Biochemical and Molecular Identification of *Acinetobacter baumannii* Isolated from Patients in Baghdad Hospitals

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Abstract

Background: *Acinetobacter baumannii* is one of the most important opportunistic bacterial pathogens that cause serious health care associated complications in hospitalized patients. This leads to prolong hospital stay which increase cost to both healthcare provider and family of the patients.

Objective: The study aimed at molecular characterization of *A. baumannii*.

Methodology: A total of 384 specimens were collected from urine, wounds, blood cultures and full depth burns from patients at different hospitals in Baghdad city. Identification of *A. baumannii* isolates was one using Phenotypic methods was confirmed by polymerase chain reaction (PCR) to amplifying 16S rRNA and bla-OXA-51 like gene. Out of 384 specimens collected 115 (29.9%) were isolated as *Acinetobacter baumannii*-calcoaceticus complex.

Results: Eighty two isolates give positive results for 16S rRNA (21.3%) and seventy six (19.79%) isolates give positive results for both rRNA and bla-OXA-51 like gene. This study showed the dominance of bla-OXA-51 in *A. baumannii* isolates from Baghdad hospitals and it is necessary to adopt appropriate strategies to control the spread of this bacteria and looking for effective drugs far from beta-lactam family.

Conclusion: Data of the current study reveal (19.79%) of isolates were positive for both biochemical and molecular detection as *A. baumannii* that was terrible percentage and it is necessary to use new strategies to treat *baumannii* infections far from ineffective drug.

Keywords: Acinetobacter baumannii, 16S rRNA, bla-OXA-51 like gene.

التشخيص الكيميوحيوي والجزيئي لبكتريا راكدة البومانية المعزولة من المرضى في مستشفيات بغداد

بكر محمد علي عبد المحسن , واثق عباس الدراغي

الملخص

الخلفية: راكدة البومانية هي بكتيريا مُمْرِضة انتهازية منتشرة على نطاق واسع في الظروف السريرية ، حيث تسبب هذه البكتيريا غالبًا مجموعة واسعة من الالتهابات ، بما في ذلك تجرثم الدم والالتهاب الرئوي والتهاب السحايا وعدوى المسالك البولية والتهابات الجروح. إلى جانب معدل الإصابة سريريا في زيادة عاما بعد عام كذلك قدرتها على تطوير مقاومة لفئات متعددة من المضادات الحيوية ، و احيانا لجميع المضادات الحيوية المعروفة جعل علاجها سريريا امرا صعبا.

الهدف: هدفت الدراسة هو تشخيص بكتريا *A. baumannii* بالاعتماد على الطريقه التقليدية (الفحوصات الكيميوحيويه) والطريقه الجزيئة باستخدام جينين (*16s RNA*و *OXA 51*) .

المنهجية: خلال فترة الدراسة من (3-1-2019 حتى 1-7- 2019) تم جمع ثلاثة مائة وأربعة وثمانون عينة سريرية من البول والجروح وزروعات الدم والحروق العميقة من كلا الجنسين ومن مختلف الأعمار لمرضى من مستشفيات مختلفة في مدينة بغداد.

النتائج : تم تشخيص مائة وخمسة عشرعزلة بالاعتماد على الصفات المظهرية والفحوصات الكيموحيوية لمعقد بكتريا راكدة البومانية وراكدة خلات الكالسيوم بعدها تم استخدام التشخيص الجزيئي باعتماد على جينين (*16s RNA OXA 51,*) لتشخيص بكتريا راكدة البومانية وحصلنا على ست وسبعون عزله مشخصه من مجموع ثلاثة مائة واربعة وثمانون عينه سريريه اي ما نسبته (19.79%) من محموع العينات.

الاستنتاج: . اظهرت هذه الدراسة النسبة المرعبه لانتشار هذه البكتريا في الظروف السريريه نتيجة لمقاومتها لطيف واسع من المضادات الحيوية وقدرتها المتميزه عن باقي افراد جنسها مما يستدعي اتباع نهج جديد وفعال في علاجها.

