The Prevalence Of Extended Spectrum Beta-lactamases In Acinetobacter baumannii Isolates From Burn Wounds In Iran

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Citation

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Abstract

Acinetobacter baumannii is a Gram-negative bacillus found in many hospital environments and its very high resistance to antimicrobial renders it as a successful nosocomial pathogen. There are many reports of Multi Drug Resistant A. baumannii from hospitals all over the world. ESBLs are beta-lactamases that hydrolyze extended-spectrum cephalosporins with an oxyimino side chain; These cephalosporins include cefotaxime, ceftriaxone, and ceftazidime. The ESBLs are frequently plasmid encoded. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (for example, aminoglycosides). Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited. This study aimed to assess the incidence of ESBLs in 48 burn isolates of A. baumannii. The susceptibilities of isolates to different antibiotics were tested by the Kirby-Bauer method. The Minimum inhibitory concentration of cefotaxime for each isolate was determined by Hicomb strips in the range of 0.001-240 µg of antibiotic. For phenotypic detection of the ESBLs double disc diffusion method. Cefazolin (100%), ciprofloxacin (100%) ofloxacin (95.8%) and kanamycin (95.8%) showed the highest rate of resistance and amikacin (52%) and imipenem (14.6%) demonstrated the lowest. 45.8% of isolates showed resistance to the 11 tested antimicrobials. 46 isolates (95.8%) were resistant to all tested concentrations of Cefotaxime (The Minimum inhibitory concentrations were above 240 µg). only one isolate (2%) has been considered as ESBL producing isolate. The high resistance of isolates to cefotaxime, ceftazidime and cefepime in companion with the low number of ESBL producing isolates, proposed another resistance mechanisms for these isolates to extended-spectrum cephalosporins.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative, nonmotile, nonfermentative bacillus, It is found in many hospital environments and can be colonize in human body in the hospital environments. The combination of its environmental colonization and its very high resistance to antimicrobial renders it as a successful nosocomial pathogen (Nordmann, 2004). There are many reports of Multi Drug Resistant (MDR) A. baumannii from hospitals in Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan and Korea, Iran, Turkey and many other areas (Barbolla et al., 2003; Houang et al., 2001; Lee et al., 2004; Liu et al., 2006; Naas et al., 2006; Nishio et al., 2004; Quale et al., 2003; Van Looveren and Goossens, 2004; Yu et al., 2004, Taherikalani et al, 2008, Metan et al, 2009). These Resistant isolates often spread to cause outbreaks throughout hospital wards. Acinetobacter is usually considered to be an opportunistic pathogen and this bacterium cause a wide range of clinical complications, such as burn wound infections, septicemia, pneumonia, urinary tract infection and meningitis, especially in immunocompromised patients. A. baumannii has emerged as a major cause of nosocomial infections. It commonly presents resistance to multiple antimicrobial agents. These strains are now ussually resistant to the rest of antipseudomonal beta-lactams and sulbactam, a beta-lactamase inhibitor with bactericide activity against A. baumannii(Garnacho-Montero and Amaya-Villar, 2010). Multi resistant A. baumannii infections tend to occur in immunosuppressed patients, in patients admitted in intensive care and burn units and in those subjected to invasive procedures and treated with antibiotics. A. baumannii frequently exhibit resistance to beta-lactam antibiotics. One of the most common causes of this resistance is the presence of extended spectrum beta-lactamases (ESBL)(Zarakolu, 2005).

Emergence of resistance to beta-lactam antibiotics began even before the first beta-lactam, penicillin, was developed. Over the last 20 years, many new beta-lactam antibiotics have been developed that were specifically designed to be resistant to the hydrolytic action of beta-lactamases. However, with each new class that has been used to treat patients, new beta-lactamases emerged that caused resistance to that class of drug. One of these new classes was the oxyimino-cephalosporins, which became widely used for the treatment of serious infections due to gram-negative bacteria in the 1980s. Resistance to these expanded-spectrum beta-lactam antibiotics due to beta-lactamases emerged quickly. The first of these enzymes capable of hydrolyzing the newer beta-lactams, SHV-2, was found in a single strain of Klebsiella ozaenae. Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended-spectrum beta-lactamases (ESBLs). Today, over 150 different ESBLs have been described. These beta-lactamases have been found worldwide in many different genera of Enterobacteriaceae, P. aeruginosa, and A. baumannii (Bradford, 2001).

