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ORIGINAL ARTICLE

Phenotypic and Genotypic Isolation and Identification of Acinetobacter baumannii from Clinical Samples at Tabriz Sina Hospital in 2011 Using Multiplex PCR Method

Mahdieh Sadeghifard[1], Hadi Alizadeh[2*], Mahtab Noorifard[3], Reza Khalili[4], Ebrahim Hazrati[5], Mohammadreza Izadpanah[6]

1. MSc. Of Micribiology, Infectious and Tropical Diseases Research Center, Tabriz University of Medical Science, Tabriz, Iran
2. Young Researchers and Elite Club, Ahar Branch, Islamic Azad University, Ahar, Iran
3. Assistant Professor, Infectious Diseases Research Center, Aja university of Medical Science
4. MSc., Department of Microbiology, Islamic Azad University, Ahar branch, Iran
5. Assistant Professor, Department of critical care and Fellowship of Critical care anesthesiology, Aja university of Medical Science
6. Phd. Student of Cell and Molecular Biology, Department of Biology, Islamic Azad University, Rasht branch, Iran
*Corresponding Author email: h-alizadeh@iau-ahar.ac.ir

ABSTRACT: Acinetobacter baumannii is one of the most important hospital opportunistic pathogen that causes wide infections in people with impotent immune system. This bacterium causes broad infections including bacteremia, sepsis, pneumonia, endocarditis, meningitis, skin and wound infections, harms to soft body tissues, and respiratory and urinary infections. Presently, unhappily, existence of strains resistant to several medications has provoked concerns in academic community of the world. Conducted in Tabriz Sina Hospital, this study intended to identify and isolate Acinetobacter Baumannii in order to prevent it from growing epidemic. Form among 1,120 samples of the patients hospitalized in different departments, a number of 95 Acinetobacter baumannii strains were identified using phenotypic and genotypic methods after biochemical tests were carried out using Multiplex PRC method.

Keywords: Acinetobacter baumannii; Pathogen Bacterium; Multiplex PRC; Epidemiology

Introduction

Acinetobacters are aerobic coccobacillus, positive catalase, negative oxidase, immobile, amorphous, and opportunistic bacteria that are unable to ferment sugars, rather use them through oxidization. In gram staining, these bacteria are observed as short, bloated, and gram-negative rods (peleg etal, 2008). Their morphological properties, however, are subject to change as they pass development phases. For example, they appear as bars in their development phase and as coccobacillus in their stationary phase. After 24 hours of incubation, acinetobacter species are observed as level colonies, usually white-gray mucoids in blood agar environment and colorless in Mac-conkey agar environment with a diameter of 1.5-3 millimeters. Acinetobacter species grow in temperatures between 20 to 37 ºC, with some of them growing at temperatures as high as 42 ºC. These organisms need a little food materials and are able to grow in different temperatures and at different PHs, unsuitable conditions, dried levels, and watery atmospheres. Usually, they grow on skin, mucous membranes, and soil (Montefour etal, 2008).

This bacterium causes broad infections including bacteremia, sepsis, pneumonia, endocarditis, meningitis, skin and wound infections, harms to soft body tissues, and respiratory and urinary infections. Outbreak of these infections is important in special care and burn units. Since it sometimes appears as strains resistant to several medications and endemic forms, Acinetobacter Baumannii is difficult to control (Sharie, 2004). From among the most important and most predisposing bacteremia factors as well as a key source of Acinetobacter Baumannii, one may refer to pneumonia, trauma, surgery operations, intravenous catheters, dialysis, long-term hospitalizations, and burns (Lai, 2006).

Molecular typing methods have essential uses for determining hospital prevalence, identification of pollution sources in staff and patients, disease transmission ways, isolation of special genotypes in conjugation with a special bacterium. Results of such experiments help eradicate Acinetobacter baumannii pathogens easily. These methods provide us with more awareness about epidemiological principles, development of many bacterial diseases, and their relation to genetics. Since Pasteur, epidemiological hypotheses in molecular typing and bacteria distinction were based on phonotypical and bio-typical methods like biotypes, serotypes, bacteriophages, and profiles sensitive to antibiotics. Currently, however, advances in molecular methods in two recent decades have been outstanding. Phonotypical hypotheses, even

though, still constitute a stronghold in microbiological laboratories. Therefore, gram staining is a unique way for identification and distinguishment of one bacterium from others. Molecular techniques, which are used for microbial typing, include PFGE, methods relying on enzyme cuttings, plasmid analyses, and PCR-based typing methods. Changes in molecular typing methods focusing on special genotypes among bacterial strains are a revolution in study of strains' diversity and monitoring infection prevalence (Higgins, 2010). Affirmation of relations among a pathogen's colons enables us to find the source of pollution (human or environment), to distinguish infectious strains from noninfectious ones, and finally to prevent from reinfections. Typing, in addition, enables us to judge an infection's source happening as a result of whether an illness' relapse or a strain except for the one which primarily caused the infection. I addition, when infection happens as a result of relapse, these methods confirm inefficiency of primary treatment regimes and rule for alternatives. Presently, molecular typing in hospitals prevent prevalence of many hospital infections, helping economy and health of different societies (Camp, 2010).

