Identification of different species of Acinetobacter Strains, and determination of their antibiotic resistance pattern and MIC of Carbapenems by E-Test

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Original Article

Abstract

Introduction: The Gram-negative emerged an important nosocomial pathogen, especially in intensive care units. These bacteria are cause of health infections, specifically in intensive care units (ICUs). Recent reports present an increase in acinetobacters resistance to carbapenems. This study set out to determine the antibiotic resistance pattern and minimum inhibitory concentration (MIC) of carbapenems in different species of acinetobacters isolated from clinical specimens of Shahid Mohammadi Hospital in Bandar Abbas.

Methods: This descriptive-cross sectional study investigated 81 acinetobacter isolates, collected from different clinical specimens, to the species level, using biochemical tests and Microgen GNA-ID System. The antibiotic resistance pattern of the species was screened by the Kirby-Bauer method performed on Mueller–Hinton agar. In addition, the MIC of imipenem and meropenem antibiotics was evaluated using E-test.

Results: Among the investigated 81 acinetobacter species, 79 (97.5%), 1, and 1 species were of acinetobacter baumannii, acinetobacter lwoffii, and acinetobacter haemolyticus families, respectively. According to the Kirby-Bauer method, the maximum antibiotic resistance of acinetobacter baumannii was to ceftriaxone (98.73%), ciprofloxacin (96.20%), and imipenem (82.28%); in addition, the maximum sensitivity was to polymyxin B and colistin (97.5%), and tigecycline (82.28%). Determination of the minimum inhibitory concentration with R-test showed that 79.75% and 78.48% of acinetobacter baumannii strains were resistant to imipenem and meropenem, respectively. The acinetobacter lwoffii with MIC=0.074µg/m and 32≤MIC µg/m were resistant to imipenem and meropenem, respectively.

Conclusion: The most prevalent species of acinetobacters in Shahid Mohammadi Hospital, Bandar Abbas, was acinetobacter baumannii. Three types of antibiotics (polymyxin B, tigecycline, and colistin) shown effective in the treatment of infections induced by these bacteria.

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Introduction:

Acintobacters include gram-negative, polymorph, non-motile, mandatory aerobic, non-fermentative, and usually encapsulated bacteria (1-3). These types of bacteria are extensively found in nature, specifically in water and soil resources (4).

Acinetobacters are opportunistic pathogens that are widely distributed in hospital settings and are responsible for nosocomial infections (2,3,5). One of the most important species of this type of acinetobacters is acinetobacter baumannii with ability for nosocomial spread (specifically in the ICU). It induces health infections such as bacteremia, urinary tract infections, wound infections, endocarditis, meningitis, surgical site infection, and pneumonia (4,6).

Acinetobacters are highly capable of developing fast antibiotic resistance, leading to its multiresistance trait (2,5). The greatest worrying capability of acinetobacters is multiresistance mechanism, emergence of new strains that are resistant to all common antibiotics, and the lack of new effective antimicrobial factors (8).

At present, the majority of acinetobacter strains are now resistant to all available antimicrobial agents. This resistance to multiple antimicrobial agents is a serious problem in infection treatment and control (4). In 1985, a vast spectrum of β-lactam antibiotics, called carbapenem, was introduced, and used for a long time in the treatment of infection induced by multiresistant acinetobacters. Currently, acinetobacter resistance to carbapenem is a global issue (3). Factors that trigger infection from antibiotic resistant acinetobacters are ICU stay for a certain time, application of pervasive devices, immune disorders, and a previous treatment with antibiotics (9).

The emergence and increase of imipenem-resistant acinetobacter baumannii in nosocomial infections have become a global issue and a threat to successful treatment of acquired infections (10).

Since carbapenem is one the most fundamental and important drugs in the treatment of acinetobacter-induced infections, the emergence of high resistant populations is a significant medical issue. This study set out to determine the species, antibiotic resistance pattern, and minimum inhibitory concentration (MIC) of carbapenems in different isolates of acinetobacters isolated from clinical specimens in Shahid Mohammadi Hospital in Bandar Abbas.

