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Sequencing Analysis of *cvaC* Gene in *Acinetobacter Baumannii* That Isolates from Different Infections

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Abstract

Acinetobacter baumannii is one of the opportunistic nurses responsible for many acquired infections in hospitals due to its ability to resist many antibiotics. This is one of the problems facing hospitals in the world. Identification of the *cvaC* gene and sequence analysis in *Acinetobacter baumannii* isolates from various infections, and mutation detection in this gene. From 1st of September to 30th of November, 2016, 200 *Acinetobacter baumannii* isolates were obtained from various clinical samples. Follow fifty isolates from blood, twenty isolates from urinary tract infections, thirty isolates from wound infections, forty isolates from burn infections and twenty-five isolates from stool samples from various hospitals (Central Children's Hospital, Al Karama Hospital, Karkh General Hospital, Al-Ameen Medical City Hospital, Educational Labs, Baghdad Teaching Hospital, Child Protection Hospital, Burns and Wounds Hospital) in Baghdad city. Identification forty isolates confirmed that they belong to *Acinetobacter baumannii*, including fourteen isolates from a stool sample, nine isolates from blood, eight isolates from burns, four isolates from wound infections, and Respiratory tract infection, , and only one isolate from urinary tract infections sample. Genotypic detection of the *cvaC gene* of *Acinetobacter baumannii* showed the presence of this gene in 16 isolates (40%) and Sequencing analysis of *cvaC* has shown seven genetic mutations and only one mutation has been converted amino acid Alanine to Valine. The amino acid Alanine was changed to Valine in Position 656 at Subject 678, resulting in silent mutations that did not affect protein translation and other mutations that resulted in a change in the amino acid arrangement and protein translation.

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1. Introduction

Acinetobacter baumannii is a Gram-negative, non-motile, strict aerobic, non-fermented lactose, coccobcilli, catalase-positive, and oxidase-negative [1,2]. *Acinetobacter baumannii* is found in nature and has been isolated from various environmental sources, including soil, water, sewage, the surfaces of living things, and medical settings. It is known for its capacity to build biological membranes [3,4]. These opportunistic bacterial pathogens cause many opportunistic infections in hospitals, including pneumonia, burns infection, wound infection, septicemia, endocarditis, and urinary tract infection.

Many of the virulence characteristics that help bacteria because disease is present in these bacteria, including capsules, biofilm development, loads the bacteria that benefit the biofilm, and protects the creature from unusual natural pressure, such as anti-infection chemicals and cleansers [5] and the production of enzymes such as lipase and protease [6,7]. *A. baumannii* is one of the opportunistic nurses responsible for many acquired infections in hospitals due to its ability to resist many antibiotics. This is one of the problems facing hospitals in the world and comes after *Pseudomonas aeruginosa* was isolated from hospital environments [8, 9, 10, 11].

Colicin is a protein produced by most *Escherichia coli* strains that affect bacteria closely related to *E. coli*. Colicin is found in members of the Enterobacteraceae family, including *Shigella* and *Citrobacter* [12]. Colicin is divided into several types A, B, D, V, and I [13]. Colicin V is a peptide antibiotic produced by members of the Enterobacteraceae family and *A. baumannii* that kills bacteria of other species. Colicin is encoded by four genes: *cvaA, cvaB, cvaC, cvaI*, and *cvaC*. Colicin, which is made up of 103 amino acids, is produced by these genes. Colicin V is a bactericidal that kills sensitive cells by altering membrane potential [14].

The mechanism of action depends on the presence of specific receptors present in or on cells sensitive to Colicin. There are different varieties of Colicin; for examples Marscin, or the term Proteuocin if Colicin is produced from *Proteus spp*. [15, 16, and 17].

This study aimed to detect the cvaC gene and sequences analysis of the cvaC gene in *A. baumannii* and detect the mutations in this gene and their effect on protein synthesis.

2. Experimental details

2.1: Collection of samples and identification of bacterial isolates

A. baumannii isolates (200) were collected from different clinical samples (blood, stool, wounds, burns, and urinary tract infections) from several hospitals in Baghdad (Central Children's Hospital, Al Karama Hospital, Karkh General Hospital, Al-Ameen Medical City Hospital, Educational Labs, Baghdad Teaching Hospital, Child Protection Hospital and burns and Wounds Hospital) during the period from 1st Sep. to 30th Oct.2016 The samples were cultured separately on MacConkey agar and blood agar then identified by using biochemical tests including oxidase and catalase tests. Conformation of identification was done by using the API20E system and VITEK-2 system [16].

2.2: Extraction of DNA

According to the manufacturer's instructions, DNA from bacterial isolates was extracted using a DNA kit (Geneaid Biotech kit system, UK).

