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In Vitro Susceptibility of Tigecycline and Colistin Against *Stenotrophomonas maltophilia*

Stenotrophomonas maltophilia Suşlarına Tigesiklin ve Kolistinin İn Vitro Duyarlılığı

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SUMMARY

Introduction: *Gram-negative bacillus Stenotrophomonas maltophilia is resistant to drugs (multi-drug resistance-MDR) and it can be isolated from nature. Treatment of the infections resulting from S. maltophilia could be problematic due to multi-resistance.*

Materials and Methods: *72 S. maltophilia strains isolated from clinical samples were included into the study. Sensitivity was determined using Tigecycline and Colistin E-test MIC method performed in the Clinical Microbiology laboratory of Baskent University, Medical Faculty between 2010 and 2014.*

Results: *In our study, colistin MIC range was found as 0.016-8 mg/L. MIC_{50} and MIC_{90} values were determined respectively as 1.5 mg/L and 12 mg/L. Tigecycline MIC range was 0-96 mg/L, and MIC_{50} was 0.19 mg/L and MIC_{90} was 1.5 mg/L. Furthermore, one tigecycline resistant strain was detected.*

Conclusion: *We believe that the determination of novel treatments and protocols and their standardization using multidisciplinary approaches can facilitate to cope with problematic and resistant nosocomial infections developed by S. maltophilia.*

Key Words: *Stenotrophomonas maltophilia; Tigecycline; Colistin; E-test*

ÖZET

Stenotrophomonas maltophilia **Suşlarına Tigesiklin ve Kolistinin İn Vitro Duyarlılığı**

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Giriş: *Stenotrophomonas maltophilia gram-negatif bir basil olup çok ilaça dirençli bir mikroorganizmadır. Tedavi seçenekleri birçok in vitro çalışma sonucu ve klinik deneyim neticesinde dikkate alınmalıdır.*

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Materyal ve Metod: *Başkent Üniversitesi Tıp Fakültesi Klinik Mikrobiyoloji laboratuvarımızda 2010-2014 yılları arasında klinik izolatlardan izole edilen 72 suşta tigesiklin ve kolistin E-test yöntemiyle minimum inhibitör konsantrasyonu (MİK) yöntemiyle duyarlılıkları belirlenmiştir.*

Bulgular: *Çalışmamızda kolitsin MİK aralığı 0.016-8 mg/L aralığında bulunmuş olup $MİK_{50}$= 1.5 mg/L ve $MİK_{90}$= 12 mg/L olarak belirlemiştir. Tigesiklin MİK aralığı 0-96 mg/L aralığında bulunmuş olup $MİK_{50}$= 0.19 mg/L ve $MİK_{90}$= 1.5 mg/L olarak belirlemiş olup bir tane dirençli suş saptanmıştır.*

Sonuç: *S. maltophilia ile gelişen hastane infeksiyonları ile mücadelede daha geniş çalışmalar ve multidisipliner yaklaşımlarla yeni tedavi seçeneklerinin belirlenmesi ve yeni protokollerin oluşturulup standardize edilmesi sorunlu ve dirençli mikroorganizmalar ile mücadelede bizlere yardımcı olabileceği kanaatindeyiz.*

Anahtar Kelimeler: *Stenotrophomonas maltophilia; Tigesiklin; Kolistin; E-test*

INTRODUCTION

Stenotrophomonas maltophilia is an important microorganism causing nosocomial infections and is an opportunistic microorganism which can be isolated from nature as well as from clinics[1]. It can be frequently isolated from the oropharyngeal and respiratory secretions of adults. *S. maltophilia* can cause health-care associated infections especially in the Intensive Care Units (ICU) of hospitals[2].

S. maltophilia, is known to have multi-drug resistance (MDR). It can lead to infections such as meningitis, ocular infections, and endocarditis particularly in patients with comorbidities. The morbidity and mortality (between 20% and 70%) rate is high with *S. maltophilia* infections and the highest rates can be observed in patients receiving inappropriate antibiotic therapy[3]. *S. maltophilia* is intrinsically resistant to various antibiotics since it contains inhibitory mechanisms such as inactivation enzymes for beta-lactamases, aminoglycoside acetyl transferase and erythromycin and genes encoding efflux pumps. The treatment of *S. maltophilia* infections is problematic due to its resistance to various types of antibiotics including carbapenems which are currently being used in hospitals[4,5]. *S. maltophilia* infections can be treated using trimethoprim-sulfamethoxazole (TMP/SMX). There are also other alternative antibiotics (such as ceftazidime, ticarcillin-clavulanate, minocycline, tigecycline, fluoroquinolones, and the polymyxins) that can be used. Treatments are being developed based on existing experiences with laboratory work, and therapeutic methods are developed on clinical trials[6]. In order to treat MDR *S. maltoph*ilia infections, alternative drugs should be investigated. In this study, using the MIC technique, we aimed to test the in vitro tigecycline and colistin activity on *S. maltophilia* strains isolated from clinical samples.