Three genes which are used in Acinetobacter baumannii molecular typing are as follows: ompA, csuE, and bla OXA-66-like. OmpA, an outer membrane A protein, is a porin that is coded by ompA gene, which induces apoptosis in epithelial cells. CsuE gene is a part of cell assembly system that plays a role in formation of biofilm. Bla OXA-66-like is an innate carbapenemase in Acinetobacter baumannii (Turton, 2006).

Materials and Methods

Phonotypical Isolation and Identification

1,120 clinical non-iterative, mostly subject-to-danger samples selected from Tabriz Sina Hospital were recorded within six months from July 2011 to Jan. 2012. These strains were identified from wound, urine, blood, and tracheal tube of individuals hospitalized in intensive care, burns, infectious, and internal isolation units. From these, a number of 145 Acintobacter strains were specified using phenotypic standard methods like gram staining, oxidase and catalase tests, and TSI agar. In order to isolate Acinetobacter baumannii from other species of Acinetobacter, O/F glucose test was applied, in which 95 Acinetobacter baumannii strains were identified and stored for long-term preservation in glycerol-included nutrient broth environment.

Genotypic Identification

To conduct molecular method, the first step is to extract genomic and plasmid DNA from Acinetobacter baumannii strains, the process which was performed based on the manufacturer's brochures using DNG TM-Plus commercial kits.

Study of blaoxa-51-like and blaoxa-23-like genes using Multiplex PCR to identify isolations in genotypic manner

Required primers were extracted and ordered to Sigma Co. after blast process was completed in NCBI site. When primers were received, primary preparations were conducted as per what was expressed hereinabove. Required primers are expressed in Table 1 to genotypically identify Acinetobacter Baumannii (Turton, 2007).

Table 1. Primers Required for Genotypic Identification of Acinetobacter baumannii

Primer's connection place	Size (bp)	Sequence	Primer
255-274	353	5'- TAA TGC TTT GAT CGG CCT TG -3'	OXA-51-likeF
588-607		5'- TGG ATT GCA CTT CAT CTT GG -3'	OXA-51- likeR
382-401	501	5'- GAT CGG ATT GGA GAA CCA GA -3'	OXA-23-likeF
863-882		5'- ATT TCT GAC CGC ATT TCC AT -3'	OXA-23-likeR

Composition used for reaction

Multiplex PCR was conducted in 25 microlters using the materials mentioned in the Table 2 with cited volumes.

Configuration of Thermal-Cycler System

After preparation of PCR reaction mixture, microtubes were subjected to thermal cycles in the following Thermal-Cycler System.

Results

Results of phenotypic identification

From among 1,120 samples collected from different departments of the Hospital, a number of 145 samples were isolated using differential methods. After O/F glucose test, a number of 95 Acinetobacter baumannii strains were identified. All obtained isolations were gram-negative coccobacillus, negative oxidase, positive catalase and citrate, immobile, showing alkaline reactions in TSI environment in both 37 and 42 ºC, and generator of acid from glucose in O/F environment.

Table 2. Composition used for study of blaoxa-51-like and blaoxa-23-like genes using Multiplex PCR

Density	Combinations
14.5 microliter	Deionized water
X1	PCR buffer
µM 200	dNTP mix
mM 1.88	Mgcl2
µM0.4	Primer OXA-51-likeF
µM0.4	Primer OXA-51-likeR
µM0.4	Primer OXA-23-likeF
µM0.4	Primer OXA-23-likeR
U2.5	Taq DNA polymerase
2 microliter	DNA pattern
25 microliter	Total

Table 3. Application of Thermal-Cycler System for study of blaoxa-51-like and blaoxa-23-like genes using Multiplex PCR

Stages		Temp.	Time
Primary denaturing		94 ºC	3 min.
	Denaturing	94 ºC	45 sec.
35 cycles	Connection	57 ºC	45 sec.
	Final proliferation	72 ºC	1 min.
Final proliferation		72 ºC	5 min.

Products underwent electrophoresis process by 1% agar gel including ethidium bromide in TBE buffer. Then, UVDoc System was used to take photograph of gel documentation in order to trace bands of gels (Sambrok and Russell, 2009).

Results of genotypic identification

To identify Acinetobacter baumannii strains in moleculat manners, Multiplex PCR method was conducted using materials cited hereinabove. All studied isolations expressed a product of 501 bp in size, showing presence of bla OXA 51 like gene. Around 81.1 percent of strains carried bla OXA 23 like gene of 353 bp in size. Presence of bla OXA 51 like gene is a proof of the fact that all stains studied herein are Acinetobacter baumannii.

