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Antibiotic Resistance Pattern of *Acinetobacter baumannii* Infections: A Five-year Epidemiological Study in Ahvaz, Iran

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Abstract

Acinetobacter baumannii is a common worldwide hospital-acquired infection that imposes significant economic and health burden due to its multi-drug resistant nature. The present descriptive laboratory data-based epidemiologic study was aimed to evaluate the relative frequency of antibiotics' resistance in *A.baumannii* isolated from the patients hospitalized in Imam Khomeini hospital in Ahvaz. The demographic data such as age, gender, underlying disease, duration of hospitalization, infection site, and type of invasive procedures performed for patients, as well as the antibiotic resistance level, were collected for all patients and statistically analyzed. The most common underlying disease was diabetes (34.8%). Urinary and intravenous catheters insertion were the most common invasive procedures performed on the patients (71.6%), the most common infection site was bronchi (tracheal depth), and the most common clinical manifestation of *A.baumannii* infection was pneumonia. The highest antibiotic resistance rate was against rifampin (100%) and levofloxacin (98.8%), and the lowest resistance rate was against ampicillin/sulbactam (47.7%). The patients who were suffering from diabetes and chronic pulmonary diseases showed a higher incidence of *A.baumannii* infection than other hospitalized patients. The infection to *A.baumannii* that is multi-antibiotic resistant is increasing.

Introduction:

Acinetobacter baumannii (*A.baumannii*) is a gram-negative, aerobic, non-fermentative coccobacillus that belongs to the Moraxella family. Different species of these bacteria are opportunistic pathogens, which resulted in a range of community-acquired infections, as well as hospital infections, and especially are the cause of infection in patients hospitalized in intensive care units. *A.baumannii* is the cause of a wide range of infections such as pneumonia associated with ventilator device; community-acquired pneumonia, endocarditis, meningitis, skin and soft tissue infections, urinary tract infection, as well as, infections related to contaminated hospital equipment and tools (Moradi *et al.*, 2015). This microorganism, also seen to causes pneumonia, urinary tract infections, septicemia, and meningitis epidemics. *A.baumannii* is the most important microorganism associated with the spread of hospital infections. It has been reported as the seventh

common pathogen isolated from samples of patients hospitalized in the intensive care unit and is the cause of 8% and 10% of bacteremia and pneumonia in patients hospitalized in the intensive care units, respectively (Magnet *et al.*, 2001). During the past 20 years, one of the major health problems was the growing number of hospital and community-acquired infections caused by antibiotic-resistant gram-negative bacteria (Gales *et al.*, 2001). Today, *Acinetobacter* species are resistant to almost all groups of common antibiotics i.e., from broad-spectrum penicillin, cephalosporin, carbapenems, and most of aminoglycosides, fluoroquinolones, chloramphenicol and tetracycline (Eliopoulos *et al.*, 2008). The high speed of becoming antibiotics resistant in this pathogen is a factor that severely limits the appropriate therapeutic choices for its effective treatment (Manikal *et al.*, 2003). The prevalence of antibiotic-resistant hospital-acquired infection caused by *A.baumannii* clearly shows the need for

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developing an infection control and prevention program for patients hospitalized in the health and therapeutic centers (Sunenshine *et al.*, 2007). The present study aims to determine the prevalence of *A.baumannii* and the rate of antibiotic-resistant *A.baumannii* isolated from the patients hospitalized in Imam Khomeini Hospital, Ahvaz, Iran during a five year period. The findings of this study could be used to choose the best therapeutic method for infection associated with *A.baumannii* microorganism.

Methodology:

This was a descriptive laboratory data-based epidemiologic study conducted on 250 patients affected from *A.baumannii* infection, referred to the Infection Control Unit of Imam Khomeini Hospital, Ahvaz, Iran from April 2012 to March 2016. The rate of antibiotic-resistance of this pathogen was determined from all patients (subjects). Triple sugar iron agar test (TSI), Sulfide, Indole, motility test (SIM), Citrate test (CIT), Lysine decarboxylation test (LDC), Methyl red-Voges-Proskauer (MR-VP), Deoxyribonuclease (DNase), broth malonite, urea broth, 3.9% salt, and Beryl-Scholin culture mediums, as well as catalase, coagulase, and oxidase tests were used to recognize and distinguish gram-positive and gram-negative bacteria. The antibiotic-resistance of the microorganism was measured using Kirby-Bauer method in the Müller Hinton culture medium. Following antibiogram and diagnostic disks (antibiotics) were used: (PadtanTeb Company: Ceftazidime, Ceftriaxone, Cefepime, Meropenem, Imipenem, Rifampin, Ciprofloxacin, Amikacin, Gentamicin, Co-trimoxazole and Rosco company: Ampicillin-sulbactam). The demographic data i.e., age, gender, underlying disease, duration of hospitalization, infection site, and type of invasive procedures performed for patients, as well as the antibiotic resistance level were collected for all patients. The data were analyzed with a statistical package SPSS (version 22). A specific checklist was prepared to collect the data from all patients. The data were then organized in the Excell and entered into the statistical package for further analyses.

Results:

Results are well summarised in 2 Tables & 3 Figures

While evaluating the patients (n=250) infected from *A.baumannii* the mean age $\pm$ standard deviation (SD) was 58.50 ± 9.53 years (age range: 20 days to 94 years old). Ten patients (4%) were under 10 years old, 4 patients (1.6%) were between 10 to 20 years old, 20 patients (8%) were between 20 to 30 years old, 22 patients (8.8%) were between 30 to 40 years old, 30 patients (12%) were between 40 to 50 years old, 89 patients (35.6%) were between 50 to 60 years old, 40 patients (16%) were between 60 to 70 years old, 20 patients (8%) were between 70 to 80 years old, and 15 patients (6%) were over 80 years old. The majority of patients (35.6%) were 50 to 60 years old. Further, among 250 patients 111

patients were female (44.4%), and 139 patients were male (55.6%). The mean of hospitalization period in the studied patients was 30.4 ± 15.223 days (range: 7 to 67 days). Further, Most of the patients (42.4%) were hospitalized from 21 to 30 days. Figure- 1 showing the frequency of the patients registered in different wards of the Hospital

Figure-1: Frequency of the patients registered in different wards of the Hospital.

Figure-2: Representing the distribution of the underlying diseases among the patients.

Figure-3 representing the anatomical site of infection in the patients. Among 250 cases, 764 samples were collected (3 to 4 samples from each patient and from different anatomical sites), and they were evaluated for antibiotic-resistance. Trachea depth was obtained from 451 samples (59%), ulcer secretions from 137 samples (17.93%), blood from 59 samples (7.72%), urine from 104 samples (13.61%), and peritoneal fluid aspiration from 12 samples (5.45%)

Figure-3: Distribution of the anatomical site of infection in the study population.

Figure-4: invasive treatment methods used in the patients with *A.baumannii* infection. VC- Venous Catheter, UC- Urinary Catheter, IT- Intubation Tube.

Figure-4 shows the invasive methods used for the treatment of infected patients. The urinary catheter was used in 9% of patients, an intravenous catheter for 5.2%, and tracheal tube in 2% of the patients and 71.6% of patients received both urinary and intravenous catheters.

The antibiotics used for the treatment of the patients assessed in this study along with the clinical outcome and the resistant to the antibiotic agent is showing in Table-2.

Table-2~ The information of antibiotics used in the infected patients along with the clinical outcomes.

Number of Isolates	Antibiotic Abbreviation	Resistance	Sensitive	Intermediate
707	AMK	86%	13%	1%
693	FEP	88%	7.80%	4.20%
9	CFM	77%	22.20%	0.80%
699	CRO	92.30%	2.30%	5.40%
621	CIP	86%	12.90%	1.10%
52	GEN	81%	15.39%	3.61%
82	IPM	90%	9.80%	0.20%
46	NAL	78%	8.69%	13.31%
46	NIT	91%	8.70%	0.30%
121	TZP	95%	4%	1%
54	RIF	100%	0%	0%
128	TCY	72%	21.90%	6.10%
617	SXT	77%	15.20%	7.80%
740	MEM	88.50%	11.39%	0.11%
184	CAZ	93.50%	5.40%	1.10%
644	SAM	47.70%	27.80%	24.50%
82	LVX	98.80%	1.20%	0%