2.3: Preparation of *cvaC* primers

The stock solution of primers was prepared according to the instructions of the manufacturer (Alpha DNA Canada) mentioned in Table (1) using sterile distilled deionized water to obtain a concentration of 100 pmol/µl. The solution of each initiator was present separately at 10 pmol/µl by adding 10µl from each stock solution to 90 µl of D.W. and mixed well with Vortex mixture, keeping with stock solutions at 20°C and mixing the initiator solution after removing it from ice using the Vortex carburetor prior to use.

Table (1): Nucleotide sequence of the primer used for detection *cvaC* gene.

Genes		Sequencing Primer sequence (5'—— 3')	Product size (bp)	Sours
cvaC	F	CACACACAAACGGGAGCTGTT	680	[18]
	R	CTTCCCGCAGCATAQTTCCAT		

The PCR polymerase reaction mixture for ***cvaC*** gene was prepared as shown in Table 2.

Table (2) PCR mixture of *cvaC* gene.

No.	PCR mixture	Volume (µl)
1	F-primer	1
2	R-primer	1
3	Template DNA	2
4	Nuclease Deionized nuclease-free water	6
5	GO Taq green master mix	10
Total		20

The contents of the PCR tubes were mixed well using the Vortex then placed in a PCR thermal cycler, as shown in Table (3).

Table (3): Optimal conditions of PCR reaction for determination of *cvaC* gene.

Step	Program	
1	Only one cycle for 5 minutes at a temperature of 94 ° C for the primary DNA denaturation.	
2	30 cycles included:	
	A	50 sec at 94 ° C for DNA template denaturation.
	B	45 sec at 55 °C for the primers to bind to DNA template annealing at 58 °C for 60 sec.
	C	45 sec at 72 ° C for the associated primers to be elongated.
3	Only one cycle for8 minute at 72 ° C for the final elongation of the double DNA strip.	

2.4: Electrophoresis of PCR products

The PCR products (5 µl) were electrophorized on 2% agarose gel with 5µl Ethidium bromide, at 100 vol. for 80 min. The DNA bands were visualized and photographed under UV light [19]**.**

3. Results and discussion

3.1: Identification

Fourteen isolates from stool, nine isolates from blood, eight isolates from burns, four isolates from wound infections and respiratory tract infections, and only one strain from urinary tract infections were identified as *Acinetobacter baumannii*.

3.2: Genotypic detection

Acinetobacter baumannii cvaC gene genotyping revealed that 16 isolates (40%) were *Acinetobacter baumannii* and had *cvaC* genes with a molecular weight of 680 bp. Figure (1). This result was consistent with a study by [17], which discovered that 17 isolates (34%) carried the *cvaC* gene. In addition, ColicinV, a peptide antibiotic that inhibits bacteria by disrupting the cell membrane and becomes a bacterium killer when it enters the inner membrane of bacteria during perplasma membranes, was formed by the *cvaC* gene [14, 20].

