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# Genotyping of *Acintobacter Baumannii* Isolated from Different Clinical Samples

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**Abstract**---A total of (150) clinical specimens were collected from patients suffering from different infections such as burns, wounds infections, urinary tract infections, and respiratory tract infection. All clinical specimens were cultured on different media. The results indicated that 22 (14.6%) of the isolates belonged to *A. baumannii*. It was found that twelve isolates (54.5%) from sputum, five isolates (22.72%) from burns, three isolates (13.63%) were detected from wounds, and two isolates (9%) from urine. In this study, used BOX and ERIC-PCR for the detection of phylogenetic diversity of *A. baumannii* isolated from different samples. In general, 20 isolates characterized using the two molecular techniques had comparable number of bands with some degree of polymorphism. *K. pneumoniae* isolates from the same source were clustered into different groups. The two molecular techniques generated 2 main clusters and the results of dendrogram of these techniques reveals that 20 polymorphic variants between *A. baumannii* clinical isolates detected by ERIC and BOX PCR.

**Keywords**--- *A. baumannii*, BOX and ERIC-PCR, phylogenetic diversity.

## Introduction

*A. baumannii* is almost be considered new pathogen and as many researchers suggested the first appearance of this pathogen was in Iraq specifically in military Iraqi War and was called “Iraqibacter”, infections of this pathogen associated with military and injured soldiers in combat due to direct environmental contamination of wounds due to considering *A. baumannii* to become the potential and serious problem in military hospital (1).

*Acinetobacter baumannii*, is a cause of hospital-associated bacterial disease (2) *A. baumannii* quickly acquires antibiotic resistance, which has led to an increased proportion of multi-drug resistant infections compared with other ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) pathogens and entry to the CDC urgent threats list for bacteria most in need of new antibiotics. Unfortunately, antibiotic development has stagnated (3). This has placed increased pressure on infection prevention to control *A. baumannii*. Environmental contamination(4) and asymptomatic patient colonization are important reservoirs for *A. baumannii* infection in clinical settings(5)

The presence of tandem DNA repeats in genomes of *A. baumannii* was confirmed by several groups (6) Based on these sequences, different methods of differentiation of *A. baumannii* strains have been developed; however, they take into account only their diversifying power of evolutionary changes of the *Acinetobacter* genus. Thus, their features responsible for drug resistance or pseudo-immunological bacterial responses, encoded in the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system, which evolved to protect the cells from exogenous phage and plasmid DNA invasion, are ignored in such analyses. On the other hand, as suggested by (7), the next step in the process of strains' classification should be focused on confrontation of the genetic and phenotypic features related to pathogenicity of bacterial species, It is based on combination of the previously described method based on analysis of repeated sequences and whole genome alignment (8).

The bacterial genome of *A. baumannii* contains recurring sequences similar to the enterobacterial repetitive intergenic consensus (ERIC) initiator sequence. This has enabled it to be used as a molecular biological tool for determining the genetic variation coding on DNA of *A. baumannii* isolates (9).

In *A. baumannii*, repetitive sequences of the gene which are called symmetric elements are often seen in the non-coding fragment of the DNA. Regarding the variable number and length of these repeat sequences, some primers have been designed. The length and number of bands obtained for each isolate are variable and the strains could be grouped according to the diversity of the bands (10).

Bacterial genomes containing Repeat sequences such as the ERIC sequence can be used as Molecular biological tools to assess the clonal variability of many bacterial isolates. ERIC-PCR fingerprinting is one of the fastest molecular typing techniques to differentiate Between *A. baumannii* and other strains of Gram-negative Bacteria responsible for hospital-acquired infections (11).

## **Materials and Methods**

### **Clinical Specimens**

The specimens were obtained from different sites of infections (burns, wounds, urine, and sputum); each swab was taken carefully from the sites of infections and transfer to the laboratory of microbiology /college of medicine. Urine (mid-stream urine) was collected from patients suffering from UTIs in sterile screw-cap

container. Swabs from burn, wound, were collected from patients before they take any antibiotics or cleaning. Each specimen was inoculated on selective media and identified by biochemical reaction according to the diagnostic procedures recommended in (12), Also isolates were tested by VITEK to be identified as *A. baumannii* using identification card of gram-negative bacteria (BioMérieux) by showing an interesting percentage of accuracy (99%)

### Ethical approval

Agreement from the family and patients for sampling and carrying out this work was obtained.

### DNA Extraction

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company (Genaid, UK).

### Primer Sequences

The primer sequences and PCR conditions that were used are listed in table 1.

