.O.; Analysis or Interpretation: S.B.; Literature Search: S.B.; Writing: S.B.

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