

ESBL- and MBL-mediated resistance in *Acinetobacter baumannii*: a global threat to burn patients

Infezioni da *Acinetobacter baumannii* produttori di ESBL e MBL nei pazienti ustionati

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INTRODUCTION

Burn wounds provide an ideal site for bacterial multiplication and are potentially richer sources of infection than surgical wounds, predominantly a consequence of the larger area involved and the longer duration of hospitalization. Infections are significant causes of morbidity and mortality in hospitalized burn patients worldwide [1]. It is currently estimated that about 75% of the mortality associated with burn injuries is due to infections rather than osmotic shock and hypovolemia [2]. For several decades, antibiotics have been crucial in the fight against infectious disease caused by microorganisms. Despite recent improvement in burn patient care and use of a wide variety of antimicrobial agents, infections that complicate the clinical course of patients who had sustained severe injuries remain the most important cause of death among burn patients. Now, most of the bacteria that cause nosocomial burn infection are resistant to at least one of the drugs most commonly given for treatment [1, 3].

Acinetobacter baumannii is an important opportunistic pathogen that can cause nosocomial infection in burns units. Resistance to extended spectrum beta-lactams in *A. baumannii* is mediated by metallo-beta-lactamase (MBL) and extended spectrum beta-lactamase (ESBL) enzymes. These enzymes constitute a real threat for treating burn victims [4-6]. In recent years,

nosocomial infections caused by ESBL- and/or MBL-resistant *A. baumannii* were significantly highly frequent in burn units [7]. The emergence of multidrug-resistant *A. baumannii* has resulted in public health problems, which have become increasingly common in burn units. Most nosocomial isolates of *A. baumannii* are currently resistant to a large variety of antimicrobial agents [4]. Nosocomial infections with *A. baumannii* remain problematic because of its high intrinsic resistance to a wide variety of antimicrobial agents. Moreover, the ability of resistant strains of *A. baumannii* to survive for prolonged periods in the hospital environment contributes significantly to antimicrobial resistance, thereby posing a difficult challenge for infection control [8].

Carbapenems used to be the drugs of choice for treating burn infections caused by *Acinetobacter baumannii* strains. Consequently, due to selective pressure on carbapenems and the increased use of this antibiotic, carbapenem-resistant *A. baumannii* has emerged [9]. This problem worsens in cases of MBL production when the drug of last choice, carbapenems, is inactive [10]. We used a method to detect on a daily basis ESBL and MBL in *A. baumannii* in all clinical specimens.

With the spread of ESBL and/or MBL positive strains in hospitals all over the world, there is a need to investigate their prevalence in a hospital so as to formulate a policy of empirical ther-

apy in high-risk units where infections due to resistant organisms are much higher. Likewise, it is also important to procure information on an isolate from a patient to avoid the misuse of extended spectrum cephalosporins, and carbapenems, which continue to be the main component of antimicrobial therapy in burn wards [2, 9]. The aim of the present study is to investigate screening of ESBL and MBL produced by *Acinetobacter baumannii* isolated from the Burn unit of the Motahari hospital in Tehran, Iran.

■ MATERIAL AND METHODS

In the present study, during a period of six months (August 2010 to February 2011), 126 isolates of *A. baumannii* were collected from patients admitted to the Burn Unit of Motahari hospital (hospitalized for at least 1 week) in Tehran in Iran. The samples were collected by swabbing the exudates of wounds, and transported under standard conditions immediately in transport culture media towards the central laboratory of the antimicrobial resistance research centre.

Identification of *A. baumannii* strains was confirmed by using standard biochemical tests such as oxidase, TSI, motility and OF test. Antimicrobial susceptibility testing was performed for all *A. baumannii* strains against cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), imipenem (10 µg), amikacin (30 µg), ticarcillin (75 µg), ticarcillin-clavulanic acid (75/10 µg), piperacillin (100 µg), piperacillin-tazobactam (100/10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), kanamycin (30 µg) and colistin (10 µg), trimethoprim (5 µg) antibiotic disks (the MAST Co, Mast Diagnostics, UK). The test was carried out following the method recommended by the Clinical and Laboratory Standards Institute (CLSI). The isolates were screened for ESBL and MBL production by using combined double disc synergy tests.

