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EVALUATION OF THE PHENOTYPIC AND GENOTYPIC DETECTION OF *ACINETOBACTER BAUMANNII* ISOLATED FROM BAGHDAD HOSPITALS

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Abstract

Phenotypic and genotypic detection of *Acinetobacter baumannii* is the commonly used methods for identification. This study evaluated the accuracy of these two detection methods. *A. baumannii* were identified based on Gram staining, API 20 EN, Vitek-2 and Polymerase chain reaction (PCR) amplification of organism specific *16S rRNA gene and bla* $_{OXA\text{-}51\ like}$ *gene*. In the present study, 100 clinical specimens were isolate from different sources, including (Sputum, Wounds, Burns, UTI and blood), 61 samples were correctly identified as *A. baumannii* according to API 20 EN, Vitek-2, whereas the PCR result showed that 45 sample of the 61 were positive by using *16S rRNA gene and bla* $_{OXA\text{-}51\ like}$ *gene* detection.

Key words : VITEK-2 System, API20 EN, PCR, *16S rRNA gene and bla* $_{OXA\text{-}51\ like}$ *gene*, *A. baumannii*.

Introduction

The *A. baumannii* is non-motile, Gram-negative coccobacilli, opportunistic extracellular human pathogen. It is emerging as an important nosocomial pathogen causing a variety of infections. It is increasingly related to serious infections among patients on these life support systems. *A. baumannii* have become resistant to almost all currently available antibacterial agents (Chaparteguí-Gonzalez *et al.,* 2018; Royer *et al.,* 2018). Up to date, the most common and widespread detection methods include characterization via a phenotypic system and commercial phenotypic methods (*e.g.,* the Vitek-2 system [Biomerieux] and the API 20 NE system). However, there are some limitations in these methods (Li *et al.,* 2015).The aim of this study was to evaluate the accuracy of phenotypic and genotypic detection of *A. baumannii.*

Materials and Methods

Patients' specimens' collection

Through the period extending from April 2018 till October 2018, 100 Clinical specimens comprising; wounds and burns were collected as swabs in sterilized containers, urine, sputum and blood were collected in sterilized lab containers from inpatients that admitted in hospitals in Baghdad (Karkh and Resafa).

Samples identification

All the bacterial isolates were examined for gram stain ability; shape and color of the cells were observed by light microscope using oil emersion, the collected specimens were streaked directly on CHROMagar *Acinetobacter* medium, MacConkey agar and blood agar then incubated for 24hours at 37°C under aerobic conditions

Identification of the bacterial isolates was conducted using Api 20 EN and Vitek-2 system (Biomerieux Vitek-2, Inc., Hazelwood, MO, USA) system for *Enterobacteriaceae* according to the procedure suggested by the manufacturing company.

DNA Extraction

DNA was extracted from all 61 *A. baumannii* clinical isolates using a commercial purification system (Genomic DNA Mini Kit (Geneaid, Taiwan)). DNA stored at 4^0C until used for molecular detection.

Conventional PCR was used for detection the following genes in this study: the 16S rRNA gene for identification the *Acinetobacter* spp and the specific gene $bla_{\text{OXA-51}}$ for identification the *A. baumannii*. Table 1 listed the sequences of the mentioned primers, table 2 shown the condition of PCR reaction for $bla_{\text{OXA-51 like}}$ *gene* detection, table 3 shown the condition of PCR reaction for *16S rRNA gene* detection.

Results and Discussion

Table 1: listed the sequences of the primers used for conventional PCR to detect *Acinetobacter baumannii 16S rRNA gene and* $bla_{\text{OXA-51 like}}$ *gene.*

Gene	Sequence	PCR product size(bp)	Reference
16S rRNA	5 -CAGCTCGTGTCGTGAGATGT-3 F	150	(Ghaima, 2016)
	5 -CGTAAGGGCCATGATGACTT-3 R		
blaOXA51	5- TAATGCTTTGATCGG CCT TG 3 F	353	(Ghaima, 2016)
	5- TGGATTGCACTTCATCTTGG -3 R		

Table 2: PCR Thermo-Cycling Conditions for $bla_{\text{OXA-51}}$ Genes (Woodford *et al.*, 2006).

Loop's steps	Temperature	Time	Number of cycle
Initial denaturation	94 ℃	5 min	1
denaturation	94 ℃	45 sec	30
Annealing	52 ℃	40 sec	
Extension	72 ℃	45 sec	
Final extension	72 ℃	6 min	1

Table 3: PCR Thermo-Cycling Conditions for 16S rRNA Genes (Higgins *et al.*, 2004).

