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ORIGINAL ARTICLE



Tigecycline susceptibility of multidrug-resistant *Acinetobacter baumannii* from intensive care units in the western Balkans

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ABSTRACT

Tigecycline can be effective to treat infections of carbapenem-resistant *Acinetobacter baumannii* (CRAB) however, no interpretive criteria have been approved so far. The objectives of this study were to evaluate the proportion of CRAB isolates and to compare gradient test with a broth microdilution (BMD) method for tigecycline susceptibility testing of *A. baumannii*.

This study included 349 multidrug-resistant (MDR) *Acinetobacter* spp. collected from Serbia, Montenegro, Bosnia and Herzegovina in 2016 and 2017. Antibiotic susceptibility testing was performed by disk diffusion, VITEK2, gradient, ComASP Colistin. Tigecycline susceptibilities were interpreted according to breakpoints of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Food and Drug Administration (FDA).

Majority of the tested isolates were CRAB (92.8%). Tigecycline MIC₅₀/MIC₉₀ values were 4/8 µg/mL by BMD and 0.5/4 µg/mL by gradient test. Essential agreement for BMD and gradient test amounted to 65.1%. With EUCAST breakpoints, categorical agreement (CA) was achieved in 38% isolates. Major discordance (MD-false susceptibility/resistance) and minor discordance (mD-false categorization involving intermediate results) were observed in 10% and 57% *A. baumannii*, respectively. With FDA breakpoints, CA, MD and mD were observed in 44%, 16% and 47% isolates, respectively. Colistin resistance was 2.1%.

The study highlights a high proportion of CRAB and several discordances between BMD and gradient test which may lead to inappropriate therapy.

KEYWORDS

Acinetobacter baumannii, tigecycline, broth microdilution, gradient strip test

INTRODUCTION

Acinetobacter baumannii has emerged as one of the most challenging nosocomial pathogens, due to its intrinsic resistance to antimicrobial agents, its propensity to acquire resistance determinants, and its ability to resist desiccation in environments typically

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found in hospitals [1]. This pathogen plays a significant role in healthcare-associated infections, especially in intensive care units (ICUs). Hence, World Health Organization has recently published its first ever list of antibiotic resistant “priority pathogens” to secure and guide research and development related to new antibiotics, among which *A. baumannii* was being selected as priority one [2]. The incidence of multidrug-resistant (MDR) *Acinetobacter* infections ranges between 47% and 93%, with mortality rates between 30% and 75% [3]. Moreover, MDR *A. baumannii* is associated with an increased length of hospitalisation and higher health care costs [4, 5]. According to published report, increase in global rates of carbapenem-resistant *A. baumannii* (CRAB) between 2006 and 2016 was observed in both developed (from 51.6 to 73.9%) and developing countries (from 72.1 to 77.8%) [6]. Additionally, a statistically significant increasing trend in isolation of *Acinetobacter* spp. was observed in a tertiary healthcare institution in central Serbia, from 2009 to 2015 [7]. Treatment options for MDR *A. baumannii* are limited since this pathogen is typically resistant to all β -lactams and fluoroquinolones. Although antibiotic therapy protocols for CRAB infections have not been well defined, they generally include tigecycline (TGC), polymyxins, or sulbactam, alone or in combination with a second agent [8]. However, toxicity (polymyxins), suboptimal pharmacokinetics and the propensity for development of resistance limit these options [9]. TGC is a broad-spectrum antimicrobial agent with *in vitro* activity against both Gram-positive and Gram-negative bacteria. It is a member of the tetracycline family and the first glycylcycline that became available for clinical use. Glycylcyclines bind to ribosomes five-fold more potently than tetracycline and minocycline, which explains the better efficacy of this drug [10]. TGC has been approved for the treatment of complicated skin and intra-abdominal infections in the United States and Europe moreover, for community-acquired bacterial pneumonia in the United States [11, 12]. The increasing clinical use of TGC necessitates accurate susceptibility testing methods however, approved criteria for TGC susceptibility testing against *A. baumannii* are still lacking. So far, several methods have been evaluated for routine TGC susceptibility testing with broth microdilution (BMD) as the reference one. Nevertheless, it has been suggested that discrepancies in the interpretation of TGC susceptibility results may exist when different testing methods are used [13, 14].

