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# Prevalence and Drug Resistance of Acinetobacter Baumannii in ICU of a Teaching Hospital

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## ABSTRACT

**Background:** Acinetobacter strains as opportunistic hospital pathogens are resistant to a large number of antibiotics and are the cause of various infections such as bacteremia, pneumonia, meningitis, urinary tract infections and surgical site infections. This study is aimed at assessing the prevalence of acinetobacter baumannii and its drug resistance in Intensive Care Unit (ICU) of a teaching hospital.
**Methodo:** This cross-sectional study was conducted on the patients admitted to the ICU for one year. Urine, wound and sputum samples of the admitted patients to the hospital were collected 48 hours later and cultured. Antibiotic resistance was measured using two methods, namely Disk Diffusion and E-test ,and the data were analyzed using SPSS, Ver. 20 and the level of statistical significance was considered to be $p < 0.05$.
**Results:** From the total positive cultures, 51% were caused by gram-negative bacteria, 42% by gram-positive bacteria and 7% by fungi, while the abundance of Acinetobacter baumannii was 17%. Among the samples affected by Acinetobacter baumannii, 45.71% were female and 54.29% were male with a mean age of 71.69%; the admissions were due to surgical problems in 34.29% of the cases and due to internal conditions in 65.71% of the cases. Totally, 90% of the patients suffered from comorbidities, the most common of which were HTN and DM; 42.9% of the patients had a history of antibiotic use before admission and the most commonly used antibiotics were Cephalosporins. Acinetobacter baumannii was most commonly isolated from sputum and then from BAL. Disk Diffusion method indicated high resistance to some antibiotics, namely Imipenem, Meropenem, Piperacillin-Tazobactam, Ciprofloxacin, Cefotaxime, Cefepime, Ceftriaxone and Ceftazidime. In this method, 100% of the samples were susceptible to Colistin. In E-test method, two cases of resistant to Colistin were reported. In this method, almost 1/3 of the samples were reported to be susceptible to Rifampicin, or showed intermediate resistance to it.
**Conclusion:** The results of this study showed that clinical isolates of Acinetobacter, especially Acinetobacter baumannii show high resistance to antibiotics, particularly to the third generation Cephalosporins and broad-spectrum Penicillins.
**KEYWORDS:** Acinetobacter, ICU, Antibiotics.

## INTRODUCTION

Acinetobacter is a gram-negative coccobacillus that can cause infections in the respiratory tract, blood, soft tissue, urinary tract, and CNS. Infections with this organism are associated with high rates of morbidity and mortality, prolonged length of stay and increased health care costs. Until 1970, Acinetobacter was considered as a rare cause for infections in the ICU, however, its prevalence has increasingly escalated and it has become a major pathogen in hospitals (1-4).

During the past decade, its resistance has increased considerably, and this has necessitated the use of broad-spectrum antibiotics such as Imipenem and Ampicillin/ Sulbactam. Currently, it seems that Acinetobacter strains are resistant to the major types of antibiotics and they seem to be responsive just to Colistin and some old toxic ones (1 and 2).

Resistance of bacteria against antibiotics is a major problem in ICU. Multidrug-resistant organisms (MDROs) cause longer length of stay, increased mortality and treatment costs; on the other hand, the use of broad-spectrum antibiotics can cause the colonization of the pathogens and serious infections (3 and 4).

Acinetobacter infections often develop into bacteremia and septicemia, and lower respiratory tract involvement with Acinetobacter progresses to bacteremia and septicemia (5).

Typically, most laboratories use Disc Diffusion method, which is a qualitative one for assessment of antibiotic susceptibility. In this method based on the size of zone of inhibition, susceptibility or resistance is reported and no number is reported in this technique.

This method is not adequate for the report of the cases of resistance associated with the increase in MIC or intermediate resistance, or for those antibiotics with insufficient disk diffusion due to the high molecular weight (6).

