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Evaluation of molecular epidemiology and antibiotic susceptibility of *Acinetobacter baumanii*, in burned patients of Motahari hospital

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Abstract

Background: Acinetobacter baumannii, as a nosocomial opportunistic pathogen, is resistant to a wide range of antibiotics and is responsible for numerous infections including bacteria, pneumonia, meningitis, urinary tract infections and wounds. The aim of this study was to determine frequency of molecular epidemiology and antibiotic susceptibility of *Acinetobacter baumanii*, in burned patients

Materials & methods:A total of 108 A. baumannii strains were collected from burn patients admitted to Motahari hospital, Iran. Antimicrobial susceptibility testing was performed according to the Kirby Bauer disk diffusion method. DNA extraction and Typing of strains was performed by PCR amplification of repetitive extragenic palindromic elements (REP-PCR).

Results: Our results showed that 98 strains were identified as A. baumannii by phenotypic tests. Also, 96 of 98 strains were PCR positive for blaOXA-51-like genes. Based on Antimicrobial susceptibility test 84 isolates (87.5%) were colistin sensitive, and 12 strains (12.5%) were resistant to colistin.

Conclusion: Our results showed that the incidence of A. baumannii strains is high in patients and blaOXA-51-like was the most prevalent gene among the burn clinical isolates of A. baumannii. Also, more than 85% of the isolates were resistant to most antibiotics.

Keywords: Acinetobacter baumannii, blaOXA-51, MDR, infection, nosocomial.

Introduction

Acinetobacter baumannii is a common Gram-negative bacillus commonly associated with aquatic environments(1). This type of bacteria , as an opportunistic nosocomial pathogen, is an increasing problem worldwide , responsible for a range of hospital-acquired infections, particularly in patients with weakened host defenses(2). This pathogen is frequently related to nosocomial infections, included pneumonia, bacteremia, meningitis and urinary tract infections(3).

considering to the prompt expansion of resistance against various antimicrobial factors due to the high capability of natural genetic alteration and the possible for extensive release because of the ability to survive on environmental surfaces, A. baumannii has currently surpassed other bacteria as the second most commonly isolated glucose non-fermenter in clinical laboratories after Pseudomonas aeruginosa with high mortality rates of 41% (4). Also due to their survival in hospital environments, it has increased the appearance of bacteria in hospital environments and increased its infection, which has resulted in mortality in patients with A. baumannii infection by approximately 75%(5, 6).

A. baumannii accounts for one of the six top-priority dangerous pathogens by the Infectious Diseases Society of America (IDSA)(7, 8). Outbreaks owing to such strains spread rapidly in any hospital through contaminated hands, contaminated fluids etc.(9). The main problem in the treatment of infections caused by A. baumannii is the ability of these bacteria to achieve antibiotic resistance to a large group of antibiotics. The emergence and spread of drug resistance A. Baumannii able to transfer genetic elements of resistance to various antibiotics has posed a major threat to hospitals(10, 11).

The extensive prescription of broad-spectrum antibiotics in hospitals has led to the rapid development of multidrug-resistant (MDR) *A. baumannii* strains(12).Currently, colistin and tigecycline are still efficient antibiotics and have become the last therapeutic options for MDR *A. baumannii* (13).

A. baumannii is hardly isolated from the natural environment(14). For the purpose of recognizing the resources of infection as rapidly as possible, a proper typing method that can differentiate epidemic strains from environmental and non-epidemic strains is needed(15).A PCR-based fingerprinting system that uses consensus primers for the repetitive extragenic palindromic (REP) sequences found in many bacterial chromosomes has been shown to be appropriate to various species and is known as repetitive extragenic palindromic polymerase chain reaction (REP-PCR)(16).

According to the above,the aim of the present study were to assess the incidence of *A. baumannii* infection over a period of 3 months, at Motahari hospital in Tehran , which is specific for burned patients, analyze their resistance pattern, and determine the molecular typing of strains by REP-PCR.

Materials and methods

Bacterial strains

A total of 108 strains were sent to laboratory of Antimicrobial Resistance Research center as identified *A. baumannii*. The clinical samples were collected from burned patients admitted to Motahari hospital,Iran and also has been registered with code 930211325242. Identifications of A. baumannii were performed using standard laboratory tests. The confirmation of strains was done by existence of blaOXA-51-like gene.