In the western Balkan region, there is a lack of data concerning the prevalence and susceptibility patterns of MDR *A. baumannii*.

The aims of present study were: (i) to detect antimicrobial resistance patterns of MDR *A. baumannii* recovered from clinical specimens of ICU patients from three western Balkan countries; (ii) to assess the proportion of CRAB; (iii) to analyse the *in vitro* activity of TGC against the tested isolates; (iv) to assess the agreement, correlation and error rates for TGC gradient strip test, compared with the standard BMD method.

MATERIALS AND METHODS

Bacterial isolates

The present multicentre study involved 349 non-redundant strains of MDR *Acinetobacter* spp. recovered from patients hospitalized in ICUs of the five tertiary care hospitals which are geographically distributed across three Western Balkan countries: Serbia (Clinical Centre of Serbia in Belgrade, Institute for Pulmonary Diseases of Vojvodina in Novi Sad, Clinical Centre of Nis in Nis), Montenegro (Clinical Centre of Montenegro in Podgorica) and Bosnia and Herzegovina (Clinical Centre of Banja Luka in Banja Luka), in the period June 2016–December 2017. The isolates included in the study were designated as MDR on the basis of resistance to at least one agent in three or more different antibiotic classes. The isolates that were non-susceptible to at least one agent in all but two or less antimicrobial classes were defined as extensively drug-resistant (XDR) *Acinetobacter* spp. The clinical materials were samples of lower respiratory tract ($n = 124$); surgical wound exudates ($n = 96$); blood ($n = 90$); peritoneal fluid ($n = 6$); surgical biopsy samples ($n = 4$); cerebrospinal fluid ($n = 4$); urine ($n = 8$).

Species identification and antimicrobial susceptibility testing

Acinetobacter spp. identification and antimicrobial activity of 18 antimicrobial agents were carried out by the VITEK2 automated system (BioMerieux SA, Marcy L' Etoile, France) according to the manufacturer's instructions. The tested antimicrobials were: ampicillin/sulbactam, ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, gentamicin, amikacin, tobramycin, ciprofloxacin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole and colistin. Besides, the disk diffusion test was done to evaluate susceptibility to ampicillin/sulbactam, ceftazidime, cefepime, amikacin and tobramycin. Additionally, MIC values of colistin and carbapenems (imipenem and meropenem) were determined by ComASP Colistin (Liofilchem, Italy) and Gradient strip test (Liofilchem, Italy), respectively. The MIC values were evaluated according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [15]. All MDR *A. baumannii* isolates were stored at -80°C in skim milk (HiMedia, India). Identification of *A. baumannii* species was done by detection of the intrinsic *bla*_{OXA-51}-like carbapenemase gene [16].

Susceptibility to TGC was evaluated by Gradient strip test and by BMD as the reference method. Gradient strip test (Liofilchem, Italy) was performed according to the manufacturer's instructions on freshly prepared Mueller Hinton Agar (HiMedia, India). The plates were inoculated with a 0.5 McF bacterial suspension. After drying the plates, TGC (0.016–256 $\mu\text{g}/\text{mL}$) strips were applied. The culture plates were then incubated in ambient air at $35 \pm 1^{\circ}\text{C}$ for 18–20 h, and the MIC values were evaluated. BMD was carried out according to the CLSI procedures [17]. Freshly prepared

cation-adjusted Mueller-Hinton broth (HiMedia, India) was used. TGC concentration ranged from 0.06 to 64 µg/mL. Panels were inoculated manually and read optically. The obtained results were expressed as MIC range, MIC₅₀ and MIC₉₀ values in µg/mL. Since European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Food and Drug Administration (FDA) have not yet determined the breakpoints for TGC susceptibility against *A. baumannii* due to insufficient evidence, the interpretation to susceptible, intermediate or resistant category was performed according to Enterobacterales breakpoints from both EUCAST ($S \leq 1$ µg/mL; $R > 2$ µg/mL) and FDA ($S \leq 2$ µg/mL; $R \geq 8$ µg/mL) [18]. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 strains were used as quality control samples.

