Investigating the Effect of Ampicillin-Sulbactam and Colistin on Acinetobacter baumannii Strains by Epsilometer Test: An In Vitro Study

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Abstract

Background: Acinetobacter baumannii is one of the most common causes of nosocomial infections. The knowledge about resistance and susceptibility of this bacterium to antibiotics is mandatory in every region. This study evaluated the susceptibility of A. baumannii strains to ampicillin-sulbactam and colistin in a total of 100 samples of A. baumannii obtained from intensive care unit (ICU) patients with nosocomial infections.

Methods: After identification of A. baumannii, susceptibility to ampicillin-sulbactam and colistin was assessed using Epsilometer test (E-test).

Results: Regarding the resistance to ampicillin-sulbactam, sensitivity, resistance, and intermediate resistance of A. baumannii strains were 62%, 16%, and 22%, respectively. The distribution of resistance among A. baumannii strains was not significantly different regarding the gender, age, duration of ICU stay, background diseases, and type of interventional procedure. The obtained strains of A. baumannii from the patients who had taken β-lactam, aminoglycoside, anti-methicillin resistant Staphylococcus aureus (anti-MRSA), and colistin were significantly resistant to ampicillin-sulbactam (P value <0.05). Overall, 16% of A. baumannii strains was significantly resistant to ampicillin-sulbactam (P value <0.05).

Conclusion: Acinetobacter baumannii strains showed resistance to ampicillin-sulbactam and only one strain was detected with colistin resistance. It is suggested that a local antibiotic resistance regarding A. baumannii infection be defined in order to improve final outcome of antimicrobial treatment and prevent further resistance.

Keywords: Acinetobacter baumannii, Ampicillin-sulbactam, Colistin, Drug resistance, E-test, In vitro

Background

Acinetobacter baumannii is appeared as a gram-negative, oxidase-negative, non-fermenting, obligate aerobic, and immotile cocobacillus. This bacterium is one of the most common causes of nosocomial infections and is known for its prolonged survival, high contamination risk, and remarkable antibiotic resistance (1-3). In many studies, the antibiotic resistance of A. baumannii has been reported as more than 80% (4,5). On the other hand, A. baumannii is one cause of infection in intensive care units (ICUs) and in the patients hospitalized in ICUs, the most common presentations of infection have been ventilator-associated pneumonia (VAP), bloodstream infection (BSI), urinary tract infection (UTI), and wound infection (6-8), with a mortality rate of 20% to 60% (9-11). Furthermore, findings suggest higher isolation rate of A. baumannii from infections caused by Gram-negative bacteria in hospital settings (12,13).

In A. baumannii strains, ampicillin-sulbactam resistance occurs through decreasing the binding ability of antibiotic to penicillin-binding protein 2 (PBP2) (14,15). In 2015, an in vitro study described the synergistic effect of ampicillin-sulbactam in combination with colistin, tigecycline, amikacin, and meropenem on A. baumannii (16). Colistin has been considered as the main antibiotic in treating the nosocomial infections caused by multi-drug resistant gram-negative bacteria, although it is usually chosen as the last option for A. baumannii (8,17). Colistin actively disrupts the lipid A component of lipopolysaccharide (LPS) in the bacterial membrane like a detergent. However, A. baumannii induces colistin resistance through changes in electric charges of lipid A (6,8,14,15,17). Despite colistin resistance in A. baumannii, treatment with this antibiotic has been considered as an empirical therapy instead of being the last choice (14,18,19). One survey on A. baumannii revealed variations in antibiotic resistance during the recent years which might be resulted from...
excessive antibiotic usage (20). The mortality rate among the patients infected with *A. baumannii* treated with only colistin, and the eradication rate of *A. baumannii* have been reported 72% and 56%, respectively. Moreover, the mortality rate of patients treated with colistin combined with ampicillin–sulbactam has been 72%, while 80% of *A. baumannii* have been eradicated (6). On the other hand, studies on *A. baumannii* indicated that there is a direct correlation between mortality rate and delay in initiating the appropriate antimicrobial therapy. Moreover, combination antibiotic therapy disturbs multiple cellular mechanisms in *A. baumannii* which lead to more satisfying outcomes (6,7,14,17).

