


Antibiotic Resistance Profile of *Acinetobacter* Species Isolated from Blood Cultures of Inpatients in Harran University Hospital

Harran Üniversitesi Hastanesi'nde Yatan Hastaların Kan Kültürlerinden İzole Edilen *Acinetobacter* Suşlarının Antibiyotiklere Direnç Profili

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Abstract

Background: *Acinetobacter baumannii* causes serious blood stream infections especially in immunocompromised and hospitalized patients. In this study, the distribution of antibacterial resistance among 92 *A. baumannii* isolates and 5 *A. Iwoffii* isolates from blood cultures collected at Harran University Hospital (Urfa, Turkey) from 2017 to 2019 was investigated.

Materials and Methods: Blood cultures were followed up in Versa-TREK (Trek Diagnostic System, USA) device; Passages were made to 5% blood agar and eosin methylene blue (EMB) agar. Passaged bacteria colonies were identified by MALDI-TOF MS (Bruker, Germany) or VITEK 2 compact system (bioMérieux, France). Sensitivities of *Acinetobacter* strains to meropenem, ciprofloxacin, amikacin, gentamicin, tobramycin, tigecycline and trimethoprim-sulfamethoxazole were tested by VITEK 2 compact system (bioMérieux France). The results were evaluated by VITEK 2 device based on EUCAST's guide.

Results: The antibiotic resistance rates of *A. baumannii* strains are as follows; ciprofloxacin 92%, imipenem and meropenem 88%, gentamicin 74%, tobramycin 67%, trimethoprim-sulfamethoxazole 63%, amikacin 53%, and tigecycline 10%. The resistance ratio of *A. Iwoffii* strains are ciprofloxacin 40%, gentamicin 40%, amikacin 40%, tobramycin 40%, trimethoprim sulfamethoxazole 20%, imipenem and meropenem 20%, and tigecycline 20%. Conclusions: Our study suggests that *A. baumannii* strains have high resistance ratios to available antibiotics. *A. Iwoffii* also has an increasing resistance profile. Tigecycline is the most sensitive antibiotic, followed by amikacin and tobramycin.

Conclusions: New antibiotics, rapid access to antibiotic sensitivity results and correct selection of empiric antibiotics has clinical importance.

Key Words: Bacteremia, Drug Resistance, *Acinetobacter baumannii*, Intensive Care Units

Öz.

Amaç: *Acinetobacter baumannii* özellikle hastanede yatan ve immün yetmezliği olan hastalarda ciddi bakteremilere sebep olabilen bir bakteridir. Bu çalışmada 2017-2019 yılları arasında Harran Üniversitesi Hastanesi'nde (Urfa, Türkiye) yatan hastaların kan kültürlerinden izole edilen 92 *A. baumannii* ve 5 *A. Iwoffii* izolatının antibiyotiklere olan direnci incelenmiştir.

Materyal ve Metod: Kan kültürü şişelerinin inkübasyonu için Versa-TREK (Trek Diagnostic System, USA) cihazı kullanıldı. Pasajlar %5 kanlı agara ve eozin metilen blue agara (EMB) yapıldı. Pasajlarda üreyen bakterilerin tanımlanmasında MALDI-TOF MS (Bruker, Germany) ve Vitek 2 compact sistemi (bioMérieux, France) kullanıldı. Meropenem, siprofloksasin, amikasin, gentamisin, tobramisin, tigesiklin ve trimetoprim-sulfametoksazole olan dirençler VITEK 2 compact sistemi ile, EUCAST önerileri temel alınarak değerlendirildi.

Bulgular: *A. baumannii* suşlarının antibiyotik direnç oranları; siprofloksasine %92, imipeneme %88, meropeneme %88, gentamisine %74, tobramisine %67, trimetoprim-sulfametoksazole %63, amikasinine %53, tigesikline %10 şeklinde bulundu. *A. Iwoffii*'nin direnç profili ise siprofloksasine, gentamisine, amikasinine ve tobramisine 40%; trimetoprim sulfametoksazole, imipeneme, meropeneme ve tigesikline 20% şeklinde idi. Sonuç: Çalışmamız, *A. baumannii*'nin yüksek antibiyotik direncine sahip olduğunu göstermektedir. Ayrıca *A. Iwoffii*'de artan bir antibiyotik direnç profili görülmektedir. Tigesiklin en duyarlı antibiyotikken, onu amikasin ve tobramisin izlemektedir.

