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Full Length Research Paper

Multi-drug resistance in *Acinetobacter baumannii* strains isolated from the clinical specimens of three hospitals in Tehran-Iran

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The aim of this study is to carry out an investigation on multi-drug resistance in *Acinetobacter baumannii* strains that are isolated from clinical samples of three highly large hospitals in Tehran-Iran. This descriptive-cross sectional study was performed in three large hospitals in Tehran on 70 samples of Acinetobacter which were isolated from patients during April to November 2010. After identifying the species level by using culture and biochemical methods, in order to determine the sensitivity of 50 isolates of *A. baumannii* to 13 antibiotics, standard methods according to CLSI guidelines were performed. In this study, resistance to three or more than three classes of antibiotics multidrug resistance was defined. In this study, 50 *A. baumannii* strains, 12 *A. lwoffii* strains and other Acinetobacter species were isolated from patients. The majority of isolates were from blood specimens. Isolates of *A. baumannii* showed the highest resistance to cefepime, ceftazidime, aztreonam, norfloxacin, ofloxacin, ciprofloxacin and amikacin. Tobramycin and meropenem were considered as effective drugs in this study. Multi-drug resistance in these strains was 55.4%, respectively. Multi-drug resistant Acinetobacters are growing and are considered as an important threat for hospitalized patients, so a change in consumption patterns of antibiotics and control of hospital infections seems to be necessary.

Key words: *Acinetobacter baumannii,* nosocomial infection, multi-drug resistance, antibiogram.

INTRODUCTION

The genus Acinetobacter comprises gram negative, oxidase negative, strictly aerobic and non motile bacteria. Various species of Acinetobacter are widespread in nature. They can be recovered from virtually all samples obtained from soil, surface water, human skin, food and waste (Gaynes et al., 2005; Leung et al., 2006; Peleg et al., 2008).

The genus known as Acinetobacter has significant taxonomic modification over the last 30 years. *Acinetobacter baumannii* is the most common species that are isolated from patients, while other species such as *Acinetobacter lwoffii, Acinetobacter Johnsonii* and *Acinetobacter haemolyticus* are rarely isolated from patients (Peleg et al., 2008).

Nowadays, due to *A. baumannii* significant clinical properties and its ability to achieve drug resistance, it is considered as one of the microorganisms that threaten antimicrobial medication. *A. baumannii* causes nosocomial infections such as bacteremia, urinary tract infections and secondary meningitis, but it has prominent role in creation of hospital pneumonia, especially pneumonia that are acquired in the upper respiratory tract in patients that are hospitalized in intensive care units (ICU) (Anstey et al., 2002; Dijkshoorn et al., 2005; Fournier and Richet, 2006; Peleg et al., 2008; Rizos et al., 2007; Villers et al., 1998).

Different studies demonstrated that various species of

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A. baumannii are resistant to wide range of antibiotics. Spread of multi-drug resistant *A. baumannii is* not limited to hospitals of one city, but is also important in national scale (Metan et al., 2007; Unal et al., 2005; Wisplinghoff et al., 2007; Wroblewska et al., 2007).

Since several factors cause resistance in *A. baumannii*, treatment of infections caused by this organism should be based on perfect antibiotics sensitivity tests. Therefore, having information regarding the prevalence and pattern of bacterial resistance to these drugs is important (Halstead et al., 2007; Scott et al., 2007; Van Dessel et al., 2004). In this study, an investigation of multi-drug resistance is done in *A. baumannii* strains that are isolated from the clinical samples of three highly large hospitals in Tehran-Iran.

MATERIALS AND METHODS

This descriptive-cross sectional study was conducted from April to November 2010. A total of 500 samples of blood, respiratory sections, urine, skin sores and trachea were collected from patients of three highly large hospitals of Imam Khomeini, Milad and Baqiyatallah, and were transferred to the laboratory by BHI broth medium.