Evaluation of concordance among BMD and gradient strip test

Categorical agreement (CA) was defined as the percentage of isolates classified in the same susceptibility category by BMD and the gradient strip test. Category discrepancies were classified as follows: (i) very major errors (VME), cases where BMD indicated resistance and the gradient strip test indicated susceptibility; (ii) major errors (ME), an isolate categorized as susceptible by BMD and resistant by the gradient strip test; (iii) minor errors (mE), one interpretation category difference between BMD and the comparative method [19]. Essential agreement (EA) was considered the percentage of MIC within ± 1 doubling dilution of the MIC determined by BMD [20].

Statistical analysis

The SPSS for Windows software package (ver. 13; SPSS Inc., Chicago, USA) was used to perform all analyses. A *P*-value of ≤ 0.05 was considered to be significant.

RESULTS

Antimicrobial patterns of MDR *Acinetobacter baumannii*

A total of 332 out of 349 (95.1%) isolates were identified as *A. baumannii*. The overall rates of antimicrobial resistance of *A. baumannii* obtained by VITEK 2 were the following: ampicillin/sulbactam, 46.9%; ticarcillin, 96.4%; ticarcillin/clavulanic acid, 93.4%; piperacillin, 97.9%; piperacillin/tazobactam, 94.8%; cefotaxime, 100%; ceftazidime, 96.3%; cefepime, 94.9%; ciprofloxacin, 97.2%; imipenem, 92.3%; meropenem, 91.8%; gentamicin, 96.6%; amikacin, 93%; tobramycin, 73.8%; tetracycline, 60.2%; trimethoprim/sulfamethoxazole, 96.9%; and colistin, 1.8%. Susceptibility test results obtained by the disk diffusion indicated the following resistance rates: ampicillin/sulbactam, 48.5%; ceftazidime, 96.6%; cefepime, 96.3%; amikacin, 93.4%; and tobramycin, 80.1%. Gradient test showed the CRAB isolates were detected in a substantial proportion of the tested sample

Table 1. Minimum inhibitory concentration (MIC) ranges, MIC₅₀, MIC₉₀, mode and susceptibility categories of tigecycline against MDR *A. baumannii* isolates (*n* = 166) using broth microdilution and gradient strip test

	Range ^a (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Mode (µg/mL)	S n (%)			I n (%)			R n (%)		
					FDA	EUCAST	FDA	EUCAST	FDA	EUCAST	FDA	EUCAST	
BMD	0.125–16	2	8	2	174 (52.4)	66 (19.9)	96 (28.92)	108 (32.53)	62 (18.67)	158 (47.59)	88 (26.51)		
Gradient strip test	0.125–16	2	4	2	244 (73.49)	108 (32.53)	86 (25.90)	136 (40.96)	2 (0.6)				

MIC = minimum inhibitory concentration; S = Susceptible; I = Intermediate; R = Resistant; EUCAST = European Committee on Antimicrobial Susceptibility Testing; FDA = Food and Drug Administration; and BMD = broth microdilution.

^a Range indicates the minimum and maximum observed value of MIC among the 166 isolates.



