Determination of Colistin and Tigecycline Resistance Profile of Acinetobacter Baumannii Strains from Different Clinical Samples in a Territory Hospital in Turkey

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Abstract:
Objective: Acinetobacter baumannii (A. baumannii) can develop resistance to various antimicrobial agents via different mechanisms. Hence, the aim of this study was to investigate, by using different methods, the resistance profiles of A. baumannii strains isolated from different clinical specimens; from colistin and tigecycline antibiotics, and also the distribution of this resistance according to the clinical samples.

Material and Methods: For this study, 1,265 clinical samples (a samples from each patient) were obtained from various clinics, between; January 2015/December 2018. Identification was conducted by VITEK® 2 compact (bioMerieux, USA) and conventional biochemical tests. Antibiotic susceptibility tests were performed by VITEK 2, and the results of colistin and tigecycline were confirmed by E test and the broth microdilution method.

Results: A. baumannii strains (1,265) were most frequently isolated from tracheal aspirate, sputum and blood samples. At the same time, strains were obtained from intensive care units (70.4%) as well as other clinics (29.6%). The rates of colistin and tigecycline-resistant strains were determined using VITEK 2, E test and the broth microdilution methods as: 3.0%, 5.7%, 9.0% and 21.7%, 24.5%, 33.0%, respectively.

Conclusion: The determination of appropriate antibiotics are important for empirical treatment. Colistin and tigecycline have become prominent as an important, alternative agent in the treatment of A. baumannii-related infections. The results of this study show that colistin and tigecycline resistance rates in intensive care units have been increasing gradually over the years. Monitoring of resistance patterns of nonfermentative bacteria, isolated from intensive care units, is important for the immediate initiation of appropriate empirical treatment. In–vitro studies with A. baumannii strains should also be supported by clinical trials.

Keywords: Acinetobacter baumannii, broth microdilution, colistin, tigecycline
Introduction

Acinetobacter baumannii (A. baumannii) is gram negative bacterium, which is mostly isolated from intensive care units. It has high clinical important in the world, and in our country, due to the continuous increase in multidrug-resistant A. baumannii.1,2

Colistin and tigecycline have become prominent as an important alternative agent used especially for the treatment of infetions related to carbapenems resistant A. baumannii strains.3-5 A. baumannii developed resistance to tigecycline has widely been used for many years6,7; however, colistin is promising for the treatment of multi-drug-resistant gram-negative bacteria. Although, the possibility of developing resistance to colistine is lower than carbapenems, it has also been reported that resistance to this agent may increase within some regions of the world over the coming years.8,9

The follow up of colistin and tigecycline susceptibility profiles are important for world health all over. The aim of this study was to investigate, by using different methods, the resistance profiles of A. baumannii strains isolated from different clinical specimens from colistin and tigecycline antibiotics in addition to the distribution of this resistance according to clinical samples.

Material and Methods

Identification and isolation of bacterial

Culture and antibiotic susceptibility results of 1,265 clinical samples, (a samples from each patient) obtained from various clinics of Afyonkarahisar Health Sciences University, between; January 2015/December 2018; according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) were evaluated. (According to the information provided by the clinicians, samples of the patients with infections were selected)

Bactec–Alert 3D (Becton Dickinson, Sparks, The United State of America) blood culture incubation system was used for the isolation of bacteria from the blood cultures. Samples from the blood culture flasks giving positive signals were cultured on 5.0% sheep blood agar, and eosin methylene blue agar medium. Pediatric blood culture samples were also cultured on chocolate agar. The medium was then incubated at 37 °C for 24–48 hours. Urine samples were cultured quantitatively on blood agar and chromogen agar medium. The media were incubated at 37 °C for 18–24 hours. A colony count of 100,000 Colony Forming Unit/milliliter (ml) was considered significant for urine samples. All other clinical samples were cultured on 5.0% sheep blood, Eozin metilen blue agar and chocolate agar, then incubated at 37 °C for 24–48 hours. Bacteria isolated from these cultures were previously identified by VITEK 2 and conventional biochemical tests (Gram stain, oxidase test, fermentation property). These obtained isolates were stored in Tryptic Soy Broth (Oxoid-England) glycerin at –20 °C until being used for this study.