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Non-fermentative gram-negative bacteria such as Pseudomonas aeruginosa and Acinetobacter baumannii need longer time to reach the cell mass as required for assessing the susceptibility because of the slower growth comparing with the family of Enterobacteriaceae. This feature makes the results of Disk Diffusion, as a qualitative susceptibility testing, to be misinterpreted (7).

In hospitalized patients in the ICU, medical and personal factors including associated comorbidities, type of pathogens and their resistance patterns cannot be modified, and the only real intervention for improving patient survival is selecting the correct medication with sufficient dose. This expected level is often achieved using a dose of the medication that is often 5 to 10 times more than MIC. (8)

E-test is among diffusion tests and is capable of measuring the approximate value of MIC (9). The rapid spread of resistance mechanisms and resistant bacteria requires accurate identification of resistant bacteria populations, which can be achieved by means of accurate antibiotic susceptibility testing (6).

Due to the differences in resistance patterns among different countries and even different regions of the same country and at different times, identifying such resistances can be of great help in selecting the most appropriate treatment options by the physicians. Furthermore, such information is an important first step to be taken in the systematic collection of epidemiological data on regional and national levels (6 and 8).

The current study has been conducted regarding the following issues: the importance of Acinetobacter strains and Acinetobacter baumannii as its most common strain in nosocomial infection, which is the cause of outbreaks of infection in different hospitals and medical centers at different times; infections triggered by Acinetobacter baumannii are resistant to multiple antibiotics and are among the leading causes of mortality and morbidity in ICU patients; they cause increased length of stay, extra costs and adverse impact on the country's health budget; and they impose emotional and psychological adverse effects on patients and their relatives.

## METHOD

This cross-sectional study was conducted at Khatam al-Anbiya Hospital in the year 2012 for one year. All patients that at least one of their CSF, urine, blood or other samples were cultured and Acinetobacter baumannii was found in their samples, and who were admitted at least 48 hours before collection of the samples were included. Demographic and clinical data of the patients including age, gender, underlying disease, use of urinary catheter, central venous catheter, pleural, mechanical ventilation, and reason for hospitalization, recent antibiotic therapy and the reason of their referral from other hospitals were collected. Collected samples of blood, urine, wound and tracheal secretions, cerebrospinal fluid (CSF), ascites, pleural effusion, or any other samples collected from the patients in ICU who were admitted at least 48 hours before collection of samples, were taken to the culture environment as soon as possible after preparing the direct smear, and the samples were kept in the incubator. Blood agar and EMB agar were used for culture of clinical samples. The cultured gram-negative samples in the environment were studied assessed based on the specific characteristics of Acinetobacter pathogen, colony morphology, and biochemical reactions and the antibiotics selected from CLSI-2006 table were assessed were studied using Disk Diffusion method. The resistant samples were studied using E-test strips manufactured by Biomerio Co., France for 6 antibiotics, namely Colistin, Meropenem, Gentamicin, Ceftazidime, Rifampin, and Piperacillin-Tazobactam and the level of MIC was determined. The data were analyzed using SPSS, Ver. 20 and the level of statistical significance was considered to be $p < 0.05$.

## RESULTS

From the total number of 411 positive cultures, 51% were caused by gram-negative bacteria, 42% by gram-positive bacteria and 7% by various species of the fungus. In Gram-negative cases, the most frequently isolated bacteria from cultures included acinetobacter baumannii (33.3%), which was found in up to 17% of the total samples.