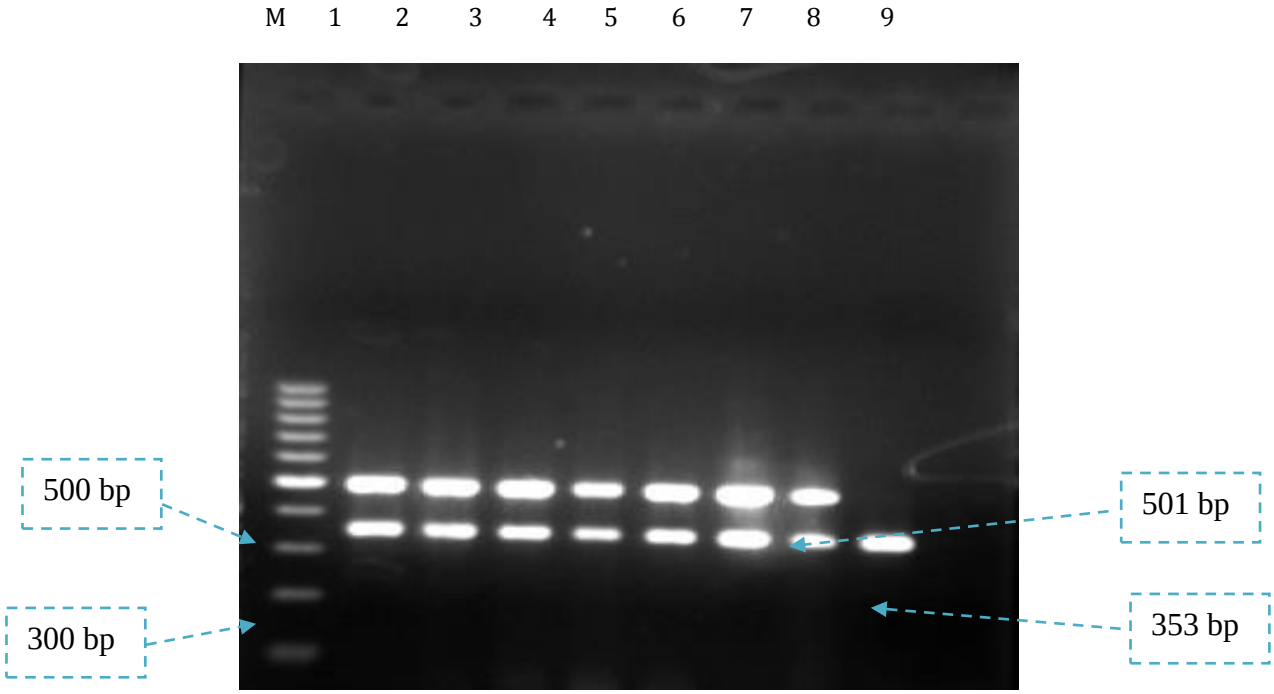

Figure 1. Identification of Acinetobacter baumannii strains using Multiplex PCR

Number M: 100 bp marker
Numbers 1-6: positive strains to bla OXA 23 like and bla OXA 51 like genes
Number 7: positive control for bla OXA 23 like and bla OXA 51 like genes (Acinetobacter baumannii ATCC 19606)
Number 8: positive strain for bla OXA 23 like gene
Number 9: negative control (devoid of DNA)

Discussion

In this study, 95 isolations from different hospital departments were collected and tested through various phenotypic and genotypic tests for affirmation of the issue whether or not samples are Acinetobacter baumannii. In biochemical tests, all strains showed such properties as gram-negative and aerobic coccobacillus features, immobility, ability to grow at 37 and 42 ºC degrees, negative oxidase, non-fermentation in TSI environment, and ability to use carbon as their energy source. In identification of genotypes, all isolations were positive to presence of bla OXA 51 like gene, which affirms that samples were Acinetobacter baumannii. These results are aligned with other studies indicating that bla OXA 51 like gene is Acinetobacter baumannii. Thus, investigation of presence of this gene among Acinetobacter baumannii isolations is applied as one of the simplest and safest ways of Acinetobacter baumannii identification. Among all isolations carrying bla OXA 51 like gene, 81.1% were positive to bla OXA 23 like gene, which shows presence of resistant Acinetobacter baumannii isolations due to oxacillin enzyme.

Conclusions

According to the results, around 34.7 and 30.5 percent of strains were respectively collected from ICU and burn unit, the finding which is confirmed by other results (Romanelli, 2009). ICU and burn unit can be regarded as high-risk locations because of severity of the context diseases, prolonged hospitalization, overuse of carbapenems or third-generation cephalosporins, and invasive procedures such as the use of urinary catheters (Shanthi and sekar, 2009).

In 2006, Jane F. Turton et al. collected 170 samples from central English laboratories within one year to study bla OXA 23 like and bla OXA 51 like genes using Multiplex PCR method that is an index confirming these samples are Acinetobacter baumannii. From among these, a number of 106 samples were positive to presence of bla OXA 51 like gene.

Suggestions

The highest prevalence rate of this bacterium is witnessed to be happening in ICU, the result which confirms importance of strict observation of hygienic principles in hospitals, particularly this unit.

Application of Multiplex PCR method offers a special advantage, since it is a quick and reliable technique which enables simultaneous identification of several resistant genes within less than 8 hours; and,

Given presence of OXA66 gene in all clinical Acinetobacter baumannii strains and inherency of this gene, this is suggested to investigate whether or not this gene can be regarded as a criterion for identification of this organism.

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