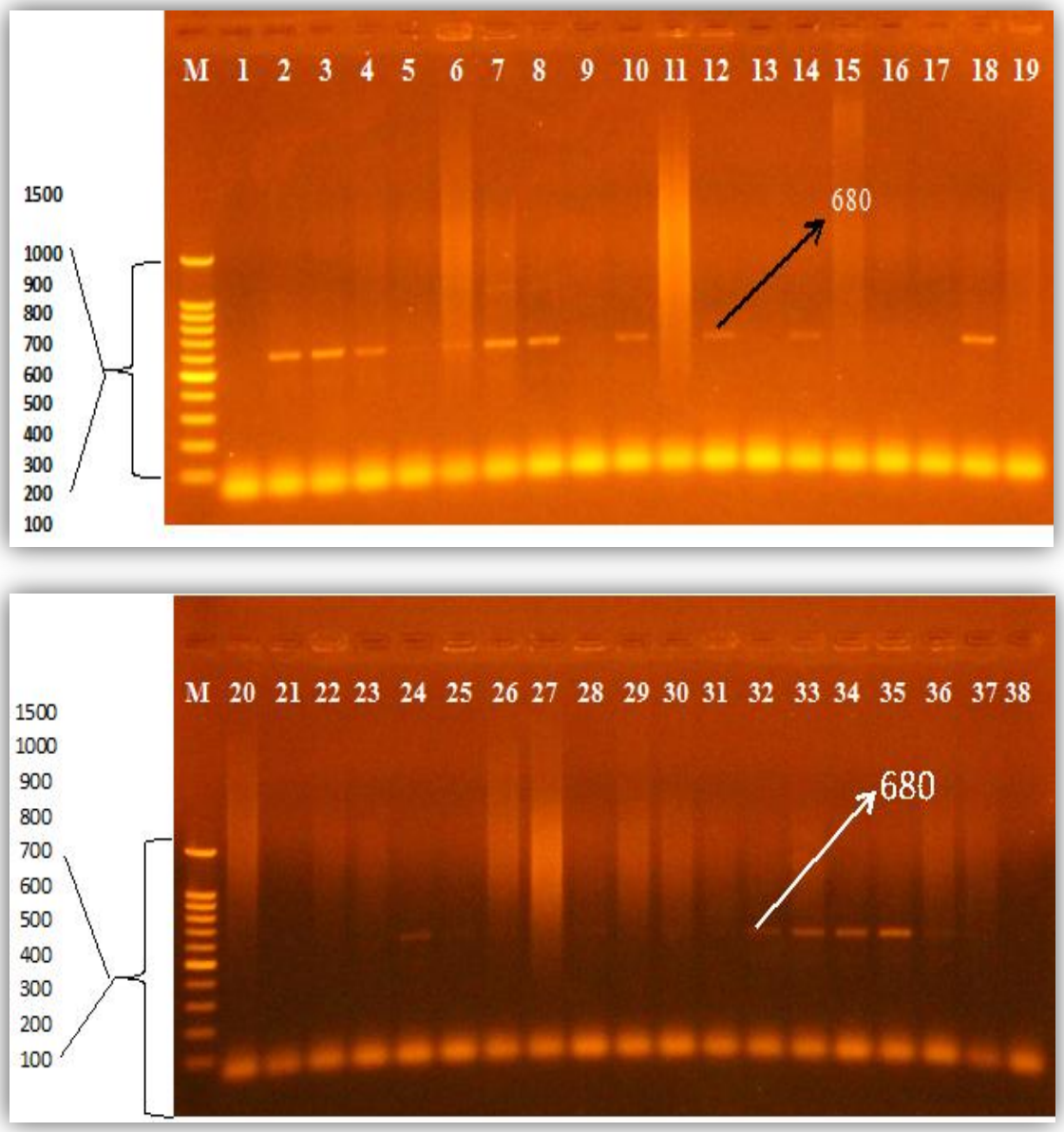

Figure 1: Electrophoresis of PCR product for detection cva C gene (680 bp) in A. baumanniii isolates on 2% agarose and 100 volts for 80 min. The M ladder (100-1500 bp); the 2, 3, 4, 6, 7, 8,10,12,14, 18, 24, 32, 33, 34, 35 and 36 were positive isolates.

3.3: Sequences analysis

Sequences analysis of *cvaC* gene in *A. baumannii* isolates (A7 isolate from stool and A12 isolate from blood samples) were done. The results showed that there are seven genetic mutations include: Mutations in Position 231 at Subject 523, in Position 231 at Subject 254, in position 266 at subject 288, in Position 453 at Subject 490, in Position 510 at Subject 547, in Position 596 at Subject 618, in Position 656 at Subject 678.

The changes in Nitrogen bases of their mutation were showed that Guanine replaced thymine at Position 231 at Subject 253 and each Thymine was replaced by Cytosine in different positions and subjects included (Position 232 at Subject 254, Position of 266 at Subject 288, Position 453 at Subject 490 and Position 510 at Subject 547 respectively). While Thymine was replaced with Adenine at Position 596 at Subject 618. Whereas Cytosine was replaced

with Thymine at Position 656 at Subject 678 all the results as shown in Table (4) and figure (2).

Analyzing the mutations' translation results revealed that there were silent mutations that had no effect on amino acid translation in all six mutations. In contrast results revealed that only one mutation changed amino acid translation, changing the amino acid Alanine to the amino acid Valine in position 656 at subject 678. Missense mutations are mutations that result in a change in amino acid in the resultant protein sequence. [21].The change in the sequence of amino acids was due to the loading of another amino acid instead of the original amino acid. The effect of a missense mutation on the resulting protein is determined by the location of the amino acid that has been changed, as follows: Acceptable, partially acceptable, or unacceptable [22].

Table (4): Changes in nitrogen bases and their effect on amino acid translation in the Gene *cva C* from *A. baumanniii* isolates A7 and A 12.