Mean and SD of the age of the patients were 71±19 years. Among 70 samples with positive result for acinetobacter baumannii, 45.71% were female and 54.29% were male; 17.10% of the patients were referred from other medical centers, 1.80% from nursing centers and 77.10% of the patients were referred by their own. The admissions were due to surgical problems in 34.29% of the cases and due to internal conditions in 65.71% of the cases. Totally, 90% of the patients suffered from previous comorbidities, and 10% had no such comorbidities. The cause of comorbidity was reported as follows: HTN (41.4%), Renal Stone (5.7%), DM (40%), IHD (24.3%), Cancer (7.1%), CVA (15.7%), RF (17.1%), COPD (12.9%), CNS surgery (10%), corticosteroid use (10%) and pressure ulcer (25.7%). The types of catheters used for the patients were as follows: Foley catheter (85.7%), NG Tube (54.3%), PEG (11.4%), CV line (38.6%), hemodialysis catheter (10%), while no catheter was used for 11.4% of the patients. From the total studied patients, 30 cases (42.85%) had a history of previous use of antibiotics and 40 patients (57.14%) had no such a history; meanwhile 18 cases (25.71%) had used antibiotics 1-10 days before admission, 8 patients (11.42%) 11-20 days before, 2 cases (2.85%) 21-30 days, 1 patient (1.42%) 51-60 days and 1 patient (1.42%) 81-90 days before admission.

The most widely used antibiotics in patients with the history of antibiotic use before admission included Cephalosporin (21.4%), Quinolones (10%), Macrolides (7.1%), Penicillin (4.3%), and Rifampin (14%). Mechanical ventilation was used for 54.29% of the patients during their stay in ICU.
The most common positive sample in terms of Acinetobacter baumannii was sputum (62.9%), followed by BAL (30%), CSF (2.9%), urine (1.4%), PEG (1.4%) and peritoneal catheter (1.4%).
Table 1 shows antibiotic susceptibility of disk diffusion method for the used antibiotics. According to the table, 100% of the samples were resistant to Cefotaxime, Cefepime, Ceftazidime and Ceftriaxone, while 100% of them were susceptible to Colistin.

**Table 1. Susceptibility and resistance of the antibiotics for Acinetobacter baumannii**

| | Susceptibility | Resistance |
|---|---|---|
| **Amikacin** | 2.90% | 94.30% |
| **Imipenem** | 5.70% | 94.30% |
| **Colistin** | 100% | |
| **Cefotaxime** | - | 100% |
| **Gentamicin** | 5.70% | 94.30% |
| **Meropenem** | 1.40% | 98.60% |
| **Cefepime** | - | 100% |
| **Ceftazidime** | - | 100% |
| **Ceftriaxone** | - | 100% |
| **Ciprofloxacin** | 1.40% | 98.60% |
| **Piperacilin-Tazobactam** | 2.90% | 97.10% |

MIC values were measured using E-test strips for 6 antibiotics, namely Colistin, Meropenem, Gentamicin, Ceftazidime, Rifampin, and Piperacillin -Tazobactam. For Ceftazidime, MIC values for all samples were reported as resistant, for Piperacillin -Tazobactam MIC was reported to be intermediate for 1 sample and resistant for all other ones. Regarding Gentamicin, MIC in 4.6% of the samples (3 cases) was susceptible, intermediate in one case and resistant for other ones. The MIC values for Meropenem included 1 susceptible case, 2 intermediate cases and all remaining resistant cases. For Colistin, among the 30 samples for which MIC was determined, one case of MIC = 4 and one case of MIC = 8 were reported which are considered to be resistant, and all other cases were reported as susceptible; meanwhile in Disk Diffusion method all samples were susceptible. Finally, from 45 samples studied for Rifampin, 3 samples were reported as susceptible (6.7%) and 15 samples as resistant (33.3%).
Table 3 shows the MIC values on the basis of CLSI.

