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# Distribution and antibiogram pattern of Acinetobacter infections in Shahid Mohammadi Hospital, Bandar Abbas, Iran

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**Original Article**

## Abstract

**Introduction:** *Acinetobacter* species are important opportunistic pathogens, widely spread in hospitals' environment and responsible for different health care associated infections. Because of its ability to rapidly develop resistance to the major groups of antibiotics, treatment of *Acinetobacter* infections is difficult and antibiotic susceptibility tests can help in choosing the best antibiotics, decreasing the cost and duration of hospitalization. The goals of this study were to determine frequency and antimicrobial susceptibility pattern of *Acinetobacter* species, clinical parameters and outcomes of patients, in Shahid Mohammadi hospital, Bandar Abbas.

**Methods:** Between April 2010 and March 2011, a total of 2132 positive cultures were obtained from various clinical specimens of hospitalized patients. Suspicious isolates of *Acinetobacter* were identified by routine microbiological methods. Antibiogram patterns of isolates for 12 currently used antibiotics were determined by Kirby-Bauer method. Clinical and microbiological data of patients was analyzed by SPSS 16 software.

**Results:** A total of 68 (3.2%) *Acinetobacter* species was isolated. *Acinetobacter* isolates was mostly obtained from ICU (24 cases, 35.8%) and emergency (12 cases, 17.9%) wards, and trachea was the major site of infection (41.2%). Colistin with 83.7% susceptibility rate was the most effective antibiotic, followed by ofloxacine 47.4% and chloramphenicol 39.5%. A high rate of resistance was observed to meropenem (98.1%), and cefepime (90.4%). Mortality rate was 14.7% in patients, mostly because of bacteremia.

**Conclusion:** Because of its serious infections and high-drug resistance, continuous monitoring of antimicrobial susceptibility and strict adherence to infection guidelines are essential to prevent and decrease *Acinetobacter* infections.

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## Introduction:

In a recent report of the Infectious Diseases Society of America, three categories of MDR gram-negative bacilli, namely, extended-spectrum cephalosporin-resistant *Escherichia coli*and *Klebsiella spp*., MDR *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter spp*. are the main concerns of antibiotic therapy (1).

*Acinetobacter*species are increasingly important nosocomial Pathogen (2). Before the 1970s, *Acinetobacter* were mostly isolated from postsurgical urinary tract infections. The significant improvement in cardiovascular recovery and the use of invasive techniques or artificial devices during the last 30 years has changed the types of infection caused by these bacteria. Since the 1980s, *Acinetobacter* species has spread rapidly among patients in intensive care units (3).

Today, *Acinetobacter* species account for 9% of nosocomial infections (4). *A. baumannii* is intrinsically resistant to some beta-lactam antibiotics and has ability to acquire resistance to all commercially available antimicrobial agents. In fact, carbapenem-resistant *A. baumannii* has been identified as one of the six pathogens *Enterococcus faecium, Staphylococcus aureus, Klebsiella*species, *Acinetobacterbaumannii, Pseudomonas aeruginosa,* and *Enterobacter* (ESKAPE) species responsible for an increasing number of nosocomial infections in the United States (5).

The six bad bugs known as ESKAPE bacteria are among the biggest threats infectious diseases physicians face today. *Acinetobacter* species are excellent biofilm producing bacteria, which facilitate their survival in hospital environments and are frequently found on the skin and in the respiratory and urinary tracts of hospitalized patients (6). Infected or colonized patients are the main reservoir of these bacteria. Because of reduced acid secretion, bacterial overgrowth in the stomach of ICU patients can lead to the development of nosocomial infections (4). The main route of transmission is the hands of hospital care workers and inanimate objects act as an intermediate reservoir between the hands of hospital workers and the patients (7). Study on 3 Army hospitals in Iran, showed that *A. baumannii* is responsible of 9% of infections in ICU patients (8).

An Iranian review study, imply an increasing in antibiotic-resistant *A. baumannii* strains from 2001 to 2013. The prevalence of MDR strains also have been increased from 50% in 2001-2007 to 74% in 2010-2011, with a mean prevalence of 71.2% (9).

The severity of *Acinetobacter* infections depend upon the site of infection and the patient's susceptibility as a result of underlying disease (10).

The purpose of this study is to provide data on the frequency, outcome, and antibiotic susceptibility of *Acinetobacter* infections, with the goal of improving its management.