Loop's steps	Temperature	Time	Number of cycle
Initial denaturation	94 ℃	4 min	1
Denaturation	94 ℃	35 sec	30
Annealing	55 ℃	45 sec	
Extension	72 ℃	40 sec	
Final extension	72 ℃	4 min	1

Api20 EN semi-automated commercial identification systems that are currently used in diagnostic microbiology. Api20 EN tests were performed for identification of the collected bacterial sample. The accurate and rapid identification of *A. baumannii* is critical for appropriate infection control in hospital settings. Up to date, the most common and widespread detection methods include characterization via a phenotypic system and commercial phenotypic methods (*e.g.*, the Vitek-2 system [Biomerieux]. which have been used to successfully identify most *Acinetobacter species*. However, there are some limitations in these methods (Lee *et al.,* 2015; Li *et al.,* 2015; Almaghrabi *et al.,* 2018).

The *A. baumannii* can be distributed depending on the site of infection that has been taken. Table 4 shown that the high percentage of isolates was obtained from sputum which represent about (75.86 %) from total number of sputum isolates. Meanwhile, moderate percentage of isolates were obtained from wounds which represent (70.37 %), of the samples (60 %) of the burns. Samples of the UTI and blood show the low percentage rate (33.33% and 20%) respectively of the samples.

These results of sputum are harmonized with (Talukdar *et al.,* 2018), which also found out that sputum isolates were the majority of total number of isolates. Meanwhile, other studies (Falagas *et al.,* 2015; Ghaima, 2016 ; Al-masaudi, 2018) mentioned to 20.6%,19.3% and 1.6% of ICU-acquired burns, wounds and UTI were obtained respectively. The differential and increasing in percentage of *A. baumannii* distribution reflected the ability to attack different human body sites. (Kamolvit *et al.,* 2015) mentioned that the high frequency of genus *Acinetobacter* among gram-negative bacteria isolated from environmental surfaces of ICUs of four hospitals located in the city of Qom, Iran.

Molecular Identification of *Acinetobacter* Species Using *16S rRNA* and $bla_{\text{OXA-51 like}}$ Genes

Table 4: Distribution of *A. baumannii* according to the site of infection.

Source of sample	No. of sample	No. of isolate	Percentage%
Sputum	29	22	75.86%
Wounds	27	19	70.37%
Burns	25	15	60 %
UTI	9	3	33.33%
Blood	10	2	20 %
Total	100	61	

The results of sixty-one (61) samples were grouped into five sites as follows (Sputum 22, Wounds 19, Burns 15, UTI 3 and blood 2).

Polymerase chain reaction (PCR) was performed for all (61) samples and the PCR result showed (45) samples positive as (Sputum 20, Wounds 13, Burns 7, UTI 3 and blood 2) by using *16S rRNA gene* and $bla_{\text{OXA-51 like}}$ gene detection. Fig. 1 and 2 shown PCR result of *16S rRNA gene* and $bla_{\text{OXA-51 like}}$ gene detection gene (150pb and 353pb), and this was done to confirm the accuracy of our tests and methods used for identification

of this genus.

Fig. 3 indicates the percentage values of our samples before and after performing the molecular detection using

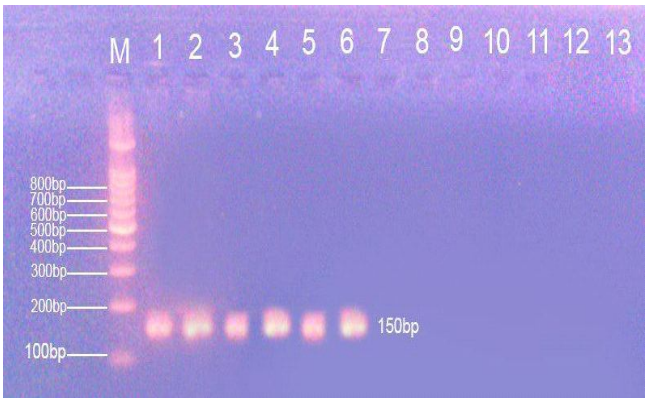

Fig. 1: 2% Agarose Gel Electrophoresis of PCR Amplified Products for *16S rRNA Gene*. Lane (M): l00bp ladder, Lane (1- 6): Positive Result with Positive Bands of 150 bp *A. baumannii*, Lane (8-13): Negative Result. (70V for 1.30hr).