Among A. baumannii strains, resistance to beta-lactams is often mediated by a AmpC-type cephalosporinase, which may confer a noticeable resistance when overexpressed. In addition, TEM-1, TEM-2, CARB-5, PER-type, CTX-M-type, VEB-1, OXA derivatives and several metalloenzymes have been found in A. baumannii. Also, the poor outer membrane permeability of A. baumannii and Penicillin Binding Protein alterations are implicated in beta-lactam resistance in this bacterium (Beceiro et al, 2008). Several phenotypic detection tests, based on the synergy between a third-generation cephalosporin and clavulanate, have been designed: the double-disk synergy test (DDST), ESBL Etests, and the combination disk method. These tests often need to be refined in order for them to detect an ESBL in some bacterial strains, such as those that also overproduce a cephalosporinase. These tests should accurately discriminate between bacteria producing these enzymes and those with other mechanisms of resistance to beta-lactams(Drieux, 2008). However it has been shown that the best phenotypic method for detection of ESBLs in Acinetobacter Sp. Is a double disk diffusion method with 30µg of cefotaxime discs at three different distances (1, 2 and 3 cm) from a disc of amoxicillin/clavulanic acid (20:10 µg) in the centre of the plate(Beceiro et al, 2008)

This study aimed to assess the incidence of extended spectrum beta-lactamases in 48 burn isolates of Multi drug Resistant A. baumannii.

MATERIALS AND METHODS

Bacterial strains and culture media:A total of 48 isolates were collected from clinical specimens from burn wards of hospitals in Tehran, Iran during a 6 months period between April and September, 2006. The isolates were further processed by the standard methods to identify as the A. baumannii (Baron and Finegold, 1990). Isolated bacteria were maintained for long storage on skimmed milk medium (BBL) by adding 10% glycerol in -60°C, cultures were maintained for daily use on nutrient agar (BBL) slants at 4°C. The Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) medium (Pronadisa) were used for detection of antibiotic resistance of strains. Acinetobacter calcuaceticus PTCC 1318 has been used as reference strain.

Determination of the strains sensitivity to antibiotics:The susceptibilities of isolates to different antibiotics were tested by the Kirby-Bauer method with NCCLS break points. To represents the different classes of antimicrobial agents commonly used for the treatment of Acinetobacter sp. infections, we used piperacillin, ciprofloxacin, gentamicin, ofloxacin, cephalotin, ticarcillin, kanamycin, imipenem, amikacin, co-trimoxazole, ceftizoxime, cefazolin and carbenicillin (Hi-media, Mumbai, India). Acinetobacter calcuaceticus PTCC 1318 has been used as reference strain.

D etermination of the isolates sensitivity to cefotaxime: Minimum inhibitory concentration (MIC) was determined by Hicomb strips (Hi-media) in the range of 0.001-240 µg of antibiotic. Muller Hinton Agar plates was prepared and inoculated with pure inoculate of each isolate with a non-toxic cotton swab, after streaking the entire agar surface of the plate with the swab, the Hi-comb strip applied to the agar surface with the MIC scale facing upwards. Plates were incubated in 37°C for 18-24h. MIC values determined as the value at which the zone of inhibition around the strip convenes the comb like projections of the strips and not at the handle. The MIC values were measured in the range of 240-0.001µg of antibiotic. Isolates were divided in three groups as Resistant (MIC≥64µg), Sensitive (MIC≤8 µg) or intermediate resistant (16 µg <MIC <32 µg).

Screening of the extended spectrum beta-lactamases: For detection of the extended spectrum beta-lactamases double disc diffusion method has been used. Double disc diffusion method with cefotaxime, ceftazidime and cefepime discs (30

mg) (Hi-media, Momby) at three different distances (1, 2 and 3 cm centre to centre) from an amoxicillin/clavulanic acid disc (20:10 mg) in the centre of the plate as previously described. Plates were incubated in 37°C for 18-24h. ESBL production is inferred when the cephalosporin zone is expanded by the clavulanate (Livermore and Brown, 2001).

RESULTS

The rates of resistance to different antibiotics for 48 isolates of Acinetobacter baumannii have been shown in Figure 1. Cefazolin (100%), ciprofloxacin (100%) ofloxacin (95.8%) and kanamycin (95.8%) showed the highest rate of resistance and amikacin (52%) and imipenem (14.6%) demonstrated the lowest (Fig 1). 45.8% of isolates showed resistance to the 11 tested antimicrobials.

Figure 1

Figure 1: The rates of resistance to different antibiotics for 48 burn wound isolates of

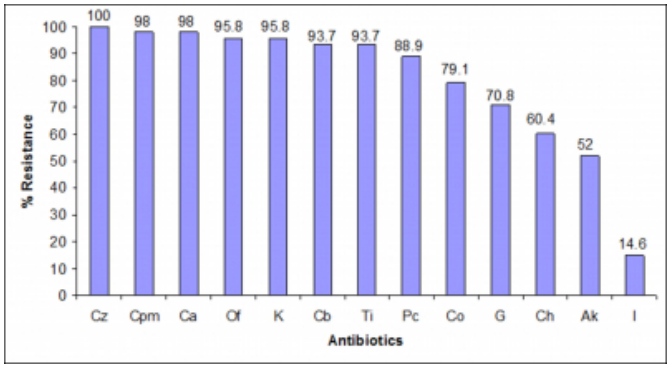

Fig 1: The rates of resistance in 48 isolates of Acinetobacter to different antibiotics (Ti: Ticarcillin, Ch: Cephalotin, Co:co-trimoxazole, K: Kanamycin, Of: Ofloxacin , Ce: Ceftizoxime, Cb: Carbenicillin, Pc: Piperacillin, G: Gentamicin, Cz: Cefazolin, I: Imipenem, Ak: Amikacin, Cpm: Cefepime, Ca: Ceftazidime).