## Methods:

This retrospective, descriptive cross-sectional study was carried out between April 2010 and March 2011, on 2132 different clinical specimens, submitted to Shahid Mohammadi Hospital (a 400-bed hospital serving of over two million people). Laboratory isolation of the bacteria was carried out by culturing the specimens on appropriate bacteriological media, including Blood agar, Chocolate agar, thioglycollate, EMB or McConkey agar. Cultures were incubated at 37°C for 24 - 48 h. Blood samples were inoculated in Trypticase soy broth bottles and incubated for at least 7 days at 37°C. Identification of *Acinetobacter* isolates was performed by routine microbiological methods using Gram staining, oxidase, TSI and motility results (11).

In vitro antibiotic susceptibility of the isolates to 12 antibiotics was determined by the Kirby-Bauer disc diffusion method, on Mueller-Hinton agar. Antibiogram discs contained the following antibiotics, at the specific concentrations indicated in parentheses: Imipenem (10µg), meropenem (10µg), colistin (25µg) ceftriaxone (30µg), cefepime (30µg), ciprofloxacine (5 µg), ceftazidime (30µg), cefazoline (30µg), ofloxasin (5µg), amikacin (30µg), gentamicin (10µg), cotrimoxazol (25µg) which were purchased from PadTanTeb, Iran. According to the guidelines of Clinical and Laboratory Standards Institute (CLSI), cell suspension inoculates were prepared from 18 - 24 h fresh, pure cultures, in 0.85% sterile saline and adjusted to match a 0.5 McFarland standard tubes. The criteria proposed by CLSI were applied for interpreting the results (12).

Leukocyte count was done by automated leukocyte analyzer method, C-reactive protein (CRP) by qualitative method of latex-CRP (ENiSon, ENiSon Lab, Tehran, Iran), and Erythrocyte sedimentation rate (ESR) by Westergren method. Results of latex-CRP tests were reported according to the presence or absence of agglutination and size of agglutinated droplets on

microscopic examination: no agglutination was considered negative; small-sized agglutinated droplets, 1+; medium-sized agglutinated droplets, 2+; and large-sized agglutinated droplets, 3+.

Other variables included in the study were age, sex, dates of admission and discharge, type of clinical specimen from which the *Acinetobacter* strains were isolated and death during hospitalization. Analysis of data was performed by SPSS 16. Significance was defined as $P \leq 0.05$.

## Results:

Out of 2132 positive cultures, a total of 68 (3.18%) *Acinetobacte*r species was isolated from various clinical specimens. 49 (72.1) of them were isolated from men and 19 (27.9%) obtained from women.

Trachea was the major site of infection (41.8%), followed by blood stream and urinary tract (16.18%) distinctly and wounds swabs (13.2%). Table 1 shows the occurrence rates of*Acinetobacter* isolates by site of infection.

**Table 1. Frequency of *Acinetobacter* isolates by body sites**

| Sample | Frequency | Percent |
|---|---|---|
| **Tracheal tube** | 28 | 41.18 |
| **Blood** | 11 | 16.18 |
| **Urine** | 11 | 16.18 |
| **Wound** | 9 | 13.24 |
| **Sputum** | 4 | 5.89 |
| **CSF** | 1 | 1.47 |
| **Ear discharge** | 1 | 1.47 |
| **Catheter** | 1 | 1.47 |
| **Drain discharge** | 1 | 1.47 |
| **Eye discharge** | 1 | 1.47 |
| **Total** | 68 | 100 |

**Table 2. Distribution of *Acinetobacter* species according to different wards of hospital**

| Wards | Frequency | Percentage |
|---|---|---|
| **General ICU** | 24 | 35.30 |
| **Internal Emergency** | 12 | 17.65 |
| **Internal 1** | 10 | 14.71 |
| **Surgery 1&2** | 5 | 7.36 |
| **Internal 2** | 4 | 5.89 |
| **Neurosurgery ICU** | 4 | 5.89 |
| **Internal 3** | 3 | 4.42 |
| **Orthopaedic** | 3 | 4.42 |
| **Cardio Surgery** | 2 | 2.95 |
| **Burn** | 1 | 1.47 |
| **Total** | 68 | 100 |

*Acinetobacter* isolates were mostly obtained from ICU (24 cases, 35.8%), followed by Internal emergency (12 cases, 17.9%) and internal 1 (10 cases, 14.7%) wards patients. The distribution of *Acinetobacter* species according to different wards of hospital is presented in Table 2.

