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Determination of Susceptibility Rates of Nosocomial *Acinetobacter baumannii* Isolates to Sulbactam by E-test Method

Nozokomiyal *Acinetobacter baumannii* İzolatlarında Sulbaktam Duyarlılık Oranlarının E-test Yöntemi ile Belirlenmesi

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SUMMARY

Introduction: *Bacteria of the genus Acinetobacter play an important role as causative agents of hospital-acquired infections. Especially in recent years, multidrug-resistant Acinetobacter infections have increasingly been observed worldwide. In parallel with the increasing rate of infections, therapeutic options are becoming limited. Although the susceptibility rates are not exactly known, sulbactam alone or sulbactam with ampicillin play a part in combination therapies against Acinetobacter infections. This study aimed to determine the minimum inhibitory concentrations (MICs) of sulbactam against multidrug-resistant Acinetobacter baumannii strains using the E-test method and to deduce the susceptibility rates based on literature data.*

Materials and Methods: *The study included 100 multidrug-resistant A. baumannii strains isolated from clinical samples obtained from patients hospitalized in intensive care units of the Ministry of Health Ankara Training and Research Hospital between June 15, 2011 and June 15, 2013. Antibiotic susceptibility testing and strain identification were performed using conventional methods and the VITEK 2 (bioMérieux SA, France) system. Resistance to three or more drugs was considered as multidrug resistance. MIC, MIC_{50}, and MIC_{90} values (µg/mL) of sulbactam against the 100 isolates were determined using the E test method. Since the breakpoint MIC of sulbactam against Acinetobacter had not been established, the susceptibility rates were estimated based on the MIC values reported in the literature (≤ 4 or 8 µg/mL).*

Results: *The MIC values of sulbactam against the Acinetobacter isolates ranged widely (between 1 and 256 µg/mL), and the MIC_{50} and MIC_{90} values were determined to be 12 and 96 µg/mL, respectively. When 8 µg/mL was considered as the susceptibility breakpoint, 44% of the isolates were found to be susceptible; however, the rate was only 21% when 4 µg/mL was considered as the breakpoint.*

Conclusion: *Based on its MIC values determined in our study, sulbactam appeared to be a promising agent for the treatment of infections caused by multidrug-resistant A. baumannii isolates. Nonetheless, more studies are needed, especially on its clinical effectiveness.*

Key Words: *Sulbactam; Acinetobacter baumannii; E-test*

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ÖZET

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Giriş: *Hastane infeksiyonlarına yol açan etkenler arasında Acinetobacter cinsi bakteriler önemli bir yer tutmaktadır. Çoklu ilaç dirençli Acinetobacter infeksiyonları dünyada artan oranlarda görülmektedir. Bu nedenle, terapötik seçenekler sınırlı hale gelmektedir. Duyarlılık oranları net olarak bilinmese de, tek başına sulbaktam veya sulbaktam-ampisilin, Acinetobacter infeksiyonlarının tedavisinde kombinasyonlarda yer almaktadır. Bu çalışmada, çoğul dirençli Acinetobacter baumannii kökenlerinde, sulbaktamın minimum inhibitör konsantrasyonu (MİK) değerleri E-test yöntemi ile incelenmiştir.*

Materyal ve Metod: *Çalışmaya, 15 Haziran 2011-15 Haziran 2013 tarihleri arasında, Sağlık Bakanlığı Ankara Eğitim ve Araştırma Hastanesinde yatan hastalardan alınan klinik örneklerden izole edilen, karbapenem direncini de barındıran çoklu ilaca dirençli 100 A. baumannii kökeni alındı. Antibiyotik duyarlılıkları ve tür düzeyinde tanımlaması konvansiyonel yöntemler ve VITEK 2 (bioMérieux SA, Fransa) sistemi ile yapılmıştır. Üç veya daha fazla ilaç grubuna karşı direnç saptanması çoğul ilaç direnci olarak kabul edildi. Yüz izolat çalışma gününe kadar -80°C'de gliserollü "brain heart" besiyerinde (Oxoid, UK) saklandı. Kontrol kökeni olarak Escherichia coli ATCC (American Type Culture Collection) 25922 kullanıldı. Sulbaktamın 100 izolata karşı E-test yöntemi ile saptanan MİK değerleri (μg/mL), $MİK_{50}$ ve $MİK_{90}$ değerleri (μg/mL) kaydedildi. Tek başına sulbaktamın Acinetobacter'e karşı belirlenmiş bir duyarlılık sınırı olmadığı için, duyarlılık oranları, literatürde rapor edilen MİK sınır değerleri dikkate alınarak hesaplanmıştır (≤ 4 μg/mL ve ≤ 8 μg/mL).*

