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Resistance and heteroresistance to colistin among clinical isolates of *Acinetobacter baumannii*

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ORIGINAL ARTICLE

ABSTRACT

Colistin is one of the most effective alternatives for treating *Acinetobacter baumannii* infections. The aim of this study was to determine colistin resistance and heteroresistance rates in *A. baumannii* from clinical samples in Hacettepe University clinical microbiology laboratory between June 2016 and January 2017. A total of 200 isolates were included in the study. *In vitro* susceptibility to amikacin, gentamicin, ceftazidime, piperacillin/tazobactam, meropenem, ciprofloxacin, and tigecycline were determined by disk diffusion test. Most isolates were multiresistant as they exhibited resistance to aminoglycosides, β-lactams, and fluoroquinolones. Colistin susceptibility was determined by broth microdilution (BMD) test (EUCAST standards) and was compared with E-test (bioMérieux, France) in 120 isolates. In 14 blood isolates that were susceptible to colistin (MIC ≤ 2 mg/L), heteroresistance was investigated with the population analysis profile (PAP) method. Overall resistance ($n = 200$) to colistin was 28% by BMD. Among the 120 isolates where the two tests were compared, resistance to colistin was 25.8% versus 4.2% with BMD and E-test, respectively. Three blood isolates (21.4%) were heteroresistant to colistin. With E-test, a majority of the resistant isolates are overlooked and *in vitro* susceptibility to colistin should be determined with broth dilution method. This is the first study in Turkey reporting heteroresistance in *A. baumannii* isolates by the PAP method and emphasizes the need to test for heteroresistance in relation to clinical outcome in serious infections due to *A. baumannii*.

KEYWORDS

Acinetobacter, colistin, resistance, heteroresistance

INTRODUCTION

Acinetobacter baumannii has emerged as an important nosocomial pathogen in recent years [1–3]. Many strains of *A. baumannii* are multidrug-resistant (MDR) to the currently available antibiotics, such as beta-lactams, fluoroquinolones, tetracyclines, and aminoglycosides. Tigecycline and colistin seem to be last resorts of treatment for MDR isolates [2, 3].

Colistin, which is also known as polymxin E, is active against Gram-negative bacteria. Its target in Gram-negative bacteria is the lipopolysaccharide (LPS) of outer membrane. Its interaction with lipid A causes destabilitation of LPS, which increases the permeability of bacterial membrane, and this leads to leakage of the cytoplasmic content, resulting in death of bacterial cell. However, the exact mode of action of polymyxins is still unclear [4]. Intrinsic or acquired mechanisms of resistance occur against colistin. Acquired resistance can be chromosomal and can be due to the modification of LPS, efflux pumps, overexpression of capsule polysaccharide, or overexpression of outer membrane proteins. Plasmid encoded resistance can also occur due to synthesis of MCR proteins [4, 5].

As reports on resistance to colistin in *Acinetobacter* spp. are increasing worldwide, recently "heteroresistance to colistin" has been reported [6, 7]. Heteroresistance is defined as the presence of resistant subpopulations in an isolate, which is susceptible (MIC ≤ 2 mg/L) to colistin by *in vitro* susceptibility tests [7].

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The frequency of heteroresistance to colistin in *A. baumannii* shows a wide range from 18.7% to 100%. This is may be due to the origin of isolates included in the study as well as the diversity of methods to detect heteroresistance [8].

It is of concern that in infections due to *A. baumannii* isolates, which are heteroresistant to colistin, treatment with colistin may result in resistance and leads to therapeutic failure [7–9]. However, as the heteroresistance cannot be determined with the conventional *in vitro* susceptibility methods, the real frequency rate is not known. Despite being one of the few alternative drugs used in the therapy of infections due to MDR *A. baumanni*, data on *in vitro* susceptibility of colistin are also scarce. Although there are some reports on colistin resistance in Turkish isolates, semi-automated tests, disk diffusion, or gradient tests have been employed to determine susceptibilities in those studies, which are not acceptable to test colistin [10, 11]. However, resistance to colistin seems to be significantly high in carbapenemase-producing *Enterobacteriaceae* in Turkey [12].