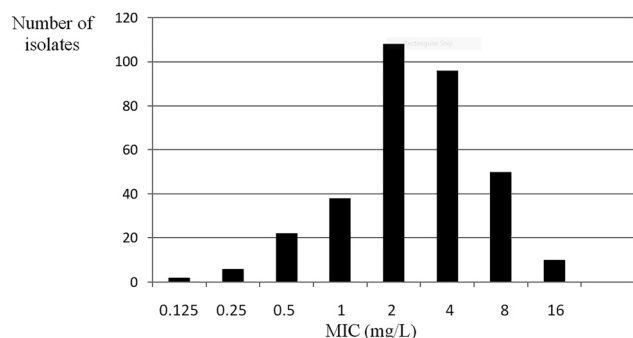


Figure 1. Minimum inhibitory concentration (MIC) distribution of tigecycline against *A. baumannii* – broth microdilution method

(92.8%). The MIC₅₀/MIC₉₀ of the tested carbapenems were 8/>32 µg/mL for imipenem and 16/>32 µg/mL for meropenem. Although carbapenem resistance was higher in non-invasive isolates (94.7%) compared to invasive strains (88.5%), statistical significance was not observed. Two hundred fifty-two isolates (75.9%) were deemed to be XDR. Moreover, eight out of 332 MDR *A. baumannii* were pan-resistant. The MIC of colistin obtained by ComASP Colistin ranged from <0.25 to 4 µg/mL. The MIC₅₀/MIC₉₀ values for colistin were <0.25/1 µg/mL. Seven out of 332 (2.1%) isolates were resistant to colistin.

Susceptibility to tigecycline, EA, CA and interpretative errors

The MIC₅₀, MIC₉₀, mode and range of MIC values for the tested isolates determined by each method ranged from 0.125 to 16 µg/mL (Table 1). Distribution of MIC values obtained with the reference method can be seen in Fig. 1, while the differences in log₂ dilutions of MICs obtained by the gradient strip test compared to BMD are presented in Table 2. Although the gradient test MIC₅₀ was identical to the BMD MIC₅₀ (2 µg/mL), MIC₉₀ obtained with gradient test (8 µg/mL) was inconsistent, compared with that obtained with BMD (4 µg/mL). By gradient strip test, 20.5% of the isolates exhibited MICs identical to those obtained with BMD and 44.6% of the remaining isolates displayed MICs within ±1 log₂ dilution. Thus, the overall essential agreement of MIC values was 65.1%.

There were no significant differences between the TGC MIC values between the countries ($P > 0.05$).

The susceptibility categories of TGC by BMD and gradient strip test are presented in Table 1. With EUCAST breakpoints, susceptibility rates were substantially lower for both BMD and gradient test.

Table 3. Categorical agreement of MIC results and types of interpretative errors produced when testing tigecycline susceptibility by gradient strip test compared to broth microdilution method

	EUCAST <i>n</i> (%)	FDA <i>n</i> (%)	<i>P</i>
Categorical agreement (CA)	176 (53%)	214 (63.9%)	$P < 0.05$
Minor errors (mE)	128 (38.6%)	86 (26%)	$P < 0.05$
Major errors (ME)	4 (1.2%)	0 (0%)	$P > 0.05$
Very major errors (VME)	24 (7.2%)	34 (10.1%)	$P > 0.05$

MIC = minimum inhibitory concentration; EUCAST = European Committee on Antimicrobial Susceptibility Testing; FDA = Food and Drug Administration.

Interpretive categorical concordance and three types of categorical interpretive errors between gradient strip test compared to the reference BMD are presented in Table 3. Discordant susceptibility rates with serious interpretative errors and rather low CA (63.9/53% with FDA/EUCAST breakpoints) were observed. Although the overall error rates were statistically higher by EUCAST ($P < 0.05$), the majority of them were mE (38.6%). Moreover, with EUCAST breakpoints gradient strip test yielded a lower rate of VVG (7.2%) than with FDA (9.3%).

DISCUSSION

A. baumannii infections represent a growing global threat and one of the most challenging healthcare-associated infections worldwide.

As expected, the vast majority of the tested *Acinetobacter* spp. in our study was identified as *A. baumannii*. However, it is worrisome that CRAB isolates comprised 92.8% of the tested MDR *A. baumannii*. A recent global survey indicated that CRAB accounted for 75% of the clinical isolates of *A. baumannii* [19]. Besides, highly resistant pathogens are more predominant in Latin America, Middle East and Asia-pacific than in North America or Europe [21].