Table 1: The primer sequences and PCR conditions

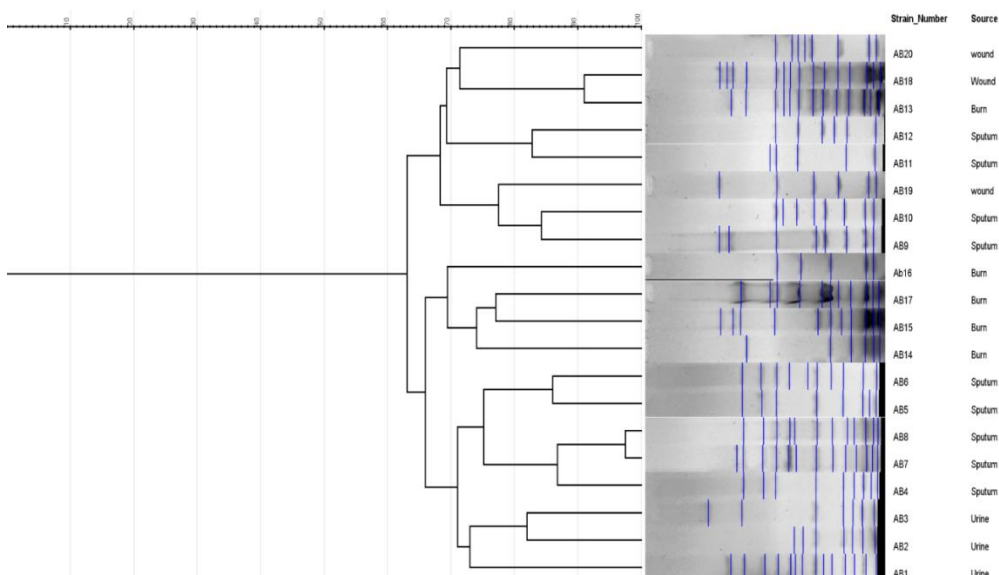
| Genes Name   | Primer sequence ( 5'- 3')    | PCR Condition                                                                                   | Ref. |
|--------------|------------------------------|-------------------------------------------------------------------------------------------------|------|
| <b>BOX</b>   | (5'CTACGGCAAGGCGACGCTGACG3') | 94°C for 5 min<br>94°C 1 min , 35 cycle<br>40°C 2 min,<br>72°C for 2 min<br>72°C for 10 min     | (13) |
| <b>ERIC1</b> | 5'TGTAAGCTCCTGGGATTAC 3'     | 94°C for 5 min<br>95°C ,1 min, 35 cycles<br>52°C for 1 min<br>72°C for 5 min<br>72°C for 10 min | (11) |
| <b>ERIC2</b> | 5' AAGTAAGTGACTGGGGTGAGCG3'  |                                                                                                 |      |

### Results

During the present study period extended from October 2021 to January 2022, a total of (150) clinical specimens were collected from patients who were attended to two main hospitals in Hilla city: Al-Imam Al-Sadiq Hospital and Babil Teaching Hospital suffering from different infections such as burns, wounds, respiratory infections ,and urinary infections. All clinical specimens were cultured on different media. The results indicated that 22 (14.6%) of the isolates belonged to *A. baumannii* It was found that twelve isolates (54.5%) from sputum, five isolates ( 22.72%) from burns ,three isolates (13.63%) were detected from wounds, , and two isolate ( 9%) from urine.

### Detection of phylogenetic diversity of *Acinetobacter baumannii* isolated from different samples by (ERIC)-PCR

ERIC -PCR finger printing grouped *A. baumannii* strains isolated from different specimens and from the same period of isolation and location. Among the (20) collected strains of *A. baumannii* the 20 strains were grouped in 7 clusters (clusters 1-7) and one unique type as shown in finger ( 1 )