Sonuç: Yeni antibiyotiklere, antibiyotik duyarlılık profillerine çabuk erişim olanağına ve ampirik tedavide doğru antibiyotiklerin seçimine ihtiyaç vardır.

Anahtar kelimeler: Bakteriyemi, ilaç direnci, *Acinetobacter baumannii*, Yoğun bakım üniteleri

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Introduction

Acinetobacter species are gram negative, glucose-non-fermentative, oxidative-negative, catalase-positive, non-motile, aerobic bacilli (1). *Acinetobacters* can live on inanimate surfaces for a long time, is a high-risk, opportunistic infection factor in hospitals (2). Among the species, *Acinetobacter baumannii* is one of the most important pathogens responsible for hospital-acquired nosocomial infections of skin, bloodstream, urinary tract and other soft tissues in the current healthcare systems (3). Carbapenem-resistant *A. baumannii* is also classified as priority 1 and critical within WHO (World Health Organization) priority pathogens list for R&D (Research And Development) of new antibiotics (4).

Most of strains of *A. baumannii* are highly resistant to clinically available antibiotics. Few antibiotics are effective for treating infections caused by *A. baumannii*. Multi-drug resistant *A. baumannii* causes severe infections and outbreaks in clinics such as intensive care units, newborn units and hematology-oncology clinics (5).

Invasive interventions, the presence of other systemic diseases in patients, and the use of multiple antibiotics may lead bacteria to cause infection (6). Because these factors are common in intensive care units (ICUs), *Acinetobacter* is commonly seen in these sites. Bacteria that gain resistance with the use of intense antibiotics can replace the hospital flora and spread from the colonies on the surfaces of clinics to the patients via health care personnel (7).

The most important infection caused by *Acinetobacter* is bacteremia. Blood culture is the gold standard technique in the diagnosis of bacteremia and sepsis (8).

Acinetobacter, which was sensitive to simple antibiotics at the time it was first identified, was easy to treat. However, as a result of the frequent use of broad-spectrum antibiotics in hospitals, multi-drug resistant *Acinetobacter* has been observed (9). This resistance against beta lactam, quinolone, carbapenem and aminoglycosides, which are frequently preferred antimicrobials makes the treatment very difficult. It causes high morbidity and mortality due to infection and leads to prolonged hospital stay and increased cost (3). Mortality rates are found 52.1% in the study of Marra et al. and 52.5% in the study of Ulu-Kılıç et al (10,11).

In blood infections, starting therapy early with appropriate antibiotics is the most important factor that reduces mortality and morbidity (12). The resistance profile of this bacterium, which develops rapid resistance to antibiotics, varies between countries and hospitals (6). This variability and high antibiotic resistance show that antibiotic resistance profile should be determined at intervals. The present surveillance should give a clue to doctors to start appropriate empirical treatment in blood circulation in cases of infections caused by *A. baumannii*, which is common in hospitals and causes high mortality (10).

In our study, antibiotic resistance profiles of *A. baumannii* and *A. lwoffii* strains grown in blood cultures of patients hospitalized in our hospital between January 2017 and December 2019 were evaluated. Their resistance changes over the three-year period were investigated.

Materials and Methods

Our study was conducted in Harran University Faculty of Medicine Hospital with 637 beds in Sanliurfa, Turkey. Blood cultures obtained from inpatients at various clinics between January 2017 and December 2019 was analyzed retrospectively. Among the bacterial cultures, 97 *Acinetobacter spp.* were observed. Antibiotic resistance profiles of these bacteria were evaluated. Blood cultures were followed up in Versa-TREK (Trek Diagnostic System, USA) device with automated blood culture bottles. Passages were made to 5% blood agar and eosin methylene blue (EMB) agar in sterile conditions from the blood culture bottles that gave a reproductive signal. Growth in the media was evaluated for 24-48 hours. The reproduced bacteria colonies were identified by MALDI-TOF MS (Bruker, Germany) or Vitek 2 compact system (bioMérieux, France). Only one result was included in the study among different cultures of the same patient reproducing the same factor. Antibiotic susceptibilities of *Acinetobacter* strains were examined with VITEK 2 compact system (bioMérieux, France). Antibiotic sensitivities of *A. lwoffii* and *A. baumannii* strains were evaluated separately. In a sterile 0.85% saline solution, a McFarland 0.5 suspension of bacteria was prepared. Meropenem, ciprofloxacin, amikacin, gentamicin, tobramycin, tigecycline and trimethoprim-sulfamethoxazole sensitivities were tested by loading on VITEK device. The results were evaluated by VITEK 2 device based on EUCAST's guide. Strains that were 'intermediate' were considered resistant.

