Int J Infect. 2023 July; 10(2): e143968. https://doi.org/10.5812/iji-143968.

Published online: 2024 July 3. **Research Article**

# Evaluation of Antibiotic Susceptibility, Carbapenemase and Metallobetalactamase-producing Strains of *Acinetobacter baumannii* Isolated from Hospitalized Patients in Zahedan During 2019 - 2022

Mojdeh Jahantigh [1], Roghayeh Javadi [2], Zakaria Bameri [1,*], Aslam Dehvari [1]

[1] Infectious Diseases and Tropical Medicine Research Center, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan, Iran

[2] Department of Microbiology, School of Medicine, Zahedan University of Medical Sciences, Zahedan , Iran

[*] *Corresponding author:* Infectious Diseases and Tropical Medicine Research Center, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan, Iran. Email: zakariabameri@yahoo.com

**Received** 2023 December 19; **Revised** 2024 May 5; **Accepted** 2024 May 20.

## Abstract

**Background:** Microbial resistance caused by *Acinetobacter baumannii*, which leads to various infectious diseases, is projected to result in ten million deaths by 2050. This is mainly due to the production of beta-lactamase enzymes, a significant concern for the World Health Organization.

**Objectives:** This study utilized phenotypic analysis to examine isolates producing metallo-beta-lactamase and carbapenemase and to determine the pattern of antibiotic resistance in *Acinetobacter baumannii*.

**Methods:** Following the isolation and identification of 372 *Acinetobacter baumannii* strains from clinical isolates using a variety of biochemical tests, the antibiotic resistance pattern was examined using the disc diffusion method. The production of beta-lactamase and carbapenemase was determined using the combined disc diffusion phenotypic test (CDDT) and the Modified Hodge Test (MHT), respectively.

**Results:** Based on this study, minocycline (7.4%) exhibited the lowest resistance rate, while carbapenems (imipenem and meropenem) showed the highest resistance. All antibiotic groups demonstrated over 80% resistance, suggesting extensive drug resistance in all samples. Furthermore, 325 (94.6%) strains of *Acinetobacter baumannii* produced metallo-beta-lactamase, 215 (57.7%) strains strongly produced carbapenemase, and 135 (36.1%) strains weakly produced carbapenemase.

**Conclusions:** Due to minimal resistance to colistin, minocycline, and ampicillin-sulbactam, it is advisable to use these drugs either individually or in combination for treatment.

*Keywords:* *Acinetobacter baumannii*,Antibioic Resistance, Carbapenemase, Metallo-beta-lactamase

## 1. Background

Today, microbial resistance is a significant global health concern. Identified as one of the most critical challenges by the World Health Organization, it is anticipated to result in ten million deaths by 2050 (1-3). Furthermore, the World Health Organization reports that antimicrobial resistance is an inherent phenomenon, wherein microorganisms develop resistance to antibiotics that were previously effective against them (4). According to a 2019 report by the Centers for Disease Control and Prevention (CDC), mortality caused by pathogens resistant to antimicrobial agents exceeded 35,000 individuals annually between 2012 and 2017 (5, 6).

In recent years, there has been an increase in multi-drug resistance among pathogens, particularly *Acinetobacter baumannii*. This gram-negative, non-fermenting coccobacillus, belonging to the Moraxellaceae family, primarily causes nosocomial infections. These include ventilator-associated and acquired pneumonia, urinary tract infections, meningitis, bacteremia, gastrointestinal infections, and wound skin infections. The presence of *Acinetobacter baumannii* strains resistant to antibiotics such as carbapenems and colistin, which are considered last-

Copyright © 2024, Jahantigh et al. This open-access article is available under the Creative Commons Attribution 4.0 (CC BY 4.0) International License (https://creativecommons.org/licenses/by/4.0/), which allows for unrestricted use, distribution, and reproduction in any medium, provided that the original work is properly cited.

resort treatments, complicates the treatment process within hospital environments (7).

Intrinsic factors responsible for *Acinetobacter baumannii's* resistance include reduced membrane permeability, increased efflux pump activity, and the production of various beta-lactamase enzymes (8). Noteworthy among these enzymes are carbapenemase and metallo-beta-lactamase, which confer resistance against beta-lactam antibiotics, including penicillins, cephalosporins, carbapenems, and monobactams. These enzymes interfere with bacterial cell wall synthesis, leading to bacterial death. The presence of these beta-lactamase enzymes is commonly observed in Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Escherichia coli* (9, 10).

