BOX- Genotyping of Carbapenem Resistant *Acinetobacter baumannii* Clinical Isolates from Diyala/Iraq

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Abstract

The aim of study is to determine the typing of Carbapenem-resistant *A. baumannii* clinical isolates from some hospitals in Diyala by the BOX-PCR technique. This study was carried out during the period from February 2020 to the end of September 2020. Out of 400 specimens, 53 isolates of *Acinetobacter baumannii* were recovered. All isolates were tested toward the different class of clinically important antibiotics (12) by using agar diffusion method. The results of resistance were as following: piperacillin 53%, Ticarcillin/ clavulanic acid 75%, cefotaxime 94% and ceftazidime 66%, cefepime 53%, gentamicin 84%, amikacin 49%, ciprofloxacin 47%, levofloxacin 38%, Aztreonam 87%, Imipenem and Meropenem 38%. Twenty (20) isolates of *Acinetobacter baumannii* were found to be resistant to carbapenems. The BOX–PCR method was used to detect the relationship among these carbapenem resistant isolates, the fingerprinting patterns of the isolates were shown 15 bands on gel electrophoresis, with molecular weight ranging between (100-3000 bp) among the 20 carbapenem resistant *Acinetobacter baumannii* isolates. Genetic relationship of different isolates of *Acinetobacter baumannii* was done by using BOX-PCR method and Dendogram analysis. The results showed the genetic relationship between *Acinetobacter baumannii* 4 clones, while 12 isolates contained different genotyping. In conclusion, the present study showed that the BOX-PCR technique is reproducible, easy, fast and cost effectiveness tool for investigating the genetic diversity of *A. baumannii* isolates.

Keywords: *Acinetobacter baumannii, BOX- PCR Method, Genotyping, Carbapenem Resistant.*

Introduction

Acinetobacter baumannii bacteria is a coccobacilli Gram negative bacterial species, aerobic, non-motile, not composed of spore, which is oxidase test negative and catalase test positive. *A.baumannii* is an emerging nosocomial pathogen causing a variety of health care-related infections, and it is associated with a significant morbidity and mortality [1]. This bacteria causes a wide range of nosocomial infections, such as ventilator-associated pneumoniae (VAP), surgical site infections, secondary meningitis, respiratory tract inflammation, wound infection, and urinary tract infection (UTI) [2]. Following the prevalence and recurrence of *A.baumannii* associated infections that occur in different wards of the hospital, such as the intensive care units (ICU), and since the strain associated with the outbreak can originate from different regions and locations [2,3], therefore, find the source of infection by different molecular methods are very important [4]. *A. baumannii* is often resistant to multiple antibiotics by upregulating or acquiring resistance determinants [5]. Carbapenem-resistant *A. baumannii* (CRAb) is considered an especially major public-health threat due to associated treatment difficulties. Outbreaks of CRAb were reported worldwide [6], and in many instances they were caused by a few international clonal lineages of *A. baumannii* [7].

Surfaces in hospitals are generally divided into densely and sparsely contaminated ones [8]. Objects in the first group, e.g. doorhandles, computer keyboards and control panels, patient charts,bed frames, are the most critical ones, as they are frequentlytouched by medical and nursing staff subsequent to patient contacts [9]. *A. baumannii* was shown to be able to survive in hospitalenvironments for prolonged periods, contaminating patient careitems, as well as non-patient care areas. Hence, these inanimatesurfaces often serve

as intermediaries in spreading microorganismsfrom one patient to another [6].

Acinetobacter baumannii bacteria has been classified in the American Society of Infectious Diseases as one of the most antibiotic-resistant microorganisms around the world that have the ability to resist many antibiotics, including cephalosporin and anti-penicillin wide-spectrum, anti-fluoroquinolones and anti-aminoglycoside[10]. Typing of bacterial isolates is an important process in the diagnosis, treatment and epidemiological research. Current methods of typing bacterial isolates can be classified into two main categories: phenotyping and genotyping. Genotyping, which refers to differences in bacterial isolates based on their genetic content, has recently been widely used for typing of bacterial isolates [11].