Methods:

This descriptive-cross sectional study was conducted on different clinical specimens including wound, chest tube, pharynx, trachea, blood, bile, urine, sputum, abdominal fluid, fixators, Foley-catheter, and Shaldon-catheter, isolated from Shahid Mohammadi Hospital patients. Each sample was isolate from one patient. The clinical specimens were incubated in blood Agar and MacConkey Agar mediums at 37°C. Initial identification of bacteria was done based on microscopic specifications and biochemical tests (oxidase, catalase, movement and culture in TSI medium). Then, Microgen GNA-ID System that includes 12 standard biochemical mediums for Lysine, arnitine, glucose, mannitol, H2S, indole, urease, topyranoside (O.N.P.G.), citrate, and tryptophan deaminase tests was used to determine the species. According to the Kit's instruction, a single colony from a 24-hour culture was emulsified in 3 mL sterile 0.85% normal saline, and then 3-4 drops of the obtained suspension were added to each well of the kit using a pasteur pipette. The kit incubated for 24 hours at 23°C, and then the positive and negative reactions were determined with the aid of the color chart. Afterwards, certain amounts of markers were added to each well and the results were read. A 4-digit code was obtained from the sum of the positive and negative reactions. This code was used by the provided software to identify the species level.

To determine the antibiotic resistance pattern for all collected species, the antibiogram test was conducted using Kirby-Bauer method with ciprofloxacin (5μg), ceftriaxone (30μg), imipenem (10μg), meropenem (10μg) (Himedia, India), calcitonin (25μg), polymyxin B (300μg), and tigecycline (15μg) (Mast, England) disks, according to standard guideline. In this technique, lawn culture technique was applied to the Mueller–Hinton medium, and the diameter of the zone of inhibition was measured after 24 hours. Results were then compared to the standard conditions and resistant isolates were identified. In addition, E. coli ATCC35218, E. coli ATCC25922, and

pseudomonas aeruginosa ATCC27853 were used as the control (11).

Results were recorded as sensitive, intermediate and resistant on the basis of Clinical and Laboratory Standard Institute (CLSI) guideline. To obtain more precise measurements of the sensitivity and resistance to carbapenem antibiotics (imipenem and meropenem), the E-test strips (Liofilchem, Italy) were used and their MICs were measured. In this method, the isolates were cultured onto the Mueller–Hinton medium, using the lawn culture method. After 24 hours, the MIC was measured based on the last digit of the E-test strip, where the zone of inhibition was created. Results were analyzed with SPSS 16.

Results:

Among 81 identified acinetobacter isolates, 64 species were male-specific and 17 species were female-specific. The mean age of 60 patients whose [medical] records were available was 41.76±2.709 years with the minimum and maximum of 5 and 82 years, respectively. According to the existing [medical] records, the mean hospital stay was 25.58±2.97 days with maximum and minimum of 85 and 3 days, respectively. The majority of isolates (37%) were obtained from trachea (Table 1).

Table 1. Frequency distribution of strains by clinical isolates

Clinical specimen	Frequency	Percentage
Trachea	30	37
Wound	2	14.8
Pharynx	9	11.1
Urine	8	9.9
Blood	6	7.4
Chest tube	4	4.9
Sputum	4	4.9
Abdominal fluids	2	2.4
Foley	2	2.4
Tracheostomy Scretions	2	2.4
Bile	1	1.2
Shaldon-catheter	1	1.2
Total	**81**	**100**

The clinical specimens were collected from different wards including ICU, Internal Units (1-3), Emergency, Burn, Surgery, Orthopedic 1 and 2, Nephrology and Neurosurgery. According to Table 2, the majority of specimens were isolated from ICU patients (58%).

Table 2. Frequency distribution of strains by hospital ward

Hospital wards	Frequency	Relative frequency
ICU	47	58.02
Internal ward 1	9	11.11
Internal ward 3	6	7.41
Internal ward 1	6	7.41
Emergency	3	3.70
Burn	2	2.47
Surgery	2	2.47
Orthopedic ward 2	2	2.47
Neurology	2	2.47
Neurosurgery	1	1.23
Orthopedic ward 2	1	1.23
Total	**81**	**100**

The acinetobacter isolates were investigated to the species level. According to the findings, 79 isolates were of acinetobacter baumannii type, and only 1 was of acinetobacter lwoffii type, and 1 was of acinetobacter haemolyticus type.

The acinetobacter lwoffii isolates were collected from a wound specimen in the Burn Ward, and the cinetobacter haemolyticus was isolated from a chest tube specimen in the ICU. According to Table 3, the maximum antibiotic resistance was observed in ceftriaxone (98.73%), and the maximum sensitivities was observed to polymyxin B and colistin (97.5%).