Phenotypic detection of ESBL

Ceftazidime-resistant isolates were screened for producing ESBL. The double disk method was used for detection of this enzyme. Then the suspension was streaked onto Mueller-Hinton agar plates (Hi Media, Mumbai, India). A disc of either ceftazidime (30 µg) or cefotaxime alone (30 µg) in combination with clavulanic acid (30 µg/10 µg) was placed at the distance of 20 mm

(centre to centre). After incubation overnight at 35°C, a positive test result was considered as a ≥ 5 mm increase in inhibition halo compared with a disk without clavulanic acid [11].

Phenotypic detection of MBL

Imipenem-resistant isolates were screened for producing MBL. The double disk method was used to detect this enzyme. Colonies from overnight cultures on blood agar plates were suspended in Mueller-Hinton broth and the turbidity standardised to equal that of a bacterial concentration of 1:100 suspension of the 0.5 McFarland standard. Then the suspension was streaked onto Mueller-Hinton agar plates (Hi Media, Mumbai, India). A disc of either imipenem alone (10 µg) or imipenem (10 µg) in combination with EDTA (750 µg / disc) was placed at the distance of 20 mm (centre to centre). After incubation overnight at 35°C, a ≥ 7 mm increase in the inhibition zone of diameter around imipenem-EDTA discs, as compared to EDTA discs alone, interpreted as indicative of MBL production [11, 12]. In addition, we reported MBL-positive strains when more than a 5 mm increase in the inhibition zone was seen in the combination of imipenem and EDTA discs in comparison with EDTA discs alone.

■ RESULTS

In our study 126 isolates of *A. baumannii* were obtained from as many burn patients (35% females and 65% males), their ages ranging between 1 and 73 years and TBSA (the total body surface area) between 6% to 92%. Antimicrobial resistance to antibiotics used in this study were determined according to the interpretive criteria of the CLSI guidelines. According to these results, all of the isolates were susceptible to colistin (Table 1).

Most antibiotyping of isolates is indicated in Table 2. Most of the ESBL-producing isolates were related to patterns 1, 2 of antibiotype (Table 2). The antibiotyping (antibiotic sensitivity profiles) of 23 isolates showed several other different patterns.

In the present study, the maximum resistance was seen against ceftazidime, aztreonam and trimethoprim (98%) and no resistance was seen against colistin. Fifty-three strains of *A. baumannii* (42%) were multi-drug resistant. This means that these 53 strains were resistant to more than three classes of antimicrobial agents and caused

Table 1 - Percentage of antibiotic resistance.

| Antibiotic | Percentage of resistance |
|-----------------------------|--------------------------|
| Ceftazidime | 98% |
| Aztreonam | 98% |
| Trimethoprim | 98% |
| Piperacillin | 97% |
| Piperacillin-tazobactam | 96% |
| Cefotaxime | 95% |
| Ciprofloxacin | 95% |
| Ticarcillin | 94% |
| Ticarcillin-clavulanic acid | 94% |
| Amikacin | 93% |
| Kanamycin | 92% |
| Imipenem | 85% |
| Gentamicin | 62% |
| Tobramycin | 58% |
| Colistin | 0% |

the greatest problem in the treatment of burn patients. The overall prevalence of ESBL-producing strains was 21% (27 of 126) in ceftazidime-resistant strains. All ESBL-positive isolates were resistant to all β -lactam drugs except one isolate that was susceptible to carbapenem and monobactam.

Among imipenem-resistant isolates 39% (42 out of 108) isolates were also MBL producers. In our research, these isolates caused the majority of mortality in the population studied. The rate of mortality in our study was 13%.

