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Full Length Research Paper

Bacteremia due to multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producing *Acinetobacter baumannii*

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Bacteremia due to *Acinetobacter baumannii* is a common problem in hospitals worldwide. Physicians in the intensive care units (ICUs) encountered a serious challenge of finding a drug to cure the extended-spectrum beta-lactamase (ESBL) producing and multi-drug resistant (MDR) *A. baumannii* bacteremia. Our aim was to determine the frequency of *A. baumannii* bacteremia in an Iranian hospital ICUs, their antibiotic susceptibility patterns, and the frequency of ESBLs by a cross-sectional study. A total of 340 patients admitted to ICUs during a 6 month period of study were investigated for bacteremia due to *A. baumannii.* Bacteria isolates from blood specimens were identified as *A. baumannii* by API 20NE system. Antimicrobial susceptibility was studied with disk diffusion method. Detection of ESBLs was done by double disk synergy test. Of the 340 patients investigated, bacteremia was found in 114 cases (33%). *A. baumannii* was diagnosed as the etiological agent of bacteremia in 69 cases (60.5%). All the isolates were multi-drug resistant. Except one, all the remaining isolates (98.6%) were resistant to at least 7 of 13 tested antibiotics. Pandrug-resistance was observed in 4 isolates (5.6%). Of all the isolated *A. baumannii,* 49 (71%) were found to be resistant to cephalosporins by screening tests and among them 27 isolates (39%) were found to be ESBL producing. Our study showed a high frequency of *A. baumannii* bacteremia occurrence in our hospital ICUs. An urgent intervention is needed to reduce the MDR bacterial load in these critical units.

Key words: *Acinetobacter baumannii,* bacteremia, multi-drug resistant, extended-spectrum beta-lactamase.

INTRODUCTION

*Acinetobacter*s are strictly aerobic, non-fermentative, pleomorphic, Gram negative coccobacilli. *Acinetobacter* species can be found everywhere in nature (soil and water) (Forster and Daschner, 1998). These micro-organisms are widely distributed in hospital environment and can be isolated readily from various surfaces and instruments such as sinks, bed rails, ventilators, and even doorknobs (Jawad et al., 1996). They are also amongst the most important opportunistic pathogens that cause nosocomial infections (Urban et al., 2003*;* Villegas and Hartstein*,* 2003).

Acinetobacter comprises seventeen validly named and fourteen unnamed species (Van Looveren and Goossens, 2004). *Acinetobacter baumannii* accounts for more than 80% of the isolates causing human diseases (Allen and Hartman*,* 2000). In hospitals, *A. baumannii* normally inhabits the skin and mucous membranes of some patients (Seifert et al., 1997; Chu et al., 1999). Although *A. baumannii* was susceptible to nearly all antibiotics in the past decades, in the recent years it has emerged as a major multidrug resistant bacterium (Fournier and Richet, 2006; Livermore, 2003). Drug resistance mechanisms in *A. baumannii* are both intrinsic and acquired. *A. baumannii* resistant strains usually contain a set of genes coding for resistance to multi-group of antibiotics at the same time (Landman et al.**,** 2002; Aygün et al., 2002).

One of the main concerns about *A. baumannii* infections is the increasing rate of isolating the strains

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Table 1. The frequency of *A. baumannii* isolates in 69 blood specimens of bacteremic patients and ESBL producing *acinetobacter*s in each ICUs.

ICUs	No. of isolates (%)	No. of ESBLs (%)
Surgery	27 (39.1)	12 (17.3)
Neurosurgery	19 (27.5)	8 (11.6)
Pediatric	13 (18.8)	5 (7.2)
Internal	10 (14.5)	2 (2.9)
Total	69 (100)	27 (39)

producing extended-spectrum beta-lactamase (ESBLs) through the world (Bergogne-Be´re´zin, and Towner 1996). These strains show considerable resistance to the antibiotics such as ceftizoxime, cefoperazone, cefotaxime, ceftriaxone, aztreonam and ticarcillin in hospitals. *Acinetobacter bacteramia*, due to pneumonia or catheter usage, accounts for a great portion of fatalities in intensive care units (ICUs) (Agodi et al., 2006). The frequency of ESBL producing *acinetobacters* in these wards has a direct impact on the result of antibiotic therapy, hospital stay and economic cost. This study aimed to determine the frequency of bacteremia due to *A. baumannii* in a major teaching hospital ICUs, their antibiotic susceptibility patterns, and the frequency of ESBLs by a cross-sectional study (Mulin et al., 1995; Scerpella et al., 1995).