As noted above, selection of an inappropriate antibiotic for the treatment of *A. baumannii*-caused infections can result in antibiotic resistance and treatment difficulty. In addition, sometimes antibiotic resistance pattern changes in some local hospitals. Therefore, when the antibiotic resistance pattern is known in a community, it would be more logical to use antibiotics in the treatment of patients; and the treatment starts with simpler antibiotics. Therefore, using a reliable antibiogram is mandatory for finding the most suitable and simpler antimicrobial strategy. On the other hand, nowadays, using Epsilometer test (E-test) is considered as a preferred method to assess the antibiotic resistance. This study was conducted in Isfahan-Iran to evaluate the antibiotic susceptibility of *A. baumannii* strains in infections observed in the ICU patients using E-test.

Methods and Materials

This retrospective study was conducted in Isfahan/Iran, from February 2017 to December 2017, on a total of 100 clinical isolates of *A. baumannii* from 137 samples with several sources obtained only from the patients admitted to the ICU, whose signs and symptoms were compatible with nosocomial infections. Nosocomial infections were defined as: VAP, UTI, BSI, soft tissue infection (STI), and central nervous system infection. Therefore, samples were obtained from urine, blood, wound, trachea, pleural fluid, and cerebrospinal fluid (CSF) on the basis of clinical diagnosis and possible kind of infection. *A. baumannii* in these patients was defined as nosocomial isolate if grown from a specimen that was sampled 48 hours after admission to the hospital.

*Acinetobacter baumannii* was identified by the appearance of Gram stained colonies, colony morphology, cytochrome oxidase reaction, and motility of the bacterium. All isolates were evaluated by amplified ribosomal DNA restriction analysis (ARDRA) and confirmed as *A. baumannii*. After selection of the samples, susceptibility of isolated *A. baumannii* strains to ampicillin-sulbactam and colistin were assessed using E-test. In addition to bacteriological techniques, the main clinical and demographic features of all selected patients including gender, age, underlying disorders (internal/surgical), and length of stay in ICU were recorded. In cases of incomplete data and doubtful evidence on sample contamination, the samples were excluded.

Finally, the collected data were entered into SPSS software (version 22.0). Frequency was used as descriptive statistics. As inferential statistics test, chi-square was used and significance level in all analyses was considered less than 0.05.

Results

In this study, 100 out of 137 samples of *A. baumannii* strains were selected based on the inclusion and exclusion criteria, from which 30% were obtained from women and 70% from men with the mean age of 54.82 ± 20.83 years and the mean ICU length of stay as 27.33 ± 33.76 days. The most common underlying cause of admission to ICU was internal diseases in 70% and the most common procedure done on these patients was intubation. The percentage of *A. baumannii* strains isolated from trachea, blood, wounds, urine, pleural fluid, and CSFs were 75%, 2%, 13%, 4%, 1%, and 5%, respectively (Table 1). The prevalence of sensitive, resistant, and intermediate *A. baumannii* strains were 62%, 16%, and 22%, respectively. The frequency distribution of resistance among *A. baumannii* strains showed no significant difference regarding gender, age, duration of ICU admission, underlying disease, and interventional procedures (P value >0.05). Nevertheless, intubation was the most frequent ICU procedure among infected patients (63%). The most frequent samples among ampicillin-sulbactam resistant *A. baumannii* were taken from trachea and the most frequent samples among ampicillin-sulbactam sensitive *A. baumannii* strains were taken from trachea, wound, urine, pleural fluid, and CSF (P value = 0.004) (Table 1).