Table 3 indicates antibiogram pattern of *Acinetobacter* species to tested antibiotics. As it is considered a high rate of resistance was observed to cefepime (90.4%), ceftazidime (89.4%), ceftriaxone (80.3%), cefazolin (75%) and amikacin (70.8%). Colistin with 83.7% susceptibility rate was the most effective antibiotic.

The data of peripheral leukocyte count (WBC), serum C-reactive protein (CRP) level and erythrocyte sedimentation rate (ESR) of the patients was extracted from their medical records, (not found for all patients) and is shown in Table 4.

The Median time of hospitalization in 88.2% of patients were more than 7 days. About 78.4% of infected patients were febrile. Ten deaths were reported in the infected patients and mortality rate was 14.7%. Septicemia was the major cause of death (5 cases). Other causes of decease were respiratory failure (2 cases) and electric shock, brain injury and pulmonary emboli (Table 5).

**Table 3. Antibiogram pattern of *Acinetobacter* species (expressed as percentage)**

| Resistant | Intermediate | Sensitive | Number | Antibiotic |
|---|---|---|---|---|
| **Ciprofloxacin** | 59 | 37.29 | 1.70 | 61.02 |
| **Imipenem** | 55 | 32.73 | 3.64 | 63.64 |
| **Meropenem** | 52 | 1.92 | 0 | 98.08 |
| **Amikacin** | 65 | 21.16 | 3.08 | 70.77 |
| **Gentamicin** | 64 | 35.94 | 6.25 | 57.82 |
| **Ceftazidime** | 47 | 8.41 | 2.13 | 89.37 |
| **Ceftriaxone** | 66 | 18.19 | 1.52 | 80.31 |
| **Cotrimoxazole** | 54 | 35.19 | 1.86 | 62.97 |
| **Colistin** | 49 | 83.67 | 0 | 16.32 |
| **Cefazolin** | 23 | 21.74 | 0 | 78.26 |
| **Cefepime** | 52 | 9.62 | 0 | 90.38 |
| **Ofloxacin** | 19 | 47.37 | 5.26 | 47.37 |

**Table 4. Infection and inflammation-related hematological parameters**

| CRP | | WBC | | ESR | |
|---|---|---|---|---|---|
| | No. | | No. | | No. |
| **Negative** | 6 (23.1%) | >5000 | 0 | ≥20 | 3 (12%) |
| **+1** | 7 (26.9%) | 5000-11000 | 15 (29.5%) | 21-30 | 2 (8%) |
| **+2** | 8 (30.8%) | 11000-20000 | 25 (49%) | <30 | 20 (80%) |
| **+3** | 5 (19.2%) | <20000 | 11 (21.5%) | | |

**Table 5. Deaths according to the final diagnosis**

| | Ward | Body temperature (°C) | Final diagnosis | Sex | Age |
|---|---|---|---|---|---|
| **1** | **ICU-General** | 38.5 | MT, Pul emboli | Male | 28 |
| **2** | **ICU-G** | 38 | MT, DAI, TE fistula | Male | 18 |
| **3** | **ICU-G** | 39 | MT, Sepsis | Male | 23 |
| **4** | **Cardiothoracic Surgery** | 38.2 | CAD,Sepsis | Female | 58 |
| **5** | **ICU-G** | 38.5 | Respiratory failure, ARDS | Female | 29 |
| **6** | **ICU-G** | 39 | Electrical injury | Male | 23 |
| **7** | **ICU-G** | 38 | MT, DAI | Male | 12 |
| **8** | **Cardiothoracic Surgery** | 37.8 | COPD,CAD, Respiratory failure | Female | 81 |
| **9** | **Internal ward** | 38.6 | MT, Sepsis | Male | 55 |
| **10** | **ICU-G** | 38 | TF, Sepsis, DIC | Male | 19 |

MT: Multiple trauma DAI: Diffuse axonal injury TE fistula: Tracheaoesophageal fistula CAD: Coronary artery disease
ARDS: Acute respiratory distress syndrome COPD: Chronic obstructive pulmonary disease TF: Tetralogy fallot
DIC: Disseminated intravascular coagulation ICU-G: ICU general

## Conclusion:

In the present study *Acinetobacter* species accounted for 3.2% of identified microorganisms, in clinical cultures, which is lower than other reports from Iran, in which 23.5% and 16.1% of isolates were found to be *Acinetobacter* species in 1993 (13) and in 2004 in Tehran (14).

