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Original Article

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

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 coccobacillus. 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.

Table 1. Susceptibility of *Acinetobacter baumannii* Strains to Ampicillin-Sulbactam Based on Demographic and Clinical 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)	54.82±20.83	53.38±17.86	51.86±23.46	56.24±20.75	0.672
ICU length of stay (day)	27.33±33.76	23.50±30.31	33.27±50.00	26.21±27.31	0.625
Specimen					
Tracheal	75 (75%)	9 (56.3%)	15 (68.2%)	51 (82.3%)	0.004
Blood	2 (2%)	2 (12.5%)	0 (0%)	0 (0%)	
Wound	13 (13%)	4 (25%)	3 (13.6%)	6 (9.7%)	
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%)	0.284
Internal disease	55 (55%)	8 (50%)	11 (50%)	36 (58.1%)	
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%)	0.227
Surgical procedure	29 (29%)	6 (37.5%)	8 (36.4%)	15 (24.2%)	
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, cephalexin, ceftriaxone, ceftizoxime, Keflin, cefepime, ceftazidime, anti-fluoroquinolones (levofloxacin, ciprofloxacin);
Aminoglycoside antibiotics including gentamicin, amikacin;
Anti-MRSA including vancomycin, teicoplanin, linezolid, rifampicin;
Anti-anaerobic antimicrobials including clindamycin, metronidazole.

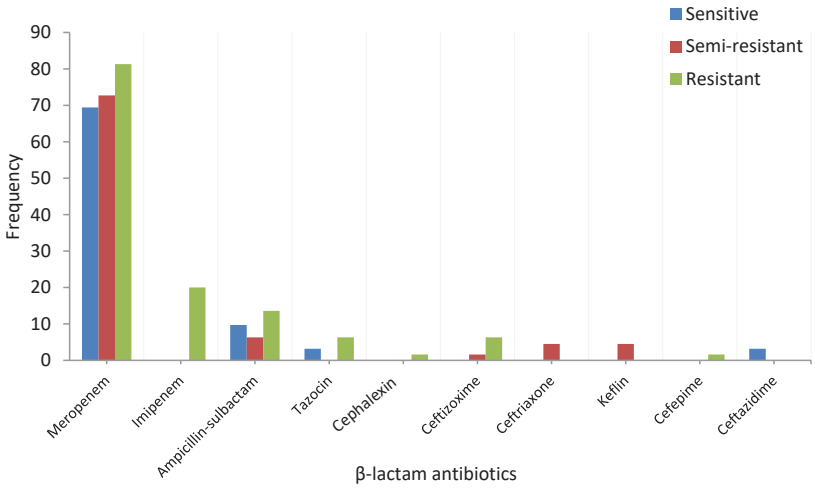

Figure 1. Distribution of *Acinetobacter baumannii* Strains Susceptible to β-lactam Antibiotics.

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, co-trimoxazole, 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.

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