MATERIALS AND METHODS

During the 6 month study period (March to August, 2010), 340 blood samples from different intensive care units of Namazi hospital (central sub-special hospital affiliated with Shiraz University of Medical Sciences, Shiraz, Iran) were collected and submitted to the hospital microbiology laboratory. All blood cultures were processed in the laboratory using the Bactec (BD-9120) system. The Samples were subcultured on sheep blood agar (SBA) and eosin methylene blue agar (EMB) and incubated in air at 37°C for 24 h. The isolates were identified as *A. baumannii* by API 20NE (bioMerieux).

Antimicrobial susceptibility of *A. baumannii* isolates against 13 selected antibiotic agents: ciprofloxacin (5 µg), amoxicillin clavulanic acid (30 µg), cefepime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), piperacilin (100 µg), amikacin (30 µg), gentamicin (10 µg), imipenem (10 µg), aztreonam (30 µg), ticarcillin/clavulanic acid (75/10 µg) and tobramycin (10 µg) were determined by using disk diffusion method on Mueller-Hinton agar according to the CLSI standards (CLSI, 2007).

ESBLs were detected by phenotypic tests containing screening and confirmatory steps. The screening step consists of testing for resistance to four different cephalosporins: cefepime, ceftazidime, ceftriaxone and cefotaxime (30 µg each). For the fact that E-test was considered as a precise method in determining the resistance to third generation cephalosporins, it was used for the screening step and the minimum inhibitory concentration (MIC) of the resistant isolates to the four beta-lactam antibiotics was determined.

MICs were interpreted with the ranges recommended by CLSI standards (CLSI, 2009). Resistant isolates were selected for the confirmatory step. The confirmatory step was based on the demonstration of synergy between the above beta-lactam agents and clavulanic acid by double-disk synergy test (DDST) and the results were interpreted as described previously (Bradford, 2001).

The reference strains (*Escherichia coli* ATCC 35218, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATTC 700603) were used as controls and included in each step.

RESULTS

During the study period, 340 patients were admitted to 4 ICUs (Pediatrics, Internal, General surgery and Neurosurgery). The average age was 63 years (16 to 71) and 59% of them were male. Of the 340 non-repeated blood samples, bacteremia with positive culture was found in 114 cases (33.5%). *A. baumannii* was diagnosed as the etiological agent of bacteremia in 69 cases (60.5%).

The frequency of *A. baumannii* isolation in each ICU is displayed in Table 1. Of the 69 isolates of *A. baumannii,* 49 (71%) were found to be resistant to cephalosporins by screening tests, and of them, 27 isolates (39%) were found to be ESBL producing by DDST. The frequencies of ESBL producing isolates in different ICUs are shown in Table 1. The most frequent ESBL producing isolates were seen in surgery ICU.

Antimicrobial susceptibility results of *A. baumannii* isolates against 13 selected antibiotic agents are shown in Table 2. The majority of the isolates showed resistance to most of the antibiotics tested. A High frequency of resistance was detected for piperacillin/tazobactam (92.8%), ciprofloxacin (91.4%) and amikacin (91.4%). Imipeneme was the most effective amongst the antimicrobials tested; 33.3% of the isolates were found to be susceptible. The imipenem resistant isolates were also resistant to piperacillin/tazobactam, ciprofloxacin and amikacin.

In this study, all the isolates were multi-drug resistant (MDR). Except one isolate that was resistant to five antibiotics, all the remaining isolates (98.6%) were resistant to at least 7 out of 13 tested antibiotics. Pandrug-resistance was observed in 4 isolates (5.6%). The MIC values of 4 selected cephalosporins for screening step of detecting ESBLs are shown in Table 3.

DISCUSSION

Hospitalized patients in ICUs are critically ill and are

Table 2. Susceptibility pattern of *A. baumannii* isolates to 13 selected antimicrobial agents by disk diffusion agar method (n = 69).