In Canadian hospitals, *Acinetobacter* made 0.7% of all isolates in intensive care unit in 2005-2006 (15). This discrepancy maybedue to thedifferent detection methods andstandards of hygiene in the hospitals.

According to our resultstrachea was the major site of infection (41.2%). In a study onmultidrug *A. baumannii* infection during an hospital outbreak, respiratory isolates were recovered from 53% of the patients (16). In Dash study maximum (56.9%) isolates of *A. baumannii* were obtained from pus

swabs of elderly age inpatients. longer duration of stay in the hospital, associated co-morbidity, and invasive procedure were found to be significant risk factors in the setup investigated. Comparable to their findings, blood (13.1%) and urine (12.4%), we detected that 16.2% of isolates were attributed to blood and urine cultures, distinctly (17).

High sensitivity of our isolates to colistin is in consistent with other studies in Iran and other countries around the word (18-22). In Amudhan study in India all of the *A. baumanii* isolates were resistant to imipenem and meropenem (23). Many studies have indicated increasing carbapenem-resistant *A. baumannii*, from 8% in 2003 to 52% and 74% in 2005 and 96% in 2007(24-25). In Iran it was reported between 49.2% to 52.5% during 2008-2011 (26-27).

Assessment of CRP, ESR and WBC are reliable methods to diagnose bacterial inflammations, CRP is an innate immune system response to acute and chronic infections and ESR gives a nonspecific measure of inflammation. The WBC total count includes information on five types that can indicate the type of infection: eosinophils, neutrophils, leukocytes, basophils, and monocytes (28).

In the present study, ESR above 30 was detected in 80% of the patients and 76.9% of patients had CRP 1+. The total count of leukocytes was also more than 11000 in 70.5% of patients, as an indication of bacterial inflammation. As it is established, one of the important quantitative factors in nosocomial infections is duration of hospitalization. Respectively, in our study 45 patients (88.2%) were hospitalized for more than 7 days.

In a study on ahospital outbreak by multi-drug resistant*A.baumannii*, 43.3% of mortalities was because of respiratory infections and the significant quantitative variable was the number of days of hospitalization (22). In another study, Pinyo and co-workers indicated that infection or colonization by *A. baumannii* was associated with an additional hospital stay of 14 days with mortality rate of 24.3% (29).

About 35.8% of isolates in our study were from ICU patients, signifying that ICU patients are at greatest risk of infection. Correspondingly a survey by the Health Protection Agency in England found that 54% of patients with *Acinetobacter* bacteraemia were hospitalized in ICUs between 1976 and 1990 (4).

In our study, mortality rate was 14.7% and septicemia was the major cause of death. Of course it is difficult to prove that all mortalities was due to *Acinetobacter* infection. In another study on clinical outcomes of patients with bloodstream infection caused by carbapenem-resistant *A. baumannii*, mortality rate was 41%. Risk factors associated with mortality were intensive care unit stay, malignancy, and presence of fever and/or hypotension (30). Data from other studies suggests that the crude or related mortality rate ranges from 20% to 60% (1). In one review paper, incidence of nosocomial bacteremia in ICU patients was reported from 1% to as high as 6.5%, compare to 0.65% in other hospitalized patients, and the mortality rate from *Acinetobacter* meningitis was between 15% to 71% (31).

Del Mar Toma study indicated that the mortality among patients with *A. baumannii* bacteremia was 19.6% (16). Munoz and co-workers founded that presence of fever and/or hypotension were associated with a higher death rate (30). These data confirms that a high long hospitalization is attributable to *Acinetobacter* infections and increase of mortality rate.

We believe that our study provides reliable information on the frequency, outcome, and antibiotic resistance pattern of *Acinetobacter* species. Our study was limited by the lack of severity scores (ie, mechanical ventilation, tracheostomy, immunesuppressions associated with transplantation, and malignancies).

As a final point, due to the important of *Acinetobacter* infections and antimicrobial resistance, the tracing of the infectious sources is of great significance for the control and prevention managements.

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