The aim of this study was (a) to determine the *in vitro* activity of colistin in *A. baumanni* isolates from clinical samples of patients admitted to Hacettepe University Hospital with broth microdilution (BMD) test, (b) to compare the BMD test with a gradient test (E-test) in randomly selected 120 isolates, and (c) to investigate colistin heteroresistance in blood isolates that were susceptible to colistin.

MATERIAL AND METHODS

Isolates

Two hundred consecutive isolates of *A. baumannii* collected from clinical samples between June 2016 and January 2017 were included in the study. Only the first isolate from each patient was accepted. Bacterial identification was confirmed with Vitek 2 ID/AST (bio-Mérieux, France) automated bacterial identification system and MALDI-TOF MS (bio-Mérieux). Isolates were stored in brain–heart infusion broth containing 10% glycerol at −20 °C until the day of study.

Antimicrobial susceptibility tests

Antimicrobial susceptibility of the isolates to amikacin, gentamicin, ceftazidime, piperacillin/tazobactam, meropenem, ciprofloxacin, and tigecycline was determined by disk diffusion test in Mueller–Hinton agar (BBL, Becton Dickinson, USA). *Escherichia coli* ATCC 25922 was used as the control strain.

Determination of susceptibility to colistin

In 200 isolates, *in vitro* susceptibility to colistin was determined with BMD using cation-adjusted Mueller–Hinton broth (Becton Dickinson) [10]. Colistin sulfate was obtained in powder form (Sigma-Aldrich, USA). In randomly selected 120 isolates, BMD and E-test (bio-Mérieux) were compared to determine the *in vitro* susceptibility to colistin. *E.coli* ATCC 25922 and *E. coli* NCTC 13846 were included in all the runs as colistin susceptible and resistant controls, respectively. The results of susceptibility tests were interpreted according to clinical breakpoint tables in EUCAST v 8.1 documents [11]. Isolates with MIC values ≤ 2 mg/L for colistin were categorized as susceptible.

Determination of heteroresistance to colistin

In 14 blood isolates, which had MIC values ≤ 2 mg/L for colistin, presence of heteroresistant subpopulations was investigated with population analysis profile (PAP) method [7]. For this purpose, cation-adjusted Mueller–Hinton agar plates containing colistin in 0.5, 1, 2, 4, 8, and 16 mg/L concentrations were inoculated with 100 µl of the bacterial suspension, which were prepared from an overnight culture and adjusted to an inoculum of 10^8 CFU/ml in saline. After 48 h of incubation at 35 °C, colonies were counted. Tests were repeated twice and a plate without colistin was added as growth control in each test. An isolate with growth in the plates with colistin >2 mg was determined as heteroresistant to colistin [7].

RESULTS

Isolates

Among *A. baumannii* isolates, 48% were from respiratory specimens (bronchoalveolar lavage, deep tracheal aspirate, and sputum), 12% were from blood, 15% from abscess, 7% from urine, and the remaining were from other sterile body fluids (CSF, bile, pericardial, peritoneal, pleural fluids, and catheter).

Susceptibility results

In vitro susceptibility of 200 *A. baumannii* isolates to 7 antimicrobials is shown in Table I. Resistance rates to colistin by clinical specimens are shown in Table II. Comparative MIC_{50}, MIC_{90} values, and resistance rates for colistin in 120 isolates by E-test and BMD are given in Table III.

Heteroresistance to colistin

Heteroresistance to colistin was investigated in colistin-susceptible 14 blood isolates by PAP method, using an inoculum of 10^8 CFU/ml. In three isolates (21.4%) with MIC values of ≤ 2 mg/L, bacterial growth was observed in plates containing 4 and 8 mg/L of colistin. These isolates were identified as "heteroresistant to colistin." Two of these isolates were susceptible to amikacin and tigecycline, whereas the third was only susceptible to tigecycline.