Resistance to carbapenems, particularly in *Acinetobacter baumannii*, is increasing and imposes a significant financial and healthcare burden (11). In 2017, this led the World Health Organization to recognize carbapenem-resistant *Acinetobacter baumannii* as a high priority on a published list of 12 antibiotic-resistant bacteria posing the greatest threat to modern medicine (12).

## 2. Objectives

Therefore, this study aimed to identify the antibiotic resistance pattern of *Acinetobacter baumannii* in Zahedan and investigate carbapenemase and metallo-beta-lactamase-producing isolates using phenotypic methods, which are crucial for diagnosing and treating infections caused by these strains.

## 3. Methods

### 3.1. Study Design and Collection of Isolates

In this cross-sectional study, 372 *Acinetobacter baumannii* strains were isolated from various clinical samples, including blood, urine, cerebrospinal fluid, burn wounds, and other body fluids, from hospitalized patients at Ali Ebne Abitaleb Hospital in Zahedan, Iran, from September 2019 to May 2022. Sampling was carried out using a simple method. To identify the genus Acinetobacter, the samples were first cultured on MacConkey agar and blood agar (Merck, Germany) media. After Gram staining, biochemical tests including carbohydrate fermentation on TSI, motility in SIM, oxidase test, growth at 42 degrees Celsius, and glucose oxidative-fermentative (O-F) test were performed for the initial identification of *Acinetobacter baumannii*.

### 3.2. Antibiotic Susceptibility Test

Antibiotic susceptibility testing was carried out using the Disc Diffusion Test (DDT) for all isolates of *Acinetobacter baumannii* according to CLSI 2022 guidelines. The isolates were categorized as sensitive, resistant, or intermediate to each antibiotic by measuring the respective zone of inhibition and interpreting the results according to CLSI 2022 guidelines (13). The discs used included piperacillin-tazobactam (100+10 µg), ceftazidime (30 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), minocycline (100 µg), and ampicillin-sulbactam (20 µg) (MAST, UK). Additionally, the *E. coli* strain ATCC 2922 was used as quality control.

### 3.3. e-Test Method

e-test strips (Liofilichem, Italy) were used to determine the susceptibility of colistin, and the results were interpreted according to CLSI 2022 guidelines (13).

### 3.4. The Combined Disk Diffusion Test (CDDT)

The production of metallo-beta-lactamases in *Acinetobacter baumannii* strains was assessed using the combined disk diffusion method. Initially, the desired bacteria were cultured on Mueller-Hinton agar medium equivalent to a 0.5 McFarland suspension for 18 to 24 hours, and then cultured on another Mueller-Hinton agar medium. Two discs were placed on the culture medium: One containing 10 µg of imipenem and the other containing imipenem and EDTA, with a minimum distance of 2.5 cm between them. The plates were then incubated at 37 degrees Celsius for 18 to 24 hours. After incubation, an increase in the diameter of the zone of growth inhibition (greater than or equal to 7 millimeters) around the imipenem+EDTA disc alone was considered indicative of metallo-beta-lactamase production (Figure 1).