**Table 2: MIC values for studied antibiotics**

| Piperacillin - Tazobactam | MIC | N (%) |
|---|---|---|
| | > 256 | 65 (98.4)% |
| | 32 | 1 (1.6%) |
| **Ceftazidime** | > 256 | 62 (96.8%) |
| | > 128 | 1 (0.02%) |
| | > 32 | 1 (0.02%) |
| **Meropenem** | > 32 | 63 (91.3%) |
| | 16, | 3 (4.3%) |
| | 12 | 2 (2.9%) |
| | 4 | 1 (1.5%) |
| **Rifampin** | > 32 | 17 (37.8%) |
| | 24 | 1 (2.2%) |
| | 16 | 1 (2.2%) |
| | 8 | 3 (6.7%) |
| | 6 | 1 (2.2%) |
| | 4 | 4 (8.9%) |
| | 3 | 6 (13.3%) |
| | 2 | 5 (11.1%) |
| | 1.5 | 4 (8.9%) |
| | 1 | 3 (6.7%) |
| **Colistin** | 0.04 | 1 (3.3%) |
| | 0.064 | 1 (3.3%) |
| | 0.094 | 1 (3.3%) |
| | 0.125 | 5 (16.7%) |
| | 0.25 | 1 (3.3%) |
| | 0.38 | 1 (3.3%) |
| | 0.5 | 5 (16.7%) |
| | 1 | 2 (6.7%) |
| | 1.5 | 7 (23.3%) |

| | | |
|---|---|---|
| | 2 | 3 (10%) |
| | 3 | 1 (3.3%) |
| | 4 | 1 (3.3%) |
| | 8 | 1 (3.3%) |
| **Gentamicin** | 1.5 | 2 (3.1%) |
| | 3 | 1 (1.5%) |
| | 12 | 1 (1.5%) |
| | 16 | 2 (3.1%) |
| | 24 | 2 (3.1%) |
| | 32 | 1 (1.5%) |
| | 48 | 2 (3.1%) |
| | 64 | 1 (1.5%) |
| | 96 | 1 (1.5%) |
| | 128 | 2 (3.1%) |
| | 256 | 50 (76.9%) |

**Table 3: MIC reference values according to CLSI**

| | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| **Gentamicin** | ≤ 4 | 8 | ≥ 16 |
| **Meropenem** | ≤ 4 | 8 | ≥ 16 |
| **Rifampin** | ≤ 1 | 2 | ≥ 4 |
| **Colsetin** | ≤ 2 | _ | ≥ 4 |
| **Piperacillin-Tazobactam** | ≤ 16 | 32.4 to 64.4 | ≥ 128.4 |
| **Ceftazidime** | ≤ 8 | 16 | ≥ 32 |

Of the patients in this study, 64.52% were finally discharged from the hospital and 35.48% of them were expired.

## DISCUSSION

Despite the advancement of medical technology, increased longevity and the possibility to provide intensive cares for the patients in ICU, we are faced with the emergence of nosocomial infection and the pathogens resistant to multiple antibiotics. Acinetobacter is one of such pathogens and the outbreak of infection caused by it has been reported in many hospitals, especially in ICUs all over the world (24 and 25). Currently, it seems that Acinetobacter strains are resistant to the major types of antibiotics and they seem to be responsive just to Colistin and some old toxic cases (1 and 2). In our study, the samples affected by Acinetobacter baumannii were only susceptible to Colsetin, followed by Rifampin, although with a much lower efficaciousness. Antibiotic resistance of bacteria is a major problem in the ICUs. Acinetobacter infections often develop into bacteremia and septicemia, and lower respiratory tract involvement with Acinetobacter progresses to bacteremia and septicemia (5). In our study, the majority of the positive samples in terms of Acinetobacter baumannii were isolated from the respiratory tract, and this finding is consistent with the findings of other similar studies.

In the present study, antibiotic susceptibility results for Acinetobacter baumannii against some antibiotics namely Ceftazidime, Cefepime, and Piperacillin - Tazobactam, Imipenem and Colistin were similar with the result of a study conducted in Italy (29).