The acinetobacter lwoffii isolates were sensitive to imipenem, meropenem, tigecycline, and polymyxin B, and resistant to colistin, ceftriaxone, and ciprofloxacin. On the other hand, acinetobacter haemolyticus strains were resistant to imipenem, meropenem, colistin, polymyxin B, ceftriaxone, and ciprofloxacin, and sensitive only to tigecycline.

The imipenem and meropenem MICs of E-test strips were somewhere between 0.002 and 32 μg/mL. According to the European Committee of Antimicrobial Susceptibility Testing (EUCAST), MIC≤2μg/mL means sensitive to imipenem and meropenem and MIC>8μg/mL means resistant to imipenem and meropenem. MICs of all isolates were measured.

Table 3. Evaluation of relative frequency of acinetobacter baumannii strains with disk fusion method based on antibiotic sensitivity and resistance pattern

Type of antibiotic	Resistant (%)	Intermediate resistance (%)	Sensitive (%)
Ceftriaxone	98.73%	1.27%	0%
Ciprofloxacin	96.20%	2.53%	1.27%
Imipenem	82.28%	0%	17.72%
Meropenem	82.28%	0%	17.72%
Tigecycline	6.33%	11.39%	82.28%
polymyxin B	2.50%	0%	97.50%
Colistin	2.50%	0%	97.50%

Table 4. Measurement of resistance to imipenem and meropenem antibiotics using disk diffusion method in this study and other studies

Resistance to imipenem	resistance to meropenem	Study	Year	Site
82.28%	82.28%	At present	2012	Iran/Banda Abbas
97.70%	_	Amini et al. [16]	2012	Iran/Iran
62%	_	Sohrabi et al. [17]	2012	Northern Iran
100%	100%	Amudhan et.al. [7]	2011	India
100%	100%	Schimith Bier et.al. [15]	2010	Southern Brazil
52.20%	52.20%	Taheri-Kalani et al. [6]	2009	Iran/Iran
49.30%	50%	Feizabadi et al. [11]	2008	Iran/Iran
25%	-	Khaltabadi-Farahani et al. [2]	2007	Iran/Kashan

According to the E-test, 79.75% and 20.25% of the acinetobacter baumannii isolates were resistant and sensitive to imipenem, respectively. In addition, 78.48% and 21.52% were resistant and sensitive to meropenem, respectively. According to the E-test, the MICs of imipenem and meropenem sensitive acinetobacter lwoffii isolates were 0.047µg⁄ml and 0.123µg⁄ml, respectively. In this method, acinetobacter haemolyticus showed resistant to imipenem and meropenem with MIC≥32µg⁄ml.

Conclusion:

More than half of ICU-acquired infections are caused by Gram-negative bacteria. Reports on the distribution of pathogenic microorganisms are different. Meric et al. in a study in Turkey reported that the acinetobacter species were the second most prevalent microorganism in the ICU (12). A study in India investigated 43 acinetobacter-infected patients for 24 months. The majority of them had ICU-acquired infections, with the prevalence of 43% for acinetobacter baumannii (13).

An increase in the resistance of acinetobacters to antimicrobial substances is a big issue in Iran (11).

In our study, 64 out of 81 collected acinetobacter isolates were specific to men (79%) and 17 specimens to women (21%), indicating higher prevalence of this bacterium among male gender. The mean age in this study was 41.76±2.709 years.

Farahani reported the prevalence of these bacteria as 58.3% in men and 41.7% in women (mean age: 39.3±19.2 years).

According to our findings, 97.5% of isolates were acinetobacter baumannii, 1.2% acinetobacter lwoffii, and 1.2% acinetobacter haemolyticus, indicating a significantly higher prevalence of the baumannii species.

This rate of prevalence was 80% in Farahani's study (2). In Feizabadi's study, the prevalence of acinetobacter baumannii, acinetobacter lwoffii, and acinetobacter haemolyticus were 84.4%, 3.9%, and 3.9%, respectively (11). According to this and other studies, acinetobacter baumannii is the most prevalent nosocomial acinetobacter species, and the main cause of nosocomial infection.

In our study, the majority of isolates (37%) were obtained from trachea culture. In a study by Hashemizadeh in Shiraz, 44.2% of isolates were obtained from the cultures of sputum and trachea (4).