AMK:Amikacin/ FEP:Cefepime/ CFM:Cefixime/ CRO:Ceftriaxone/ CIP:Ciprofloxacin/ GEN:Gentamicin/ IPM:Imipenem/ NAL:Nalidixic Acid/ NIT:Nitrofurantoin/ TZP:Tazocin/ RIF:Rifampin/ TCY:Tetracycline/ SXT:Co-Trimoxazole/ MEM:Meropenem/ CAZ:Ceftazidime/ SAM:Ampicillin-Sulbactam/ LVX:Levofloxacin

Discussion:

In the study in Brazil, the mean age of patients was noted to be 53.3 years where most of them (42.5%) were of 60 to 70 yrs old (Castilho *et al.*, 2017). Direkel *et al.* (2012) in Turkey found mean age of patients was 68 years where most of them (45%) were 75 to 85 yrs whereas the mean age of patients was 61 years and most of them (44.5%) were in the age group of 65 to 75 yrs old in Poland (Krzysciak *et al.*, 2016) and it resembles our result. This difference could be attributed to different statistical populations of the two studies.

As per Castilho *et al.* (7), neoplasm (34.4%) and AIDS (17.2%) were the most common underlying diseases which were in contrast with our findings. In the studies of Krzysciak *et al.* (2017) and Maraki *et al.* (2016) conducted on the hospitalized patients with *A.baumannii* infection, the most common underlying diseases were diabetes (42%) and chronic pulmonary disease (34%), which were agreement with our results. Similarly results of Rezaei *et al.* (2016) from Isfahan, Sohail *et al.* (2016) from Pakistan, Direkel *et al.*

(2016) from Turkey reported bronchus and surgical wounds as the most common anatomical site for infection and are in agreement with our results.

In the present study, three types of invasive procedures were used for hospitalized patients; most of them (71.6%) had both urinary and intravenous catheters. In the study of Krzysciak *et al.* (2017) and Maraki *et al.* (2016), most common invasive procedures which were performed for patients with *A.baumannii* infection were urinary and intravenous catheters. However, in the study of Castilho *et al.* (2017), the most commonly used procedure was the tracheal tube. As the foregoing study was only performed on ICU hospitalized patients that most of them had VAP, the difference of the type of invasive procedure between such study and the present study was reasonable. In the study of Gao *et al.* (2014) from China and the study of Maraki *et al.* (2016), the most common invasive procedure in patients with *A.baumannii* infection was also urinary and intravenous catheters. A strong point of our study which could be underlying for future studies was taking into account the issue of invasive procedures type that was certainly important in *A.baumannii* infection development in patients, however, this issue was not considered in the previous studies.

In the study of Krzysciak *et al.* (2017), about 81% of patients were hospitalized in the intensive care unit, as per Maraki *et al.* (2016), 53.9% of patients were hospitalized in the intensive care unit, 27% of patients were hospitalized in the internal diseases ward, and 18% of patients were hospitalized in the surgery ward, Gao *et al.* (2017), reported that 61.7% of patients were hospitalized in different wards of the hospital, and 38.4% of patients were hospitalized in ICU. We found in our study, 73.6% of patients with infections were hospitalized in the intensive care unit and 26.4% of them were hospitalized in different sections of the hospital. Results of this study wherein agreement with studies of Krzysciak *et al.* (2017) and Maraki *et al.* (2016). Moradi *et al.* (2015) in a study in Tehran from 2001 to 2013, reported that resistance rate to carbapenems and aminoglycosides has been rising constantly that was in agreement with our findings. In the study of Hamzeh *et al.* (2012) from Syria, stated the highest antibiotic activity was against colistin and carbapenems. In the present study, the resistance rate for carbapenems (imipenem and meropenem) was very high and this difference could be due to the sudden increase in the resistance rate of *A.baumannii* during recent years, and the high prevalence of MDR, XDR-AB species. In the study of Manikal *et al.* (2000), reported from USA, most of the samples were resistant to the routine antibiotics such as cephalosporin, aminoglycosides, and fluoroquinolones that were in agreement with our study. In the study of Izadpoor *et al.* (2013) from Ahvaz, the highest resistance rate for samples containing *A.baumannii* was related to ciprofloxacin (96.9%) and co-trimoxazole (95.2%). In the present study,