MATERIALS and METHODS

The strains were isolated from clinical samples in Baskent University Medical Faculty Clinical Microbiology laboratory between 2010 and 2014 and were included into the study. Bacteria identification was performed using either classical methods (growth and morphological features observed upon culturing bacteria in a 5% sheep blood agar and eosin methylene blue (EMB) agar, features of bacteria observed by staining them with Gram stain, and performing the catalase and oxidase tests) or novel techniques such as using gram-negative bacteria identification cards in the Vitek2 (bioMerieux) fully automated microbial identification system. Blood culture samples were studied in the fully automated BacT/Alert 3D (bioMerieux) blood culture system. Clinical Laboratory Standards Institute (CLSI) disk diffusion method was used to determine antibiotic sensitivity[7]. As recommended by CLSI, plates were checked after 16-20 hours of incubation at 35 $\pm$ 2°C. They were incubated for an additional 24 hours at 35 $\pm$ 2°C and checked again in the end of the incubation. *Pseudomonas aeruginosa* ATCC 27853 strains were used as control strains and MIC sensitivity was between the range of 0.5-4[7].

E-Test

Isolates were cultured at 37°C for 18 hours in Eosin-Methylene Blue (EMB) agar (Becton Dickinson, Sparks, USA). The 0.5 (10^8 cfu/mL) McFarland was inoculated on BBL Mueller-Hinton agar

Table 1. Examples of clinical isolates

Clinical sample	Wound swab culture	Blood culture	Respiration secretion (deep tracheal aspirates)	Abscess culture	Urine culture	Sputum culture	Body fluid	Total
N	38	17	5	4	4	2	2	72

(MHA) (Becton Dickinson, Sparks, USA) plates. Furthermore, E-test strips for tigecycline or colistin (bioMŕrieux SA, France) were incubated with isolated bacteria. The MIC value of the inhibition zone in the agar culture was determined after 18 hours of incubation at 35°C. Even though the European Committee on Antimicrobial Susceptibility Testing (EUCAST) states that the MIC threshold of the Enterobactericeae for tigecycline antibiotic is > 2 µg/mL, there is no value determined for *S. maltophilia* strains. According to the literature, the sensitivity threshold value was detected as ≤ 2 µg/mL for *S. maltophilia*[7,8].

RESULTS

72 *S. maltophilia* strains were isolated from the clinical sample cultures of hospitalized patients (collected for bacteriological examination) in Baskent University Medical Faculty hospital between January 2010 and December 2014. The distribution of these strains according to clinical samples can be seen in Table 1.

In our study, colistin MIC range was found as 0.016-8 mg/L. MIC_{50} and MIC_{90} values were determined respectively as 1.5 mg/L and 12 mg/L. Tigecycline MIC range was 0-96 mg/L, and MIC_{50} was 0.19 mg/L and MIC_{90} was 1.5 mg/L. Furthermore, one tigecycline resistant strain was detected. MIC sensitivities of the microorganisms and the MIC ranges of the control strains were shown in Table 2.

DISCUSSION

S. maltophilia infections have been diagnosed particularly in the ICU of hospitals and the pathogen is known as opportunistic. The number of antibiotics which can be used to treat these infections is limited due to resistance[9]. *S. maltophilia* infections are commonly observed in patients who are severely debilitated or immunocompromised due to some kind of comorbidity[10]. There are some factors (such as advanced age, prematurity, previous operations, diabetes mellitus, malignancy, implementation invasive interventions, stay in ICU, previously used broad-range beta-lactam, aminoglycoside or fluoroquinolone antibiotics) which can facilitate *S. maltophilia* infections in in-patients[11]. The rate of *S. maltophilia* strains isolated from a hospital is generally between 4% and 8%[12]. The mortality rate is quite high (between 20% and 70%) in patients who have *S. maltophilia* infection[3]. The mortality rate is over 50% particularly in bacteremia[11].

Although antibiotic resistance is a diverse problem, the combination of TMP/SMX is primarily preferred to treat *S. maltophilia* infections. Piperacillin, fluoroquinolones (e.g., levofloxacin and moxifloxacin), and tetracycline derivatives (e.g., minocycline) can also be used. Antibiotic treatment should be arranged upon antibiograms[13]. The TMP/SMX resistance is

Table 2. Tigecycline and colistin MIC_{50}, MIC_{90} and control values determined by E-test

	E-test		
		MIC (µg/mL)	
	N	Range	MIC_{50}	MIC_{90}
S. maltophilia				
Tigecycline	72	0.016-8	0.19	1.5
Colistin	72	0-96	1.5	12
Escherichia coli (control strain)				
Tigescycline		0.64		
Colistin		0.64		
Pseudomonas aeruginosa (control strain)				
Tigecycline		----		
Colistin		0.50		

reported as around 10% in Europe[14]. The limitation of our study was we that could not differentiate colonization from infection.