Regarding the classification of previously used antibiotics, it was found that the most commonly used previous antibiotics in patients were β-lactams, fluoroquinolones, and anti-methicillin resistant *Staphylococcus aureus* (anti-MRSA) with the percentages of 88%, 40%, and 66%, respectively. Other antibiotics were aminoglycosides, cotrimoxazole, colistin, and antianaerobics (Table 2). Considering the effects of previously used antibiotics on *A. baumannii* resistance pattern, ampicillin-sulbactam resistant *A. baumannii* was seen in case of drug history with β-lactam, aminoglycoside, anti-MRSA, and colistin (P value <0.05). In other words, the highest frequency of susceptibility to ampicillin-sulbactam was detected in the patients receiving other antibiotics (Table 2). Figure 1 demonstrates the frequency of *A. baumannii* strains, susceptible to ampicillin-sulbactam in terms of used β-lactam antibiotics. As shown, a great percentage of patients who had taken imipenem and meropenem were highly or intermediately susceptible to ampicillin-sulbactam.
Effect of Ampicillin-Sulbactam and Colistin on A. baumannii Strains

Table 1. Susceptibility of Acinetobacter baumannii Strains to Ampicillin-Sulbactam Based on Demographic andClinical Features of Patients

| Variables                  | Total (n=100) | Resistant (n=16) | Intermediate (n=22) | Sensitive (n=62) | P Value |
|----------------------------|---------------|------------------|---------------------|------------------|---------|
| Female                     | 30 (30%)      | 5 (31.3%)        | 6 (27.3%)           | 19 (30.6%)       | 0.950   |
| Male                       | 70 (70%)      | 11 (68.8%)       | 16 (72.7%)          | 43 (69.4%)       |         |
| Age (y)                    | 54.8±20.8     | 53.8±17.8        | 51.8±23.14          | 56.2±20.75       | 0.672   |
| ICU length of stay (day)   | 27.3±33.7     | 23.5±30.3        | 33.2±50.0           | 26.2±27.31       | 0.625   |

| Specimen                   |              |                  |                     |                  |         |
|----------------------------|--------------|------------------|---------------------|------------------|---------|
| Tracheal                   | 75 (75%)     | 9 (56.3%)        | 15 (68.2%)          | 51 (82.3%)       |         |
| Blood                      | 2 (2%)       | 2 (12.5%)        | 0 (0%)              | 0 (0%)           |         |
| Wound                      | 13 (13%)     | 4 (25%)          | 3 (13.6%)           | 6 (9.7%)         | 0.004   |
| Urine                      | 4 (4%)       | 1 (6.3%)         | 0 (0%)              | 3 (4.8%)         |         |
| Pleural fluid              | 1 (1%)       | 0 (0%)           | 0 (0%)              | 1 (1.6%)         |         |
| CSF                        | 5 (5%)       | 0 (0%)           | 4 (18.2%)           | 1 (1.6%)         |         |

| Intervention               |              |                  |                     |                  |         |
|----------------------------|--------------|------------------|---------------------|------------------|---------|
| No intervention            | 11 (11%)     | 2 (12.5%)        | 2 (9.1%)            | 7 (11.3%)        | 0.724   |
| Intubation                 | 63 (63%)     | 9 (56.3%)        | 14 (63.6%)          | 40 (64.5%)       | 0.828   |
| Craniotomy                 | 11 (11%)     | 1 (6.3%)         | 4 (18.2%)           | 6 (9.7%)         | 0.441   |
| Laparotomy                 | 12 (12%)     | 3 (18.8%)        | 2 (9.1%)            | 7 (11.3%)        | 0.682   |
| Tracheostomy               | 20 (20%)     | 1 (6.3%)         | 3 (13.6%)           | 16 (25.8%)       | 0.153   |
| Chest tube                 | 7 (7%)       | 2 (12.5%)        | 1 (4.5%)            | 4 (6.5%)         | 0.614   |
| Shunt                      | 3 (3%)       | 0 (0%)           | 2 (9.1%)            | 1 (1.6%)         | 0.156   |
| NIV                        | 1 (1%)       | 0 (0%)           | 0 (0%)              | 1 (1.6%)         | 0.734   |
| Debridement                | 1 (1%)       | 1 (6.3%)         | 0 (0%)              | 0 (0%)           | 0.071   |
| Vascular surgery           | 2 (2%)       | 1 (6.3%)         | 0 (0%)              | 1 (1.6%)         | 0.373   |
| EVD                        | 2 (2%)       | 0 (0%)           | 2 (9.1%)            | 0 (0%)           | 0.057   |
| Low limb surgery           | 2 (2%)       | 1 (6.3%)         | 0 (0%)              | 1 (1.6%)         | 0.577   |