Introduction

Acinetobacter baumannii is a kind of lactose-non-fermenting, gram-negative coccobacillus, which is widely exist in in natural environment and become endemic in hospital. Besides, *A. baumannii* has strong environmental adaptability and drug resistance, [1] and it is included in the 'ESKAPE' list of the most common and serious MDR pathogens that are the major cause of nosocomial infections throughout the world [2]. In recent years, it has been found clinically that the infection rate of *A. baumannii* has increased year by year, and according to the data of Chinese bacterial resistance monitoring results in 2012, the detection rate of *Acinetobacte*r has exceeded that of *Pseudomonas aeruginosa*, ranked the first place among lactose fermenting bacteria [1] Moreover, *A. baumannii* is also known for its ability to develop resistance to multiple classes of antibiotics, the emergence of multi-drug resistant *A. baumannii*, pan-resistant *A. baumannii* or even fully resistant *A. baumannii* have become a difficult problem in clinical anti-infective treatment [3]

A. baumannii has strong resistance to hot and humid ultraviolet rays and chemical disinfectants, and it can survive for more than 25 days on the surface of dry objects. It is the most commonly isolated gram-negative coccobacilli in medical personnel, medical equipment and surface of objects, which often shows the characteristics of multi-drug resistance, extensive drug resistance and pan-drug resistance [4] they are responsible for a number of hospital acquired infections. To control the spread of *A. baumannii* in the hospital, it is necessary to distinguish the outbreak strain from epidemiologically unrelated *Acinetobacter*. This requires the comparison of isolates at the subspecies level which is done by epidemiological typing methods. Phenotypic typing systems based on biochemical profiles (biotyping), molecular typing such as 16S rRNA gene [5].Several characteristics of the 16S rRNA gene, such as its essential function, ubiquity, and evolutionary properties, have allowed it to become the most commonly used molecular marker in microbial ecology [6].

This bacteria produces metallo-beta-lactamase (IMP-VIM and types of SIM) causing resistance to most beta-lactam [7]. *A. baumannii* harbored enzymes such as Oxacillinase OXA-51 which hydrolyze carbapenems. Also, three groups of irrelevant enzymes of OXA-23, OXA-24, and OXA-58 have been defined. The aim of our study was to identify phenotypic and molecular characteristics of *Acinetobacter baumannii*.

Materials and Method

During a period of this study from 3-1-2019 to 1-7-2019, a total of 384 specimens were collected from urine, wounds, blood cultures and full depth burns from patients at different

hospitals in Baghdad city, to isolate Acinetobacter baumannii. Clinical relevance were determined by the clinical microbiologists and attending physicians. The specimens were inoculated initially on blood agar and MacConkey agar then incubated for 24h at 37 °c.

The biochemical methods as Figure 1. Shown were used to identify Acinetobacter baumannii-calcoaceticus complex [8]. The isolates then stored in BHI slants with 15% glycerol at -20 °c