In the laboratory, each sample was cultured on blood agar and MacConkey agar (Merck Co., Germany) and incubated for 24 h in 37℃. Blood specimens was cultured in Trypticase Soy Broth (TSB) (Merck, Germany) and sub-cultured on chocolate agar. After 24 h, with direct examination (gram staining), the presence of gram negative coccobacillus was confirmed by the microscopic approach. In order to recognize different species of Acinetobacter, all suspected colonies were identified by colonial morphology, gram-staining, positive catalase, negative oxidase, growth in 37 and 42℃ and other biochemical reactions.

After identification of Acinetobacter species, in order to determine the drug resistance phenotype, disk diffusion method as recommended by clinical laboratory and standards institute (CLSI) was performed. In this study, 13 different antibiotic disks from Oxoid Ltd. (Basingstoke, UK) were used, which included amikacin (30 µg), ampicillin/sulbactam (10/10 µg), aztreonam (30 µg), cefepime (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamycin (10 µg), imipenem (10 µg), meropenem (10 µg), norfloxacin (10 µg), ofloxacin (1 µg), piperacillin / tazobactam (100/10 µg) and tobramycin (10 µg). In addition, the antibiotic potency of the disks was standardized against the reference strains of *Escherichia coli* (ATCC 25922) as negative control and the reference strain of *A. baumannii* (ATCC 19606) as positive control was used.

According to different studies, isolates of *A. baumannii* that show resistance to three or more than three categories, including quinolone antibiotics (ciprofloxacin), broad spectrum cephalosporins (ceftazidime and cefepime), combined lactam/lactamase inhibitor (ampicillin/sulbactam), aminoglycosides (amikacin, tobramycin) and carbapenems (imipenem, meropenem) are considered as multi-drug resistant strains. Finally, for statistical analysis, data were entered into a database using SPSS 16 for Windows (SPSS Inc., Chicago, IL) and then, results were analyzed by using chi-square test.

RESULTS

During this study, a total of 70 samples of Acinetobacter were isolated from 500 collected samples. 50 samples of patients were identified as *A. baumannii* (71%), 12 were *A. lwoffii* (17.1%) and 8 (11.4%) were other Acinetobacter species. The results showed that 50 samples of *A. baumannii* were isolated from 19 blood samples (38%), while 15 trachea tube aspiration samples (30%), 6 wound samples (12%), 4 urine samples (8%), 1 oral sample (2%) and 5 samples (10%) had unknown origin (Table 1). The majority of strains were isolated from ICUs (20/50) and the remaining strains were from infectious ward (15/50), emergency ward (10/50) and other wards.

Table 1. Frequency of *A. baumannii* according to the type of clinical samples.

Samples	No (%)
Blood	19 (38)
Trachea tube aspiration	15 (30)
Wound	6 (12)
Urine	4 (8)
Oral samples	1 (2)
Unknown samples	5 (10)
Total	50 (100)

In this study, almost all samples were resistant to ceftazidime and cefepime, while high resistance to aztreonam, norfloxacin, ciprofloxacin, amikacin, imipenem, gentamycin and ampicillin-sulbactam were observed. Tobramycin and meropenem were considered as effective drugs in this review (in Table 2, patterns of antibiotic resistance in *A. baumannii* were shown).

Results of this study showed that 27 samples of *A. baumannii* (54%) were resistant to three or more than three antibiotics (Table 3) and 16 samples (32%) showed resistance to two antibiotics. Also, none of the resistant strains showed complete resistance to all antibiotics.

Conclusion

A. baumannii is an important opportunistic pathogen to high virulence. It is responsible for severe nosocomial infections over the last 30 years. This bacterium, particularly multi-drug resistant strains has been implicated as the cause of serious infectious disease in different parts of the hospitals and treatment of such infections because of their broad resistance to antibiotics is difficult (Anstey et al., 2002; Gaynes et al., 2005; Leung et al., 2006; Peleg et al., 2008). Moreover, since environmental factors and different patterns of antimicrobial agents play important role in creation and expansion of these strains in different parts of the world, in this study, the incidence of multi-drug resistant *A. baumannii* from clinical samples of three hospitals in Tehran-Iran was performed.

In this investigation, 71.5% of isolates were *A. baumannii* and 28.5% were identified as A*. lwoffii* and other Acinetobacter species. A similar finding was also observed by Constantiniu and colleagues during years

Table 2. Frequency of antibiotic resistance in *A. baumannii* isolates.

