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Antimicrobial resistance patterns of *Acinetobacter baumannii* using the E-test method.

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Abstract

The opportunistic pathogen, Acinetobacter *baumannii* is a gram negative, obligate aerobic, non-fermentative coccobacillus responsible for a variety of infections. It has the ability to develop resistance to many antibiotics. Antimicrobial resistance of this organism has become a worldwide problem that limited therapeutic options. Surveillance of antimicrobial drug resistance is substantially a great issue to guide empirical treatment. This study aimed to determine the antimicrobial resistance pattern of Acinetobacter *baumannii*. In this retrospective study, the antibiotic resistance of 88 Acinetobacter isolates to 12 antibiotics was measured during one year using the E-Test (MIC, France) method in Mueller Hinton agar (Conda, Spain) plates. Species identification was determined by VITEK2 automated system (bioMerieux, Inc. Durham, NC27712 USA). The data was analyzed using Spss version 22 software (SPSS Inc. Chicago, IL). The most isolated Acinetobacter *baumannii* was isolated from the wound (98.9%). The frequency of antibiotic resistance in Acinetobacter isolates was as follows: Ticarcillin (96.6%), Ceftazidime (96.6%), Cefepime (96.6%), Piperacillin/Tazobactam (95.5%), Meropenem (94.3%), Ciprofloxacin (94.3%), Levofloxacin (93.1%), Trimethoprim/Sulfamethoxazole (89.8%), Gentamicin (86.4%), Tobramycin (79.5%), Rifampicin (38.7%), Colistin (7%). Of all the isolates 97.7% were identified as having a MDR phenotype based on the definition that 86 isolates of 88 Acinetobacter *baumannii* isolates exhibited resistance to carbapenem or resistance to at least one agent in three or more antibiotic classes. Acinetobacter *baumannii* isolates showed the highest sensitivity to Colistin and the lowest sensitivity to Ticarcillin, Ceftazidime and Cefepime. It has high resistance [96.6%] to: Ticarcillin, Ceftazidime and Cefepime in Acinetobacter *baumannii* isolates. According to this study we suggest that we could use Colistin and rifampicin to empirical treatment infections caused by Acinetobacter *baumannii*.

Keywords: Acinetobacter baumannii, Antimicrobial resistance, E-Test method, Ahvaz, Iran.

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Introduction

Acinetobacter baumannii is a gram-negative, nonmotile, obligate aerobe, non-fermentative coccobacillus, important opportunistic pathogen responsible for a variety of infections including bacteremia, ventilator-associated pneumonia, urinary tract infections, CSF infection, meningitis, blood stream infection, skin and soft tissue infection and dirty wound infections [1-9]. Since recent years this bactrium has caused severe nosocomial infections [2,10]. Moreover, over the past years, clinical isolates of *Acinetobacter baumannii* have increased [10]. Recent reports in our region have indicated that *Acinetobacter baumannii* prevalence in patients was 8.5% and 9.6% in two consecutive years respectively [11]. According to CLSI guidelines, *Acinetobacter baumannii* has inherent resistance against Amoxicillin, Ampicillin/Sulbactam, Amoxicilline/Clavulanic acid, Aztreonam, Ertapenem, Trimethoprim, Chloramphenicol, Fosfomycin and may be sensitive to Ampicillin/Sulbactam [12] and now it has become resistant to many antimicrobial agents through multiple mechanisms that reduced therapeutic options [2-5,7-9,13-18]. High prevalence of MDR *Acinetobacter baumannii* has been reported in studies in Iran [10,16]. *Acinetobacter baumannii* isolates were considered to be multidrug resistant [MDR] if they exhibit resistance to Carbapenem or resistance to at least one agent in three or more antibiotic classes [19].

In these circumstances, Polymyxins [including Colistin and Polymyxin B] remain as the major active antibacterial agent for the treatment of MDR *Acinetobacter baumannii* infections [20]. In some cases, Colistin is one of the only clinically available antibiotics that maintains active against these isolates [8,21]. But increased use of this antibiotic had led to progress of Colistin resistant strains [1] though; some strains are resistant to Colistin [4,8,20-22]. Unfortunately, the disk diffusion method is inaccurate for the examination of *Acinetobacter baumannii* resistance also broth micro dilusion and agar dilusion are cumbersome to perform and infeasible to implement as usual tests in many clinical laboratories [12]. And there is a lack of well-documented data on *Acinetobacter baumannii* resistance pattern with the E-Test method for antimicrobial resistance in the Ahvaz, south-west of Iran. The information obtained from this study can determine the frequency of antibiotic resistance of *Acinetobacter baumannii* and can reduce the use of antibiotics with high resistance. The purpose of this study was to show the antimicrobial resistance pattern of *Acinetobacter baumannii* isolates with the E-test method from patients referred to the laboratory from Ahvaz hospitals.