No.	Nitrogen bases	Changes in Nitrogen bases	Position	Subject	Amino acid	Changes in amino acid
1	Thymine	Guanine	**231**	**253**	-	-
2	Thymine	Cytisine	**231**	**254**	-	-
3	Thymine	Cytisine	**266**	**288**	-	-
4	Thymine	Cytisine	**453**	**490**	-	-
5	Thymine	Cytisine	**510**	**547**	-	-
6	Thymine	Adenine	**596**	**618**	-	-
7	**Cytosine**	**Thymine**	**656**	**678**	**Alanine**	**Valine**

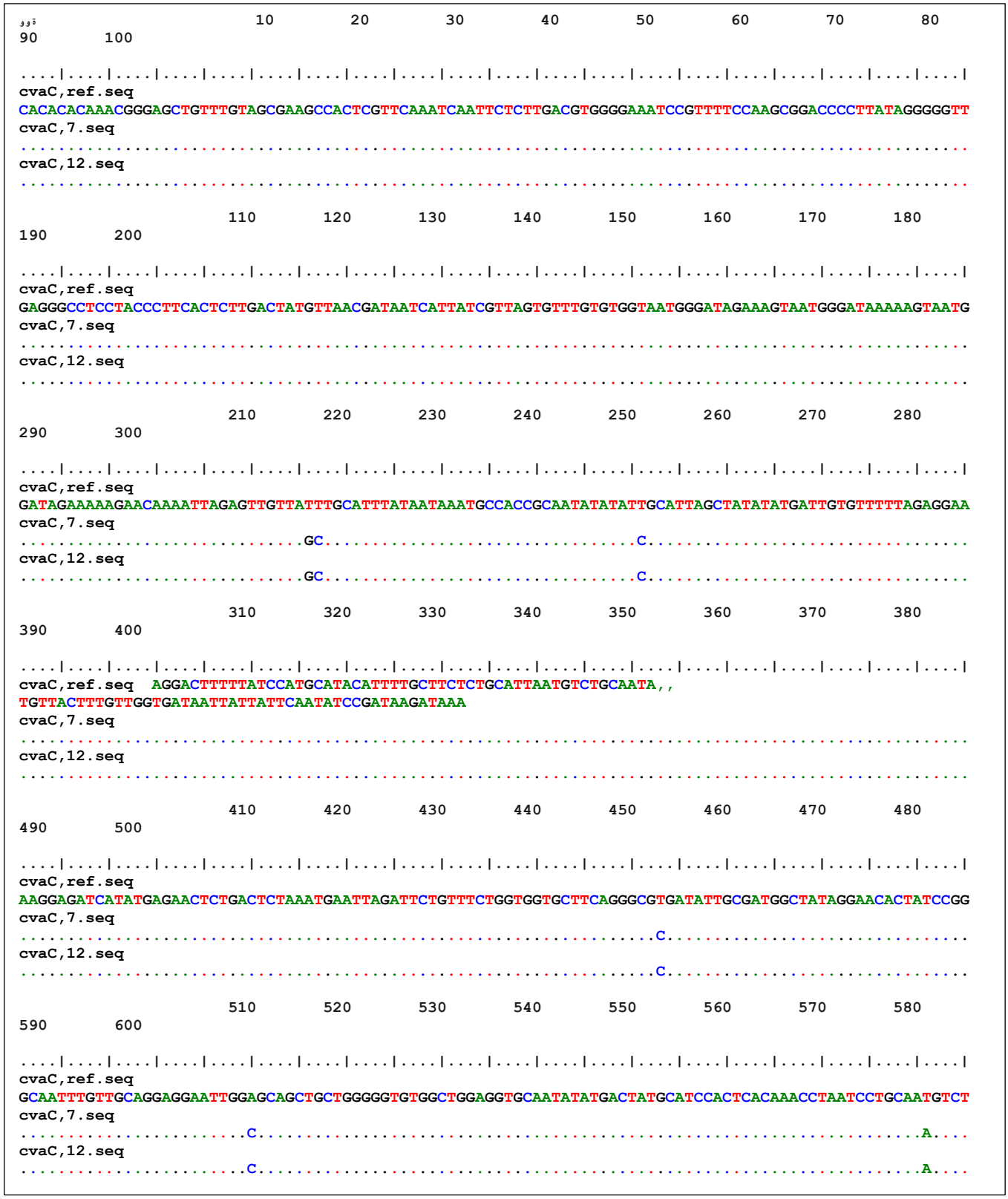

Figure 2: Analysis of the multiple sequences of the reference *cvaC* gene with two changes in *A. baumanii* isolates A7 and A12 using the BioEdit Sequence Alignment Editor Software.

4. Conclusions

Sequencing analysis results of *cvaC* gene showed silent mutations that did not affect protein translation, and only one mutation that led to change in the amino acid Alanine was converted Valine in Position 656 at Subject 678.

Conflict of Interest: The authors declare that they have no conflict of interest.

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