Susceptibility test

Antimicrobial agents which were used include piperacillin-tazobactam, ceftazidime, amikacin, tobramycin, imipenem, tigecycline, cefepime, ciprofloxacin and colistin. Antimicrobial susceptibility test was performed according to the Kirby Bauer's disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines(17) using the Muller Hinton agar (Merck) and antimicrobial discs (Rosco) .

Tigecycline disc diffusion criteria are not yet established by CLSI or EUCAST, therefore the FDA criteria were applied(18). MIC of colistin (Mast) was determined according to standard methods using broth microdilution test (19) using colistin sulfate powder (Sigma-Aldrich).

DNA extraction

In order to obtain extract DNA, the strains of *A. baumannii* strains were grown overnight at 37 ºC on Mueller–Hinton agar plates. A colony of each pure isolates was then suspended in 300 ml sterile distilled water and boiled for 10-15 min. After 5 min of centrifugation (10000 rpm), the supernatant was used as the DNA template for PCR amplifications.

Molecular typing

Typing of strains was performed by PCR amplification of repetitive extragenic palindromic elements (REP-PCR). The primers used for REP-typing include REP F: (5'-ICGIC1TlATCIGGCCTAC-3'), and REP R: (5'-IIIICGICGICATCIGGC-3'). PCR amplification was performed in a reaction mix containing REP1 and REP2 primers (50 pmol), dNTPs mixture (0.2 mM), MgCl2 (3 mM), 2 unit Taq DNA polymerase, 2.5 µl of 10X PCR buffer and 7 µl of bacterial DNA template in a final volume of 25 ml. The following temperature profile was used: initial denaturation at 95 ºC for 5 min, 30 cycles of denaturation at 90 ºC for 30 sec, annealing at 45 ºC for 1 min, extension at 72 ºC for 1 min and a final extension at 72 ºC for 16 min. PCR products were subjected to electrophoresis in 1.5% agarose gels. The 100-bp DNA ladder was applied to assess PCR product size.

Results

Phenotypic tests

Our results showed that 98 strains were identified as *A. baumannii* by Gram staining, cell and colony morphology, positive catalase test, negative oxidase test and absence of motility, glucose oxidation, gelatin liquefaction, beta hemolysis, growth at 37°C and 42°C, arginine hydrolysis and susceptibility to chloramphenicol(20).

Identification of blaOXA-51-like

All isolates were subjected to PCR to detect blaOXA-51-like genes. 96 of 98 strains were PCR positive for blaOXA-51-like genes. Two strains that were PCR negative for blaOXA-51-like, identified as other *Acinetobacter* species. (Figure 1.)

Antimicrobial susceptibility test

The percentage of antibiotic susceptibility is presented in figure 2. Colistin MIC was determined by broth microdilution (BMD). 84 isolates (87.5%) were colistin sensitive, and 12 strains (12.5%) were resistant to colistin.

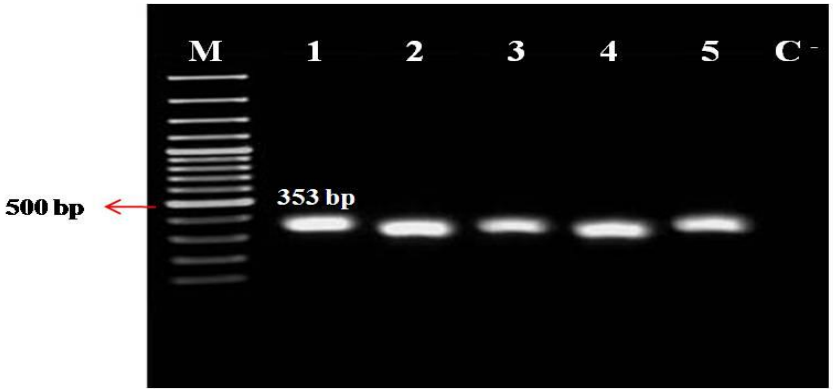

Figure1. M: DNA size marker, 1-5: selected strains of *Acinetobacter Baumanii*, C: *Acinetobacter lowfii* as control negative

