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Molecular technique for identification and characterization of *Acinetobacterbaumannii* *

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Abstract

The present study involved isolation and characterization of *Acinetobacter baumannii* from patients suffering from urinary tract infection (UTI), wound infection and vaginitis . A total of 200 clinical samples were collected from patients admitted to Al-Zahraa hospital, Al- Hakeem hospital and Al – Sadder teaching city from September 2011 to January 2012. Identification of isolates has been carried out depending on biochemical test and polymerase chain reaction technique (PCR) . Our study proved that PCR technique depending on *rpoB* gene was a specific method for identification of *A.baumannii* compared with *gyrB gene* in which 26 isolates have been identified which included 13 (36%) isolates from UTI, 7 (19%) isolates from wounds infection and 5 (13%) isolates from vaginitis.
Key word : (*Acinetobacter baumannii* , urinary tract infection , PCR , *gyrB gene* , wounds infection).

Introduction:

Acinetobacter is a gram negative coccobacilli that is strictly anaerobic, catalase positive and oxidase negative. It is widely distributed in nature, being found in soil and water. The taxonomy of *Acinetobacter* revealed at least 32 named and unnamed species that have been described [1], of those *A.baumannii* and closely related un named genomic species 3 and 13TU which were the most clinically relevant. These three species are commonly grouped together with the environment species *A.calcoaceticus* in the *A.calcuaceticus* – *A.baumannii* complex because of the difficulty in separating these species by using phenotypic method [9; 11].

Manual and semi – automated commercial identification system such as API 20NE, and VITEK2 don't differentiate among these species , so that gene sequences including *gyrB, recA* gene have been proposed to delineate species within the genus *Acinetobacter* [2; 3; 4].The use of protein-encoding genes as phylogenetic markers is now a common approach[4; 5; 6]. Many investigations have demonstrated that sequences from protein-encoding genes can accurately predict genome relatedness and may replace DNA–DNA hybridization for species identification and delineation in the future [7; 8].The use of *gyrB* as a means to identify *Acinetobacter* isolates to the species level is not new, it was demonstrated over a decade ago that nucleotide and amino-acid sequences can both be used for taxonomic purposes, and that they correlate with DNA–DNA hybridization[8].The rate of molecular evolution inferred from *gyrB* gene sequences is faster than that inferred from16S rRNA gene sequences [3]. Several molecular studies which depend on amplification of rDNA restriction

analysis is recognized as providing good discrimination, but can be time-consuming, while tRNA fingerprinting does not discriminate between *A. baumannii* and genomic sp. 13TU .

Sequencing of the *rpoB* gene and its flanking spacer regions, and of the 16S, 23S rRNA gene spacer region, have been proposed for identification of *Acinetobacter* isolates to the species level [11; 12]. Various protein-encoding genes such as *recA*, *groEL* , *rpoB, rpoD* and *gyrB* have been used for the classification of bacteria at the intrageneric level [2;4]. To focus the light on an important pathogenic bacteria this study was carried out. to explain a perfect method in identification and diagnosis of *A. baumannii*.

Materials and Methods:

* Collectionof samples:

A total of 200 samples were recorded from patients suffering from burn infection (50 sample), vaginal infection (50 sample) and urinary tract infection (100 sample) who admitted to AL-Zahar Teaching hospital , AL-Hakem hospital and AL-SaderTeaching city, during a period from September 2011 to January 2012.All samples were collected in a proper way to avoid any possible contamination .

* Laboratorydiagnosis:

Primary identification and characterization of *A.baumannii* from other isolates were performed according to depending on the morphological properties of colonies growing on blood agar base after incubation at 44^{O}C for 24 hr. These properties involved: colony shape, size, color, translunacy, by biochemical test which include: oxidase , catalase , Indole MR-VP, simmon citrate, KIA and hemolysin tests.*Molecular identification by PCR.

DNA extracted from bacterial cell by alkaline lysis method described by Sambrook and Russell (2001) was used as a template in a specific PCR for diagnosis of *A.baumannii* by *gyrB* and *rpoB* gene. Aset of primer (table1) was used for amplification of *gyrB* and*rpoB* gene using reactionmixture(table2)with final volume of 25 µl for*gyrB* and 50 µl for *rpoB* gene .