The method of genotyping has become a pattern for understanding the essential mechanisms of infection and the relationship between bacterial strains and their geographical spread in the field of infection control. It is an important tool in determining the source of infection in hospitals. As well as genotyping acts to differentiate between bacterial isolates on the basis of genetic content [12]. There are several methods of genotyping that are important in the finding of genetic kinship between bacterial isolates, BOX-PCR is the easiest to perform and less expensive.The BOX method is a useful method of epidemiological studies of many species of Gram positive and negative bacteria [11,12] . Therefore, due to the lack of comprehensive studies in this field, this study aimed to determine the typing of Carbapenem-resistant *A. baumannii* clinical isolates from some hospitals in Diyala by the BOX-PCR method.

Materials and Methods

Isolation and Identification of Bacterial Isolates

All clinical samples were collected forms wounds ,urin , blood and burns infection from different hospitals in Baquba/Diyala, Iraq during the period of beginning of February 2020 to the end of September 2020.The isolates were identified by culturing all sample on MacConkey agar, blood agar and also used biochemical tests including oxidase and catalase tests and further identification were done by using Vitek 2 system (BioMerieux, France) [13].

Antibiotic Susceptibility Testing

To estimate potential resistance of *Acinetobacter baumannii* isolates against 12 items of antibiotics from different classes, all isolates had been subjected to antibiogram test according to (CLSI-2017)[14], for Pipracillin, Ticarcillin/clavulanic acid, Cefotaxime, Ceftazidime, Cefepime, Ciproflxacin, Levofloxacin, Gentamicin, Amikacin, Aztreonam, Imipenem and Meropenem.The isolates were initially screened for carbapenems susceptibility by disk diffusion method using imipenem, and meropenem (10 µg each) antibiotic disks. Results were interpreted according to guidelines recommended by CLSI guidelines.

DNA Extraction

Genomic DNA was extracted from bacterial isolates using extraction Kits of Genomic DNA, Purification depending on instruction of manufacturing company (Promega USA). The DNA concentration was mishearing by used Quantus Florometer (Promega, USA).

BOX-PCR

The *A.baumannii* isolates were fingerprinted by BOX-PCR using BOX primer (52–CTACGGCAAGGCGACGCTGACG –32) which produces a PCR product with variable bands (bp) [15]. Amplification was performed with a 25µL reaction mixture that consisted of 5µL GO Taq Green Master Mix), 5µL template DNA, 4 µL Primer (10 pmol/µL) and 11µL deionized sterile D.W. (Promega USA). PCR reaction tubes were transferred into thermal cycler that was programmed as following: initial denaturation for 5 mints at 95 °C, (the conditions for each cycle were: 1 min. at 94 C̊, 30 sec. at 46 °C and 1min. at 72 °C), and final extension at 72 °C for 5 mints. Amplified PCR products were detected by agarose gel electrophoresis using a 1% agarose gel in TBE buffer (Promega, USA) with 5µL Ethidium bromide (10mg/mL) (Promega, USA), at 100 vol./ cm2 for 90min. The DNA bands were visualized and photographed under UV light. The size of the products was analyzed in comparison to a MW100-3000 bpDNA ladder (Promega, USA) [16].

Statistical Analysis

Dendogram analysis was done by using PAST program (version 0.45) to determine the genetic

relationship between all carbapenem resistant *A.baumannii* isolates.

Results and Discussion

Results of distribution of different clinical isolates showed that most isolates obtained, were from burns (49%) while other isolates distributed among wound 13(24.5%), isolates from blood 9(17%) , and the last 5 (9.5%) isolates from urin (Table 1). Out of 400 clinical samples, only 53 isolates (13.25%) were belonged to *A. baumannii*.