Table I. Comparative *in vitro* activity of seven antimicrobials against 200 *A. baumannii* isolates

Antimicrobial agent	*n* (%)		
	Susceptible	Intermediate	Resistant
Amikacin	90 (45.0)	4 (2.0)	106 (53.0)
Gentamicin	73 (36.5)	6 (3.0)	121 (60.5)
Ceftazidime	34 (17.0)	5 (2.5)	161 (80.5)
Piperacillin/ tazobactam	27 (13.5)	9 (4.5)	164 (82.0)
Meropenem	36 (18.0)	1 (0.5)	163 (81.5)
Ciprofloxacin	38 (19.0)	0 (0)	162 (81.0)
Tigecycline	105 (52.5)	35 (17.5)	60 (30.0)

Table II. Resistance to colistin and *A. baumannii* isolates by specimens

Specimens	Number of isolates	Resistance [*n* (%)]
Respiratory	95	21 (22.1)
Blood	24	10 (41.7)
Pus	30	10 (33.3)
Urine	15	4 (26.7)
Other	36	11 (30.6)
Total	200	56 (28.0)

Table III. Comparative MIC_{50}, MIC_{90} values, and resistance rates for colistin in *A. baumannii* by E-test and BMD tests ($n = 120$)

	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	Resistance [*n* (%)]
E-test	0.064	0.5	0.015–64	5 (4.2)
BMD	0.50	≥32	0.015–≥32	31 (25.8)

Note: BMD: broth microdilution; MIC: minimum inhibitory concentration.

DISCUSSION

A. baumannii is recognized as a significant cause of hospital infections and is identified as one of the most dangerous nosocomial microorgansisms by the Infectious Diseases Society of America [6]. *Acinetobacter* spp. causes a broad range of nosocomial infections, such as ventilator-associated pneumonia, urinary tract infections, endocarditis, sepsis, meningitis, skin and soft tissue infections, and wound infections [2]. With the emergence of MDR *A. baumanni* isolates worldwide, colistin and tigecycline have become the only alternatives for the treatment of infections due to this microorganism [6]. According to the results of this study, most isolates were MDR as they exhibited resistance to aminoglycosides, β-lactams, and fluoroquinolones (Table I). In recent years, colistin has been used extensively against MDR strains of *A. baumannii* and as expected, reports of resistance to colistin began to appear with increasing frequency. In 2006, colistin heteroresistance was first reported, followed by others [6, 13–15]. In this study, *in vitro* activity of colistin was investigated in 200 consecutive isolates of *A. baumannii*, which were isolated from patients hospitalized in the Hacettepe University Hospital, from June 2016 through January 2017. The majority of the isolates (25%) originated from respiratory tract specimens from patients who were in intensive care units (ICUs). In a recent SENTRY report, 13,752 *A. baumanni* isolates collected from different parts of the world between 1997 and 2016, it is reported that in Europe, most of the isolates were from ICUs [14]. It is indicated in several studies that *A. baumanni* isolates were isolated more frequently from respiratory specimens; similarly, most of the isolates were recovered from respiratory samples in this study (47.5%) [3, 14]. BMD method is considered the gold standard for determining colistin MICs [11, 16]. At present, lower MIC values are obtained with the gradient tests and even if the quality control strains are in the accepted range, they are not appropriate to determine colistin susceptibility. Semi-automated tests were also reported to give major errors [11, 16].

In this study, overall resistance to colistin was 28% by BMD. In randomly selected 120 isolates, susceptibility to colistin was investigated employing BMD and E-tests simultaneously and major discrepancies were recorded. Resistance to colistin was found as 4.2% by E-test and 25.8% by BMD tests. In more than 20% of the isolates, resistance to colistin was missed by E-test, which is a very major error. As it was suggested in several studies, performing gradient tests in the routine microbiology laboratories may be easier but it may be misleading for the clinican with undesirable results in the treatment with colistin [16–18]. Until the recent warning by EUCAST that only BMD tests are acceptable in the determination of colistin susceptibility, many investigators have employed different methods to determine colistin susceptibility in *A. baumanni* and hence data on resistance rates are scarce for colistin. In the SENTRY survey, colistin resistance rate in *A.baumanni* in European countries is around 6.1% and it has increased up to 10.4% in the period between 2013 and 2016. Turkey is among the countries with highest level of resistance [14]. In studies from several countries, resistance to colistin is generally lower [6, 19]. Meanwhile, in a study from Korea where BMD test was employed, it is reported as 30.6% [20]. Data on the susceptibility of colistin to *A. baumannii* are scarce in Turkey as elsewhere, since in many studies, BMD tests have not been employed; hence, actual resistance rates may be higher than the previous reports for colistin.