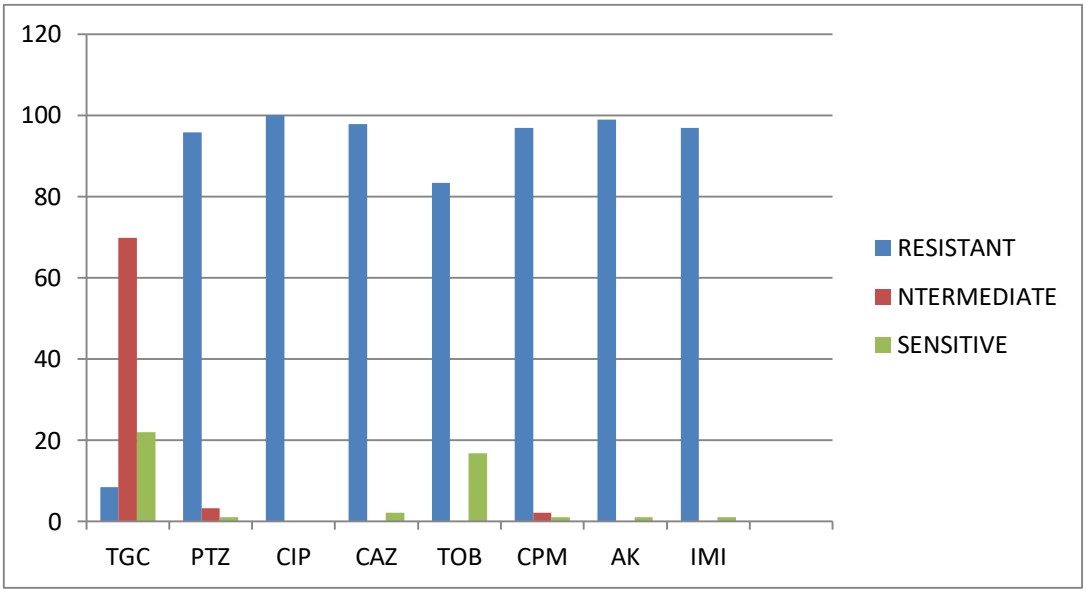

Figure 2. Antibiotic susceptibility of Acinetobacter baumanii

TGC: tigecycline, PTZ: piperacillin-tazobactam, CIP: ciprofloxacin, CAZ: ceftazidime, TOB: tobramycin, CPM: cefepime, AK: amikacin, IMI: imipenem.

Genotyping

A total of 98 strains were submitted for molecular typing by REP-PCR. According to the observations from the electrophoresis of REP-PCR products on 1% gel agarose. The analysis results classified the isolates into 7 main patterns from group A to G. Group A, with 37% of isolates was the predominant group, other groups: 5.3% to group B, 18.4% to group C, 18.5% to group D, 13% to group E, 5.2% to group F, and 2.6% to group G.

Discussion

Acinetobacter baumannii, as a significant pathogen involved in nosocomial infections, particularly in immunocompromised patients such as the group with burned injuries(21). In recent years, nosocomial infections of A. baumannii, as an opportunistic pathogen, have been on the rise. Acinetobacter species are difficult to identify due to the current classification and use of commercial identification systems in clinical microbiology laboratories. Meanwhile, A. baumannii has emerged as a MDR microorganism in hospitals around the world. The treatment of these bacteria, especially MDR and beta-lactamase strains with a wide range, is of major concern(22). Furthermore, their capacity for long-term survival in the hospital with antibiotic resistance and the potential for dynamic genomic regeneration under selective pressure make A. baumannii one of the important hospital pathogens(23).