| Underlying disease         |              |                  |                     |                  |         |
|----------------------------|--------------|------------------|---------------------|------------------|---------|
| No underlying disease      | 34 (34%)     | 6 (37.5%)        | 6 (27.3%)           | 22 (35.5%)       |         |
| Internal disease           | 55 (55%)     | 8 (50%)          | 11 (50%)            | 36 (58.1%)       | 0.284   |
| Surgical procedure         | 5 (5%)       | 0 (0%)           | 3 (13.6%)           | 2 (3.2%)         |         |
| Internal-surgical procedure| 6 (6%)       | 2 (12.5%)        | 2 (9.1%)            | 2 (3.2%)         |         |

| Cause of hospital admission|              |                  |                     |                  |         |
|----------------------------|--------------|------------------|---------------------|------------------|---------|
| Internal disease           | 70 (70%)     | 10 (62.5%)       | 13 (59.1%)          | 47 (75.8%)       |         |
| Surgical procedure         | 29 (29%)     | 6 (37.5%)        | 8 (36.4%)           | 15 (24.2%)       | 0.227   |
| Internal-surgical procedure| 1 (1%)       | 0 (0%)           | 1 (4.5%)            | 0 (0%)           |         |

Abbreviations: CSF, cerebrospinal fluid; NIV, non-invasive ventilation; EVD, external ventricular drain.

Table 2. Distribution of Acinetobacter baumannii Strains Susceptible to Ampicillin-Sulbactam Based on Taken Antibiotics

| Antibiotics* | Total (n=100) | Sensitive (n=62) | Intermediate (n=22) | Resistant (n=16) | P Value |
|--------------|---------------|------------------|---------------------|------------------|---------|
| β-Lactam antibiotic | 88 (88)       | 43 (69.3%)       | 20 (90.9)           | 15 (93.8)        | 0.028   |
| Fluoroquinolones   | 40 (40)       | 26 (41.9%)       | 6 (27.3%)           | 8 (50)           | 0.325   |
| Aminoglycoside antibiotics | 4 (4)        | 0 (0)            | 4 (18.2)            | 0 (0)            | 0.001   |
| Anti-MRSA          | 66 (66)       | 36 (58.1)        | 16 (72.7)           | 14 (87.5)        | 0.020   |
| Antianaerobic antimicrobials | 6 (6)        | 5 (8.1%)         | 1 (4.5%)            | 0 (0)            | 0.456   |
| Co-trimoxazole     | 3 (3)         | 2 (3.2%)         | 0 (0)               | 1 (6.3)          | 0.529   |
| Colistin           | 15 (15)       | 7 (11.3%)        | 4 (18.2)            | 4 (25)           | 0.026   |

Data are shown as No. (%)

*β-lactam antibiotics including meropenem, imipenem, ampicillin-sulbactam, tazocin, cephalaxin, ceftriaxone, cefotaxime, Keflin, ceftipime, ceftazidine, anti-flooroquinolones (levofloxacin, ciprofloxacin);
Aminoglycoside antibiotics including gentamicin, amikacin;
Anti-MRSA including vancomycin, tescoplanin, linezolid, rifampicin;
Anti-anaerobic antimicrobials including clindamycin, metronidazole.
On the other hand, colistin-resistant *A. baumannii* was only detected in a 21-year-old male participant, a known case of renal transplant, who had been admitted to ICU with a diagnosis of pneumonia and convulsion. He had been intubated during the ICU admission due to respiratory distress (internal disease causes). The formerly used medications for the patient were meropenem, vancomycin, levofloxacin, ganciclovir, tamiflu, cotrimoxazole, and colistin. As a matter of fact, his tracheal samples, *A. baumannii* strains, were resistant to both colistin and ampicillin-sulbactam. Unfortunately, the patient died 20 days after the ICU admission for multi-organ failure and uncontrolled infection.