Table 2 - Antibiotype of isolated Acinetobacter.

| Pattern | Number of isolates antibiotics | Antibiotyping | | | | | | | | | | | | | ESBL | MBL | |
|---------|--------------------------------|--|-----|----|-----|-----|-----|----|------|----|----|----|---|----|------|------------------|------------------|
| | | CTX | CAZ | AT | IMI | PTZ | PRL | TC | TC-C | GM | AK | TO | K | TM | CI | pos. (total: 27) | pos. (total: 42) |
| 1 | 46 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 8 | 12 |
| 2 | 46 | R | R | R | R | R | R | R | R | S | R | S | R | R | R | 4 | 30 |
| 3 | 11 | R | R | R | S | R | R | R | R | R | R | R | R | R | R | - | - |
| 4 | 23 | several different antibiotic susceptibility patterns, data not shown | | | | | | | | | | | | | 15 | - | |

CTX: cefotaxime, CAZ: ceftazidime, AT: Aztreonam, IMP: imipenem, PTZ: piperacillin-tazobactam, PRL: piperacillin, TC: ticarcillin, TC-C: ticarcillin-clavulanic acid, GM: gentamicin, AK: amikacin, TO: tobramycin, K: kanamycin, TM: trimethoprim, CI: ciprofloxacin.

DISCUSSION

Prolonged duration of patients staying in burn wards and the serious threat of the spread of multidrug-resistant bacterial pathogens, which cause nosocomial infection, are the prime concerns of physicians [7, 13, 14]. All the members of Enterobacteriaceae initially produced MBL and ESBL enzymes, but currently few other gram-negative bacilli such as *Acinetobacter baumannii* produce these enzymes [15, 16]. ESBL and MBL enzymes are capable of hydrolyzing extended spectrum cephalosporins and monobactams, but cannot hydrolyze cephamycins and imipenem (11, 17, 18). In Iran, due to overconsumption of new broad-spectrum β -lactams and appropriate therapeutic choice for treatment of nosocomial infections in a burn unit, extensive resistance of microorganisms to these antibiotics has developed [17]. In our study, 27 isolates (21%) of ceftazidime-resistant strains were identified as ESBL producers based on the results of phenotypic tests that compared the study of patients in a burn ward. One study on *Acinetobacter baumannii* in Iran showed 2% ESBL-producing isolates. An increased frequency of ESBL-producing *Acinetobacter baumannii* strains gives cause for great concern regarding treatment among burn patients [19]. In another Iranian study on *Escherichia coli* from various clinical specimens (blood, stool, urine, wound swabs, etc.) ESBL production was detected in 43.6% and 64% of the isolates. The differences in results may be related to different used methods, samples and variations of bacterial species [11, 17]. Two different studies in Turkey and Korea showed an incidence of 46% and 54.6% ESBL producers, respectively [15, 22].

By contrast, in a study performed in France, Pino et al. showed 10% MBL production in *A. baumannii* and in another study performed in Saudi Arabia, they reported that 8.1% of *A. baumannii* strains isolated from burn patients were ESBL producers [23, 24]. The variations are related to utilization of different protocols for treatment of the patients and different sites of the specimens used. *Pseudomonas aeruginosa* was the most frequent ESBL producer followed by other gram-negative bacilli such as *A. baumannii* [12, 13, 25].

In addition to the intrinsic resistance to cephalosporins and aztreonam, ESBL-producing organisms have shown that co-resistance to many other classes of antibiotics like quinolones and aminoglycosides was largely due to a limitation of therapeutic options.

The most significant risk factors for colonization or infection with ESBL-producing organisms in burn patients include long-term antibiotic exposure, longer hospitalization, increasing rates of third-generation cephalosporin use and invasive procedures [19, 26].

Treatment of ESBL-producing strains of *A. baumannii* in burn patients has emerged as an important challenge. Many factors contribute to determine the choice of antibiotics and management of burn infections. Even though β -lactamase inhibitors have important activity against ESBL *in vitro*, their clinical effectiveness against serious infections due to ESBL-producing organisms is controversial. The antibiotics for the treatment include carbapenems, aminoglycoside and β -lactamase inhibitor combinations [27-29].

MBL, an important enzyme, mediates resistance to β -lactam antibiotics, including carbapenems. MBL has been reported from several countries specifically in multidrug resistance (MDR) pathogens like *Acinetobacter* species and *P. aeruginosa* [11, 12, 30].

Aztreonam or potentially toxic polymyxin B and colistin considered as a viable therapeutic option in the treatment of MBL production in *A. baumannii* from burning infection. Thirty-nine percent of IPM-resistant *Acinetobacter* isolates were MBL producer.