Three clones of *A. baumannii* are identified that are prevalent in hospital outbreaks worldwide; international

clonal lineages (ICLs) ICL1 and ICL2 are MDR [2, 18, 21]. In addition, new ICLs have been reported, which are MDR and carry carbapenemase genes [21–23]. In a study by Gur et al. [24], it was shown that all isolates of *A. baumannii*-producing OXA-58-like carbapenemases had identical or similar pulsed-field gel electrophoresis patterns, indicating the clonal dissemination of OXA-58-like carbapenemase-producing isolates in this center. Clonal dissemination may account for the high rate of resistance to colistin in this study.

Although heterogenous antimicrobial resistance was reported in *Haemophilus influenzae* in 1947 and subsequently in Gram-positive bacteria, the term "heterogenous" was first reported in 1970. Heterogenous resistance and methods to define this phenomenon have not been clearly characterized and to determine heteroresistance, several methods may be employed; at present, the gold standard is the PAP method [7, 25]. Li et al. [7] define heteroresistance as the presence of subpopulations of resistant bacteria in an isolate having an MIC, which is considered susceptible by standard testing methods. Such that, in *A. baumannii*, in a culture of bacteria with an MIC value of <2 μg/ml for colistin, isolates which can grow in the presence of >2 mg/ml colistin, is considered heteroresistant. With a more recent definition, an isolate is considered heteroresistant when there is a >8 fold difference between the MIC and the concentration inhibiting the entire population in PAP assay [25]. In this study, PAP test was applied for 14 blood isolates, which were susceptible to colistin (MIC < 2 mg/L) by BMD, and colistin heteroresistance was detected in three isolates. The colistin MICs of these isolates were 0.06, 0.125, and 0.5 μg/ml and in all three isolates, subpopulations grew in plates containing 4 and 8 μg/ml colistin in PAP test. Therefore, these three isolates were considered as colistin heteroresistant by both aforementioned definitions.

There are several reports on the rate of colistin heteroresistance. Yau et al. [8] reported 23% of heteroresistance in 30 isolates collected between 1998 and 2006 from Western Pacific region countries. Li et al. [7] reported heteroresistance in 15 of the 16 isolates tested. Srinivas et al. [26] observed heteroresistance in 83% of the 24 isolates. Gazel and Tatman Otkun [27] investigated heteroresistance in 31 blood isolates of *A. baumannii* and did not observe any heteroresistance.

Impact of heteroresistance on the clinical outcome is a concern, since it is one of the last resorts of treatment in MDR *A. baumannii* infections. Some authors believe that infections occurring with heteroresistant isolates may result in resistance *in vivo* as a result of exposure to colistin [9, 25, 28, 29]. In contrast to the views of some investigators who report heteroresistant isolates in patients with no history of colistin use, Hawley et al. [9] suggested that although heteroresistance may be observed in isolates from patients not receiving colistin previously, it is more frequent in patients who had a history of colistin use. Favorable clinical outcomes with colistin therapy in patients infected with heteroresistant isolates have been attributed to the use of aggressive colistin dosing and combination therapy [26].

CONCLUSIONS

The standard method to detect *in vitro* susceptibility to colistin is BMD; however, it is not sufficient to detect heteroresistance. PAP assay, which is the gold standard to detect heteroresistance, cannot be performed in routine laboratories. Rate of colistin heteroresistance is a new area of research and this is the first study showing heteroresistance to colistin with PAP assay in Turkish *A. baumannii* isolates. We believe that further studies are needed to determine the frequency of colistin heteroresistance and the association of *in vitro* heteroresistance with clinical outcome in infections due to *A. baumannii*.