Bulgular: *Acinetobacter izolatlarına karşı sulbaktam MİK değerleri geniş bir aralıkta dağılmıştı (1 μg/mL ile 256 μg/mL arasında); $MİK_{50}$ ve $MİK_{90}$ değerleri ise sırasıyla 12 μg/mL ve 96 μg/mL saptandı. Duyarlılık sınırı 8 μg/mL kabul edildiğinde, izolatların %44'ü duyarlı saptanmışken, sınır 4 μg/mL kabul edildiğinde bu oran %21 ile sınırlı kaldı.*

Sonuç: *Çalışmamızdaki sulbaktam MİK değerleri göz önüne alındığında, çoklu ilaca dirençli A. baumannii tedavisinde sulbaktam umut verici bir ajan olarak görülmektedir. Ancak, özellikle klinik etkinlik konusunda farklı çalışmalara ihtiyaç vardır.*

Anahtar Kelimeler: *Sulbaktam; Acinetobacter baumannii; E-test*

INTRODUCTION

Bacteria of the genus *Acinetobacter* are important agents causing hospital-acquired infections[1]. High incidences of nosocomial infections caused by these pathogens are due to their tolerance of environmental conditions and ability to easily become resistant to antibiotics. *Acinetobacter baumannii* is a species commonly isolated from patients and hospital environments[2].

In recent years, *Acinetobacter* species have become resistant to antibiotics, especially as the use of broad-spectrum antibiotics increased. Particularly in intensive care units (ICUs), where invasive interventions (such as intubation and urinary or intravenous catheterization) are frequently performed, multidrug-resistant *Acinetobacter* infections are becoming increasingly more troublesome[3].

Due to the escalation of antimicrobial resistance among microorganisms, attempts have been made to develop new treatment protocols. Combination therapy, development of new antibiotics, and using obsolete antibiotics are just some examples of these studies.

Sulbactam is a semisynthetic compound with the chemical name penicillanic acid sulfone. It is a specific inhibitor of beta-lactamases produced by several gram-positive and gram-negative aerobic and anaerobic microorganisms. In particular, this drug inhibits chromosomal enzymes of *Citrobacter*

diversus, *Klebsiella* spp., *Proteus vulgaris*, and *Bacteroides* spp. as well as beta-lactamases produced by staphylococci and extended-spectrum beta-lactamases. In addition to some class-D beta-lactamases, chromosomal class-C beta-lactamase of *Morganella morganii* is also inhibited by sulbactam. [4,5]. However, sulbactam does not inhibit many chromosomal beta-lactamases in bacteria[4].

In addition to beta-lactamase inhibition, sulbactam also has intrinsic bactericidal activity against some multidrug-resistant *Acinetobacter* species through penicillin-binding protein 2[6]. Sulbactam alone displays direct antimicrobial activity against *Bacteroides fragilis* and *Acinetobacter* species[7]. The efficacy of sulbactam has been confirmed in several studies documenting successful treatments of *Acinetobacter*-related serious infections, including meningitis and ventriculitis. However, the incidence of resistance to sulbactam is also gradually increasing[6].

In this study, minimum inhibitory concentrations (MICs) of sulbactam were determined against multidrug-resistant *A. baumannii* strains to investigate the potential of sulbactam as a treatment option.