Table(1): Distribution of *A. baumannii* isolates among different clinical samples

Type of Clinical Sample	No. (%) samples	No. (%) of A. baumannii isolates
Burn swab	100 (25%)	26(49%)
Wound swab	100 (25%)	13(24.5%)
Blood	100 (25%)	9(17%)
Urin	100 (25%)	5(9.5%)
Total	400 (100%)	53(13.25%)

Antimicrobial sensitivity test

Fifty three *A. baumannii* isolates were screened for their resistance to 12 different types of antibiotics. Results in table (2) show that isolates varied in their resistance to the antibiotics. It was found that 53% and 75% of the isolates were resistant to piperacillin and ticarcillin-clavulanic acid,. The rates of resistance to the cephalosporins were as follows: cefotaxime 94%, ceftazidime 66% and cefepime 53% respectively.

Table (2): Antibiogram susceptibility of *A. baumannii* isolates toward antimicrobial agents

Antibiotic	Resistant isolates No. & %
Pipracilin	28(53%)
Ticarcillin/clavulanic acid	40(75%)
Cefotaxime	50(94%)
Ceftazidime	35(66%)
Cefepime	28(53%)
Ciprofloxacin	25(47%)
Levofloxacin	20(38%)
Gentamicin	45(84%)
Amikacin	26(49%)
Aztreonam	46(87%)
Imipenem	20(38%)
Meropenem	20(38%)

Aminoglycosides and quinolones resistance were variable among *A.baumannii* isolates; 84% to gentamicin and 49% to amikacin, however, resistance to quinolones ciprofloxacin was detected as 47% and levofloxacin 38% among isolates.Resistance to monobactam was high when 87% of isolates being resistant to aztreonam. Results revealed that 20 *A.baumannii* isolates (37%) were found to be carbapenem (imipenem and meropenem) resistant.

BOX Fingerprinting

Initially, the genetic variability of 20 carbapenem resistant *A. baumannii* strains tested were investigated with the BOX-PCR method described previously [17]. The BOX-PCR genotyping method was used to detect the relationship between isolates of carbapenem resistant *A. baumannii* that isolated from burns cases, wonds, blood and urin. The genetic fingerprint 15 bands with molecular weights ranging from (100-3000) bp between the isolates of carbapenem resistant *A.baumannii* 100 bp, 200 bp, 300 bp, 350 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 950 bp, 1000 bp, 1500, 2000 bp, 3000 bp the percentage were 10%,5%, 20%, 15%, 30%, 50%, 55%, 65%, 45%, 40%, 60%, 50%, 30%, 10% at respectively as shown in table (3). Two main groups (**A** and **B**) were observed. BOX-PCR typing showed 4 groups of genotypes and 10 unique isolates. In spite of differences in the location and isolation sources of these isolates, a clear clonality was observed, which indicated the epidemiology of these isolates figure (1). The results showed a genetic affinity between four groups of carbapenem resistant *A. baumannii* isolated from various sources from several hospitals in Diyala, while 12 isolated had a different genotype as illustrated in figure (1). This agreed with another study found a genetic affinity between four groups of *A. baumannii* isolated from various sources from several hospitals in China [18].