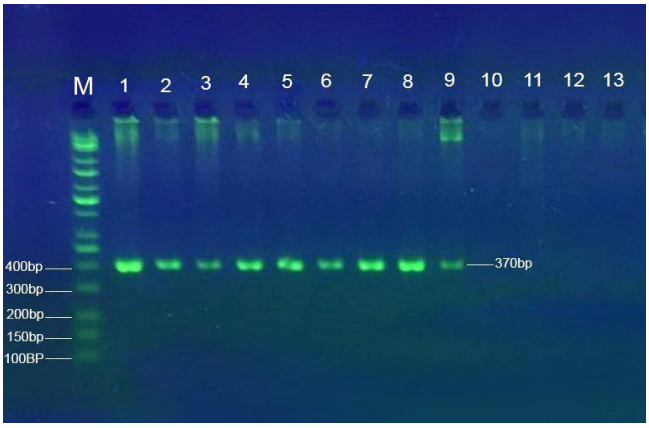

Fig. 2: 1% Agarose Gel Electrophoresis of PCR Amplified Products for *bla* $_{\text{OXA-51 like}}$ Gene. Lane (M): l00bp ladder, Lane (1- 9): positive Result with Positive Bands of 353 bp *A. baumannii*, Lane (10-13): Negative Result. (70V for 1.30hr).

16S rRNA gene and *bla* $_{\text{OAX-51-like}}$ gene.

The positive results of *16S rRNA gene* and *bla* $_{\text{OAX-51-like}}$ gene for our samples were as follows (Sputum 44.44%, Wounds 28.89%, Burns 15.56%, UTI 6.67% and blood 4.44%). Only samples containing *16S rRNA gene* and *bla* $_{\text{OAX-51-like}}$ gene (45) were considered for further molecular analysis. *A. baumannii* isolates carried the chromosomally encoded *16S rRNA gene* and *bla* $_{\text{OAX-51-like}}$ gene. These findings support those of other studies demonstrating that the detection of the *16S rRNA* gene can be used as a supplementary tool to identify the organism at the genus and *bla* $_{\text{OAX-51-like}}$ gene for species level, confirmed by additional methods (Elabd *et al.,* 2015).

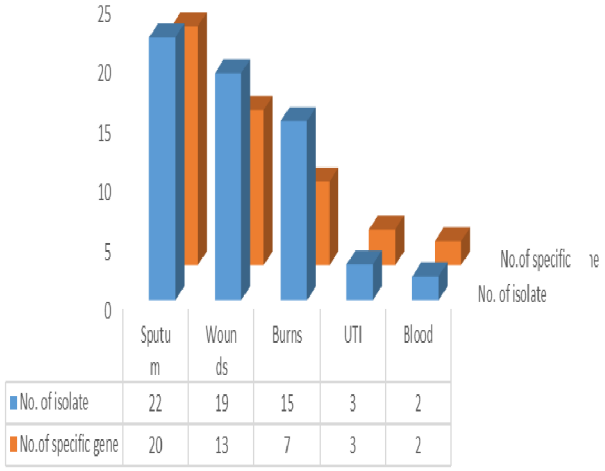

Fig. 3: Comparison of *A. baumannii* Isolates Before and After Molecular Detection.

Ghaith *et al.,* (2017) who revealed *A. baumannii* harboring *bla*$_{\text{OXA-51-like}}$ gene has been identified as a marker for species identification. An intrinsic *bla*$_{\text{OXA-51-like}}$ gene detected in all isolates in this study supports the use of this gene as a surrogate marker of *A. baumannii* identification.

Mosavat *et al.,* (2018) who stated that the production of *β-lactamases* was the most prevalent mechanism of β-lactam drug resistance in *A. baumannii,* and this enzyme coded by OXA gene so this gene has been used as a molecular diagnostic kits, which is in agreement to the present result.

The presence of *16S rRNA gene* and *bla* $_{\text{OAX-51-like}}$ gene is restricted for *A. baumannii* and this has been reported to be useful for molecular characterization of *A. baumannii* (Ghaima *et al.,* 2016; Kamolvit *et al.,* 2015).

Conclusion

By the result of the recent study, we can conclude that the genotypic detection was more accurate and controlled than the phenotypic method.

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