MATERIALS and METHODS

This study was conducted at the Department of Infectious Diseases and Clinical Microbiology of the Ministry of Health Ankara Training and Research Hospital between June 15, 2011 and June 15, 2013. Our study included 100 multidrug-resistant (including carbapenem-resistant) *A. baumannii* isolates that were obtained from clinical samples sent to our microbiology laboratory from hospital ICUs. Isolates were collected over a 2 year period and originated from the urinary tract, blood and respiratory tract. All isolates were identified from different patients. Of these isolates, 59 were from the patients in Anesthesiology and Reanimation Department, 23 from the Neurology Department, 11 from the Neurosurgery Department, and seven from the Internal Diseases Department.

The isolates were tested by conventional methods and using the VITEK 2 (bioMerieux SA, France) system for antibiotic susceptibility testing and species-level identification. Resistance to at least three drug groups functional in the treatment of *Acinetobacter* infections was considered as multidrug resistance. Isolates were carefully selected from different wards and different dates, and only one clinical isolate was included per patient. The 100 isolates were preserved at 80°C in the brain heart infusion broth (Oxoid, UK) containing glycerol.

For the study, the *A. baumannii* isolates were taken out of the deep freezer and subcultured on pre-cast EMB and sheep blood agar media. After 18-28 h of incubation in an aerobic atmosphere at 35 ± 2°C, bacterial colonies from fresh subcultures were used.

Bacterial suspensions equivalent to 0.5 McFarland turbidity standard were prepared for each isolate and evenly spread on Mueller-Hinton agar with sterile cotton swabs. The stored Etest strips (bioMerieux SA, France) were taken out of the 80°C freezer, allowed to stay at room temperature for 30 min, and then placed on the inoculated Mueller-Hinton agar plates. Plates were placed in an incubator and assessed after 18-24 hours. MIC values of the antibiotic tested were determined based on the point where the zone of complete growth inhibition intersected the Etest strip.

The MIC, MIC_{50}, and MIC_{90} values (µg/mL) of sulbactam against the 100 isolates, which were determined with the Etest method, were recorded, and the susceptibility rates were deduced. None of the "Clinical and Laboratory Standards Institute (CLSI)", "European Committee on Antimicrobial Susceptibility Testing (EUCAST)", and "Food and Drug Administration (FDA)" guidelines provides the breakpoint MIC values for sulbactam alone. Therefore, the susceptibility rates were calculated based on the MIC limit values reported in the literature (≤ 4 and ≤ 8 µg/mL)[8]. Moreover, estimations were done by taking as a reference sulbactam in the ampicillin-sulbactam combination provided in the CLSI guidelines, similar to other studies (Table 1)[9,10]. *Escherichia coli* ATCC 25922 was used as a control strain.

RESULTS

We evaluated 100 *A. baumannii* isolates from clinical samples obtained from hospitalized patients. Most of the strains were isolated from tracheal-aspirate culture. The second was isolated from the urine culture and then the blood culture.

Table 1. The limit of MIC values of *Acinetobacter baumannii* strains as suggested by CLSI

Antimicrobial drug	MIC (µg/mL) interpretation criteria		
	Susceptible	Intermediate	Resistant
Sulbactam*	≤ 4	8	≥ 16

MIC: Minimum inhibitory concentration, CLSI: Clinical and Laboratory Standards Institute.
* A range of MIC for sulbactam within ampicillin-sulbactam combination was used as indicated in CLSI guideline.

The sulbactam MIC ranges, MIC_{50} and MIC_{90} values (µg/mL), and the susceptibility rates (based on the MIC values provided in the CLSI guidelines for sulbactam in the ampicillin-sulbactam combination) for the isolates included in this study are shown in Table 2.

Depending on whether 4 or 8 µg/mL was used as the susceptibility breakpoint, 21 (21%) or 44 (44%) isolates were found to be susceptible to sulbactam, respectively.