Table (1): Primers of gene amplification

Primer	Sequence	PCR products
Sp4F	CACGCCGTAAGAGTGCATTA	
Sp4R	AACGGAGCTTGTCAGGGTTA	294 bp
Sp2F	GTTCCTGATCCGAAATTCTCG	490 bp
Ac696F	ATAYCGYAAAGAYTTGAAAGAAG	397 bp
Ac1093R	CMACACCYTTGTTMCCRTGA	

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Table(2): Volume of reaction in a PCR mixture for *gyrB*, *rpoB* gene

Component	*gyr B* gene	*rpoB* gene
	Volume (µl)	Volume (µl)
Go Tag Green Master Mix,	12.5	25
Upstream primer	5*	1.0
Downstream primer	2.5	1.0
DNA template	5	2.5
Nuclease – free water	ـــ	18
DMSO	_____	2.5
Total	25	50

PCR amplification was performed inan PCR thermo cycler (Biometra ,USA) using the programexplained in table (3). PCR products and 100 bp DNA ladder (Promega, USA) were visualized by 2 % agarose gel electrophoresis and stained with ethidium bromide. The electrophoresis was performed under 60 Volts for 80 minutes .UV-transilluminator was used to visualized DNA band and gel was photographed using digital camera [7].

Table (3): PCR condition for amplification of *gyrB* and *rpoB* gene

Primer	No. of cycle	PCR condition		
		denaturation	Annealing	Extension
*Sp4F Sp4R Sp2F	25	94ºC 2 min	94ºC for1 min 60ºC for 30 S 72 ºC for 1 min	72 ºC for 10 min
**Ac696F Ac1093R	35	94ºC 5 min	94ºC for1 min 52 ºC for 1 min 72 ºC for 1 min	72 ºC for 10 min

*Primers of *gyrB*gene , **Primers of *rpoB* gene.

Result and discussion:

A total of 200 samples were collected from patients suffering from Urinary tract infections (100) , vaginal infections (50) , and wound infections (50) . All samples were cultured on blood agar base under 44 ºC for 24 hrs. A biochemical test and

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molecular method by using specific gene *gyrB* and *rpoB* gene were used for the identification of *Acinetobacter* spp. According to the biochemical test100isolates were suspected to be *A.baumannii*. The results of amplification by PCR for *gyrB* gene were shown in figure(1). *A.baumannii* was identified when two amplicons were described with molecular weight (294) bp and (490) bp, while *A. genomics* was identified when one amplicon with (294) bp was observed. Our results showed that 36 (36%) isolates were identified as *A.baumannii* while 15 (15%) isolates were identified as *A. genomics*, other isolates show no PCR product

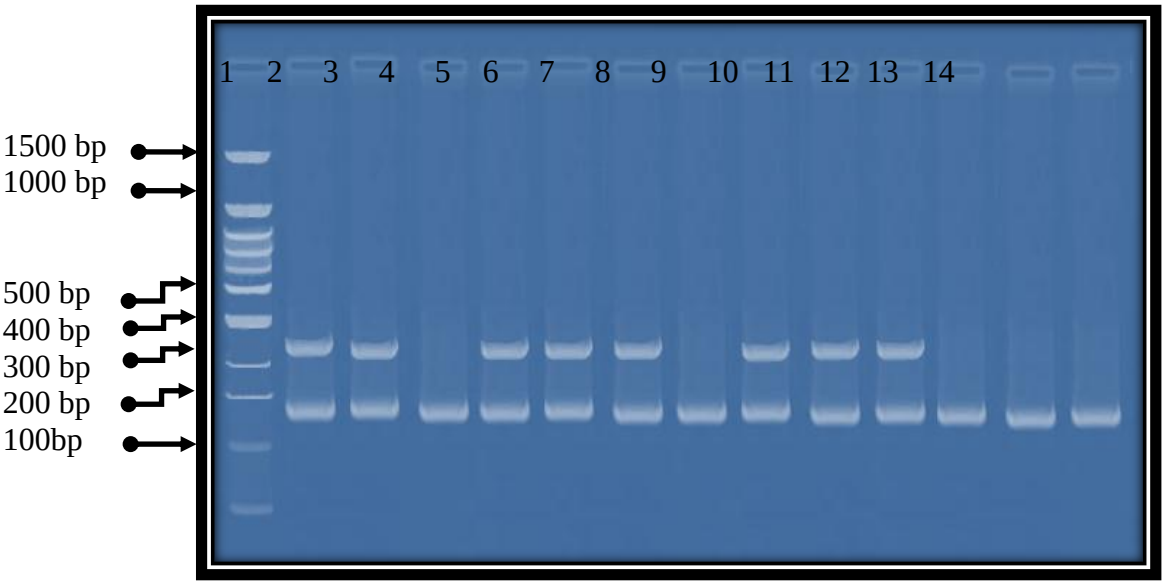

Figure (1): Identification of *A.baumannii* by amplification of *gyr B* gene by PCR (amplicons size 294 bp and 490 bp). Line 1: Ladder DNA (100bp). Line 2,3,5,6,7,9,10,11: *A. baumanni* Line 4,8,12,13,14: *A. genomics* .