**Discussion**

*Acinetobacter baumannii* is an opportunistic pathogen which has been known as the fundamental cause of nosocomial infections especially in ICUs over the last 30 years. *A. baumannii* is universally recognized for its significant feature in being able to acquire antibiotic resistance as multidrug resistant (MDR) and extensive drug resistant (EDR). Although new inventions have been reported in the field of antibiotic therapy, *A. baumannii* infection still poses serious mortality risk besides higher costs of hospitalization. Higher risk of antibiotic resistance will pose challenges not only for the patients but also for hospital settings and their staff (1,2,22). Some environmental factors and antimicrobial agents contribute to the development and spread of these MDR strains around the world. For instance, colistin resistance can vary based on the geographical features of the study (23,24). Out of respect for the previous studies, a number of factors including a clinical intervention such as recent surgical procedures, using central venous catheter, urinary catheter, and tracheal intubation have been declared as the risk factors of *A. baumannii*-caused nosocomial infections. Moreover, some studies reported length of stay in hospital and patient bed location as risk factors (6,14,15). Most importantly, *A. baumannii* is a common cause of VAP which is non-responsive to a routine antibiotic therapy (8,17). In our study, more than 50% of the strains had been taken from tracheal samples. The most frequent involved clinical procedure was intubation and the most frequent underlying disease in these patients was internal disease.

Based on our results, no significant correlations were observed between *A. baumannii* resistance pattern to ampicillin-sulbactam and each of the following factors including intervention (i.e. intubation), underlying disease (i.e. internal disease), length of stay in hospital, age, and gender.

On the other hand, multiple in-vitro studies on *A. baumannii* drug resistance claimed that early administration of inappropriate antibiotics would lead to antibiotic resistance, higher mortality rate, poor prognosis, and increased severity of infection (8,25). β-lactam is one of the antibiotics which is widely used to treat *A. baumannii* infections, but β-lactamase is the main core structure in demolishing and corrupting the β-lactam which leads to drug resistance (6,26,27). Interestingly, β-lactamase enzyme produced by the bacteria is highly mutated through replacing active site of amino acids, which leads to the production of new and highly diverse β-lactamase (28). El Salabi et al suggest that plasmid and nuclear chromosome are responsible for high diversity of β-lactamases presenting in *A. baumannii*. Indeed, they carry a set of genes encoding resistance to several families of antibiotics concurrently and even transmit their resistance to other strains (27). Viehman et al declared that a combination therapy of ampicillin-sulbactam with colistin might lead to a decreased mortality and increased likelihood of *A. baumannii* eradication (6). In our study, only 16% of obtained *A. baumannii* strains were resistant to ampicillin-sulbactam. The most frequently used medications were also β-lactams, fluoroquinolones, and anti-MRSA antibiotics. The frequency distribution
of A. baumannii resistance pattern demonstrated that a higher prevalence of resistance to ampicillin-sulbactam was seen in the patients who had taken β-lactams, aminoglycosides, anti-MRSA antibiotics, and colistin. In fact, a significant correlation was found between prescription of β-lactams, aminoglycosides, and anti-MRSA antibiotics and ampicillin-sulbactam resistance. Furthermore, previous consumption of colistin may cause resistance to ampicillin-sulbactam. Nevertheless, the role of combination therapy of ampicillin-sulbactam with colistin in producing drug resistance was not investigated in our study.

In this regard, Willemsen et al in their study showed that the use of ciprofloxacin (CIP) was associated with a stronger increase in resistance than the use of β-lactams (29). Furthermore, according to the results of many studies, a significant increase in antimicrobial resistance to CIP, co-amoxicillin + clavulanic acid (AMCL), and cefuroxime (CFRX) was observed over a relatively short period of time, with the increase of resistance to CIP being stronger than that to AMCL and CFRX (30,31).

Colistin is the last option in the treatment of MDR A. baumannii infection which is our light on the horizon for critically ill ICU patients (32-35). Some ways out to lower colistin resistance among A. baumannii strains are: less antibiotic prescription by physicians, less over the counter drug abuse by the community, and only use in life-threatening situations (32,33). However, Vakili et al showed the frequency of colistin-resistant strains as 11.6% (36). Another study declared 14.2% of colistin resistance among their A. baumannii strains (37). Although colistin is often the only treatment in these MDR strains (38), the resistance has increased worldwide recently (39-41). Actually, some isolated strains showed hetero-resistance to colistin; in these strains, an apparently colistin-susceptible strain carried a small proportion of colistin-resistant genes (42). Under abuse of colistin, both in vitro (43) and in vivo (40) hetero-resistance strains rapidly construct high level of colistin resistance (40,43,44). In our study, only one patient showed colistin resistance. The patient was a known case of renal transplant, critically ill, who was prescribed with meropenem, vancomycin, levofloxacin, ganciclovir, tamiflu, and co-trimoxazole.