Pearson chi-square test was used for statistical evaluation of antibiotic resistance changes.

Ethical approval for this study was obtained from Harran University Clinical Research Ethical Committee (21/02/2020-E.9317 * 76244175-050.04.04)

Results

97 *Acinetobacter spp.* strains which grew in blood cultures of 97 patients from various clinics in Harran University in 2017-2019 were analyzed. Among them, 5 strains were *A. lwoffii* and 92 strains were *A. baumannii*.

Considering the antibiotic resistance rates of *A. baumannii* strains; resistance to ciprofloxacin 92%, imipenem %88, meropenem 88%, gentamicin 74%, tobramycin 67%, trimethoprim-sulfamethoxazole 63%, amikacin 53%, and tigecycline 10% were observed (Figure 2).

Regarding the resistance profile of *A. Iwoffii* strains; resistance to ciprofloxacin (40%), gentamicin 40%, amikacin 40%, tobramycine 40%, trimethoprim sulfamethoxazole 20%, imipenem %20, meropenem 20%, and tigecycline 20% were seen.

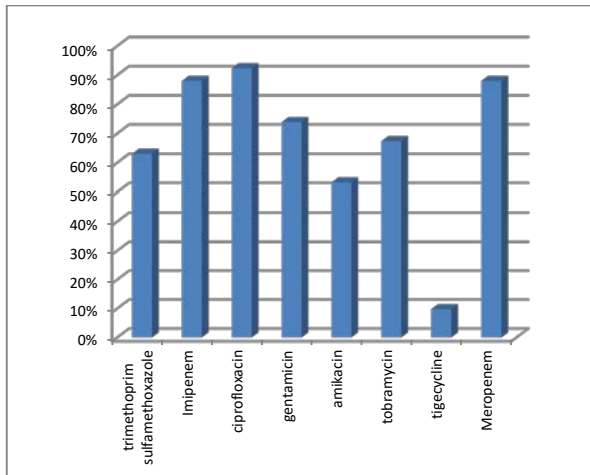


Figure 1. Percentage of Resistance of *A. baumannii* to Different Antibiotics

The annual change resistance of *A. baumannii* to antibiotics between years of 2017-2019 was examined by Pearson Chi-square test in SPSS program. The increase in the number of strains found resistant to amikacin was statistically significant ($P < 0.05$). This significance originated from 2017. In other antibiotics tested, no significant change in resistance was detected over a three-year period (Table 1).

Table 1. Antibiotic Resistance Percentages of *A. baumannii* by Years

Year	SXT	IPM	CIP	GEN	AMK	TOB	TGC	MEM	Number of patients
2017	57%	95%	95%	81%	29%	81%	14%	95%	21
2018	56%	81%	88%	66%	56%	63%	3%	81%	32
2019	72%	90%	95%	77%	64%	64%	13%	90%	39

Abbreviations: AMK, amikasin; GEN, gentamisin; CIP, ciprofloxacin; IPM, imipenem; MEM, meropenem; SXT, trimethoprim sulfamethoxazole; TGC, tigecycline; TOB, tobramicin.

Looking at the clinics where *A. baumannii* strains were isolated; 64% in adult intensive care units (including 53% internal intensive care, 11% surgical intensive care), 13% in pediatric intensive care unit, 11% in neonatal intensive care unit, 7% in adult services, 5% in pediatric services. Considering all the clinics, it was seen that the clinic where *A. baumannii* was isolated the most was general intensive care unit with a high rate of 27% (Figure 2).

Two of *A. Iwoffii* strains were isolated from neonatal intensive care, one from pediatric metabolism service and two from pediatric intensive care.