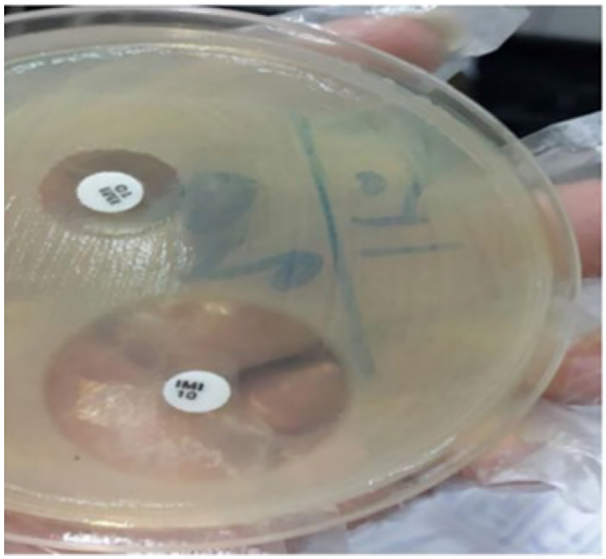

**Figure 1.** Combined disc diffusion phenotypic test (CDDT) phenotypic result

### 3.5. The Modified Hodge Test (MHT)

This test was used to detect the production of carbapenemase in *Acinetobacter baumannii* strains. According to the CLSI guidelines for the Hodge test, a 0.5 McFarland standard suspension of *E. coli* ATCC 25922 (a carbapenem-sensitive *E. coli* strain) was prepared and diluted with sterile saline at a 1: 10 ratio. The diluted *E. coli* suspension was uniformly inoculated on a Mueller Hinton Agar (MHA) medium plate, and allowed to dry for about 5 minutes. A disc containing 10 µg of ertapenem was placed at the center of the inoculated MHA plate. Using a sterile swab, 3 - 5 colonies of freshly cultured *Acinetobacter baumannii* (test organisms) and *Klebsiella pneumoniae* ATCC BAA-1705 (positive control) were streaked in a straight line from the edge of the disc to the edge of the plate. The plate was then incubated at 35 (± 2) °C for 16 - 24 hours. Following incubation, enhanced growth of the *E. coli* and the formation of clover leaf-like indentations at the intersection of the streak of the test organism (and controls) and the zone of inhibition of the *E. coli* were observed.

### 3.6. Statistical Analysis

Statistical analysis was performed using the Student *t*-test and Tukey HSD with SPSS version 23.

## 4. Results

Among the 372 isolates of *Acinetobacter baumannii*, 206 (55.4%) were collected from male patients and 166 (44.6%) from female patients. The most frequent clinical samples were blood (39.5%), endotracheal tube (ETT) (34.4%), and wound (20.7%). As shown in Figure 2, most samples were collected from patients hospitalized in the ICU ward (59.7%), followed by the burn ward (12.1%) and the emergency ward (4.8%). The antibiotic susceptibility test showed the highest resistance to ceftazidime (92.4%), cefepime (91.7%), and meropenem (91.4%). Resistance to minocycline (7.4%) was the least detected among the isolates. According to the MIC test, all isolates were sensitive to colistin (Figure 3).

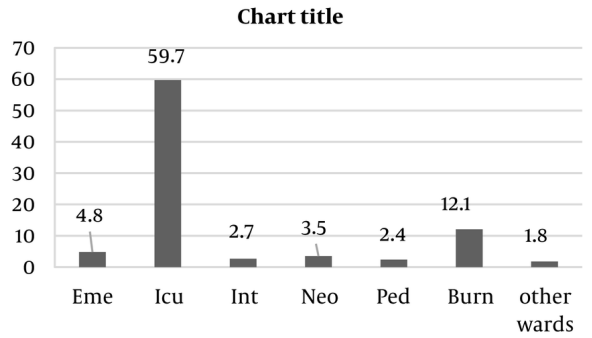


**Figure 2.** Frequency of *Acinetobacter baumannii* isolates based on different clinical. Eme, emergency; ICU, Intensive care unit; Int, internal; Neo, neonatology; Ped, Pediatric.

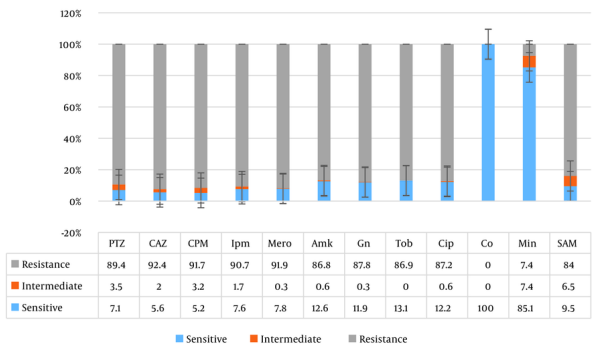


**Figure 3.** Antibiotic susceptibility pattern in clinical isolates of *Acinetobacter baumannii*

### 4.1. The Results of the Combined Disk Diffusion Test (CDTT)

Out of 372 strains of *Acinetobacter baumannii*, 352 strains showed a ≥ 7 mm increase in the zone diameter

with the Imipenem + EDTA disk compared to the Imipenem disk alone. This result indicates that 94.6% of the isolates produced the metallo-beta-lactamase enzyme.