**Conclusions**

Overall, 16% of our obtained A. baumannii strains showed resistance to ampicillin-sulbactam and only 1 strain was detected with colistin resistance. After careful consideration of our results and aforementioned surveys on A. baumannii, getting a suitable opinion on the antibiotic resistance pattern is highly dependent on environmental factors and the usual antimicrobial regime prescribed for A. baumannii infection. Considering the particular importance of colistin as one of the last options in treatment, more studies with larger sample sizes and broader time windows are required to assess the prevalence of resistance in A. baumannii strains-induced nosocomial infections. Considering the challenges that MDR A. baumannii infected patients face, it is advised to investigate the antibiotic resistance pattern regularly to decrease the drug resistance and prevent potential threat to the public health.

**Conflict of Interests**

None declared.

**References**

1. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21(3):538-82. doi: 10.1128/cmrr.00058-07.
2. Gaynes R, Edwards JR. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis. 2005;41(6):848-54. doi: 10.1086/432803.
3. Fournier PE, Richet H. The epidemiology and control of Acinetobacter baumannii in health care facilities. Clin Infect Dis. 2006;42(5):692-9. doi: 10.1086/500202.
4. Bassetti M, Righi E, Esposito S, Petrosillo N, Nicolini L. Drug treatment for multidrug-resistant Acinetobacter baumannii infections. Future Microbiol. 2008;3(6):649-60. doi: 10.2217/17460913.6.649.
5. Michalopoulos A, Falagas ME. Treatment of Acinetobacter infections. Expert Opin Pharmacother. 2010;11(5):779-88. doi: 10.1517/14656561003596350.
6. Viehmeyer JA, Nguyen MH, Doi Y. Treatment options for carbapenem-resistant and extensively drug-resistant Acinetobacter baumannii infections. Drugs. 2014;74(12):1315-33. doi: 10.1007/s40265-014-0267-8.
7. Zalts R, Neuberger A, Hussein K, Raz-Pasteur A, Gefen Y, Mishachi T, et al. Treatment of carbapenem-resistant Acinetobacter baumannii ventilator-associated pneumonia: retrospective comparison between intravenous colistin and intravenous ampicillin-sulbactam. Am J Ther. 2016;23(1):e78-85. doi: 10.1097/MJT.0000000000000324.
8. Thi Khanh Nhu N, Riordan DW, Do Hoang Nhu T, Thanh DP, Thwaites G, Huong Lan NP, et al. The induction and identification of novel colistin resistance mutations in Acinetobacter baumannii and their implications. Sci Rep. 2016;6:24291. doi: 10.1038/srep24291.
9. Maragakis LL, Perl TM. Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis. 2008;46(8):1254-63. doi: 10.1086/529198.
10. Jung YJ, Park MS, Kim SE, Park BH, Son JY, Kim HY, et al. Risk factors for multi-drug resistant Acinetobacter baumannii bacteremia in patients with colonization in the intensive care unit. BMC Infect Dis. 2010;10:228. doi: 10.1186/1471-2334-10-228.
11. Doughari HJ, Ndakidemi PA, Human IS, Benade S. The ecology, biology and pathogenesis of Acinetobacter spp.: an overview. Microbes Environ. 2011;26(2):101-12.
12. Irfan S, Idees F, Mehraj V, Habib F, Adil S, Hasan R. Emergence of carbapenem-resistant gram negative and vancomycin resistant gram positive organisms in bacteremic isolates of febrile neutropenic patients: a descriptive study. BMC Infect Dis. 2008;8:80. doi: 10.1186/1471-2334-8-80.
13. Sopirala MM, Mangino JE, Gebreyes WA, Biller B, Bannerman T, Balada-Llasat JM, et al. Synergy testing by Etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother. 2010;54(11):4678-83. doi: 10.1128/aac.00947-10.
14. Lee HJ, Bergen PJ, Bullita JB, Tsuji B, Forrest A, Nation RL, et al. Synergistic activity of colistin and rifampin combination against multidrug-resistant Acinetobacter baumannii in an in...
vitro pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother. 2013;57(8):3738-45. doi: 10.1128/aac.00703-13.