Antimicrobial susceptibility testing

Antibiotic susceptibility tests were performed by VITEK 2, whilst results of colistin and tigecycline were confirmed by E test and broth microdilution (BMD) method alone. A. baumannii isolates that were resistant to more than three of the existing antibiotics determined were identified as multi drug resistance (MDRs). However, pan drug resistance (PDRs) were defined as resistant to all available antibiotics. Extreme drug resistance (XDRs) were defined as resistant to all antibiotics; with the exceptions of colistin, tigecycline or one or two antibiotics.10 Antibiotics used for this purpose were determined as: gentamicin, amikacin, tobramycin, imipenem, meropenem, cefepime, ceftazidime, ampicillin–sulbactam, piperacillin, piperacillin–tazobactam, ciprofloxacin, levofloxacin, trimetoprim–sulfametoksazole, netilmicin, tigecycline and colistin. These antibiotics were determined according to EUCAST recommendations.11 For VITEK 2 system external (One-world Ocuracy Company, Turkey), internal (E. coli American
Type Culture Collection (ATCC) 25922, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 strains were used) quality control studies were performed regularly, so as to check the accuracy of the work. The results of antibiotic susceptibility from VITEK 2 were evaluated according to EUCAST recommendations.11

The BMD method; the bacterial suspensions were adjusted according to EUCAST recommendations for the BMD method. As described in EUCAST, *P. Aeruginosa*; ATCC 27853 were used for quality control of the BMD test. The microplates were incubated at 35 °C for 20 hours, then visually evaluated.

For the E test method; the 0.5 McFarland turbidity suspension of *A. baumannii* strains were prepared, and strains were cultured on a Muller hinton agar surface. After the plates were dried, colistin and tigecycline strips (AB Biodisk, Sweden) were placed. The plates were incubated at 35 °C for 24 hours, minimum inhibitory concentration (MIC) is defined as the lowest concentration of antibiotic capable of inhibiting the growth of the microorganism. Therefore, the concentrations required to inhibit 50.0% and 90.0% of the strains (MIC 50 and MIC 90, respectively) were calculated for colistin and tigecycline.

**MIC breakpoints**

Colistin sensitivity breakpoint for *Acinetobacter*; the MIC breakpoint of ≤2 mg/L, is interpreted to be sensitive by EUCAST.11 There are no MIC breakpoint values approved by EUCAST for tigecycline. For this reason, MIC breakpoints recommended for Enterobacteriaceae by the United States Food and Drug Administration (USFDA) (≤2 μg/ml susceptibility, ≥8 μg/ml resistance) were interpreted, and then based on these.11,12

**Ethical approval**

Ethical approval for this retrospective study was obtained from the local ethics committee of Afyonkarahisar Health Sciences University.

**Statistical analysis**

Data obtained were entered and analysed in Microsoft Excel 2010. Statistical analysis was performed using the IBM Statistical Package for the Social Science (SPSS) Statistics 20. The results were analyzed by using chi-square method, with p-value<0.05 being accepted as statistically significant.