### 4.2. Results of the Modified Hodge Test (MHT)

According to the results of this test, 215 strains (57.7%) of *Acinetobacter* baumannii were strongly positive, 135 strains (36.1%) were weakly positive, and 23 strains (6.2%) were completely negative (Figure 4).

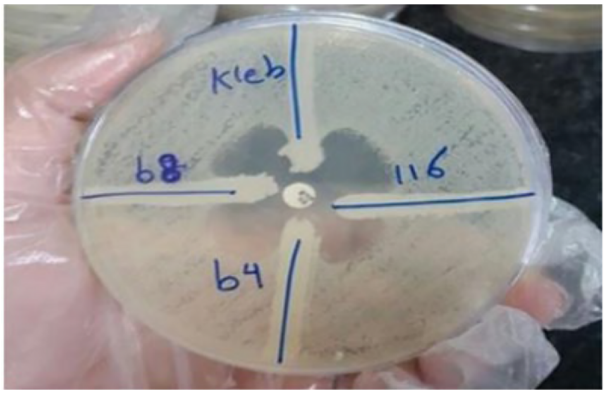


**Figure 4.** Results of the modified Hodge test (MHT) for *Acinetobacter baumannii*. Erapenem (10 µg) was used as the substrate. Enhanced Growth (formation of clover leaf-like pattern) = Positive Result (Carbapenemase production).

## 5. Discussion

In this study, antibiotic resistance and the production of carbapenemase and metallo-beta-lactamase in *Acinetobacter baumannii* strains isolated from clinical samples in Zahedan were investigated using phenotypic methods. Although carbapenems have been the drug of choice for treating infections caused by MDR strains, their widespread use in recent years has led to an increase in carbapenem-resistant strains (14). Recent research has explored the use of antibiotics such as amikacin, polymyxin, tigecycline, minocycline, tobramycin, piperacillin/tazobactam, carbapenems (imipenem, meropenem, doripenem), and colistin to treat infections caused by *Acinetobacter baumannii* (15).

Additionally, some studies showed that resistance to carbapenems was 90 to 100% during 2016 to 2017 in Iran (16). In other countries, the resistance rate has been within the range of 85 to 98% (17). The results of this study showed that the highest sensitivity was to minocycline (85.1%), and the highest resistance was to ceftazidime (92.4%). Therefore, prescribing cephalosporin antibiotics would not be suitable for the therapeutic management of these infectious conditions.

Salehnia and Nojomi in Guilan demonstrated that 65% of *Acinetobacter baumannii* strains were resistant to gentamicin and 60% of isolates were resistant to piperacillin/tazobactam. These results differ from those of the present study (18). Pachon-Ibanez et al. in Spain showed a 78% resistance to imipenem during their study (19). Additionally, Gao et al. recorded a 93.75% resistance to imipenem in their study (20).

Initial studies in the 1970s showed that minocycline was effective for treating infections caused by *Acinetobacter baumannii*. However, at that time, *Acinetobacter* species were sensitive to most common antibiotics available, so minocycline was not used. The prevalence of MDR strains led to a reassessment of old but effective drugs for treatment, such as minocycline, resulting in the CLSI reclassifying this antibiotic as a group B effective antibiotic for treating Acinetobacter. The study by Adibhesami et al., conducted from 2011 to 2013 in Tehran, indicates that minocycline is still an effective treatment option for *Acinetobacter* infections (21). Denys et al. in the United States reported that 98 to 100% of *Acinetobacter baumannii* strains isolated from 2005 to 2011 were sensitive to minocycline (22).

Furthermore, in the present study, the highest number of strains were isolated from the ICU ward. Therefore, it is suggested to design some preventive strategies to reduce nosocomial infections caused by *Acinetobacter baumannii* in the ICU.

### 5.1. Conclusions

According to the results of the present study, all strains were sensitive to colistin, and the lowest resistance was observed with minocycline (4.7%). Therefore, considering the limitations of colistin (being expensive) and minocycline (not recommended for pregnant women and children), the use of these drugs, or a combination of them, is recommended for treatment. Additionally, it is crucial to supervise the use of antibiotics in hospitals, especially in ICU wards.