15. Housman ST, Haghigha M, Nicolaou DP, Kutl JL. In vitro pharmacodynamics of human-simulated exposures of ampicillin/sulbactam, doripenem and tigecycline alone and in combination against multidrug-resistant Acinetobacter baumannii. J Antimicrob Chemother. 2013;68(10):2296-304. doi: 10.1093/jac/dkt197.

16. Kuo SC, Lee YT, Yang Lauderdale TL, Huang WC, Chuang MF, Chen CP, et al. Contribution of Acinetobacter-derived cephalosporinase-30 to sulbactam resistance in Acinetobacter baumannii. Front Microbiol. 2015;6:231. doi: 10.3389/fmicb.2015.00231.

17. Araujo AA, Garcia-Sj, Kim HS, Sung HJ, Kim MN, et al. In vitro synergetic activity of antimicrobial agents in combination against clinical isolates of colistin-resistant Acinetobacter baumannii. Antimicrob Agents Chemother. 2016;60(11):6774-9. doi: 10.1128/aac.00839-16.

18. Tripodi MF, Durante-Mangoni E, Fortunato R, Utilti R, Zarrilli R. Comparative activities of colistin, rifampicin, imipenem and sulbactam/ampicillin alone or in combination against epidemic multidrug-resistant Acinetobacter baumannii isolates producing OXA-58 carbapenemases. Int J Antimicrob Agents. 2007;30(6):537-40. doi: 10.1016/j.ijantimicag.2007.07.007.

19. Çetus ES, Tekeli A, Özoğuz AG, Us E, Aridogan BC. Determination of in vitro activities of polymyxin B and rifampin in combination with ampicillin/sulbactam or ceftazidime/ sulbactam against multidrug-resistant Acinetobacter baumannii by the E-test and checkerboard methods. Jpn J Infect Dis. 2013;66(6):463-8.

20. Nectari Hakimeyem I, Kucukbayrak A, Tas T, Burcu Yikilgan A, Akyaya A, Vasaayaca A, et al. Nosocomial Acinetobacter baumannii infections and changing antibiotic resistance. Pak J Med Sci. 2013;29(5):1245-8.

21. Mostofi S, Mirnejad R, Masjedian F. Multi-drug resistance in Acinetobacter baumannii strains isolated from clinical specimens from three hospitals in Tehran-Iran. Afr J Microbiol Res. 2011;5(21):3579-82. doi: 10.5897/AJMR11.139.

22. Babay HA, Kambal AM, Al-Anazy AR, Saidu AB, Aziz S. Acinetobacter baumannii blood stream infection in a teaching hospital- Riyadh, Saudi Arabia. Kuwait Med J. 2003;35(3):196-201.

23. Bou G, Oliver A, Martinez-Beltran J. OXA-24, a novel class D beta-lactamase with carbapenemase activity in an Acinetobacter baumannii clinical strain. Antimicrob Agents Chemother. 2000;44(6):1556-61.

24. Hirakí Y, Yoshida M, Masuda Y, Inoue D, Tsuji Y, Kamimura H, et al. Successful treatment of skin and soft tissue infection due to carbapenem-resistant Acinetobacter baumannii by ampicillin-sulbactam and metronidazole combination therapy. Int J Infect Dis. 2013;17(12):e1234-6. doi: 10.1016/j.ijid.2013.05.002.

25. Diomi-medri JA. [Acinetobacter baumannii pandrug-resistant: update in epidemiological and antimicrobial managing issues]. Rev Chilena Infectol. 2005;22(4):298-320.

26. El Salabi A, Walsh TR, Chouchani C. Extended spectrum beta-lactamases, carbapenemases and mobile genetic elements responsible for antibiotics resistance in Gram-negative bacteria. Crit Rev Microbiol. 2013;39(2):113-22. doi: 10.3109/1040841X.2012.691870.

27. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother. 2010;54(3):969-76. doi: 10.1128/aac.01009-09.