Methods

The study was approved by the ethical committee of Ahvaz Jundishapur University of medical sciences (code: IR.AJUMS.REC.1398.654). A retrospective study performed on laboratory data of patients during March 2018 and March 2019 from *Acinetobacter baumannii* isolates of patients referred to the laboratory from hospitals in Ahvaz. Of all the patients referred to the laboratory during one year, the clinical specimen of *Acinetobacter baumannii* isolates were collected from 88 patients (wound specimens=87, blood specimens=1). All the isolates were identified as *Acinetobacter baumannii* species with use of VITEK2 automated system (bioMérieux, Inc. Durham, NC27712 USA).

Susceptibility of the isolates to 12 antimicrobial agents including Piperacillin/Tazobactam, Meropenem, Gentamicin, Colistin, Rifampicin, Trimethoprim/Sulfamethoxazole, Ceftazidime, Cefepime, Ticarcillin, Tobramycin, Ciprofloxacin, Levofloxacin was determined by E-Test according to manufacturer's recommendation (MIC, France). E-Test susceptibility test were performed in accordance with manufacturer's instructions on Mueller-Hinton Agar plates [Conda, Spain]. Mueller Hinton Agar plate were inoculated and were incubated for 20-24 hours in an aerobic atmosphere at 35°C ± 2°C according to the instructions for a bacteriostatic antimicrobial provided by the manufacturer (Table 1).

Table 1: *Sites of isolation.*

Site	Number (%)
Wound	87 (98.9 %)
Blood	1 (1.1%)
Total	88 (100%)

Breakpoint for defining susceptibility, intermediate and resistance were applies in accordance with CLSI guidelines, and the results were interpreted according to CLSI guidelines [12]. Escherichia coli ATCC25922 and Pseudomonas Aeruginosa ATCC35218 were used as control. Data was analysed with descriptive method using Spss version 22 software [SPSS Inc. Chicago, IL].

Results

In this study, 88 *Acinetobacter baumannii* were analyzed. The most commonly identified site of *Acinetobacter baumannii* was the wound [98.9%] and blood [1.1%]. The frequency of antibiotic resistance in Acinetobacter isolates was as follows: Ticarcillin (96.6%), Ceftazidime (96.6%), Cefepime (96.6%), Piperacillin/Tazobactam (95.5%), Meropenem (94.3%), Ciprofloxacin (94.3%), Levofloxacin (93.1%), Trimethoprim/Sulfamethoxazole (89.8%), Gentamicin (86.4%), Tobramycin (79.5%), Rifampicin (38.7%), Colistin (7%). *Acinetobacter baumannii* isolates showed the highest sensitivity to Colistin.

Of the isolates of *Acinetobacter baumannii* 96.6% were resistant to Ticarcillin, Ceftazidime, and Cefepime. Most of them [93.2%] were sensitive to Colistin. From a total of 88 isolates a baumannii, the most active agent was Colistin with 93.1%sensitivity followed by rifampicin with 34.1% sensitivity, respectively. *Acinetobacter baumannii* showed the highest resistance to Ticarcillin and Ceftazidime and Cefepime antibiotics (Table 2). According to the standardized definition of MDR strains, 86 (97.7%) isolates exhibited the MDR pattern.

Table 2: *Antimicrobial resistance of Acinetobacter baumannii to 12 antimicrobial agents.*

Susceptibility			
Antimicrobial agent	**Resistant**	**Intermediate**	**Sensitive**
Ticarcillin	85(96.6%)	2(2.3%)	1(1.1%)
Piperacillin/ Tazobactam	84(95.5%)	1(1.1%)	3(3.4%)
Ceftazidime	85(96.6%)	0(0%)	3(3.4%)
Cefepime	85 (96.6%)	0(0%)	3(3.4%)
Meropenem	83(94.3%)	0(0%)	5(5.7%)
Gentamicin	76(86.4%)	6(6.8%)	6(6.8%)
Tobramycin	70(79.5%)	11(12.5%)	7(8%)
Ciprofloxacin	83(94.3%)	1(1.1%)	4(4.6%)
Levofloxacin	82(93.1%)	2(2.3%)	4(4.6%)
Colistin	6(7%)	0(0%)	82(93.2%)
Rifampicin	34(38.7%)	24(27.2%)	30(34.1%)
Trimethoprim/ sulfamethoxazole	79(89.8%)	0(0%)	9(10.2%)

According to the standardized definition of MDR strains, 86 (97.7%) isolates exhibited the MDR pattern.