Antimicrobial agent	Resistant (%)	Intermediate (%)	Susceptible (%)
Ceftazidime	57(82.7)	7(10.1)	5(7.2)
Cefotaxime	60(87.0)	5(7.2)	4(5.8)
Ceftriaxone	49(71.0)	14(20.3)	6(8.7)
Cefepime	49(71.0)	13(18.9)	7(10.1)
Aztreonam	62(89.9)	4(5.8)	3(4.3)
Imipenem	46(66.7)	0(0.0)	23(33.3)
Piperacillin or tazobactam	64(92.8)	1(1.4)	4(5.8)
Ticarcillin or clavulanic acid	59(85.6)	5(7.2)	5(7.2)
Ciprofloxacin	63(91.4)	1(1.4)	5(7.2)
Amikacin	63(91.4)	1(1.4)	5(7.2)
Gentamicin	61(88.4)	0(0.0)	8(11.6)
Tobramycin	51(74.0)	7(10.1)	11(15.9)
Trimethoprim or sulfamethoxazole	62(89.9)	5(7.2)	2(2.9)

Table 3. MIC values of 4 selected cephalosporins and frequency of antimicrobial susceptibility amongst *A. baumannii* isolates (n=69).

Antimicrobial agent	MIC range (µg/ml)	MIC[a] 50 (µg/ml)	MIC[b] 90 (µg/ml)	No. of susceptible isolates (%)
Cefotaxime 17	32-128	64	128	17(24.6)
Ceftazidime	32-128	64	128	10(14.5)
Cefepime	4-128	32	128	30(43.4)
Ceftriaxon	2-64	32	64	19(27.5)

[a] Minimum inhibiting concentration 50% of isolates, [b] Minimum inhibiting concentration 90% of isolates.

always at higher risks of developing nosocomial infections with antibiotic resistant bacteria. The emergence and spread of multidrug resistant *A. baumannii* with ability to survive in various harsh environments and genetic potential to transfer resistant determinants is a major concern in hospitals (Sehulster et al., 2004).

The most surprising finding in this study was the high frequency of bacteremia due to *A. baumannii* (60.5 %) in ICUs through a 6 month period of the study. Previous studies in different areas of the world have shown different frequencies of *A. baumannii* bacteremia in ICUs. For example, Seifert et al. (1995) reported a frequency of 8.1% of *Acinetobacter* spp. bacteremia in some hospitals' ICUs in Germany, and *A. baumannii* was the most isolated species (57%). Wareham et al. (2008) in UK identified 399 episodes of *Acinetobacter* spp. bacteremia during 1998 to 2006, with *A. baumannii* as the most frequently isolated species. Most episodes occurred in critical care and were associated with multidrug resistance (52 %). Choi et al. (2006), during a 6 year study period in Korea, observed 378 episodes of *Acinetobacter* spp. bacteremia in 344 patients (2.4% of all bacteremia episodes). Of those episodes, 92.3% were due to *A. baumannii* and 49.1% of all *A. baumannii* were in ICU.

Another important finding in our study was that all the isolates of *A. baumannii* in ICUs were MDR (100%). Of those isolates, 5.6% were pandrug resistant. Data on the frequency of MDR *A. baumannii* bacteremia in ICUs in different parts of the world is scarce. A few previous studies have also shown a high frequency of MDR *A. baumannii* in ICUs. Wisplinghoff et al. (2000) in a study in the USA found that 30% of all *A. baumannii* isolates from blood samples of bacteremic patients in ICU were resistant to ≥ 4 different classes of antimicrobial agents.

The frequency of ESBL producing *A. baumannii* isolates in our ICUs was 39%. As we know, there was only one study done on this subject. Gopal Katherason et al. (2010) in Malaysia detected 16 isolates of *Acinetobacter* spp. as the cause of bacteremia in ICU, 5 of them were ESBL producing.

In conclusion, our study showed a high frequency (60.5 %) of *A. baumannii* bacteremia occurrence in our hospital ICUs. All of them were multidrug resistant and 39% were ESBL producing. It is obvious that our main concern in ICUs is MDR bacteria that limit our capacity to manage the clinical manifestations of patients with available antibiotics. So it seems that an urgent intervention is needed to reduce the MDR bacterial load in the ICUs.

The use of Strict sterile techniques in the insertion of central venous catheters (Eggimann and Pittet, 2002), frequent in-service education for the procedures of insertion and maintenance of intravascular devices were used to prevent intravascular device-related infections,

the proper use of antiseptic solution for blood drawing, teaching and auditing the proper hand washing practices (Little et al., 1999) and using sterile gloves and masks, are necessary practices for reducing the bacteremia in ICUs.

Avoidance of the empirical broad spectrum antibiotic therapy for ICU patients and prescription of the appropriate antibiotics according to the antimicrobial susceptibility test results, mandatory use of specific sterilized hospital apron for each patient by physicians and nurses can be our immediate response to decreasing the selective pressure for MDR bacteria in ICUs.

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