DISCUSSION

In ICUs in Turkey, *Acinetobacter*-associated infections have become the most frequently observed and most difficult to treat infections[11,12]. The *Acinetobacter* strains used in our study were also isolated from patients hospitalized in ICUs and included isolates resistant to carbapenem. The most frequent hospital-acquired infection in our ICUs is ventilator-associated pneumonia. Therefore, most of the strains used in this study were isolated from tracheal-aspirate culture. In this study depending on whether 4 or 8 µg/mL was used as the susceptibility breakpoint, 21 (21%) or 44 (44%) isolates were found to be susceptible to sulbactam, respectively.

High rates of resistance to antibiotics in *A. baumannii* isolates lead to difficulties in the treatment of related infections and need for alternative therapeutic options. Due to the inefficiency of the current treatment, combined use of antibiotics was proposed. First studies demonstrating direct antimicrobial activity of sulbactam against *Acinetobacter* species were performed in the 1980s[7,13]. It was also demonstrated that the efficacy of sulbactam against carbapenem-resistant *Acinetobacter* species was higher than that of colistin[14]. Nonetheless, sulbactam alone is not recommended as a treatment option, and it is usually administered in combination treatments, namely, with ampicillin and cefoperazone. A combination of sulbactam and carbapenem was reported to show a high level of synergistic activity[15].

A limited number of studies have been conducted on the efficacy of sulbactam alone, with two of them being of most interest. Swenson et al. assessed 195 *A. baumannii* isolates by the microdilution method and determined MIC_{50} and MIC_{90} values for sulbactam to be 8 and 128 µg/mL, respectively[16]. In a study by Hawley et al., which included 95 *A. baumannii* isolates, MIC_{50} and MIC_{90} values were determined to be 16 and 64 µg/mL, respectively, by the microdilution method[17]. In our study, the MIC_{50} and MIC_{90} values were similarly found to be 12 and 96 µg/mL by using the Etest method. Due to the lack of an established susceptibility breakpoint in this study, similar to other studies, the resistance pattern could not be inferred.

The fact that there are no established breakpoint MIC values for sulbactam in the CLSI, EUCAST, and FDA guidelines makes interpretation of the test results difficult. Although direct bactericidal

Table 2. MIC range, MIC_{50} and MIC_{90} values, and rate of susceptibility of sulbactam against *Acinetobacter baumannii* isolates as determined with E-test

Antibiotic	Bacteria (n= 100)	MIC range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Susceptibility rates* (%)		
					Susceptible	Intermediate	Resistant
Sulbactam	100	1-256	12	96	21	38	41

MIC: Minimum inhibitory concentration, CLSI: Clinical and Laboratory Standards Institute.
* A range of MIC for sulbactam within ampicillin-sulbactam combination was used as indicated in CLSI guideline.

activity of sulbactam against *A. baumannii* is recognized, there are no specific data on an efficient therapeutic dose and correlation of MIC values with a clinical response[18]. Therefore, the MIC ranges for sulbactam were determined using as a reference the sulbactam data in an ampicillin-sulbactam combination, provided in the CLSI guidelines, as done in similar studies. Consequently, it was determined that susceptible isolates constituted 21% (21/100), while 38% (38/100) were intermediate, and 41% (41/100) were resistant. When the MIC value of ≤ 8 µg/mL was used as a susceptibility breakpoint for sulbactam, 44% of the isolates were found to be susceptible. Despite the discrepancies between the numbers of isolates susceptible to sulbactam, the data confirm that some multidrug-resistant *Acinetobacter* strains are susceptible to sulbactam. Colistin is currently the only choice for carbapenem-resistant strain infections, and resistance to colistin is alarming. Beside these, side effects of colistin especially on renal functions are limiting the use of it[19]. It is not expected that new and efficient antimicrobial drugs will appear in the near future. Therefore, sulbactam alone or in combination may be a today's option to treat some infections caused by multidrug-resistant *Acinetobacter* strains. Consequently, we believe that sulbactam should be promoted in clinical studies to determine its MIC values for *Acinetobacter* species and the efficacy of single or combined administration.

The most important limitation of this study is that its results could not be applied to clinical practice due to the lack of established MIC values for sulbactam.

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