**Results**

Firstly, the distribution of 1,265 *A. baumannii* strains, according to samples included in this study, were analyzed. During this time, the distribution of *A. baumannii* strains were also determined according to the clinics. The percentages of observed strains, isolated from the different units were: intensive care unit (17.7%), anesthesia intensive care unit (15.5%) and neonatal intensive care unit (12.6%), respectively. However, *A. baumannii* strains were also isolated from; neurosurgery (5.8%), orthopedics and traumatology (3.9%) and general surgical (3.0%) clinics, respectively (Table 1). *A. baumannii* strains were most often isolated from tracheal aspirate, sputum and blood samples (Figure 1). The resistance of *A. baumannii* isolates to colistin and tigecycline were examined by comparing the intensive care unit to other clinics (Figure 2). The rates of colistin and tigecycline resistant strains were determined by using VITEK 2, E test and BMD as; 3.0%, 5.7%, 9.0% and 21.7%, 24.5% and 33.0%, respectively. In addition, a comparison was made of the interpretative results, MIC 50 and MIC 90 for colistin/tigecycline as well as susceptibility testing methods (Table 2). At the same time, resistance rates for other antibiotics used in the treatment of *A. baumannii* – related infections was found to be quite high. According to the antibiotic resistance profile, especially; ceftazidime (95.3%), cefepime (95.6%), ampicillin–sulbactam (94.5%), piperacillin (99.6%), piperacillin–tazobactam (98.9%), imipenem (95.3%), meropenem (96.1%), ciprofloxacin (94.3%) and levofloxacin (95.5%), are very noticeable with high resistance rates.
According to VITEK® 2 compact (bioMerieux, USA) and BMD results, all 1,265 *A. baumannii* isolates were determined as MDR with 9.0% as PDR and XDR. When the resistance rates of *A. baumannii* strains to colistin and tigecycline were examined by years, it was observed that the rates gradually increased (Table 4).

**Table 1** Distribution according to clinics of *Acinetobacter baumannii* isolates

<table>
<thead>
<tr>
<th>Clinics</th>
<th>Number of investigated strains (n)</th>
<th>Rate of investigated strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intensive care units</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%) 891 (70.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest Diseases Intensive Care</td>
<td>224</td>
<td>17.7</td>
</tr>
<tr>
<td>Anesthesia Intensive Care</td>
<td>197</td>
<td>15.5</td>
</tr>
<tr>
<td>Neonatal Intensive Care</td>
<td>160</td>
<td>12.6</td>
</tr>
<tr>
<td>Neurosurgery Intensive Care</td>
<td>108</td>
<td>8.5</td>
</tr>
<tr>
<td>Neurology Intensive Care</td>
<td>78</td>
<td>6.2</td>
</tr>
<tr>
<td>General Surgical Intensive Care</td>
<td>51</td>
<td>4.0</td>
</tr>
<tr>
<td>Internal Intensive Care</td>
<td>44</td>
<td>3.5</td>
</tr>
<tr>
<td>Coronary Intensive Care</td>
<td>21</td>
<td>1.7</td>
</tr>
<tr>
<td>Pediatric Intensive Care</td>
<td>8</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Other clinics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%) 374 (29.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurosurgery</td>
<td>74</td>
<td>5.8</td>
</tr>
<tr>
<td>Orthopedics and Traumatology</td>
<td>50</td>
<td>3.9</td>
</tr>
<tr>
<td>General Surgical</td>
<td>38</td>
<td>3.0</td>
</tr>
<tr>
<td>Chest Diseases</td>
<td>37</td>
<td>2.9</td>
</tr>
<tr>
<td>Nephrology</td>
<td>31</td>
<td>2.5</td>
</tr>
<tr>
<td>Physical Therapy and Rehabilitation</td>
<td>24</td>
<td>1.9</td>
</tr>
<tr>
<td>Medical Oncology</td>
<td>20</td>
<td>1.6</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>18</td>
<td>1.4</td>
</tr>
<tr>
<td>Neurology</td>
<td>15</td>
<td>1.2</td>
</tr>
<tr>
<td>Infectious Diseases</td>
<td>13</td>
<td>1.0</td>
</tr>
<tr>
<td>Internal Medicine</td>
<td>13</td>
<td>1.0</td>
</tr>
<tr>
<td>Pediatric Health and Diseases</td>
<td>11</td>
<td>0.9</td>
</tr>
<tr>
<td>Hematology</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>Other</td>
<td>24</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,265</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Figure 1 Distribution according to clinical sample types of *Acinetobacter baumannii* isolated (n/%)