Antibiotic	Sensitivity patterns (%)		
	Resistant	Intermediate	Sensitive
Cefepime	96	4	0
Ceftazidime	96	4	0
Aztreonam	95	3	2
Norfloxacin	95	1	4
Ofloxacin	88	4	8
Ciprofloxacin	88	4	8
Amikacin	85	5	10
Imipenem	76	2	22
Gentamycin	61	3	36
Ampicillin-sulbactam	59	3	38
Piperacillin-tazobactam	40	8	52
Meropenem	31	4	65
Tobramycin	26	2	72

Table 3. Frequency of multi- drug resistance in *A. baumannii.*

	Resistance to one or several antibiotics					Total
Number of antibiotics	1	2	3	4	>4	50
Number of isolates resistant *A. baumannii*	7	16	13	5	9	

2001 to 2004. They also detected 71% *A. baumannii* and 29% *A. lwoffii* from 24 clinical isolates (Constantiniu et al., 2004).

Hujer et al. (2006), in their study that was conducted on military and civilian patients in Iraq and Afghanistan, reported that 15% of the strains were resistant to all nine antibiotics which were tested and 89% of the strains showed resistance to at least three antibiotic classes. In their study, more than 90% of the isolates were resistant to ciprofloxacin, less than 80% were resistant to cephalosporins with a broad spectrum, 40% were resistant to ampicillin-sulbactam, 20% were resistant to imipenem and 81% were resistant to at least one of the aminoglycosides (amikacin or tobramycin) (Hujer et al., 2006). In this study, more than 90% of isolates were resistant to ciprofloxacin, ofloxacin and cephalosporins (ceftazidime and cefepime), 76% to imipenem, 59% to ampicillin / sulbactam, and less than 35% to meropenem and tobramycin (Table 2). Also, in this study, more than 50% of isolates were resistant to at least three antibiotic classes (Table 3). Differences observed between the two studies could be due to methods and resistance patterns that were influenced by environmental factors and the antimicrobial patterns which were used. It is necessary to say that international travels are also important in the development of multi-drug resistant strains.

Acinetobacter strains with antibiotic resistance have been reported from all around the world (Brink et al., 2007). In the study performed by Ayan et al. (2003), out of 52 strains, all isolates were resistant to piperacillin, piperacillin-tazobactam, ticarcillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ceftriaxone and gentamycin. Also, they were resistant to tobramycin, ciprofloxacin, ampicillin-sulbactam, co-trimoxazole and amikacin, whose results highly conform to the results of this research (Ayan et al., 2003).

Rahbar and colleagues in the year 2005 to 2006 determined that, *A. baumannii* showed high percentage of resistance to ceftriaxone (90.9%), piperacillin (90.9%), ceftazidime (84.1%), amikacin (85.2%) and ciprofloxacin (90.9%), whose results partly conform with the results of this research. They also conducted a research, which showed that imipenem was the most effective agent against these organisms (resistance 4.5%), and the result was in conflict with the results of this study (Rahbar et al., 2010).

In a study conducted by Karlowsky et al. (2003), they showed that in year 1998 to 2001, 90% of *A. baumannii* strains were sensitive to meropenem, but in this investigation, only 44% of strains showed resistance to meropenem. However, the usage of this antibiotic with tobramycin could be more effective against *A. baumannii* strains (Karlowsky et al., 2003).

In a study performed by Hoe Koo et al. in years 2007 to 2008, they determined amikacin as the most effective drug among nine antimicrobial agents, unlike this study,

where tobramycin and imipenem were the most effective agents among the 11 antimicrobial agents that were used (Hoe Koo et al., 2010).

Overall, the results indicate that among the common Acinetobacter species, *A. baumannii* is more responsible for nosocomial infections and more than 50% of the strains are multi-drug resistant, so control of hospital infections seems to be necessary among the three hospitals which were investigated. In this regard, in response to the uncontrolled use of antibiotics, multi-drug resistant *A. baumannii* in hospital environment increased; so, control of antibiotics usage in hospitals play an important role in preventing the emergence of such strains and infections caused by them.

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