This study shows the *in vitro* activity of TGC and a panel of other antimicrobial agents against MDR *A. baumannii*. TGC has a relatively safe therapeutic profile compared to that of other agents that often have activity against MDR *A. baumannii* (e.g. polymyxins and aminoglycosides) [22]. The current study utilized BMD and gradient strip test both to establish the activity profile of TGC against MDR *A. baumannii* isolates and to allow for a direct assessment of the potential variability between the two testing methods.

Table 2. Differences in log₂ dilutions of MICs obtained by gradient strip test compared to the reference broth microdilution method

Test method	<i>n</i> (%) of isolates showing a MIC difference (in log ₂ dilutions) of:							
	>−3	−3	−2	−1	0	+1	+2	+3
Gradient strip test	0 (0)	18 (5.4)	54 (16.2)	94 (28.3)	152 (45.8)	8 (2.4)	6 (1.8)	0 (0)

MIC = minimum inhibitory concentration.



Overall, TGC exhibited potent *in vitro* activity against the tested isolates. The TGC MIC₅₀ obtained by each method (2 µg/mL) seems to be concordant with other reports [23, 24]. Conversely, high prevalence of TGC resistance in MDR *A. baumannii* was reported in Tel Aviv in 2003 [25]. The MIC₅₀ and MIC₉₀ values were found to be 16 and 32 µg/mL, respectively, with a wide range of 1.0–128 µg/mL [25]. In the present study, the gradient strip test yielded slightly lower MIC values than the reference method. The gradient test MIC₉₀ was 1 log₂ dilution lower than MIC₉₀ obtained by BMD, although the MIC₅₀ were equal by each method. In a recent study, Tas et al. also found that the MIC values differed between these methods. Indeed, MIC₅₀ (2 µg/mL) and MIC₉₀ (4 µg/mL) for BMD as well as MIC₅₀ (2 µg/mL) and MIC₉₀ (6 µg/mL) for gradient strip test are comparable to the results obtained in the present study [26]. Likewise, discrepant MIC values of TGC obtained by gradient strip test and BMD have been reported [27]. Some reports indicated association between the variation in MIC results and different compositions of the Mueller–Hinton medium used for gradient testing (particularly the manganese concentration) [28]. It is well known that tetracycline efflux pumps, which are present in some *A. baumannii* strains, require divalent cations for their function.

In the present study, essential agreement for BMD and gradient test was observed in only 65.1% of the tested *A. baumannii*. Categorical agreement was lower by EUCAST (53%) compared with FDA (63.9%) recommendations and was largely attributed to a relatively high mE rate of the gradient strip test, most of which represented a shift from the intermediate category to the susceptible category. Many of these mE could be attributed to incremental MIC differences afforded by the granularity of the gradient strip test readings. Major and very major error rates were rather low.

Since the search for novel antibiotics has still been challenging, the effectiveness of some older antibiotics such as colistin has been evaluated in MDR microorganisms. Our results showed only 2.1% to be resistant to polymyxin, supporting the possible use of this drug for treating *A. baumannii* infections. This finding is similar to that reported for the period 2006–2016 (pooled prevalence for developing and developed countries: 1.4% and 1.3%, respectively) [6]. Nevertheless, its systemic use is limited due to the adverse effects such as nephrotoxicity, neuromuscular blockade and neurotoxicity.

Although the number of bacterial isolates is a limitation of this study, obtained data are valuable for both treatment and laboratory processing of these strains.

The current study depicts that the majority of MDR *Acinetobacter* spp. isolated from ICU patients were XDR and CRAB. As shown above, it is quite challenging to obtain reliable results of *Acinetobacter* susceptibility testing to TGC as there are no standardized guidelines. The discordances between MICs determined by BMD and gradient strip test are cause for concern, as many clinical microbiology laboratories utilize gradient test for TGC testing of *A. baumannii* isolates. However, the obtained data suggest that TGC could be an attractive option for the treatment of serious infections caused by *A. baumannii*, though further investigations are

warranted so that treatment of MDR *A. baumannii* could be guided by validated *in vitro* data.

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