Figure 2 Distribution according to clinical types and resistant number of *Acinetobacter baumannii* isolated (n)
**Table 2** Comparison of interpretative results, and minimum inhibitory concentration 50 and minimum inhibitory concentration 90 for colistin/tigecycline, and susceptibility testing methods

<table>
<thead>
<tr>
<th>Antibiotics and methods</th>
<th>n (%) of colistin/tigecycline-resistant/susceptible</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. baumannii</td>
<td>50</td>
</tr>
<tr>
<td>Colistin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD</td>
<td>110 (9.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>E test</td>
<td>72 (5.7)</td>
<td>0.50</td>
</tr>
<tr>
<td>VITEK 2</td>
<td>38 (3.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>Tigecycline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD</td>
<td>417 (33.0)</td>
<td>4.00</td>
</tr>
<tr>
<td>E test</td>
<td>311 (24.5)</td>
<td>1.50</td>
</tr>
<tr>
<td>VITEK 2</td>
<td>275 (21.7)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

MIC=minimum inhibitory concentration, BMD=broth microdilution, µg/ml=microgram/milliliter

**Table 3** The antibiotics resistance rates of *Acinetobacter baumannii* strains (n=1265)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Number of resistant strains (n)</th>
<th>Resistance rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tigecycline</td>
<td>275</td>
<td>21.7</td>
</tr>
<tr>
<td>Colistin</td>
<td>38</td>
<td>3.0</td>
</tr>
<tr>
<td>Amikacin</td>
<td>705</td>
<td>55.7</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>846</td>
<td>66.9</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>601</td>
<td>47.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>1,206</td>
<td>95.3</td>
</tr>
<tr>
<td>Cefepime</td>
<td>1,210</td>
<td>95.6</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>1,195</td>
<td>94.5</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>1,260</td>
<td>99.6</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>1,251</td>
<td>98.9</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1,206</td>
<td>95.3</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1,216</td>
<td>96.1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1,193</td>
<td>94.3</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>1,205</td>
<td>95.5</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>872</td>
<td>68.9</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>548</td>
<td>43.3</td>
</tr>
</tbody>
</table>
In accordance with the data of our study, there was no statistically significant differences between the intensive care unit and other clinics, in terms of resistance of \textit{A. baumannii} to colistin (p-value=0.061). However, there was a statistically significant difference between the intensive care and other clinics, in terms of the resistance of \textit{A. baumannii} to tigecycline (p-value=0.001). In addition, colistin and tigecycline resistance of \textit{A. baumannii} isolates were compared with imipenem resistance. There was no statistically significant relationship between resistance of isolates to colistin and resistance of isolates to imipenem (p-value=0.696). Although, there was a statistically significant relationship between resistance of isolates to tigecycline and resistance of isolates to imipenem (p-value=0.001).

As to the comparison of BMD and E test methods, for determining the resistance of \textit{A. baumannii} strains to colistin, the difference between the methods was not significant (p-value=0.500). In contrast, the determination of \textit{A. baumannii}'s sensitivity to tigecycline, the difference between BMD and E test methods was found to be statistically significant (p-value=0.000).

Table 4 The rates of resistance to colistin and tigecycline of \textit{Acinetobacter baumannii} strains, according to years

<table>
<thead>
<tr>
<th>Years (n)</th>
<th>Colistin Number (%)</th>
<th>Tigecycline Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015 (136)</td>
<td>8 (5.8)</td>
<td>30 (22.0)</td>
</tr>
<tr>
<td>2016 (267)</td>
<td>18 (6.7)</td>
<td>72 (26.9)</td>
</tr>
<tr>
<td>2017 (407)</td>
<td>33 (8.1)</td>
<td>142 (34.8)</td>
</tr>
<tr>
<td>2018 (457)</td>
<td>51 (11.1)</td>
<td>173 (37.8)</td>
</tr>
<tr>
<td>Total (1265)</td>
<td>110*</td>
<td>417*</td>
</tr>
</tbody>
</table>

*Based on broth microdilution method results

Discussion
In recent years, \textit{A. baumannii} has exhibited high resistance to some antibiotics, causing infections that are difficult to treat, especially in hospitalized patients. \textit{A. baumannii} causes severe nosocomial infections, such as; ventilator–associated pneumonia, urinary tract infections, endocarditis, sepsis and meningitis, particularly in immunocompromised patients.