The MBL producer rates from this study were similar to the results of Kaleem et al. and Anuradha et al. showed 37% and 36%, respectively (26, 31). The rate of MBL producers in our study is lower than the result reported by Mohamed et al. and Yong et al. [32, 33]. This difference in the prevalence of MBL-producing *A. baumannii*

strains seems to be the result of the variation among the different patients studied and the different rates of antibiotic uses in different hospitals. For instance, 68% of patients infected with MBL producer *A. baumannii* received at least one of the members of the carbapenem class of antibiotics (Imipenem or Meropenem). Emergence of resistant *A. baumannii* and other bacteria such as KPC-producing *Klebsiella* resulted in considerable overuse of broad-spectrum β -lactams [29, 34, 35].

Although some studies showed that MBL-producing strains could be susceptible to monobactam (aztreonam), in this study we indicated that all MBL-producing strains were resistant to aztreonam [12].

Therefore, genetic research will be needed for the detection of genes that are associated with the resistance enzymes. The global spread of MBL- and ESBL-mediated resistance in *A. baumannii* is one of the emerging problems among burn patients that we are currently facing. Thus, studies determining these enzymes would appear crucial [36, 37].

In conclusion, the current study highlights the high prevalence of MBL and ESBL enzymes among *A. baumannii* isolates in burn infections [36]. It also reflects the strained future of the therapeutic options available for burn patients, especially for infections caused by *A. baumannii*. The high incidence of MBL and ESBL production due to multiple mechanisms of burn infection is alarming, and critical action needs to be taken from the standpoint of both therapy and infection control.

Clinical microbiology laboratories should perform the monitoring methods to detect MBL and ESBL enzymes routinely, so that the proper antimicrobial therapy can be instituted and the dissemination of these isolates may be prevented by employing suitable control measures [9, 10, 26, 38]. These data suggest the utility of an accurate surveillance for ESBL and MBL in *A. baumannii* isolated from burn patients as an important step for successful antimicrobial treatment in the future [39-44].

Keywords: *Acinetobacter baumannii*, extended-spectrum β -lactamase (ESBL), metallo- β -lactamase, burn patients.

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SUMMARY

Acinetobacter baumannii is an important pathogen causing infections, especially in burn patients. The aim of this study was to determine prevalence of Extended Spectrum Beta-Lactamase (ESBL) and Metallo-Beta-Lactamase (MBL) in isolated strains of *A. baumannii* from burn patients. In all, 126 *A. baumannii* strains were isolated from both male and female burn patients admitted to the burn unit in Motahari hospital, Tehran. The susceptibility test was done by disk combination, and the disc test and disc diffusion method were performed to con-

firm the production of ESBL and MBL isolates in accordance with CLSI standard guidelines. While 21% of ceftazidime-resistance *A. baumannii* isolates were found to be ESBL producers, 39% of imipenem-resistant isolates produced MBL. Prolonged duration of hospitalization of burned patients made an important contribution to the incidence of resistant bacteria. The utility of an accurate surveillance for ESBL and MBL in *A. baumannii* isolated from burned patients as an important step for successful antimicrobial treatment in the future.

RIASSUNTO

Acinetobacter baumannii è un microrganismo patogeno responsabile di gravi infezioni, soprattutto nei pazienti ustionati. Questo studio è stato condotto al fine di determinare la prevalenza della produzione di beta-lattamasi a spettro esteso (ESBL) e metallo-beta-lattamasi (MBL) in ceppi di *A. baumannii* isolati da pazienti ustionati. Complessivamente, sono stati analizzati 126 isolati provenienti da pazienti di ambo i sessi ricoverati presso l'Unità per ustionati del Motahari Hospital di Tehran. I test di sensibilità e la produzione di ESBL e MBL sono stati con-

dotti in accordo alle linee guida del CLSI. La produzione di ESBL è stata rilevata nel 21% degli isolati resistenti a ceftazidime; la produzione di MBL è stata rilevata nel 39% degli isolati resistenti a imipenem. La degenza prolungata cui sono sottoposti i pazienti ustionati ha costituito un fattore che ha contribuito notevolmente all'elevata incidenza di isolamento di batteri resistenti. Si conferma la necessità di una attenta sorveglianza degli isolati di *A. baumannii* produttori di ESBL e MBL nei pazienti ustionati al fine di istituire la migliore terapia antibiotica.

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