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Molecular Detection of Acientobacter Baumannii Isolated From Nosocomial Infections in Baghdad Hospitals

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Abstract

The present study is an attempt for detection of *A. baumannii* by conventional and PCR methods using species-specific primers for these *A. baumannii*. A total of 87 samples were collected from hospitals in Baghdad (Al-Rasafa and Al-Karkh Hospitals) during the period from 2019 to 2020.The samples included: 40 specimens, from wounds, respiratory infections (sputum), burns, CSF and 47 samples from the hospital environment (swabs), while samples collected from intensive care unit including patient beds, surgical instruments and appliances, emergency lobby and baby incubators**.** *A. baumannii* isolate identification depending on the morphologic characteristics on the culture media including, blood agar, MacConkey agar, as well as the biochemical tests including the manual biochemical tests that include catalase, oxidase and tests, and the automated biochemical tests such as API 20E,VITEK 2 system. The genomic DNA of *A. baumannii* isolates were extracted using wizard genomic DNA purification kit, the extracted genomic DNA was analyzed using 1% agarose gel electrophoresis, and then the concentration and purity of the extracted genomic DNA were determined using Nanodrop spectrophotometer device.To detect the *A. baumannii* isolates by molecular methods, the extracted genomic DNA of these isolates was submitted for amplification to detect the *blaOXA-51* gene by the PCR method using species-specific primers for *A. baumannii*, out of 47 samples (35) showed positive results for *A. baumannii* by observing the PCR product of *bla*OXA-51 gene with ~353bp, in the agarose gel electrophoresis.

Keywords: Molecular detection, Acientobacter baumannii, nosocomial infections

INTRODUCTION

Acinetobacter baumannii is one of the most important agents of hospital infections. Rapid and accurate identification and genotyping of *A. baumannii* is very important, especially in burn hospitals in order to prevent the spread of related nosocomial infections and to further epidemiological studies (Maleki *et al*., 2017). Multi Drug resist of *A. baumannii* infections tend to occur in immunosuppressed patients, in patients with serious underlying diseases, and in those subjected to invasive procedures and treated with broad-spectrum antibiotics. Thus, infections due to *A. baumannii* are frequently found in intensive care units (ICUs), where they are implicated as the cause of ventilator-associated pneumonia (VAP), urinary tract infections, and bacteremia. *A. baumannii* also causes, albeit less frequently, complicated skin and soft tissue, abdominal, and central nervous system infections (AmirMoezi *et al*., 2016; Aljindan *et al*., 2018). Recent importance is that *A. baumannii* has become a major pathogen

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found in combat-associated wounds. The factors contributing to colonization, virulence, and invasion are being defined. Today, different molecular methods have been developed to better understand the epidemiology and clinical significance of *Acinetobacter* species. However, most of them are very difficult for routine diagnostic applications in the microbiology laboratory and their uses are limited to reference laboratories (Karah *et al*., 2016, Ghaith *et al*., 2017). A simple and useful molecular technique for identifying *A. baumannii* isolates is the identification of the *blaOXA-like-51* carbapenemase gene by PCR method (Wang *et al*., 2018) *OXA*-TYPE genes, especially the subgroup *OXA-51*, have been studied in *A. baumannii* isolates around the world. This gene has a chromosomal position and can be used as a molecular reagent of *A. baumannii*. In other words, to differentiate the associated strains of *Acinetobacter* that are not interdependent, it is necessary to compare the isolates at the subtype level. Some typing methods have been designed to achieve this goal. Different typing systems are based on phenotypic tests and molecular techniques (Turton *et al*., 2006; Couvé-Deacon *et al*., 2019). The aim of the current study was the molecular detection of *A. baumannii* isolated from nosocomial infections in Baghdad hospitals.

MATERIALS AND METHODS

The study was conducted at Baghdad University, Institute of Genetic Engineering and Biotechnology for postgraduate. A total of 87 samples were collected from hospitals in Baghdad (Al-Rasafa and Al-Karkh Hospitals) during the period from 2019 to 2020.The samples included: 40 specimens, from wounds, respiratory infections (sputum), burns, CSF and 47 samples from the hospital environment(swabs),while samples collected from intensive care unit including patient beds, surgical instruments and appliances, emergency lobby and baby incubators.

The specimens were transferred to the laboratory (by transport media) and cultured on selective blood agar and MacConkey agar then incubated at 37°C for 24 hours under aerobic conditions.

PCR was performed based on the identification of *blaOXA-51* gene for the molecular detection of *A. baumannii* isolates. In the first step, the genomic DNA extraction of bacterial isolates using a commercial wizard genomic DNA purification kit according to manufacturer's instructions (Promega, USA) with some modification,t hen the extracted DNA from the *A. baumannii* isolates was quantified spectrophotometrically at O.D. 260/ 280 nm with ratios 1.4-1.5. The sensitivity of the *A. baumannii* -F and *A. baumannii* -R primers was evaluated by PCR amplification for serial diluted concentrations (10-100 ng) of purified genomic DNA isolated from *A. baumannii* .

The primers used for detecting these genes by PCR is 5-TAATGCTTTGATCGGCCTTG-3Fand5- TGGATTGCACTTCATCTTGG-3 R**.(7)** PCR was performed in a 20-µl reaction mixtures containing 5 µl PCR Master Mix Reagent ,1 µl of forward primer, 1 µl of reverse primer, 2.5 µl of DNA template and 12.5 µl nuclease-free H2O. Reaction mixtures were initially heated to 94°C for 4 min, followed by 30 cycles at 94°C for 35 s, 55°C for 45 s, and 72°C for 40 s. The final extension step was performed at 72°C for 6 min. The PCR-amplified products of *blaOXA-51* gene of *A. baumannii* were analyzed by 1% agarose gel electrophoresis.The 100 bp DNA ladder (Promega, USA) was used and the gel was run at

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100 volt for 45 minutes at room temperature. The PCR products were stained with ethidium bromide and visualized by an image analyzer (ChemiImager 5500, Alpha Innotech, USA).