It has been reported to increase the rates of resistant strains over the years, due to the intense and uncontrolled use of antimicrobial agents against \textit{A. baumannii}, which has the property of being able to survive in a hospital environment. This resistance confines treatment options considerably.

Hence, the clinical importance of detecting antibiotic resistance profiles has increased, due to the fact that bacteria develops resistance to many antibiotics, including carbapenems in a short time. Resistance rates may vary regionally according to the antibiotics administered.

The distribution rates of \textit{A. baumannii} strains, according to the clinics, have been examined in some regions of the world. For example; in a study by Odewale et al.\textsuperscript{14}, \textit{A. baumannii} strains were most frequently isolated from intensive care units (72.7%), surgical clinics (18.2%) and pediatrics (9.1%). Sivarajanji et al.\textsuperscript{15} demonstrated \textit{A. baumannii} strains were most frequently isolated from intensive care units (36.0%), general surgical (25.0%) and Obstetrics and Gynaecology (18.0%). Biglari et al.\textsuperscript{16} isolated 38.6% of \textit{A. baumannii} strains from intensive care units, 18.9% from surgical, and 15.1% from orthopedics and traumatology. World data has therefore exhibited that \textit{A. baumannii} strains have been isolated from many different clinics and clinical samples.

In our study, 70.4% of the strains were isolated from the intensive care units, with 29.6% being isolated from the samples sent from other clinics. Thus, these
strains were most frequently isolated from chest diseases within the intensive care unit, anesthesia intensive care units and neonatal intensive care units, respectively. In addition, these strains were mainly isolated from neurosurgery, orthopedics and traumatology and chest disease clinics, respectively (Table 1). Of the 110 colistin-resistant A. baumannii strains, 87 were isolated from intensive care units, with 23 isolated from other units. Of the 417 tigecycline-resistant A. baumannii strains, 278 were isolated from intensive care units, and 139 from other units. The results of our study have shown, once again, that A. baumannii is often isolated from intensive care units. This can be explained by the follow-up of critical patients in intensive care units, and by the more frequent use of invasive interventions, such as mechanical ventilation, tracheostomy, intubation, central catheterization and urinary catheters.

Isolated clinical samples that were carbapenem resistant A. baumannii have been determined in various studies. Biglari et al.16 mentioned that: A. baumannii strains were mostly isolated from wounds (43.3%), tracheal aspirate (31.2%), urine (8.5%), blood (5.7%) and sterile body fluids (2.8%). Ferdous et al.17 reported that: A. baumannii strains was determined in blood (67.7%), urine (12.9%), tracheal aspirate (8.9%) and wounds (3.3%). In our study, A. baumannii was isolated from tracheal aspirate (32.1%), sputum (24.5%), blood (19.4%), wounds (8.7%) and urine (7.3%) (Figure 1). In particular, it makes one think that mechanical ventilation, nasogastric catheters and tracheostomy applied in intensive care units are risk factors for A. baumannii, which are mostly isolated from trachel aspirate and sputum.

Colistin and tigecycline have been proven to be effective against A. baumannii infections, and have been used more frequently in recent years. However, isolates that are resistant to these two antibiotics have also started to be reported.18 In our study, the rates of colistin and tigecycline-resistant strains were determined using VITEK 2, E test and BMD methods as: 3.0%, 5.7%, 9.0% and 21.7%, 24.5%, 33%, respectively. In our study, it was found that the E test results, VITEK 2 and Broth microdilution results was quite different from each other (Table 2).