In our study more than 85% of isolated strains were resistant to most of antibiotics. Although surprisingly, regarding to tigecycline, 65% of strains showed intermediate resistance against this novel antibiotic. Apart from colistin which is still effective against *A.baumannii*, tigecycline could be considered as another option of antibiotic therapy for treatment of multi resistant *A.baumannii.*

In a study by Farivar et al, of the 100 samples collected in the intensive care unit of Rasoul Akram Hospital, Iran 21 (21%) were A. Baumannii(24). In another study by Rit et al in 2012, among 4180 clinical isolates were identified as 0.74% A.baumannii and 25.98% other types of Acinetobacter(25). In addition, Sadeghifard et al in 2010 reported that all A. baumannii isolates were 100% resistant to azetronone, cefotaxime, ceftazidime, ceftriaxone, meropenem, and ticarsalin-clolanate(26). Another study showed that the isolated bacteria had 100% tetracycline resistance, 95.2% to gentamicin, amikacin and 90/5% to ceftazidime(24). Ayan et al reported that of 52 isolates, all strains were resistant to piperacillin, cefepime, cefotaxime, ceftazidime, cefriaxone, gentamicin and azetronam(27).

The difference in resistance pattern of A. baumannii may be due to variation in clinical specimens, time of study, and treatment modalities in each geographic region(28). According to researchs, resistance to broad-spectrum antibiotics can be due to carbapenemse-producing bacteria. On the other hand, the most common carbapenemase genes are contained in removable genetic elements and can be transmitted to other bacteria. Patients in the intensive care unit are at risk of infection by hospital agents (4,5) and may eventually lead to burn wound infection. Recent studies on carbapenem resistant due to the production of oxa have become increasingly popular(29-31).

In Iran, the frequency of isolation of MDR A. baumannii increased from 50% in 2001-2007 to 74% in 2010-2015, with an average prevalence of 71%. The possible causes of this 24% increase include the following: Cross-border exchanges between countries such as Iran, Iraq and Turkey that have the highest number of MDR cases, use of sensitive methods for MDR detection in diagnostic laboratories, increased number of patients with chronic immunosuppression with prolonged hospitalization and also widespread access to services such as bronchoscopy procedures, which may lead to increased rates of unintended complications associated with A. baumannii infections in clinics and hospitals(32).

In recent times, various molecular methods are widely used for epidemiological evaluations, defining the source of infection, and relatedness of different genotypes to different patterns of antibiotic resistance and virulence factors(16). The repetitive elements are available in the genome of various groups of bacteria, which are advantageous in genotyping techniques. Repetitive element sequence based –PCR (REP) is one of the most beneficial methods for determining genotype of isolates. This PCR based technique is more time-effective and low cost compared to other methods such as RFLP and PFGE methods. In our study, epidemiologic assessment of 98 strains of *A.baumannii,* based on their similarity pattern by REP-PCR, showed 7 clones among the species isolated from a large hospital in Tehran specific for burned patients. Most of the A. baumannii isolates (37%) belonged to genotype A. other studies in other countries such as Malaysia and China indicated similar results of REP patterns but not the same, as expected(33, 34).

Among the five main phylogenetic subgroups, the chromosomal-like blaOXA-51 gene used as a species-specific marker was used to identify A. baumannii. However, plasmid-mediated OXA-51 has also been identified in A. baumannii isolates. Generally, there are two types of OXAs within *Acinetobacter*: intrinsic (chromosomal) OXAs and acquired OXAs. The first intrinsic *A. baumannii* OXA carbapenemase gene was discovered in 2005 and was named blaOXA-51. Also, to date, three subgroups of acquired OXA have been characterized: OXA-23-like, OXA-24/40-like, and OXA-58-like(2). According to reviews, The prevalence of blaOXA-51 in A. baumannii clinical isolates was reported as 77.8% in Turkey and 85.3% in South Africa(35, 36). Iranian studies have used blaOXA-51 as a tool to identify A. baumannii and have shown 100% gene carrying(37).

Overall, although there is no evidence of hospital design and prevention of nosocomial infections, other factors, particularly inadequate health of medical staff, appear to be more effective.

Conclusion

According to the present study, the prevalence of A. baumannii species is high in patients and blaOXA-51 is the most common gene among A. baumannii clinical isolates. Also, more than 85% of the isolates were resistant to most antibiotics. Therefore, these results are crucial for the control of the Hospital Infectious Diseases Committee in health care systems.

Conflict of Interest

The author has no conflicts of interest to declare.

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