On the other hand, Figure (2) showed the results of amplification of *rpoB* gene ,it explained that out of 36 isolates which were identified by *gyerB* gene, only 26 (72%) isolates were identified as *A. baumannii* with PCR product (397) bp

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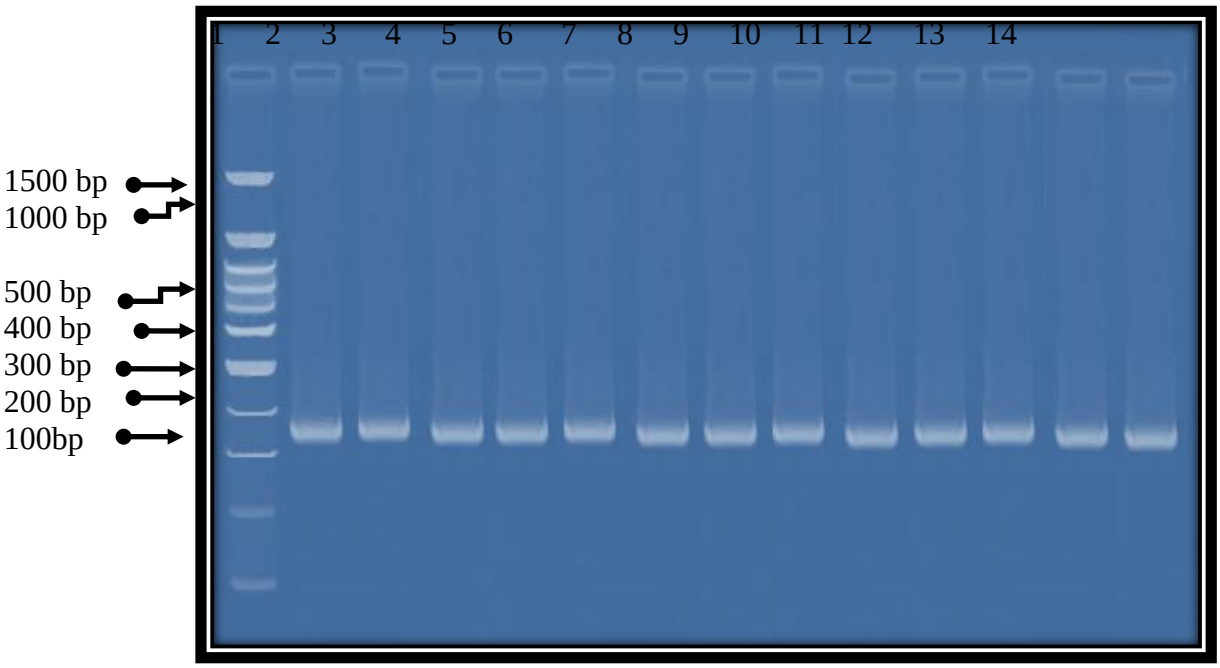

Figure (2): Identification of *A. baumannii* by amplification of rpoB gene by PCR (amplicon size 397 bp).
Line 1: Ladder DNA (100bp).Line 2 - 14*: A.baumannii* isolates.

Because of increasing pathogenic importance of *Acinetobacter spp*, a development of reliable identification methods for these strain was required. Many identification schemes depending on DNA homology, phenotypic characters , and compression of cell envelope and outer membrane protein pattern yield in consistent result [9 ; 13 ; 14 ; 15].

Phenotypically many geno-species are very similar and are not readily distinguishable [8].The currently used 16S r RNA gene sequencing determination have failed to distinguished closely related genomic species of *Acinetobacter* due to its extremely low polymorphic nature [9; 13].

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تقنيات جزيئية لتشخيص وتوصيف بكتيريا *Acinetobacte rbaumannii**

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الخلاصة
تضمنت الدراسة الحالية عزل وتشخيص بكتريا Acinetobacterb *aumannii* من إخماج المجاري البولية ، الحروق، والمهبل . جُمعت 200 عينة سريرية من المرضى المراجعين لمستشفى الزهراء والحكيم ومدينة الصدر التعليمية للفترة من سنة 2011 ولغاية 2012 . وتم الاعتماد على الاختبارات البايوكيمياوية وتقنية تفاعل السلسلة المتبلمر PCR في تشخيص العزلات وقد اثبتت الدراسة الحالية ان افضل طريقة في التشخيص هي تقنية الـ PCR باستخدام الجين التشخيصي rpoB اذ تم عزل وتشخيص 26 عزلة وبواقع (7) من اخماج الجروح, (13) عزلة من اخماج الادرار و (5) عزلات من اخماج المهبل.