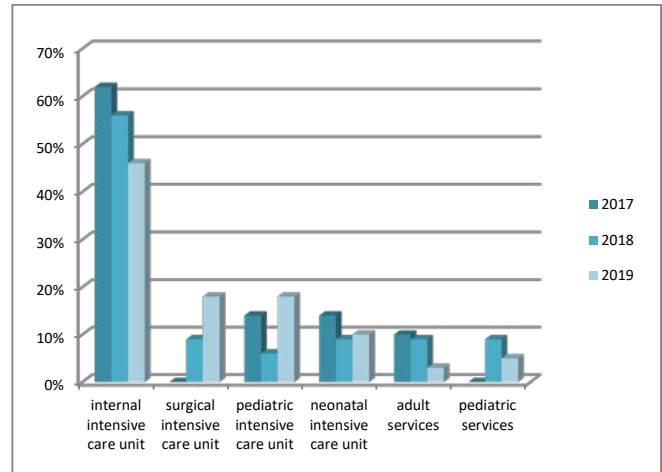


Figure 2. Percentage of isolation of 92 *A. baumannii* from clinics of Harran University Hospital.

Discussion

A. baumannii is frequently seen in inpatient services of hospitals, especially in intensive care units. Due to multiple antibiotic resistance, infections progress with high morbidity and mortality. Multidrug resistance is defined as the resistance against more than one of the five groups of drugs, consisting of antipseudomonal cephalosporins, carbapenems, quinolones, aminoglycosides and a combination of β -lactam and β -lactamase inhibitor (7).

In our study, high carbapenem and quinolone resistances were encountered among *A. baumannii* strains of our hospital. Tigecycline was determined as the most sensitivity antibiotic and amikacin and tobramycin followed tigecycline. It was statistically significant that the resistance of amikasin rose from 29% to 64% in 3 years. Resistance to trimethoprim sulfamethoxazole increased from 57% to 72% in 3 years, but this increase was not significant. Ghajavand et al. examined *A. baumannii* which were isolated from intensive care units. They collected 350 samples including urine, catheter, wound, blood, eye swabs, sputum, and cerebrospinal fluid. Examination of drug resistance of 43 isolates showed that 100% were resistant to ciprofloxacin; 93% were resistant to meropenem, imipenem, ampicillin-sulbactam, and cefepime; 91% were resistant to trimethoprim-sulfamethoxazole; 86% were resistant to ceftazidime; 84% were resistant to tetracycline; and 54% were resistant to amikacin (13).

In a study conducted by Çolakoğlu et al. with *A. baumannii* strains isolated from blood cultures in Adana, they found 83% resistance to ciprofloxacin, 82.3% to meropenem, 80% to imipenem, 74.1% to gentamicin, and 68.1% to amikacin (12). Coşkun found 100% resistance to imipenem and meropenem, 98.1% to ciprofloxacin, 80.8% to gentamicin and 75% to amikacin in a study conducted in Tokat (14). These results are compatible with the high ciprofloxacin and carbapenem resistances seen in our study.

Resistance in *A. baumannii* strains in the study of Şirin et al. was; ciprofloxacin 95.2%, imipenem 90.4%, meropenem 90.4%, gentamicin 73.1%, tigecycline 72.1%, amikacin 63.5%, trimethoprim sulfamethoxazole 54.8% (15). In the study of Taşçı et al. covering the years 2014-2015, resistance was as follows; trimethoprim sulfamethoxazole 50%, ciprofloxacin 40%, gentamicin 40%, amikacin 30%, imipenem 30% and meropenem 30% (8).

In a study by Türk Dağ et al., they saw the resistance profile as 79% to gentamicin, 75% to imipenem, 75% to ciprofloxacin, 59% to amikacin. Furthermore, they found that colistin and amikacin are more effective than other antibiotics (16). Uzun et al. encountered a resistance of gentamicin 93%, imipenem and meropenem 86%, ciprofloxacin 81%, and amikacin 32% in *A. baumannii* strains in ICUs in Izmir, and they did not encounter resistant to tigecycline (7).

Considering these studies conducted in our country, high carbapenem resistance (88%) and quinolone resistance (92%) were found in our study. Resistances are similarly observed with our study. The highest sensitivity among the antibiotics examined in our study was tigecycline (90%), and this sensitivity was considered as a common result with other reports when excluding colistin. Due to this high resistance to carbapenems and ciprofloxacin, it seems more appropriate to use tigecycline and colistin in the empirical treatment of *A. baumannii*.