In the present study, ICU-acquired acinetobacter had the highest prevalence and the majority of isolates were obtained from ICUs. Amudhan in a study in India (2011) reported that the majority of isolates (95.6%) were from ICUs (7). This high prevalence of acinetobacter baumannii in ICUs may be due to the use of pervasive devices and weak immune systems of ICU patients.

Reports suggest that the acinetobacter baumannii is highly resistant to ceftriaxone and ciprofloxacin. For example, Amudhan and Schimith Bier in a study found that all strains were resistant to ciprofloxacin (7,12). Feizabadi (2008) reported that 83.5% of strains were ceftriaxone-resistant and 87.9% were ciprofloxacin-resistant (11).

Hashemizadeh reported that 87% of strains were resistant to ceftriaxone and 65% to ciprofloxacin (4). In our study, 98.73% of acinetobacter baumannii strains were ceftriaxone-resistant and 2.96% were ciprofloxacin-resistant.

Carbapenems have been the medication of choice for acinetobacter-induced infections. Nevertheless, the population of carbapenems-resistant strains has been globally increased in recent years (7). The emergence of imipenem-resistant acinetobacter baumannii is a global issue. This is because it threatens the successful arrays of acinetobacter baumannii infection treatment. In our study, the resistance of acinetobacter baumannii to carbapenem (imipenem and meropenem) was very high (82.28%). In Jafari et al.'s study, resistance of acinetobacter baumannii isolates to imipenem (40.9%) and meropenem (60%) was lower than our study (10). The prevalence of carbapenem-resistance is not equal in different regions. This is also true in Iran; in addition, the resistance to carbapenem antibiotics has been increased with time (Table 4).

Typically, the carbapenem resistant acinetobacter baumanii (CRAB) strains are resistant to many antibacterial agents. At present, polymyxin B and tigecycline may be good choices for treating infections caused by these pathogens (14).

Resistance to colistin was significantly lower than other antibiotics. As a result, colistin seems a very good choice for curing acinetobacter-induced infections. This may be because of less administration of this antibiotic in recent years (15).

In the present study, 82.28%, 11.39%, and 6.33% of acinetobacter baumannii isolates were sensitive, semi-sensitive, and resistant to tigecycline, respectively. In addition, the sensitivity to polymyxin B and colistin was 97.5%.

Amudhan reported that the sensitivity of acinetobacter strains to polymyxin B and tigecycline was 97.4% and 93.1%, respectively (7). In Kalani's study (2009), 91.2% of acinetobacter strains were sensitive to tigecycline and polymyxin B, and only 8.8% of them were resistant to these antibiotics (6). Shahcheraghi reported that 93.8% of acinetobacter strains were sensitive to colistin (15,16).

In the aforementioned studies, the sensitivity to polymyxin B was higher than 90%. This high rate of sensitivity may be due to the less administration of polymyxin B, which is because of its associated complications (neurotoxicity and nephrotoxicity).

Rahimzadeh reported that 84% of strains were sensitive to polymyxin B and 77% to colistin (17).

These statistics were lower than those provided by aforementioned studies. This indicates that the need for the conduction of further investigations. The lack of access to antibiogram disks from a single manufacturer was among research limitations.

The observation of hygiene and prevention practices plays a significant role in reduction of infectious diseases. Due to the importance of nosocomial infections, and their financial and life-threatening damages, physicians and medical staff should decide on appropriate solutions to prevent this problem in hospital settings. The improvement of the quality of healthcare services and provision of proper services to these patients are probably a good step to promote health and hygiene status in society (12).

The frequency of infection with carbapenems-resistant acinetobacter baumannii is high in Shahid Mahmoudi Hospital in Bandar Abbas. The degrees of resistance to imipenem and meropenem are 79.75% and 78.48%, respectively. Three types of antibiotics (polymyxin B, tigecycline, and colistin) are effective in the treatment of infections induced by these bacteria. Further control and sensitivity are required to prevent an increase the prevalence of these infections.