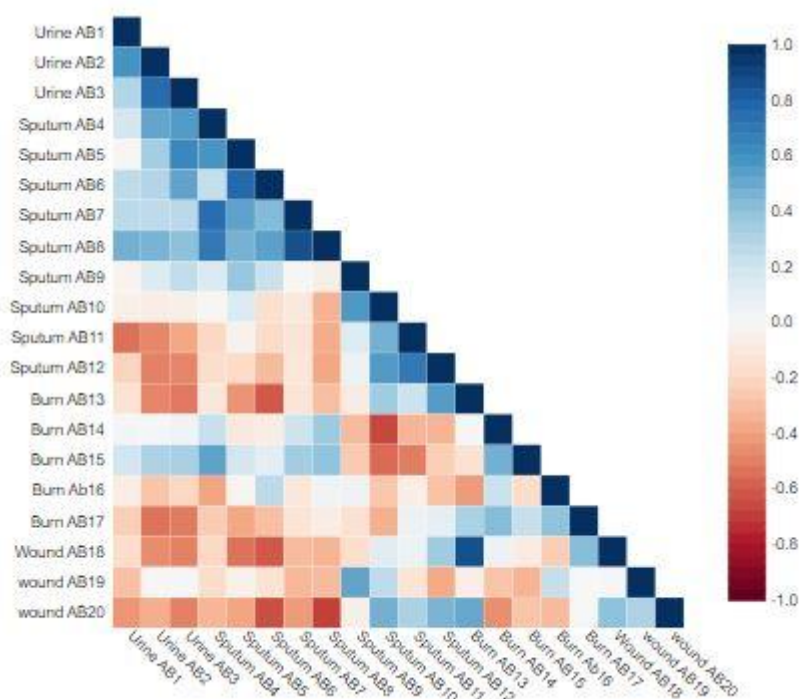


**Fig (1) The ERIC-PCR-derived cladogram representing the relationship among 20 of *A. baumannii* in patients with different sample sources. Bar (above) represents the distance values. This cladogram was generated by Unweighted Pair Group Method with Arithmetic mean (UPGMA).**

In this study, the ERIC Primer showed DNA polymorphism among *A. baumannii* isolates from different clinical sample, either in the occurrence of amplified fragment or in the variable genetic similarities of each isolates with the other. The diversity could be due to the fact that they all were obtained from different sources, or due to the genetic instability of *A. baumannii*.

The first cluster (1) contained (3) isolates obtain from urine, the second cluster included 3 isolates obtain from sputum, the third cluster contain 2 isolates collected from sputum the fourth cluster contain 3 isolates obtain from burn samples,the fifth cluster contain 5 isolates obtain how 2 sputum and one you wound,The sixth cluster contain two isolates obtain from sputum and finally the seventh cluster contain, 3 isolates, two isolates obtain from wound and one from burn.

In the present study the genotyping of clinical *A. baumannii* isolates by ERic-PCR identified (20) distinct genetic profiles with different similarities value ranging from (20-80)% as shown in Fig. (2):



**Fig. (2): A Heat map represent the Genetic correlation similarity matrix among the studied 20 of *Acinetobacter baumannii* strains using ERIC-PCR assay.**

According to the results, this method indicated genetic diversity and heterogeneity among clinical isolates. Bacteria genome containing repeat sequence such as the ERIC sequence can be used as molecular biological tools to assess the Clonal variability of many bacteria isolates (11)

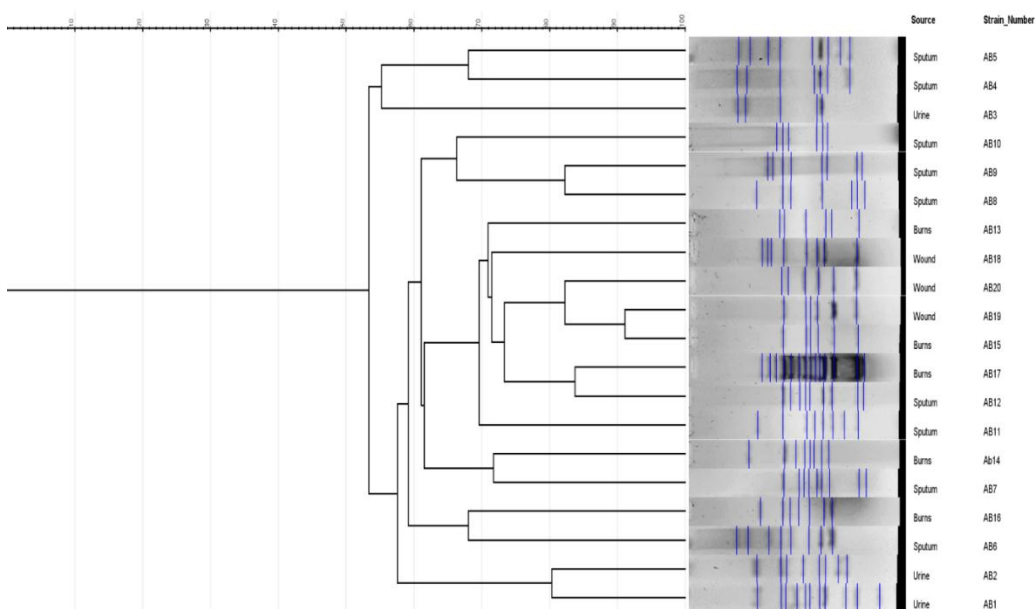
ERIC - PCR finger printing is one of the fastest molecular typing techniques to differentiate between *A. baumannii* and other strain of Gram negative bacteria responsible for hospital-acquired infections. ERIC- PER focused on the ability to discriminate between strains of the same or closely related species and also able to discriminate between members of different species. also the result of ERIC-PCR may play a role as a bacterial identification tool, providing more sensitive typing results than basic phenotypic typing methods (14)

By using ERIC-PCR fingerprinting genotype analysis (12) found that 51 strains of *A. baumannii* were clustered into seven groups, while the remaining 8 were single strains. The genetic relatedness of *A. baumannii* isolated from admitted patients was high, indicating cross-transmission within the hospitalized patients. Several studies have reported to identification of profiles in *A. baumannii* isolates as (15) who identified 14 ERIC-PCR patterns among 80 isolates; and (16) who identified 20 ERic-PCR types among 75 isolates.