The resistance rates of carbapenem resistant isolates to these two antibiotics were examined in a lot of regions around the world. Henwood et al.19 reported that both colistin and tigecycline resistance were determined in 11 of 13 imipenem-resistant isolates. Abdulzara et al.20 demonstrated that colistin resistance was associated with a high level of resistance to other antimicrobials.

In our study, it was determined that the rate of colistin-resistance of carbapenem-resistant isolates was lower. However, 95.3% and 96.1% of A. baumannii strains were found to be resistant to imipenem and meropenem, respectively. Thus, all colistin resistant (110) and tigecycline resistant (417) strains were found to be imipenem resistant (Figure 2). This study found a statistically significant relationship between resistance of isolates to tigecycline and resistance to imipenem. In the light of this information, according to the data, we believe that carbapenem-resistant A. baumannii strains tend mostly to develop resistance to colistin and tigecycline.

Elabd et al.21 pronounced that: 4.6% of A. baumannii strains were found to be resistant to colistin, by using the automated system and E test method. According to the results of Rossi et al.22, which reported that 1.4% of A. baumannii strains were found to be resistant to colistin, by using VITEK 2 and E Test methods; whilst, Asif et al.23 mentioned that 0.8% of A. baumannii strains were found to be resistant to colistin, by using the E test method. The resistance rates of carbapenem-resistant Acinetobacter strains to tigecycline are higher than colistin resistance rates. However, tigecycline is still also an active drug against all A. baumannii, including strains.
that are resistant to imipenem, and various studies have been conducted in regards to this. In the study performed by Alhaddad et al., the tigecycline resistance of A. baumannii tested by the VITEK 2 method, the resistance was determined as 12.5%. Our rates of colistin and tigecycline-resistant strains, obtained by VITEK 2, were consistent with the rates of colistin and tigecycline-resistant strains rates in the world. In many studies, using the BMD method, the rates of colistin and tigecycline-resistant strains were significantly higher than the rates obtained by VITEK 2 and E test methods. In addition, VITEK 2 has several limitations in terms of the reliability of the results. VITEK 2 tigecycline results require confirmation by BMD or E test, for multi drug-resistant pathogens. The performance of VITEK 2 and E test is also poor for colistin susceptibility testing. Thus, colistin resistant isolates should be confirmed by reference to the BMD method.

From a study conducted in Spain, 20 (19.1%) out of 115 A. baumannii strains were resistant to colistin and the remaining 93 (80.9%) strains were susceptible, by the BMD reference method. In the study of Deng et al., tigecycline resistance of A. baumannii was tested by BMD, and this resistance was determined as 86.0%. A study conducted by Casal et al., revealed 20 (20.0%) out of 100 A. baumannii strains were resistant to tigecycline, 60 (60.0%) strains were intermediate and the remaining 20 (10.0%) strains were susceptible, by the BMD reference method. Furthermore, a study conducted in Greece, referenced 18 (90.0%) out of 20 A. baumannii strains were resistant to colistin, and the remaining 2 (10.0%) strains were susceptible, by the BMD reference method. In same study, it was found that all A. baumannii strains were resistant to tigecycline by the VITEK 2. As can be seen from the results of these studies, and our study, VITEK 2 results and BMD results differ significantly.

Conclusion
According to our results along with world data, the high rates of colistin and tigecycline resistance of A. baumannii isolates, isolated in intensive care units, showed that infection control measures in hospitals and antibiotic usage policies in intensive care units should be revised.

However, the susceptibility of empirically initiated antibiotics; such as colistin and tigecycline, in cases suspect of infection should be evaluated in-vitro conditions by the BMD method. Additionally, it is necessary to renew the culture and antibiogram requests by considering that resistance may develop even during treatment.

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Conflict of interest
The authors declare no conflicts of interest.

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