## Acknowledgements

The authors are grateful to Zahedan University of Medical Sciences for their support in conducting research. (This work was supported by a research grant from ZAUMS with ethical approval code IR.ZAUMS.REC.1402.134). The data used to support the findings of this study in order to protect data are

available from Zahedan University of Medical Sciences for researchers who mention the names of authors in their manuscripts.

## Footnotes

**Authors' Contribution:** Study concept and design: Mojdeh Jahantigh, acquisition of data: Mojdeh Jahantigh, Roghayeh Javadi, analysis and interpretation of data: Mojdeh Jahantigh, drafting of the manuscript: Mojdeh Jahantigh, critical revision of the manuscript for important intellectual content: Zakaria Bameri, Aslam Dehvari, statistical analysis: Mojdeh Jahantigh, study supervision: Zakaria Bameri.

**Conflict of Interests Statement:** The authors declare no conflicts of interest.

**Data Availability:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Ethical Approval:** This study is approved under the ethical approval clinical code IR.ZAUMS.REC.1402.134 .

**Funding/Support:** The authors are grateful to Zahedan University of Medical Sciences for their support in conducting research. This work was supported by a research grant from ZAUMS with ethical approval code IR.ZAUMS.REC.1402.134.

## References

1. Jahantigh M, Larypoor M. Review on the production of effective plant biopolymers in reducing microbial infections. *J Res Bulletin of Med Sci*. 2022;**27**(1):e12.
2. Zahedani SS, Jahantigh M, Amini Y. Determining a pattern for antibiotic resistance in clinical isolations of Pseudomonas aeruginosa. *Tehran Univ Med Sci J*. 2018.
3. Jahantigh M, Tahmasbi H, Bakaeiyan M. Antibiotic Resistance Pattern of Pseudomonas aeruginosa Strains Isolated from Burn Patients. *J Med Mashhad Univ Med Sci*. 2018;**60**(6):1121-31. https://doi.org/10.22038/MJMS.2019.14778.
4. Qayum I. Top ten global health threats for 2019: the WHO list. *J Rehman Med Institute*. 2019;**5**(2):1-2.
5. Kadri SS. Key Takeaways From the U.S. CDC's 2019 Antibiotic Resistance Threats Report for Frontline Providers. *Crit Care Med*. 2020;**48**(7):939-45. [PubMed ID: 32282351]. [PubMed Central ID: PMC7176261]. https://doi.org/10.1097/CCM.0000000000004371.
6. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol*. 2015;**13**(1):42-51. [PubMed ID: 25435309]. https://doi.org/10.1038/nrmicro3380.
7. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. *Clin Microbiol Rev*. 2008;**21**(3):538-82. [PubMed ID: 18625687]. [PubMed Central ID: PMC2493088]. https://doi.org/10.1128/CMR.00058-07.
8. Levy-Blitchtein S, Roca I, Plasencia-Rebata S, Vicente-Taboada W, Velasquez-Pomar J, Munoz L, et al. Emergence and spread of carbapenem-resistant Acinetobacter baumannii international clones II and III in Lima, Peru. *Emerg Microbes Infect*. 2018;**7**(1):119. [PubMed ID: 29970918]. [PubMed Central ID: PMC6030224]. https://doi.org/10.1038/s41426-018-0127-9.
9. Nasr P. Genetics, epidemiology, and clinical manifestations of multidrug-resistant Acinetobacter baumannii. *J Hosp Infect*. 2020;**104**(1):4-11. [PubMed ID: 31589900]. https://doi.org/10.1016/j.jhin.2019.09.021.
10. Ayobami O, Willrich N, Harder T, Okeke IN, Eckmanns T, Markwart R. The incidence and prevalence of hospital-acquired (carbapenem-resistant) Acinetobacter baumannii in Europe, Eastern Mediterranean and Africa: a systematic review and meta-analysis. *Emerg Microbes Infect*. 2019;**8**(1):1747-59. [PubMed ID: 31805829]. [PubMed Central ID: PMC6913636]. https://doi.org/10.1080/22221751.2019.1698273.