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resistance to ciprofloxacin was 86% and it was 77% for cotrimoxazole which showed a slight decrease in resistance to these two antibiotics. In the study of Bahador *et al.* (2014), conducted from 2006 to 2011 in Tehran, almost all samples were sensitive to minocycline. In the study of Direkel *et al.* (2012) carried out in Turkey, and the study of Bataresh *et al.* (2013) from Jordan, resistance rate toward various antibiotics were different with the results of the present study. In these two studies, the resistance rate to ciprofloxacin was higher than our study. Direkel *et al.* (2016) reported the percentage of resistance to levofloxacin was 86%, but in our study, it was 98.8%. These two differences in results could be related to the issue that today, many of physicians in different communities are using fluoroquinolones as the first therapeutic line for several infections. The result of resistance to the fluoroquinolones could be related to the high usage frequency of these antibiotics along with improper usage of them and based on the types of antibiotics of this group, the resistance rate against them will be increased. Castilho *et al.* (2010) reported from Brazil, the highest antibiotics resistance rate was related to cefepime (96.4%) and quinolones (91.1%), and the most effective antibiotics used against *A.baumannii* were polymixin-B, tigecycline, and amikacin. In this study, the resistance rate for ampicillin/sulbactam was reported to be 60.7%, while in the present study, the resistance rate to ampicillin/sulbactam was 47.7% (24.5% had medium sensitivity). In the study of Maraki *et al.* (2014) from Greece, samples were resistant (with more than 90% resistance rate) to most of the antibiotics including carbapenems, piperacillin/tazobactam, ticarcillin / clavulanic, imipenem, ceftazidime, ciprofloxacin, meropenem, and tetracycline. From aminoglycosides, the least resistance rate was reported for amikacin (60%). Colistin was the most effective antibiotic of this study. In the study of Rezaei *et al.* (2016) from Isfahan, the most resistance rate was reported for carbapenems and quinolones (more than 98% for ertapenem/meropenem/imipenem and ciprofloxacin).

An overall review about these studies and their comparison to our study showed is that antibiotic-resistance rate in the study of Rezaei *et al.* (2018) was higher than ours. This difference was due to the studied statistical sample that included patients hospitalized in ICU, and as the review of similar articles showed, frequency of MDR-AB and XDR-AB species in samples of ICU patients was significantly higher than other hospital sections. The results of Sohail *et al.* (2016), was completely in agreement with the results of the present study. In the study of Jasemi *et al.* (2013) from Tehran, 97% of samples were resistant to at least two antibiotics of carbapenem family that support our findings. However, the rate of antibiotic-resistance for gentamicin and ampicillin/ sulbactam was 90% and 60%, respectively, which was in contrast with our findings (81%

resistance to gentamicin, and 47.7% resistance to ampicillin /sulbactam). The difference could be attributed to the issue that in the study of their first evaluation performed on MDR-AB and XDR-AB in which the antibiotic-resistance rate was certainly higher. Totally, patients with diabetes and pulmonary chronic diseases that have received invasive procedures including intravenous and urinary catheters and those whose hospitalization duration was more than 20 days had the highest *A.baumannii* infection rate. On the other hand, the most common anatomical site for this infection was bronchus and the most common clinical demonstration of *A.baumannii* was pneumonia. It should be noticed that antibiotic-resistance of *A.baumannii* to all common antibiotics that were used for treating gram-negative bacilli was significantly increasing. Our findings showed that, among the present antibiotics, only polymixins and tigecycline, and in some cases minocycline were suitable for treatment of infection with this microorganism.

Conclusion:

Our findings showed that susceptibility to *A.baumannii* infection among elderly patients undergoing procedures such as urinary and venous catheter insertion with long hospitalization (more than 20 days) and patients with chronic pulmonary diseases such as asthma and bronchitis and diabetes as underlying diseases was higher than other patients. On the other hand in earlier studies including our study, the most common anatomical site of infection was bronchus and the most common clinical manifestation of *A.baumannii* infection was pneumonia. Our findings in support of earlier studies showed that antibiotic resistance of *A.baumannii* is rising against almost all antibiotics, which are commonly used for the treatment of resistant gram-negative bacterial infections. Among different types of antibiotics, polymyxins (polymyxin-B, colistin), monocycline and tigecycline have the most antibacterial activity against *A.baumannii*. For efficient management of high antibiotic-resistance of *A.baumannii* two options are suggested. First, preventing or reducing hospital-acquired infections of *A.baumannii* through establishing a robust infection control program. Second, using new antibiotic regimens, combinations of different antibiotics, to treat the associated infections according to the susceptibility patterns of *A.baumannii*.

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