References:

1. Khosrishahi N, Sharifi M. Isolation of carbapenem resistant Acinetobacter baumannii (CRAB) strains from patients and equipments of Intensive care units (ICUs) at Qazvin between 2005-2006. Iranian Journal of Medical Microbiology. 2008;10(35):11-15. [Persian]
2. Farahani Kheltabadi R, Moniri R, Shajari G, Nazem Shirazi M, Musavi S, Ghasemi A, et al. Antimicrobial Susceptibility patterns and the distribution of resistance genes among Acinetobacter species isolated from patients in shahid Beheshti hospital, Kashan. Feyz Journal. 2009;12(4):61-67. [Persian]
3. Zarrilli R, Giannouli M, Tomason F, Triassi M, Tsakris A. Carbapenem resistance in *Acinetobacter boumannii*: the molecular epidemic features of an emerging Probkem in health care facilities. J Infect Dev Ctries. 2009;3(5):335-347.
4. Hashemizadeh Z, Zargani AB, Emami A, Rahimi MJ. Acinetobacter antibiotic resistance and frequency of ESBL-producing strains in ICU patients of Namazi Hospital (2008-2009). Journal of Qazvin University of Medical Sciences. 2010;14(2):47-53. [Persian]
5. Yang HY, Lee HJ, Suh JT, Lee KM. Outbreaks Imipenem resistant *Acinetobacter baumannii* Producing OXA-23 β-Lactamase in a Tertiary Care Hospital in Korea. Yonsei Med J. 2009;50(6):764-770.
6. Taherikalani M, Fatholahzadeh B, Emaneini M, Soroush S, Feizabadi M. Distribution of different carbapenem resistant clones of *Acinetobacter baumannii* in Tehran Hopital. New Microbiolo. 2009;32(3):265-271.
7. Amudhan SM, Sekar U, Arunagiri K, Sekar B. OXA beta-Lactamase-mediated carbapenem resistance in *Acinetobacter baumannii*. IJMM. 2011;29(3):269-274.
8. Munoz-Price LS, Weinstein RA. Acinetobacter infection. NEJM. 2008;358(12):1271-1281.
9. Ghibu L, Miftode E, Olivia D, Dorobat C. Carbapenem-resistant *Acinetobacter baumannii* postoperative meningitis. Jurnalul de Chirurgie. 2011;7(1):109-113.
10. Jafari S, Najafipour S, Kargar M, Abdollahi A, Mardaneh J, Fasihy Ramandy M, et al. Phenotypical Evaluation of Multi-Drug Resistant Acinetobacter Baumannii. Journal of Fasa University of Medical Sciences. 2011;2(4):8-12. [Persian]
11. Feizabadi MM, Fatholah Zadeh B, Taherikalani M, Rasoolinejade M, Sadeghifard N, Aligholi M, et al. Antimicrobial Susceptibility Patterns and Disteribution of blaOXA Genes among Acinetobater spp. Isolated from Pationts at Tehran Hospitals. Jpn J Infect Dis. 2008;61(4):274-278.
12. Meric M, Willke A, Caqlayan C, Tokerk CC. Intensive care unit–acquired Infection: Incidence, riskfactors and associated mortality in a Turkish university hospital. JPN J Infect Dis. 2005;58(5):297-302.
13. Proshonth K, Badrinoth S. Epidemiological investigation of nosocomial Acinetobacter infections using orbitrarity primed PCR and pulse field gel electrophoresis. Indian J Med Res. 2005;122(5):408-418.
14. Schimith bier KE, Luiz SO,Scheffer MC, Gales AC, Paganini MC, Nascimento AJ, et al. Temporal evolution of carbapenemresistant *Acinetobacter baumannii* in Curitiba, southern Brazil. AMJ Infect Control. 2010;3(8):308-314.
15. Amini M, Davati A, Golestanifard M. Frequency of Nosocomial Infections with Antibiotic Resistant Strains of Acinetobacter spp. in ICU Patients. Iranian Journal of Pathology. 2012;7(4):241-245.
16. Shahcheraghi F, Akbari Shahmirzadi N, bbasalipour Bashash M, Jabbari H, Amirmozafari N. Detection of blaCTX ,blaTEMbeta-lactamase genes in clinical isolates of Acinetobacterspp. From selected Tehran hospitals, Iranian Journal of Medical Microbiology. Iran J Med Microbiol. 2009;3(1):1-9.
17. Rahimzadeh A, Farajnia S, Pourbabaee MA, Ansarin KH, Zolfaghari MR, Masoudi N. Detection of Prevalence of OXA-2 and OXA-10 Type ESBL and Class I Integron among Acinetobacter Bumanii Strains Isolated from Patients of Tabriz City (Iran) by PCR Technique. Journal of Babol University of Medical Sciences. 2012;14(5):56-63. [Persian]