11. Holt K, Kenyon JJ, Hamidian M, Schultz MB, Pickard DJ, Dougan G, et al. Five decades of genome evolution in the globally distributed, extensively antibiotic-resistant Acinetobacter baumannii global clone 1. *J Microbial Genomics*. 2016;**2**(2). e000052.
12. Wareth G, Linde J, Hammer P, Nguyen NH, Nguyen TNM, Splettstoesser WD, et al. Phenotypic and WGS-derived antimicrobial resistance profiles of clinical and non-clinical Acinetobacter baumannii isolates from Germany and Vietnam. *Int J Antimicrob Agents*. 2020;**56**(4):106127. [PubMed ID: 32750418]. https://doi.org/10.1016/j.ijantimicag.2020.106127.
13. World Health Organization. *performance sandards for antimicrobial susceptibiliy testing*. 32st ed. 2022. M100 p.
14. Kim UJ, Kim HK, An JH, Cho SK, Park KH, Jang HC. Update on the Epidemiology, Treatment, and Outcomes of Carbapenem-resistant Acinetobacter infections. *Chonnam Med J*. 2014;**50**(2):37-44. [PubMed ID: 25229014]. [PubMed Central ID: PMC4161759]. https://doi.org/10.4068/cmj.2014.50.2.37.
15. Fishbain J, Peleg AY. Treatment of Acinetobacter infections. *Clin Infect Dis*. 2010;**51**(1):79-84. [PubMed ID: 20504234]. https://doi.org/10.1086/653120.
16. Agarwal S, Kakati B, Khanduri S, Gupta S. Emergence of Carbapenem Resistant Non-Fermenting Gram-Negative Bacilli Isolated in an ICU of a Tertiary Care Hospital. *J Clin Diagn Res*. 2017;**11**(1):DC04-7. [PubMed ID: 28273965]. [PubMed Central ID: PMC5324410]. https://doi.org/10.7860/JCDR/2017/24023.9317.
17. Lee K, Yum JH, Yong D, Lee HM, Kim HD, Docquier JD, et al. Novel acquired metallo-beta-lactamase gene, bla(SIM-1), in a class 1 integron from Acinetobacter baumannii clinical isolates from Korea. *Antimicrob Agents Chemother*. 2005;**49**(11):4485-91. [PubMed ID: 16251286]. [PubMed Central ID: PMC1280121]. https://doi.org/10.1128/AAC.49.11.4485-4491.2005.
18. Salehnia A, Nojomi F. [Phenotypic study of Extended-spectrum Beta lactamase (ESBL) producing isolates of Acinetobacter baumannii in patients reffered to a military hospital in Guilan province]. *J Cellular Molecular Res*. 2020;**32**(4):454-8. Persian.
19. Pachon-Ibanez ME, Docobo-Perez F, Lopez-Rojas R, Dominguez-Herrera J, Jimenez-Mejias ME, Garcia-Curiel A, et al. Efficacy of rifampin and its combinations with imipenem, sulbactam, and colistin in experimental models of infection caused by imipenem-resistant Acinetobacter baumannii. *Antimicrob Agents Chemother*. 2010;**54**(3):1165-72. [PubMed ID: 20047914]. [PubMed Central ID: PMC2825983]. https://doi.org/10.1128/AAC.00367-09.
20. Gao J, Zhao X, Bao Y, Ma R, Zhou Y, Li X, et al. Antibiotic resistance and OXA-type carbapenemases-encoding genes in airborne Acinetobacter baumannii isolated from burn wards. *Burns*. 2014;**40**(2):295-9. [PubMed ID: 23886986]. https://doi.org/10.1016/j.burns.2013.06.003.
21. Adibhesami H, Douraghi M, Rahbar M, Abdollahi A. Minocycline activity against clinical isolates of multidrug-resistant Acinetobacter

baumannii. *Clin Microbiol Infect*. 2015;**21**(11):e79-80. [PubMed ID: 26197214]. https://doi.org/10.1016/j.cmi.2015.07.007.

22. Denys GA, Callister SM, Dowzicky MJ. Antimicrobial susceptibility among gram-negative isolates collected in the USA between 2005 and 2011 as part of the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.). *Ann Clin Microbiol Antimicrob*. 2013;**12**:24. [PubMed ID: 24006892]. [PubMed Central ID: PMC3851274]. https://doi.org/10.1186/1476-0711-12-24.