ERIC-PCR are valuable typing methods for non- fermentative gram-negative bacilli, and has pivotal role in understanding the essential mechanisms of *A. baumannii* infecting and discovering the relationship between bacterial species, also this method provides great potential to study bacterial Sequences because the sequences are longer and do not base on a specific region of the genome (17)

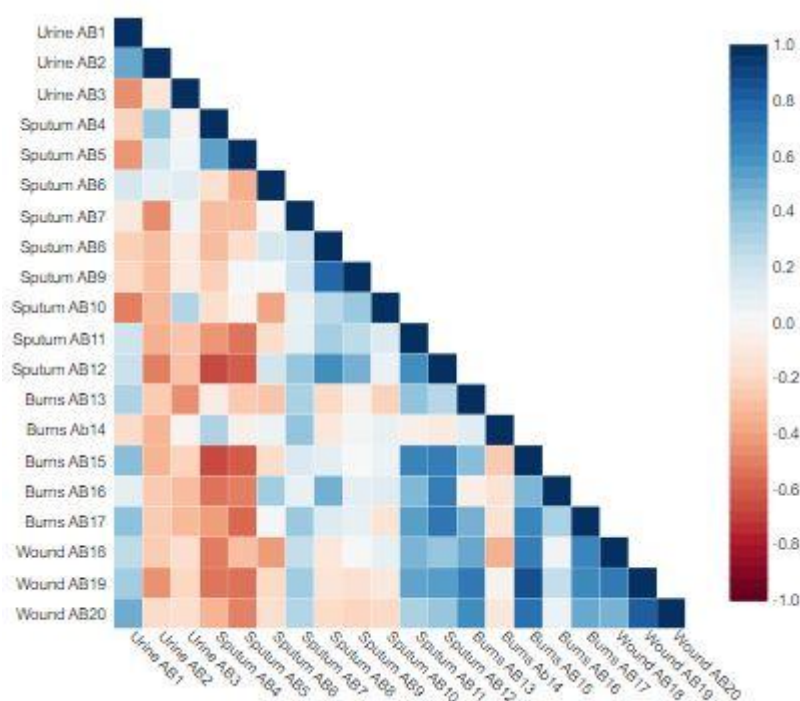
### Detection of Box- element fingerprint in *A. baumannii*

By using Box primer ,*A. baumannii* performed different DNA patterns with amplicons that gave a polymorphic band varied in size from (100-3000) bp, the finger printing Patterns of the isolates were shown 15 bands on gel Electrophoresis as shown in figure(3)



**Fig. (3): The Box-PCR-derived cladogram representing the relationship among 20 of *Acinetobacter baumannii* in patients with different sample sources. Bar (above) represents the distance values. This cladogram was generated by Unweighted Pair Group Method with Arithmetic mean (UPGMA).**

The data from Box Dendro gram of *A. baumannii* isolates were grouped into two main group, and among (20) collected strains of *A. baumannii*., the strains were grouped into 7 clusters, while the group A contain one subgrouped, while group B contain 6 subgrouped and 3 unique isolates as shown in figure (4 )



**Fig. (4): A Heat map represent the Genetic correlation similarity matrix among the studied 20 of *Acinetobacter baumannii* strains using Box-PCR assay.**

Most of the box sequences were encounter in close proximity to genes, suggesting there potential role as a regulatory element controlling coordinate virulence or competence - related gene expression, Box elements are the key element in adaptive bacterial evolution(18). Study done by (19) found that among the 20 *A. baumannii* isolates, the results showed the genetic relationship between *A. baumannii* clones, while 18 isolates contained different genotyping. It was found that in spite of differences in the location and isolation sources of these isolates, a clear clonality was observed.

Genotyping method is a useful for detecting vector strains as well as identifying the epidemic between isolation and genetic relationships among isolates (20) The heterogeneity difference may demonstrate of environmental factor and the level of hospital hygiene on the distribution and genetic clonal formation variation. Studies was demonstrate that Box - PCR method is a highly differentiated Power in study clinical isolation in the same genetic group indicating the transmission of pathogens from the hospital environment to patients as well as the distribution of pathogens in hospital environment (19) The different similarity in isolates in the same site of isolation due to they are genomically different from each other which makes them distinguishable from one another. The differences in similarity ratios between subspecies of the same species may be due to the different sources of sampling as well as possible mutations that result in genetic variation over time (9).

## Conclusion

Molecular techniques such as BOX-PCR and ERIC-PCR are very useful for identifying *A. baumannii* and for studying the genetic linkages of clinical isolates of *A. baumannii* in hospital acquired infection

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