RESULT AND DISCUSSION

Acinetobacter baumannii is responsible for hospital-acquired infections and are recently two of the most important healthcare-associated infections in hospitals. Infection caused by these bacteria often lead to significant mortality and morbidity (Fallah et al., 2014, Poorabbas et al., 2015). The results of forty-seven (47) samples were grouped into five sites as follows (Sputum, Wounds, Burns, UTI and blood).

On MacConkey agar *A. baumannii* colonies appeared as as small, pale yellow to pink and non/partially lactose fermented, but the colonies were red color after short period (2-3) weaks (old colony) (Figure 1). The Gram staining of *A. baumannii* was showed When examined under light compound microscope (oil immersion, 100 X), bacterial isolates showed as a Gram–negative bacteria, coccobacilli and occasionally arranged in diplococci. (Figure 2). The results of biochemical tests were used for further identification of *P. mirabilis* isolates showed positive reactions for catalase, citrate utilization but was negative for oxidase test,Indol production , voges-proskauer (VP). The Catalase test was carried out to distinguish between the bacteria producing of catalase enzyme (which has ability to broken toxic hydrogen peroxide (H2O2) to oxygen and water). The result of this test was positive for the bacterial isolates diagnosed by released oxygen (O2) as bubble on slides. the result of growth on the Citrate utilization test was positive, due to capability of bacteria to utilize citrate as a sole carbon and energy source, this led to change the color of medium from green to blue. In the oxidase test, the bacteria gave a negative result where the violet color did not appear on the filter paper, indicating that the bacterial isolates not produced oxidase enzyme. The bacteria gave a negative result to the indole test by not showing a red ring on the surface of the peptone water after the reagent was added, due to the inability of the isolates to release the tryptophanase enzyme, which destroys the amino acid (tryptophan). As gave a negative result to a methyl red because inability of isolates to release the enzymes that give acidic products from glucose fermentation, therefore the color of the medium did not change to the red when the red methyl reagent was added. Also the result was negative to the Voges proskuer examination where the isolates showed inability to ferment glucose, therefore didn't change the color of the medium to red The results of biochemical tests of current study agree with study conducted by(Atlas *et al*., 1996).

Genomic DNA was successfully extracted from *A. baumannii* isolates using a commercial genomic DNA purification kit (Promega,USA), The concentration and purity of extracted DNA were determined, and the concentration of the extracted DNA ranged between (50-239) ng/µL. DNA bands were confirmed and analyzed by gel electrophoresis. The results of DNA **extraction from all isolates are exemplified by the samples shown in (F**igure 3).

Polymerase chain reaction (PCR) was performed for all (47) samples and the PCR result showed (35) samples with a positive result by using bla-OXA-51-like gene detection. Figure (4) show PCR result of blaOXA-51-like gene detection gene (353pb), and this was done to confirm the accuracy of our tests and methods used for identifying of *A.baumannii*. The

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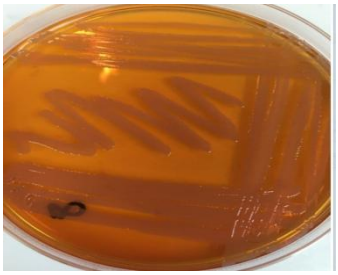
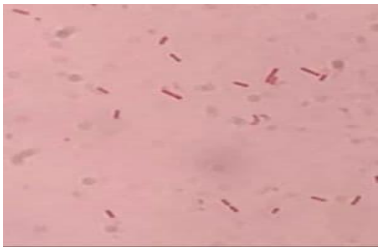
results were in agreement with most previous studies which indicated the presence of this gene all clinical isolates of *A.baumannii* and not detected among any other *Acinetobacter* spp., consequently the *bla*OXA-51-like gene normally considered to be species-specific to *A.baumannii* (Brown and Amyes).



Figure(1)Colonies morphology of *A. baumannii* culture on MacConkey agar medium at 37ºC for 24hrs.



Figure(2): Microscopic examination of *A. baumannii* (Gram stain).

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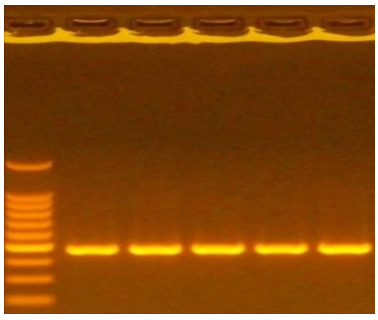

Figure (4): Agarose gel electrophoresis of PCR products for the *blaOXA*-51-like Gene. (70V for 2hr).

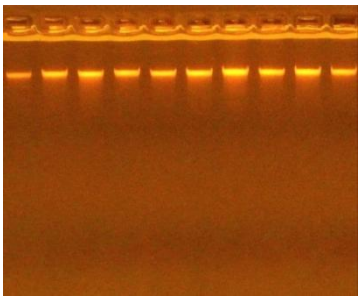

Figure (3): Gel electrophoresis of extracted genomic DNA of *blaOXA*-51-like Gene isolates using 1% agarose gel at 7volt/cm for 1 hour. Lane 1-10: Extracted genomic DNA.

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