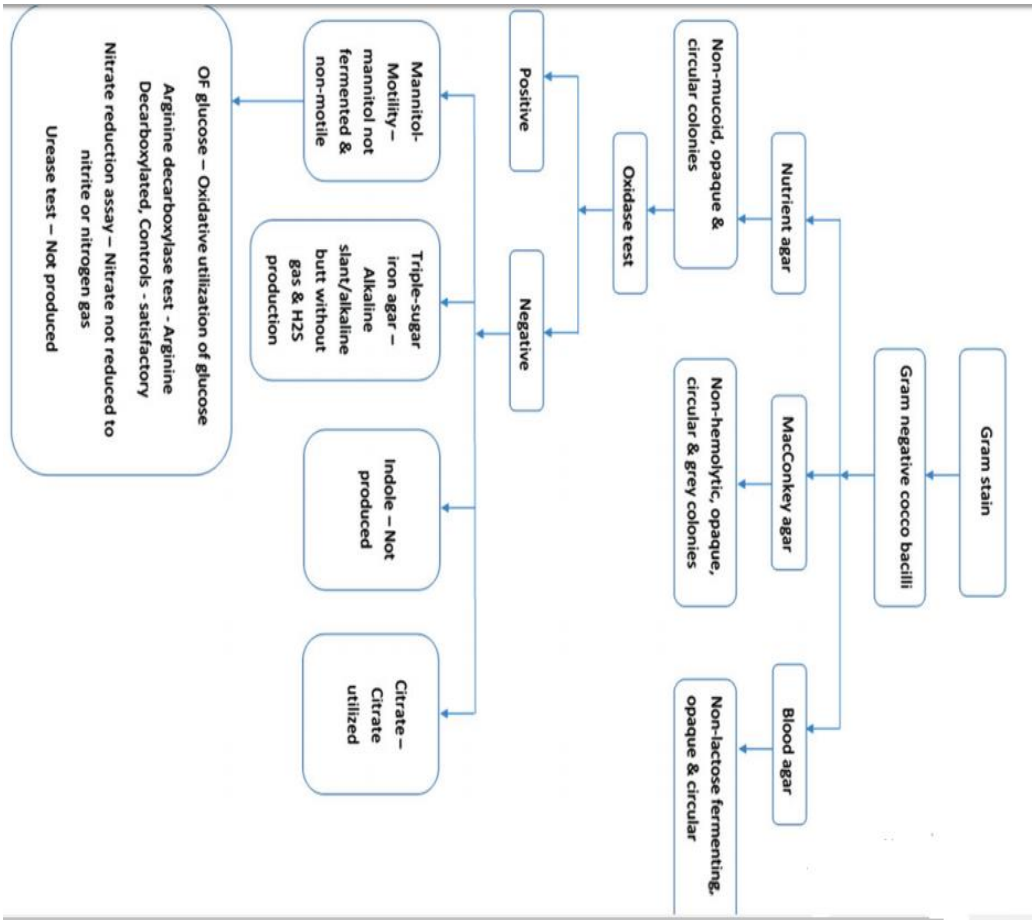

Figure 1. : Illustrate the biochemical test Algorithm for the identification of *Acinetobacter baumannii-calcoaceticus* complex, the purpose of the test and the perspective results.

Polymerase chain reaction assay was performed to detect *Acinetobacter baumannii* by amplifying *16S rRNA* and *bla-OXA-51* like gene. DNA of the isolates was extracted using bacteria genomic DNA Mini Kit (Geneaid) according to manufactures' instructions followed by measuring DNA concentration and purity using Nano drop device .The primer sets using to amplify as shown in Table 1.

Table 1. : The primers used in this study.

No.	Gene	Primer sequence (forward/ reverse)	Amplicon size	References
1	*16s rRNA*	F: 5'-CAGCTCGTGTCGTGAGATGT-'3	150bp	[9]
		R: 5'-CGTAAGGGCCATGATGACTT-'3		
2	*Bla-OXA-51 like*	F: 5' –TAATGCTTTGATCGGCCTTG-'3	353	[10]
		R: 5' –TGGATTGCACTTCATCTTGG-'3		

AccuPower® PCR PreMix kit was used to prepare 20 µL reaction size according to company instruction including DNA template (5 µL), each primer (10 pmol) (1.5 µL) and Nuclease free water (12 µL).

The components of the PCR mixture were placed in the PCR tubes fitted with the kit and contain the rest of the PCR components (Taq) DNA polymerase, dNTPs, Tris-HCl, KCl, MgCl2, Stabilizer and tracking dye) .the tubes were then mixed by vortex for 5 seconds finally transferred to thermocycler which programmed as shown in Table 3. For *16s rRNA* gene detection and in Table 3. *Bla-OXA-51* like gene detection.

Table 2. : Programs of thermocycler conditions for *16s rRNA* gene detection.

Steps	Temperature	Time	Number of cycles
Initial denaturation	94 °c	4 min.	1
Denaturation	94 °c	35 sec.	30
Annealing	55 °c	45 sec.	
Extension	72 °c	40 sec.	
Final extension	72 °c	4 min.	1

Table (3): Programs of thermocycler conditions for *Bla-OXA-51 like* gene detection.

Steps	Temperature	Time	Number of cycles
Initial denaturation	94 °c	5 min.	1
Denaturation	94 °c	45 sec.	30
Annealing	52 °c	40 sec.	
Extension	72 °c	45 sec.	
Final extension	72 °c	6 min.	1

PCR products were separated by gel electrophoresis performed at 100volt and 80 mA for 45 min. on 1.5 % agarose gel and visualized by UV light.