28. Willemsen I, Bogers-Hofman D, Winters M, Kluytmans J. Correlation between antibiotic use and resistance in a hospital: temporary and ward-specific observations. Infection. 2009;37(5):432-7. doi: 10.1007/s15010-009-8325-y.

29. Kunin CM. Resistance to antimicrobial drugs—a worldwide calamity. Ann Intern Med. 1993;118(7):557-61.

30. Neu HC. The crisis in antibiotic resistance. Science. 1992;257(5073):1046-7.

31. Xu X, Kong F, Cheng X, Yan B, Du X, Gai J, et al. Integron gene cassettes in Acinetobacter spp. strains from South China. Int J Antimicrob Agents. 2008;32(5):441-5. doi: 10.1016/j.ijantimicag.2008.05.014.

32. Japoni S, Japoni A, Farshad S, Ali AA, Jamalidoust M. Association between existence of integrons and multi-drug resistance in Acinetobacter isolated from patients in southern Iran. Pol J Microbiol. 2011;60(2):163-8.

33. Shahcheraghi F, Akbari P, Shahmirzadi N, Abbas Alipour Bashash M, Jahbari H, Amir Mozafari N. Detection of b blaCTX, blaTEMbeta-lactamase genes in clinical isolates of Acinetobacter baumannii spp. from selected Tehran hospitals. Iran J Med Microbiol. 2009;3(1):1-9. [Persian].

34. Smolyakov R, Rorer A, Riesenberg K, Schlaefeer F, Alkan M, Porath A, et al. Nosocomial multi-drug resistant Acinetobacter baumannii bloodstream infection: risk factors and outcome with ampicillin-sulbactam treatment. J Hosp Infect. 2003;54(1):32-8.

35. Vakili B, Fazeli H, Shoaei P, Yaran M, Ataei B, Khovash F, et al. Detection of colistin sensitivity in clinical isolates of Acinetobacter baumannii in Iran. J Res Med Sci. 2014;19(Suppl 1):S67-70.

36. Bahador A, Taheri M, Pourakbari B, Hashemizadeh Z, Rostami H, Mansoori N, et al. Emergence of rifampicin, tigecycline, and colistin-resistant Acinetobacter baumannii in Iran; spreading of MDR strains of novel International Clone variants. Microb Drug Resist. 2013;19(5):397-406. doi: 10.1089/mdr.2012.0233.

37. Li J, Nation RL, Turmidje JD, Milne RW, Coulthard K, Rayner CR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant gram-negative bacteria. Lancet Infect Dis. 2006;69(9):589-601. doi: 10.1016/s1473-3099(06)70580-1.

38. Gales AC, Jones RN, Sader HS. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of Gram-negative bacilli: report from the SENTRY antimicrobial surveillance programme (2001-2004). Clin Microbiol Infect. 2006;12(4):315-21. doi: 10.1111/j.1469-0691.2005.01351.x.

39. Rodriguez CH, 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. 2009;65(2):188-91. doi: 10.1016/j.diagmicrobio.2009.05.019.

40. Park YK, Choi JY, Jung SJ, Park KH, Lee H, Jung DS, et al. Two distinct clones of carbapenem-resistant Acinetobacter baumannii isolates from Korean hospitals. Diagn Microbiol Infect Dis. 2009;64(4):389-95. doi: 10.1016/j.diagmicrobio.2009.03.029.

41. Hawley JS, Murray CK, Jorgensen JH. Colistin heteroresistance in acinetobacter and its association with previous colistin therapy. Antimicrob Agents Chemother. 2008;52(1):351-2. doi: 10.1128/AAC.00766-07.

42. Tan CH, Li J, Nation RL. Activity of colistin against heteroresistant Acinetobacter baumannii and emergence of resistance in an in vitro pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother. 2007;51(9):3431-5. doi: 10.1128/aac.01571-06.

43. Goldstein FW, Ly A, Kitisz MD. Comparison of Etest with agar dilution for testing the susceptibility of Pseudomonas aeruginosa and other multidrug-resistant bacteria to colistin. J Antimicrob Chemother. 2007;59(5):1039-40. doi: 10.1093/jac/dkm046.