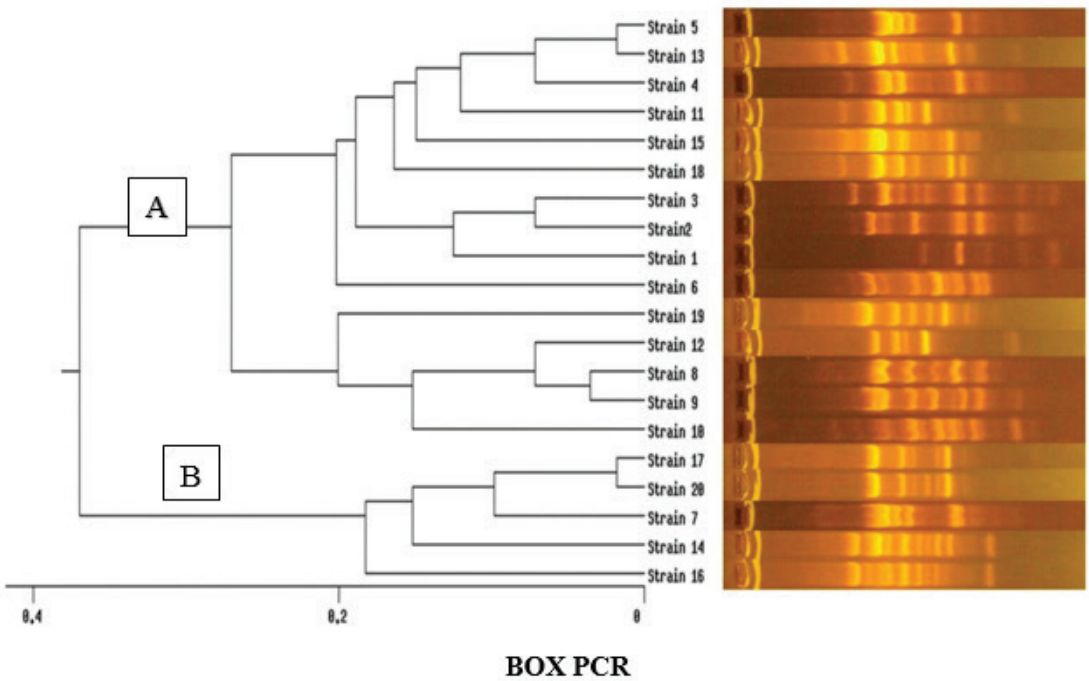

Figure (1). BOX-PCR generated dendrogram showing genetic relatedness of 20 *Acinetobacter baumannii* isolates.

Genotyping method is a useful for detecting vector strains as well as identifying the epidemic between isolation and genetic relationships among isolates and can be categorized into different groups using genotyping methods. This *A. baumannii* heterogeneity differences in several studies from different parts of the world, demonstrate the impact of environmental factors and the level of hospital hygiene on the distribution

and genetic clonal formation variation. As the results demonstrated, our studied carbapenem resistant *A. baumannii* clinical isolates were genetically diverse and heterogeneous, suggesting that multiple subtypes of the species are involved in infection. Moreover the findings of present work suggest that genotyping by BOX-PCR, may play an important role in routine epidemiological surveillance, and in the identification of the source of transmission of *A. baumannii* in the hospitals.Many studies was demonstrated that BOX-PCR method is a highly differentiated power in the 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 the hospital environment [19].

Table (3): Molecular weight and percentages of Box Bands.

Band	Molecular weight (bp)	No. of isolate & percentage %
BOX1	100	2(10%)
BOX2	200	1(5%)
BOX3	300	4(20%)
BOX4	350	3(15%)
BOX5	400	6(30%)
BOX6	500	6(30%)
BOX7	600	10(50%)
BOX8	700	11(55%)
BOX9	800	13(65%)
BOX10	900	9(45%)
BOX11	950	8(40%)
BOX12	1000	12(60%)
BOX13	1500	10(50%)
BOX14	2000	6(30%)
BOX15	3000	2(10%)

Additionally the results of study by Al-Shwalkh *et al*. (2018)[20] who found a genetic affinity between the isolates of *Pseudomonas aeruginosa* used the method of BOX-PCR, and identified 16 genotypes and molecular weights ranging from (140-1000) between isolates of *Pseudomonas aeruginosa* isolated from different sources. Finally, the results of current study in agreement with Abdulla (2020)[21] who found a genetic affinity between the isolates of *Escherichia coli* used the method of BOX-PCR, and identified 12 genotypes and molecular weights ranging from (150-1500) between isolates of *Escherichia coli* isolated from different sources .

Conclusions

High isolation rate of carbapenem resistant *A.baumannii* (CRAB) was detected in this study. In conclusion, the present study showed that the BOX-

PCR technique is reproducible, easy, fast and cost effectiveness tool for investigating the genetic diversity of *A. baumannii* isolates.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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