Results and Discussion

In recent years, nosocomial infections of *A. baumannii*, as an opportunistic pathogen, are increasing. Treatment of this bacteria especially MDR and broad-spectrum beta-lactamases producing strains is of major concern .in our study out of 384 specimens isolates 115 (29.9%) were as *Acinetobacter baumannii-calcoaceticus complex*. However, these phenotypic characteristics were performed as preliminary identification tests for *A. baumannii* which were not considered a sufficiently reliable identification method [11] these may not because the insufficiently of the test systems but due to some properties of *Acinetobacter baumannii*.

Molecular identification was considered more accurate in identification of bacteria and eliminates the variable phenotypic problem. The using of *16S rRNA* gene in bacterial species classification have been well established because *16S rRNA* genes are highly conserved among species and all organisms possess various unique species-specific region that allow for bacterial identifications .In this study , the presence of a specific *16S rRNA* was used as a confirmation process. Eight two isolates (21.3%) gave positive results with product size equal to ~ 150 bp as shown in Figure 2 .

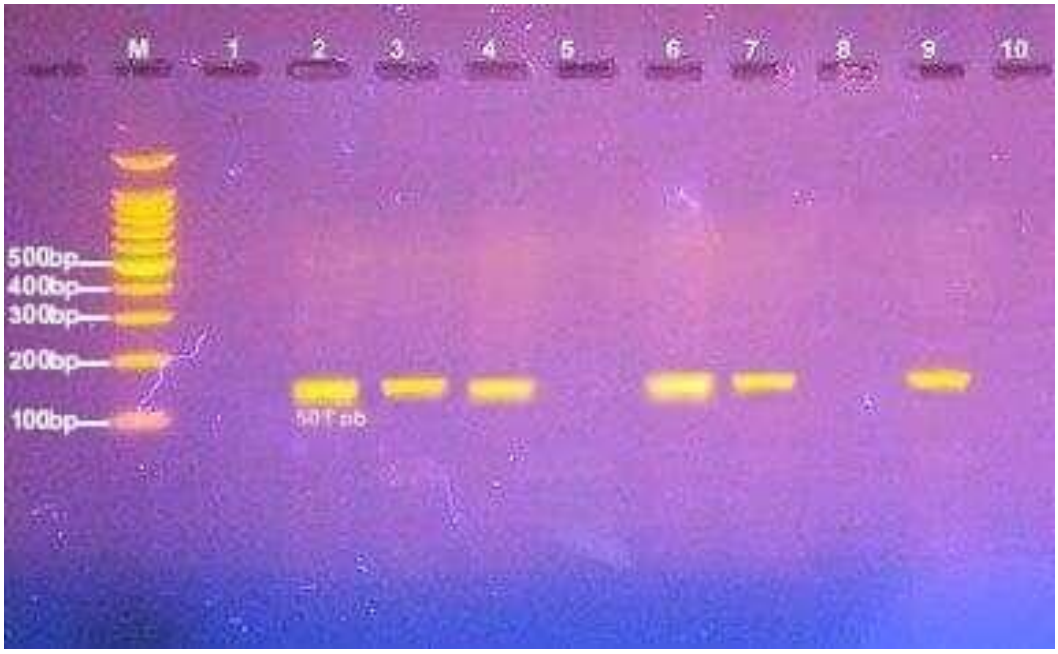

Figure 2. : Electrophoresis result of *16S rRNA* amplification: lane M: Size marker 100 bp; lane 1, 5, 8, 10: Clinical isolates show negative results for 16S rRNA; lanes 2, 3, 4, 6, 7, 9: Clinical isolates show positive results for 16S rRNA.