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Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., Scheld, M., Spellberg, B., Bartlett, J.: Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis **8**, 1–12 (2009).
2. Antunes, L. C. S., Visca, P., Towner, K. J.: *Acinetobacter baumannii*: Evolution of a global pathogen. Pathog Dis **71**, 292–301 (2014).
3. Qureshi, Z. A., Hittle, L. E., O'Hara, A. J., Rivera, J. I., Syed, A., Shields, K. R., Pasculle, A. W., Ernst, R. K., Doi, Y.: Colistin-resistant *Acinetobacter baumannii*: Beyond carbapenem resistance. Clin Infect Dis **60**, 1295–1303 (2015).
4. Poirel, L., Jayol, A., Nordmann, P.: Polymyxins: Antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev **30**, 557–596 (2017).
5. Olaitan, A. O., Morand, S., Rolain, J. M.: Mechanisms of polymixin resistance: Acquired and intrinsic resistance in bacteria. Front Microbiol **5**, 1–18 (2014).
6. Cai, Y., Chai, D., Wang, R., Liang, B., Bai, N.: Colistin resistance of *Acinetobacter baumannii*: Clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother **67**, 1607–1615 (2012).
7. Li, J., Rayner, C. R., Nation, R. L., Owen, R. J., Spelman, D., Tan, K. E., Liolios, L.: Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother **50**, 2946–2950 (2006).
8. Yau, W., Owen, R. J., Poudyal, A., Bell, J. M., Turnidge, J. D., Yu, H. H., Nation, R. L., Li, J.: Colistin hetero-resistance in multidrug-resistant *Acinetobacter baumannii* clinical isolates from the Western Pacific region in the SENTRY antimicrobial surveillance programme. J Infect **58**, 138–144 (2009).
9. Hawley, J. S., Murray, C. K., Jorgensen, J. H.: Colistin heteroresistance in *Acinetobacter* and its association with previous