In a study conducted with *A. baumannii* strains in Taiwan, Liu et al. found resistance rates as 100% to gentamicin, 96% to amikacin and 94% to tobramycin (17). In the study conducted by Zhang et al. in the scope of the Tigecycline Evaluation and Surveillance Trial, covering the years 2012-2016, they found amikacin and meropenem resistance as 49.1% and 66.8%, respectively (18).

In a retrospective study conducted by Alagesan et al. in South India, the resistance of *A. baumannii* strains changed from 67% to 74% in meropenem, from 0% to 20% in tigecyclin, from 65% to 75% in amikacin in the years 2009-2013 (19).

Among our *A. lwoffii* strains, we found the resistance as follows: 40% to ciprofloxacin, 40% to gentamicin, 40% to amikacin, 40% to tobramycin, 20% to trimethoprim sulfamethoxazole, 20% to imipenem, 20% meropenem, 20% to tigecycline. In a study published in Public Health England, *A. lwoffii*'s resistance rates in 2018 were seen as 1% to ciprofloxacin, 1% to meropenem and 1% tobramycin, while amikacin and gentamicin resistant strains were not found (20). Tega et al. found the resistance as trimethoprim sulfamethoxazole 60%, gentamicin 60%, tobramycin 50%, ciprofloxacin 40%, amikacin 10%, and no resistance to imipenem and meropenem (21).

In the light of these studies, it is seen that the resistance of *A. lwoffii* to antibiotics varies regionally, but it does not seem to have high resistance like *A. baumannii*.

Penicillins, cephalosporins and colistin were not included

in our study. In the EUCAST guideline, it has been stated that susceptibility tests for penicillins are unreliable in *Acinetobacter* spp. It is added that this bacterium is resistant to penicillins in most cases. No limit values for penicillin group drugs and cephalosporins are given in the guideline. Also it is recommended to use only the liquid microdilution method in determining the sensitivity of colistin in EUCAST (22). These antibiotics were excluded from our study due to the use of VITEK-2 device, which evaluates according to EUCAST in determining antibiotic susceptibilities in our study.

In *Acinetobacter*-induced bacteremia, the availability of colistin is very important for clinics due to multiple drug resistance. For this information we need to use liquid micro dilution but due to the cost and personnel requirements, this method is not used in most laboratories. In some studies conducted due to the importance of colistin, the sensitivity of colistin from the semi-automated systems was compared with the reference method (liquid micro dilution), and it was argued that every result, which was borderline and sensitivity from the semi-automated system, should be controlled by the liquid micro dilution method (23,24). The lack of colistin in our sensitivity panel is a limitation of our study.

Uncontrolled and incorrect use of antibiotics is the most important reason for increased resistance (12). Blood stream infections and mortality rates due to *A. baumannii* can be reduced by avoiding unnecessary antibiotic treatment and taking preventive measures against infection (11). Consequently, considering the antibiotic resistance changes in the sensitivity or resistant direction over the years and the first treatment of bloodstream infections is given empirically, monitoring the resistance profiles of *Acinetobacter* in each hospital is important in order to prevent the mortality and high treatment costs, especially in the ICU.

Conclusion

In our study, it was determined that *A. baumannii*'s resistance to carbapenem and quinolones is very high. Tigecycline is the most sensitivity antibiotic, followed by amikacin and tobramycin). *A. lwoffii* is less frequent but emerging species of *acinetobacters* and appears that it has increased antibiotic resistance. Observing the resistance of bacteria to antibiotics is a task of microbiology laboratories. Regularly determining and reporting antibiotic susceptibility results enable physicians to determine their own antibiotic use policies and to take infection control measures. Also, the resistance profile in regions, countries and even world can be observed with these data (12).

Ethical Approval: Ethical approval for this study was obtained from Harran University Clinical Research Ethical Committee (21/02/2020-E.9317 * 76244175-050.04.04)

Author Contributions:

Concept: H.A.

Literature Review: H.A.

Design : H.A. , M.B., F.Y.Z.

Data acquisition: H.A.

Analysis and interpretation: H.A. , M.B.

Writing manuscript: H.A.

Critical revision of manuscript: M.B., F.Y.Z.

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