In order to compare the antibiotic activity of different bacteria (for *Enterobacteriaceae*, ≤ 2/≥ 8 µg/mL for S/R determined by USA-FDA), tigecycline was given to *Acinetobacter* spp. and *S. maltophilia*[15]. In our study, we examined the effectivity of tigecycline and colistin antibiotics on *S. maltophilia* using the MIC method. The activity of tigecycline against *S. maltophilia* was indicated (MIC_{50} 0.5 µg/mL and MIC_{90} 2 µg/mL). The activity of tigecycline against *S. maltophilia* was between 89.3% and 98.3% and it could be inhibited at ≤ 2 µg/mL and furthermore, the activity of both TMP/SMX and colistin against *S. maltophilia* were respectively as 94.5% and 98.5%[16]. It was shown that the inhibition rate of the tigecycline activity against *S. maltophilia* was %92.3 at ≤ 2 µg/mL and the colistin activity against *S. maltophilia* was around %94.5[17]. Betriu et al. have shown that the MIC range of tigecycline for *S. maltophilia* was 0.25-8 mg/L, the MIC_{50} and MIC_{90} values were found as 1 mg/L and 4 mg/L respectively and there was no resistant strain.

In a study of nosocomial pneumonia in which *S. maltophilia* was the causative agent, sensitivity to tigecycline in 102 strains was 80.4%[18]. In another study, all 40 *S. maltophilia* strains obtained in contact lens-using cases were found to be susceptible to tigecycline[19]. In a study conducted by Renteria et al., MIC_{50} value has been detected as 0.25 µg/mL and MIC_{90} value as 1 µg/mL for tigecycline in *S. maltophilia* strains[20]. In a Hungarian study, 160 *S. maltophilia* strains have been found to have an MIC_{50} value of 0.5 µg/mL and an MIC_{90} value of 2 µg/mL[21]. In our study, these values were 0.19 and 1.5 µg/mL, respectively.

In a study of antimicrobial susceptibility of 30 *S. maltophilia* strains resistant to TMP/SMX, only 37% of strains have been reported to be sensitive to levofloxacin and moxifloxacin, and all were resistant to colistin and tigecycline[22]. In our study, all strains were susceptible to TMP/SMX. In a study investigating the change of in vitro colistin resistance according to years, 641 *S. maltophilia* clinical isolates were evaluated. In this study, the resistance rate of colistin was 8% in 1996, whereas it was 54% in 2013, an increase of 11.4 times[23]. In a Hungarian study, colistin MIC values were very high (MIC_{50} value), and our results were quite satisfactory.

Colistin; a member of polymyxins, has been shown to be used in the treatment of the infections related to MDR gram-negative bacteria. There is no MIC range for colistin and tigecycline according to CLSI and EUCAST guidelines. In line with the literature, we showed that the MIC range of tigecycline was 0-96 mg/L, MIC_{50} and MIC_{90} values were determined respectively as 0.19 mg/L and 1.5 mg/L and there was only one resistant strain. Researchers have observed the tigecycline activity against *S. maltophilia* bacteria[24]. Unlike other studies, we demonstrated that colistin MIC range was 0.016-8 mg/L, and we calculated the MIC_{50} and MIC_{90} values respectively as 1.5 mg/L and 12 mg/L. Furthermore, we also detected one resistant strain.

Even though the susceptibility testing of colistin is not reliable, it is still important in the treatment of infections associated with MDR gram-negative bacilli[25]. Regarding colistin, MIC values of *P. aeruginosa* and other non-*Enterobacteriaceae* (susceptible MIC, ≤ 2 mg/L; intermediate MIC, 4 mg/L; resistant MIC, ≥ 8 mg/L) are determined by CLSI. On the other hand, colistin treatment procedures have not yet been clarified by CLSI; particularly for the colistin-resistant *S. maltophilia* isolates[7,25]. In the literature, the MacABCsm efflux pump in *S. maltophilia* has recently been shown to confer intrinsic resistance to antimicrobials [aminoglycosides, macrolides, and polymyxin B and polymyxin E (colistin)] and to play an important role in regulating oxidative and envelope stress tolerance and biofilm formation[26].

Due to increased resistance to antibiotics, colistin has become popular in the treatment of infections due to MDR pathogens. Tan et al. have indicated that all isolated *S. maltophilia* strains were resistant to colistin (MIC_{90} ≥ 128 mg/L)[27].

To conclude, tigecycline can be a new active agent that can be used for infections associated with gram-negative, gram-positive as well as anaerobic pathogens[24]. We generally support the idea that the determination of novel treatments and new protocols and their standardization should be ensured by using multidisciplinary approaches that can facilitate to cope with problematic and resistant nosocomial infections developed by *S. maltophilia*.

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