colistin therapy. Antimicrob Agents Chemother **52**, 351–352 (2008).
10. International Organization for Standardization: ISO 20776-1:2006: Clinical laboratory testing and in vitro diagnostic test systems-Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part I: Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. ISO, Geneva, Switzerland, 2006.
11. The European Committee on Antimicrobial Susceptibility Testing: Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 8.1, 2018.
12. Süzük Yıldız, S., Kaşkatepe, B., Şimşek, H., Sarıgüzel, F. M.: High rate of colistin and fosfomycin resistance among carbapenemase-producing *Enterobacteriaceae* in Turkey. Acta Microbiol Immunol Hung **66**, 103–112 (2019).
13. Charretier, Y., Diene, S. M., Baud, D., Chatellier, S., Santiago-Allexant, E., van Belkum, A., Guigon, G., Schrenzel, J.: Colistin heteroresistance and involvement of the PmrAB regulatory system in *Acinetobacter baumannii*. Antimicrob Agents Chemother **62**, 1–28 (2018).
14. Gales, A. C., Seifert, H., Gur, D., Castanheira, M., Jones, R. N., Sader, H.: Antimicrobial susceptibility of *Acinetobacter calcoaceticus – Acinetobacter baumannii* complex and *Stenotrophomonas maltophilia* clinical isolates: Results from the SENTRY Antimicrobial Surveillance Program (1997–2016). Open Forum Infect Dis **6**, 34–46 (2019).
15. Hernan, R. C., Bombicino, K., Granados, G., Nastro, M., Vay, C., Famiglietti, A.: Selection of colistin-resistant *Acinetobacter baumannii* isolates in postneurosurgical meningitis in an intensive care unit with high presence of heteroresistance to colistin. Diagn Microbiol Infect Dis **65**, 188–191 (2009).
16. Matuschek, E., Åhman, J., Webster, C., Kahlmeter, G.: Antimicrobial susceptibility testing of colistin-evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. Clin Microbiol Infect **24**, 865–870 (2018).
17. Chew, K. L., La, M. V., Lin, R. T. P., Teo, J. W. P.: Colistin and polymyxin B susceptibility testing for carbapenem resistant and mcr-positive *Enterobacteriaceae*: Comparison of sensititre, MicroScan, Vitek 2, and Etest with broth microdilution. J Clin Microbiol **55**, 2609–2616 (2017).
18. Bardet, L., Rolain, J. M.: Development of new tools to detect colistin-resistance among *Enterobacteriaceae* strains. Can J Infect Dis Med Microbiol **2018**, 3095249 (2018).
19. Lai, C. C., Chen, Y. S., Lee, N. Y., Tang, H. J., Lee, S. S., Lin, C. F., Lu, P. L., Wu, J. J., Ko, W. C., Lee, W. S., Hsueh, P. R.: Susceptibility rates of clinically important bacteria collected from intensive care units against colistin, carbapenems, and other comparative agents: Results from Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART). Infect Drug Resist **12**, 627–640 (2019).
20. Ko, K. S., Suh, J. Y., Kwon, K. T., Jung, S. I., Park, K. H., Kang, C. I., Chung, D. R., Peck, K. R., Song, J. H.: High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. J Antimicrob Chemother **60**, 1163–1167 (2007).
21. Fonseca, É. L., Caldart, R. V., Freitas, F. S., Morgado, S. M., Rocha, L. T., Santos, R. C. D., Vicente, A. C. P.: Emergence of XDR International Clone IC-6 *Acinetobacter baumannii* carrying $bla_{\text{OXA-72}}$ and $bla_{\text{CTX-M-115}}$ in the Brazilian Amazon Region. J Glob Antimicrob Resist (2019). [Epub ahead of print]
22. Munoz-Price, L. S., Arheart, K., Nordmann, P., Boulanger, A. E., Cleary, T., Alvarez, R., Pizano, L., Namias, N., Kett, D. H., Poirel, L.: Eighteen years of experience with *Acinetobacter baumannii* in a tertiary care hospital. Crit Care Med **41**, 2733–2742 (2013).
23. Mayanskiy, N., Chebotar, I., Alyabieva, N., Kryzhanovskaya, O., Savinova, T., Turenok, A., Bocharova, Y., Lazareva, A., Polikarpova, S., Karaseva, O.: Emergence of the uncommon clone ST944/ST78 carrying $bla_{\text{OXA-40-like}}$ and $bla_{\text{CTX-M-like}}$ genes among carbapenem-nonsusceptible *Acinetobacter baumannii* in Moscow, Russia. Microb Drug Resist **23**, 864–870 (2017).
24. Gur, D., Korten, V., Unal, S., Desphande, L. M., Castanheira, M.: Increasing carbapenem resistance due to the clonal dissemination of oxacillinase (OXA-23 and OXA-58)-producing *Acinetobacter baumannii*: Report from the Turkish SENTRY Program sites. J Med Microbiol **57**, 1529–1532 (2008).
25. El-Halfawy, O. M., Valvano, M. A.: Antimicrobial heteroresistance: An emerging field in need of clarity. Clin Microbiol Rev **28**, 191–207 (2015).
26. Srinivas, P., Hunt, L. N., Pouch, S. M., Thomas, K., Goff, D. A., Pancholi, P., Balada-Llasat, J. M., Bauer, K. A.: Detection of colistin heteroresistance in *Acinetobacter baumannii* from blood and respiratory isolates. Diagn Microbiol Infect Dis **91**, 194–198 (2018).
27. Gazel, D., Tatman Otkun, M.: Investigation of colistin heteroresistance and some factors affecting heteroresistance in carbapenem-resistant *A. baumannii* strains. Mediterr J Infect Microb Antimicrob **6**, 1–9 (2017).
28. Rodriguez, C. H., Barberis, C., Nastro, M., Bombicino, K., Granados, G., Vay, C., Famiglietti, A.: Impact of heteroresistance to colistin in meningitis caused by *Acinetobacter baumannii*. J Infect **64**, 119–121 (2012).
29. Tan, C. H., Li, J., Nation, R. L.: Activity of colistin against heteroresistant *Acinetobacter baumannii* and emergence of resistance in an in vitro pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother **51**, 3413–3415 (2007).