Discussion

Major site of *Acinetobacter baumannii* isolation is varied in different studies [5,7,23]. Most of *Acinetobacter baumannii* isolates were isolated from the wound in this study [98.9%], Similar to surgical patients (47.4%) in the study of Dent et al. [23].

Most (40%) of *Acinetobacter baumannii* samples were collected from wound infection in the study of Darvishi [24], Although in studies done in Qatar, Iran, USA, wound isolates of *Acinetobacter baumannii* was 10.5% and 3% and 13% respectively, In the study of Al Samawi et al. Ahdi Khosroshahi et al. and Dent et al. the most commonly identified site of *Acinetobacter baumannii* isolation was respiratory tract [5,7,23]. The difference between these data could be due to *Acinetobacter baumannii* isolated of this study collected from only one ward of hospitals and didn't collect from respiratory ward. Perhaps, it is because their isolates were larger than our study and their results were more reliable than our results. Our study demonstrated a high resistant to cephalosporin's like Cefepime (96.6%) and Ceftazidime (96.6%). Our findings was near to other studies reported by Moogahi et al and Goudarzi M and Azimi H. and Farshadzadeh et al and Biglari et al. and Moosavian et al. studies [11,25-28].

Resistance to Ceftazidime in study of Ahdi Khosroshahi et al. and Ansari et al. was high (above 90%), that is similar to our study [7,9]. Our finding compared to those of other studies shows that resistance to these antibiotics is high [25]. The results of our study is in contrast to that of Al Samawi et al. (Qatar, 2012), because in our study resistance to Cephalosporins is higher than their results [5]. The difference between the results of the two studies could be due to that fact that antimicrobial resistance has increased in recent years and they performed their studies five years ago or they used another antimicrobial resistance method or Cephalosporins isn't available in their country.

According to most of these finding it seems that Ceftazidime and Cefepime is not an appropriate choice for empirical prescription of *Acinetobacter baumannii* isolates. In many cases, Carbapenems has been used in empirical therapy of severe infecion. Resistance to Carbapenems for the treatment of infections caused by *Acinetobacter baumannii* is being observed [9,26,27]. Development of resistant to these antimicrobial agents in *Acinetobacter baumannii* has been rising during recent years [29]. Determined resistance rates of 79% against Meropenem with E-test method in *Acinetobacter baumannii* isolates from malayzia, biglari et al. [25]. In our study it was found that 83 isolates (94.3%) was resistant to Meropenem. Resistance to Meropenem in the study of Moogahi et al. was 86% and in the study of Moosavian et al. was 96% near to our study [11,28]. This fact indicates that Carbapenem Resistances among *Acinetobacter baumannii* isolates are increasing. But in study of Mirnejad et al. One of the most effective antimicrobial agents against *Acinetobacter baumannii* was Meropenem [30], this could be because of the difference between region and date of studies. Also it has been shown that Imipenem and Doripenem are more potent than Meropenem against *Acinetobacter baumannii* [29]. We suggest that in future studies we can use of these Carbapenems instead of Meropenem. While Carbapenems have long been considered as an effective antibiotic against *Acinetobacter baumannii* infection, our study and aforementioned studies show that this antimicrobial agent isn't a good choice for empirical treatment. Resistance to Aminoglycosides is high in the studies performed in Iran in recent years [7,24].

In the present study isolates resistant to aminoglycosides such as gentamicin [86.4%] is similar to the finding of Goudarzi and Azimi study, and similar to our study resistance to gentamicin is higher than tobramycin [26]. Moogahi et al. and Moosavian et al. showed that60% and 96% of *Acinetobacter baumannii* were resistant to Gentamicin respectively [11,28]. In the study of Asadollahi et al., Gentamicin has a good effect on MDR *Acinetobacter baumannii* [6], Also in study of Mirnejad et al. Tobramycin was one of the most effective agent against *Acinetobacter baumannii* [30] However, further control is required to prevent the increase of resistance to these antibiotics in our study.