The findings of the present study were also in line with the results of the study in Brooklyn, USA regarding antibiotic susceptibility of Acinetobacter baumannii using MIC method, except for Imipenem. The results of our study also were consistent with those reported by Rahbar et al. in the hospitals of Tehran regarding isolation origin, resistance to Ceftriaxone and Piperacillin - Tazobactam and Ciprofloxacin, and somewhat for Ceftazidime and Amikacin; however, regarding Imipenem, there is a discrepancy with the above study where the most samples were isolated from respiratory tract and the resistant was reported as: Ceftriaxone (90.9%), Amikacin (85.2%), Ciprofloxacin (90.9%), and Imipenem (5/4%) (12). In a study conducted in Turkey, 51.4% of the studied patients who showed positive culture for Acinetobacter baumannii had the history of consumption of antibiotics, 81.1% of the patients suffered from comorbidities, and ventilator was used for 83.7% of them and the results of the study are consistent with those of this study. Meanwhile the highest resistance of Acinetobacter baumannii was reported to be against Piperacillin - Tazobactam, Ciprofloxacin and Ampicillin - Sulbactam (100%), and these are quite similar to our study. However, resistance to Meropenem and Ceftazidime has been reported to be 55% and these are inconsistent with our results (14). In the study by Thean Yen Tan et al. in Singapore, the synergetic effect of antibiotics against extensively drug-resistant Acinetobacter baumannii was investigated. From 16 isolated samples, the combined effect of Polymyxin B and Rifampin for killing bacteria in the lab was better than the combined effect of Polymyxin B and Tigecycline or Tigecycline and Rifampin. (15)

According to our study, the organisms showed considerable susceptibility to Rifampin, so may be the combination of Colistin and Rifampin can be promising in XDR cases, as mentioned in the study conducted in Singapore (23).

In our study 100% of the samples were susceptible to Colistin using Disk Diffusion method, while in E-test method, one case of MIC = 4 and one case of MIC = 8 were reported and it seems that E-test method should necessarily be performed for Colistin. In the Disk Diffusion method, the susceptibility of the Meropenem was reported as 1.4%, however in determining MIC, two cases of intermediate resistance (2.9%) and one case of susceptibility (1.5%) were reported, while in the disk diffusion method, intermediate resistance was not reported. According to the above issues, determining MIC for Meropenem seems to be useful for identifying intermediate resistant cases.

In the study by Wang et al in 2003 on the epidemic of drug-resistant Acinetobacter baumannii in ICUs, all samples were resistant to Aztreonam, Amikacin, Ampicillin - Sulbactam, Ceftazidime, Cefepime, Ciprofloxacin, Gentamicin, Imipenem, Meropenem, Piperacillin - Tazobactam and Ticarcillin - Clavulanic acid and susceptible to Polymyxin, and these are consistent with the findings of the present study.

In our study, determining the susceptibility using Disk Diffusion method and determining MIC using E-test strips provided similar results in the case of Ciprofloxacin, Piperacillin - Tazobactam and Gentamicin; However, for Colistin a case of intermediate resistance and a case of resistance was observed using E-test strips, and also two cases of intermediate resistance were reported for Meropenem, none of which reported in Disk Diffusion method. Interestingly, the result of determining MIC and susceptibility using E-test for Rifampin showed that from 45 samples tested for Rifampin, there were 3 susceptible cases with MIC = 1 (6.7%) and 15 cases with intermediate resistance $1 \leq$ MIC $<4$ (33.%). Given the high level of resistance to other antibiotics, this is remarkable. However, there are few studies conducted for combined therapy using Rifampin for Acinetobacter baumannii and it seems reasonable that some studies be conducted in this regard.

Given that almost all Acinetobacter baumannii strains are susceptible to Colistin, it is better to avoid inappropriate treatment with antibiotics, especially in the case of colonization and to sustain such susceptibility in our country and region.

The main limitation of this study was for the procurement of E-test strips for other antibiotics and even for the studied antibiotics, especially Colistin, since these are not available in Iran and their procurement is too difficult for various reasons. Another limitation was differentiating colonization from infection, and it is necessary to overcome this limitation in future studies.

In a sensitive and risky department such as ICU, one of the most effective methods for preventing from infection is planning and making a policy on disinfection and sterilization of the equipment. The physicians should prescribe antibiotics to the patients in ICU only after obtaining the antibiogram as the simplest and cheapest way to prevent antibiotic resistance.

Given the high incidence of nosocomial infections, especially in ICUs, there is an urgent need to organize a nosocomial infection surveillance system in order for identifying the important factors causing infections and identifying the existing problems leading to complications in our country.

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