Determination of the strains sensitivity to cefotaxime:46 isolates (95.8%) were resistant to all tested concentrations of Cefotaxime, the MIC Not be determined, because these isolates were resistant to all tested concentrations (MICs were above 240 µg). The Minimum inhibitory concentration of Cefotaxime for two remained isolates were 30 and 0.1 microgram, so According to the test criteria they were considered as intermediate resistant and sensitive respectively(Table 1). However Acinetobacter calcuaceticus ATCC 23055 was sensitive to cefotaxime (MIC=1 µg).

Figure 2

Table 1: The percentage of Sensitive, intermediate resistant and resistant isolates of to cefotaxime.

No. of *Acinetobacter* isolates	Sensitive(%)	intermediate resistant(%)	Resistant(%)
48	(%2) 1	(%2) 1	(%95.8) 46

Screening of the extended spectrum beta-lactamases: For detection of the extended spectrum beta-lactamases double disc diffusion method has been used. From 48 Acinetobacter isolates only in one isolate (2%) synergy has been seen between clavulanic acid and cefotaxime, so it has been considered as ESBL producing isolate. Acinetobacter calcuaceticus ATCC 23055 determined as ESBL negative strain.

DISCUSSION

Nosocomial outbreaks of infection by A. baumannii have been reported worldwide and most isolates of this bacterium are resistant to many different classes of antibiotics usually used in patient treatment. Sulbactam and polymyxin B are sometimes the only usable therapeutic agents for these kinds of infections(Beceiro, 2008). -Lactamases continue to be the main cause of resistance to -lactam antibiotics among many kinds of bacteria, especially gram negative bacteria. There has been an increased incidence of ESBLs wordwide in the recent years(Bradford, 2001). Several different methods for the detection of ESBLs in clinical isolates have been suggested, however because of the sensitivity of some Acinetobacter isolates to clavulanic acid the best recommended phenotypic method for detection of ESBL producing Acinetobacter is double disc diffusion method (Beceiro, 2008). Present study showed that the most useful antibiotics for infections caused by A. baumannii were imipenem, amikacin and cephalotin. Resistance to some antibiotics such as gentamicin, ciprofloxacin and co-trimoxazole showed high increases in comparison with the previous studies by Guardabassi et al. (1998). Also, in present study burn wound isolates of Acinetobacter baumannii showed high resistance to other tested antibiotics (Fig 1).

Shakil and Khan (2010) during a one-year study isolated 6 carbapenem-resistant Acinetobacter baumannii isolates from 920 urine cultures of patients attending an Indian hospital. Four from six(66.6%) isolates were identified as ESBL producers, so they suggests that in regions with known prevalence of bacteria simultaneously resistant to carbapenems and advanced generation cephalosporins, the drugs of choice for empiric treatment could be amikacin and

ampicillin/sulbactam, however in our study in compare with the Shakil and Khan(2010) the prevalence of ESBL producing isolates was low(2%) in spite of very high resistance of isolates to cefotaxime(95.8%)(Fig 1).

Mohammadtaheri et al 2010 conducted a study to determine the antimicrobial susceptibility patterns among common pathogens in the intensive care unit (ICU) of a university hospital in Iran between 2006 and 2009. They reported that the most clinical isolates of bacteria were A. baumannii (22.4%); they reported that only 97.6% of Acinetobacter isolates were sensitive to cefotaxime, these finding is in accordance with our data about the prevalance of resistant isolates (98%). However the rate of resistance to Imipenem reported by Mohammadtaheri et al was 1.2% while in our study its relatively higher (14.6%). We found that 45.8% of isolates were resistant to the 11 tested antimicrobials and only were sensitive to Imipenem, also all the isolates were multi-drug resistant (MDR), in the other studies MDR phenotypes were also commonly reported(Mohammadtaheri et al 2010, Shete et al, 2010).

In a retrospective study was carried out of gram-negative isolates from the adult ICU of King Fahad National Guard Hospital (KFNGH) between 2004 and 2009, the most frequently isolates were Acinetobacter baumannii, clinical isolates of Acinetobacter are frequently resistant to most antimicrobial agents, and evidence of pan-drug resistance among isolates has been observed, but they did not reported any ESBL producing isolates belong to Acinetobacter Spp(Al Johani et al, 2010).

In conclusion the high rate of antibiotic resistance in our burn isolates re emphasizes the essential need for the applying of the new strategies for the prevention and control of Acinetobacter caused MDR infections. Also epidemiological informations helps to design better programms for infection control in different hospital wards, especially burn wards. Therefore, developing nationaland regional antibiotic policy and is essential due to increasing resistance patterns in Acinetobacter isolates. Also, developing a local antibiogram database will improve the information of antimicrobial resistance patterns in Iran and will also help to improve empiric treatment strategies based on ward-specific data.

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