These results were in agreement with those results of Al-Radi *et al.,* (2018), in which the same primers have been used to detect *Acinetobacter spp.* As fuks *et al .,* (2018) mention that *16S rRNA* gene is still not sufficient and more precise and accurate identification is required to detect *A. baumannii blaOXA-51* like genes are present in at least the vast majority of isolates of *A. baumannii*, there has been some debate as to whether they are present in all isolates of these species . If they are consistently found and are also unique to this species, then their detection could provide a simple and convenient method of identifying *A. baumannii* which could more easily be carried out than current definitive methods, such as amplified *rRNA* gene restriction analysis (ARDRA), and which would be more reliable than biochemical identification (e.g., by API), which is most commonly used [13]. Seventy six isolates (19.79%) gave positive results for containing *bla-OXA-51* like gene with product size equal ~ 350 bp as shown in Figure 3.

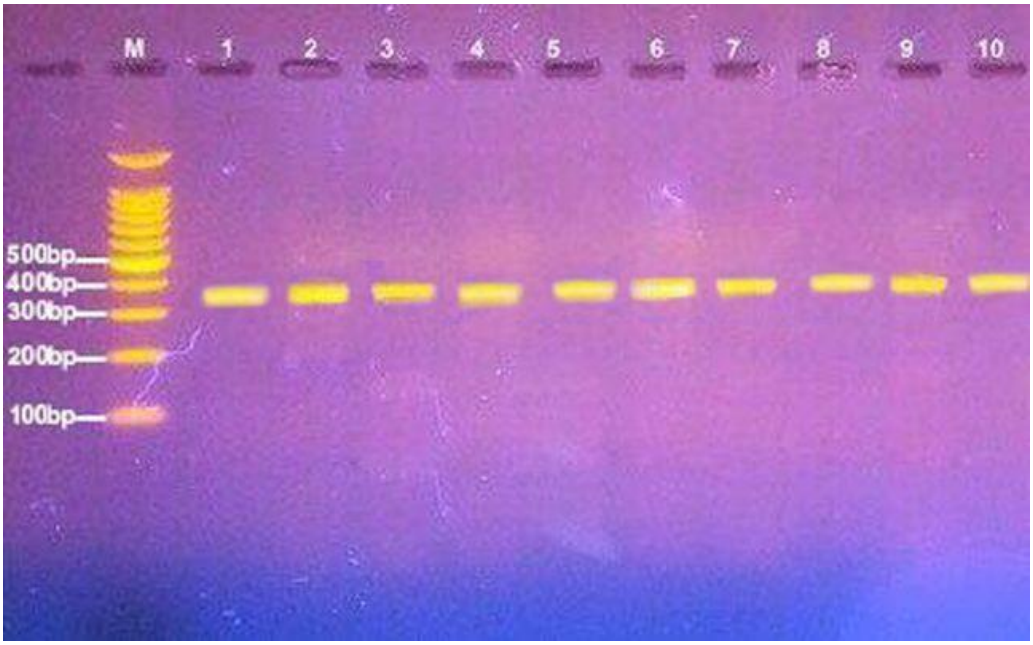

Figure 3. : Electrophoresis result of bla-OXA-51 amplification: lane M: Size marker 100 bp; all lanes contain clinical isolates shown positive results for bla-OXA-51.

The isolates (19.79%) that give us positive result for both genes were adopted as *A. baumannii*, this terrible percentage possibly due to the ability of *A. baumannii* to cause nosocomial infections and resistance to a wide range of antibiotics. In addition to that *A. baumannii* survives desiccation better than other *Acinetobacter spp.*with its ability to form biofilm that involved in cell attachment on epithelial cells and smooth surfaces of medical instruments like urinary catheters and lung tubes [14]. The percentage of the current study is higher than, study conducted by Al-Radi *et al.,* (2018) that evaluated dissemination of *A. baumannii.* On the other hand, the presence of *bla-OXA-51* like gene in all *Acinetobacter baumannii* isolates which is one of the various mechanisms to resist different types of beta-lactam. those with carbapenemase activity are the most concern for drug resistance and include the serine oxacillinase (belonging to Ambler class D OXA type) and the metallo-b- lactamases (Ambler class B) [5]. So it is necessary to adopt appropriate strategies to control the spread of this bacteria and looking for effective drugs far from beta-lactam family

Conclusions

Data of the current study reveal (19.79%) of isolates were positive for both biochemical and molecular detection as *A. baumannii* that was terrible percentage and it is necessary to use new strategies to treat *baumannii* infections far from ineffective drug.

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