More than 79% Acinetobacter isolates resistant to Quinolones nearly coresponds to the findings of Vakili et al. study and Goudarzi, Azimi and Moosavian et al. and Biglari et al. [10,25,26,28]. The results of this study confirmed previous reports. The prevalence of Piperacillin/ Tazobactam resistance (95.5%) is very similar to that found in the study of Vakili et al. in Iran (98.5%) [10], and was higher than those reported in Qatar by Al Samawi et al. [5]. Similar to another study result in Iran our study demonstrated that more than84% of *Acinetobacter baumannii* isolates is resistant to Trimethoprim/ Sulfamethoxazole [11]. In contrast to the study conducted by Goudarzi and Azimi in Iran, resistance to Trimethoprim/ Sulfamethoxazole in MDR *Acinetobacter baumannii* was (43.8%) higher than our results [26].

Determined resistance rate of 100% against Ticarcillin in Khosroshahi et al. study is nearly comparable to our results [7]. In the current study 65.9% isolates were non susceptible to Rifampicin, but, in study of Farshadzadeh et al. 97% of Carbapenem-Resistant *Acinetobacter baumannii* are non-susceptible to rifampicin [27]. In this study Resistance rates to Rifampicin was (38.7%) and in the study of Ahdi Khosroshahi et al. it was 27%. These results are similar to each other; however, in our study susceptibility of *Acinetobacter baumannii* to Rifampicin (34.1%) is worse than demonstrated by another method by Khosroshahi et al. (73%) in Iran [7]. Difference in number of samples collected, method of experiment and geographical area where the sample collected could be the main factors for difference in this results. The results in the present study agree with the studies have demonstrated that resistance to most antimicrobial drugs have increased during recent years [11,29]. Colistin is an effective antibiotic against *Acinetobacter baumannii* isolates in our finding.

Several studies have shown that Colistin demonstrates potent invitro activity against *Acinetobacter baumannii* isolates [6,8,27,31]. This fact suggests that a high percentage of patients with *Acinetobacter baumannii* isolation would require a Colistin based regimen to treat infections, But Colistin has a number of side effects and isn't suitable for treating all of the infections caused by *Acinetobacter baumannii* [13], But keep in mind that according to the

study of Garcia-Quintanilla et al. and Ansari et al. and according to this fact that 7% isolates in our study was resistant to Colistin in an increasing number of patients infected with Colistin-resistant strains [9,22]. The highest susceptibility rates were found to Colistin (93.2%) and Rifampicin (34.1%) in *Acinetobacter baumannii* isolates in our study thus we suggest that Colistin and Rifampicin could be used for empirical treatment of infections caused by *Acinetobacter baumannii*. Extensive use of antibiotics within hospitals has contributed to the emergence of *Acinetobacter baumannii* strains which are resistant to a wide range of antibiotics [6].

The emergence of MDR isolates significantly limits effective therapeutic options. In the present study the prevalence of MDR *Acinetobacter baumannii* isolates accounted for 97.7% of the total. Results of recent studies showed prevalence of MDR *Acinetobacter baumannii* in Iran is 100% [26]. These findings are higher than those reported in previous studies [5,23,25]. It could be due to different volumes and patterns of antimicrobial consumption in distinct areas. The transmission of MDR *Acinetobacter baumannii* through hands of healthcare workers and from hospital facilities might contribute to the wide dissemination of MDR *Acinetobacter baumannii* in the hospital [25]. The increasing prevalence of *Acinetobacter baumannii* MDR strains in hospital environment results in the need to modify therapeutic options.

Conclusion

As most of other studies, we came to the conclusion that *Acinetobacter baumannii* is resistant to many of available antibiotics; therefore it is prescribe antibiotics to treat this infection. Vigilance is needed by committee of antibiotic resistance control to prevent outbreak of this pathogen. Since the prevalence of MDR *Acinetobacter baumannii* cannot be determined with this method in Ahvaz in 2017, our data can be used as a reference to assess any increase in the prevalence of resistance *Acinetobacter baumannii* in the future. This study has several limitations that need to be considered when interpreting these data. The study was retrospective and the study period was short, PCR for the detection of antibiotic resistance genes and virulence factors and mutations were not performed. The current study did not address the role of gender, age, occupation. Our results are based only on invitro findings with E-test method on little sample and need to be confirmed by another available method like a dilusion method and on larger samples of *Acinetobacter baumannii* isolates from various sites of body.

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