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Characterization of *bla*OXA variants of *Acinetobacter baumannii* isolated from burns and wounds infections in Baghdad, Iraq.

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ABSTRACT

In this study, five isolates (KKG1, KKG2, KKG3, KKG4 and KKG5) of *Acinetobacter baumannii* were selected for analysis of their sequences, these isolates were contained 2 genes (*bla*OXA23 and *bla*OXA24) and only one of the isolates was carried *bla*OXA58, and also all the isolates were borne species-specific gene (*bla*OXA51). The five local isolates were resisting to the antibiotics Imipenem and Meropenem with minimal inhibitory concentrations (MICs) (≥ 16 µg/ml). *Bla*OXA genes sequencing analysis was conducted by using NCBI tools, MEGA6 and BioEdit softwares, and also 21 sequences of these genes were submitted to GenBank. The results confirmed the findings of gel electrophoresis of *bla*OXA genes and detection the presence of some the variants for these genes in our local isolates. Using BLASTn and Phylogeny analysis showed the presence of some variants for *bla*OXA51 such as *bla*OXA69, *bla*OXA98, *bla*OXA107 and *bla*OXA110. *Bla*OXA72 was detected as variant for the gene *bla*OXA24. Several insertion mutations were recorded in *bla*OXA58 gene of the local isolate KKG5. Also, it was found a very high identity and phylogenetic relationship of the studied genes with those in Asian countries such as Iran and China which was found in strains isolated from burns and nosocomial infections. In conclusion, the presence of variations in *A. baumannii* isolates requires the investigation of resistance during the use of antibiotics for the treatment burns infections by carbapenems.

Keywords: *Acinetobacter baumannii, bla*OXA, Variants

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INTRODUCTION

Acinetobacter baumannii has become an important pathogen in hospitals worldwide, also outbreaks and nosocomial infections caused by this pathogen due to multidrug-resistant strains, especially in the intensive care units and burn units [1, 2]. Carbapenems are the drugs of choice for treatment of *A. baumannii* infections, but there was a progress of resistance to this class of antibiotics because of the increasing of clinical use of these antibiotics. High prevalence of Carbapenem-resistant *A. baumannii* has become a globally problem [3, 4]. There are a different mechanisms for carbapenems resistance, including loss of outer membrane protein change of penicillin binding protein, efflux pump, and carbapenem-hydrolyzing enzymes. It was found that the Class D carbapenemase (OXA enzymes) are the main mechanism of resistance in *A. baumannii* [5]. OXAs, which are not inhibited by EDTA and/or clavulanic acid, are subdivided into six families, as follows: the OXA-23-like, OXA-24/40-like, OXA-51-like, OXA- 58-like, OXA-143-like, and OXA-182-like families [6]. Clonal outbreaks of carbapenem-resistant and OXA-23–producing *A. baumannii* have been reported in many countries, such as Bulgaria [7], Brazil [8], Iraq and Afghanistan [9]. Therefore, the aim of this study is Analyzing the sequence of the *bla*OXA genes of *A. baumannii* isolated from burns and wounds infection in some Baghdad hospitals in order to detect the variations of the local isolates.

MATERIALS AND METHODS

Out of our previous studies conducted in Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq. We obtained 96 *A. baumannii* isolates from patients with burns and wounds infections with high prevalence of *bla*OXA-24-like and *bla*OXA-23-like resistance genes among multi-drug resistant (MDR) *A. baumannii* strains in Baghdad hospitals, The five local isolates were resisting to the antibiotics Imipenem and Meropenem with minimal inhibitory concentrations (MICs) (≥ 16 µg/ml) [10, 11].

Five multidrug resistant isolates were selected to conduct the nucleotide sequence of *bla*OXA genes. PCR products for *bla*OXA-23 like, *bla*OXA-24 like, *bla*OXA-51 like and *bla*OXA-58 like genes were detected by agarose gel electrophoresis and the sequencing was carried out using the Applied Biosystem (AB) capillary system (Macrogen Research, Seoul, Korea). PCR products were subjected to direct sequencing, both strands of PCR products were sequenced with an automatic sequencer. DNA sequences were analyzed and similarity searches were carried out with the Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov). The nucleotide sequences of the *bla*OXA genes of *A. baumannii* reference strains reported from different parts of the world (available in public database: GenBank) were downloaded and aligned using the ClustalW method of MEGA6 program. The phylogeny was conducted using UPGMA (Unweighted Pair Group Method with Arithmetic mean) method of the same program.

RESULTS AND DISCUSSION

The nucleotide sequences of *bla*OXA genes (*bla*OXA-51-like, *bla*OXA-58-like,*bla*OXA-23-like and *bla*OXA-24-like) of our local isolates (KKG1, KKG2, KKG3, KKG4 and KKG5) reported in this study have been submitted to the NCBI/GenBank database under accession numbers as showed in Table 1.

Table 1: GenBank accession numbers for the nucleotide sequences of the *bla*OXA genes of the local isolates.

	Accession numbers for the nucleotide sequences			
Isolate code	***bla*OXA51**	***bla*OXA23**	***bla*OXA24**	***bla*OXA58**
KKG1	**LC093477 LC093482**	**LC096086**	**LC100126**	-

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KKG2	**LC093478 LC093483**	**LC096087**	**LC100127**	-
KKG3	**LC093479 LC093484**	**LC096088**	**LC100128**	-
KKG4	**LC093480 LC093485**	**LC096089**	**LC100129**	-
KKG5	**LC093481 LC093486**	**LC096090**	**LC100130**	**LC133497**

Aligning of the obtained sequences with the of reference strains in GenBank confirmed the correct identification of *bla*OXA-23 like, *bla*OXA- 24like, *bla*OXA-51 like and *bla*OXA-58 like genes by PCR. Also, these sequences were analyzed for the presence of variants of these genes, detection the differences in the nucleotides (mutations) and the genetic relationships by using the phylogeny via the phylogenetic tree.

The results of the alignment of the *bla*OXA51 sequences of the local isolate KKG5 with the reference strain TUMS/BTRF/125 with accession number JX305943, isolated from patient in ICU in Iran, revealed the presence of some differences in the nucleotides of our queries in the positions 172, 184, 247, 257 and 286 of the subject as shown in the Figure 1.

Figure 1: Alignment of *A. baumannii bla*oxa51 gene sequence from this study with reference strain *A.baumannii* TUMS/BTRF/125 available in GenBank.

By using nucleotide BLAST (BLASTn) from NCBI database of *bla*OXA51 sequences of the local isolates, it was found that our isolates possessed many variants of the gene *bla*OXA51 resulted from some differences in nucleotides which may lead to change in some of amino acids in the produced enzyme. The most important variants in the current study were *bla*OXA69, *bla*OXA98, *bla*OXA107, *bla*OXA110, *bla*OXA112, *bla*OXA144 and *bla*OXA117. The Figure 2, demonstrated an example of the high identity (99 %) between the nucleotides sequence of *bla*OXA51 gene of the local isolate KKG1 with the *bla*OXA69 gene with accession number (KJ187473) from strain B-300 isolated from nosocomial infection, Russia.

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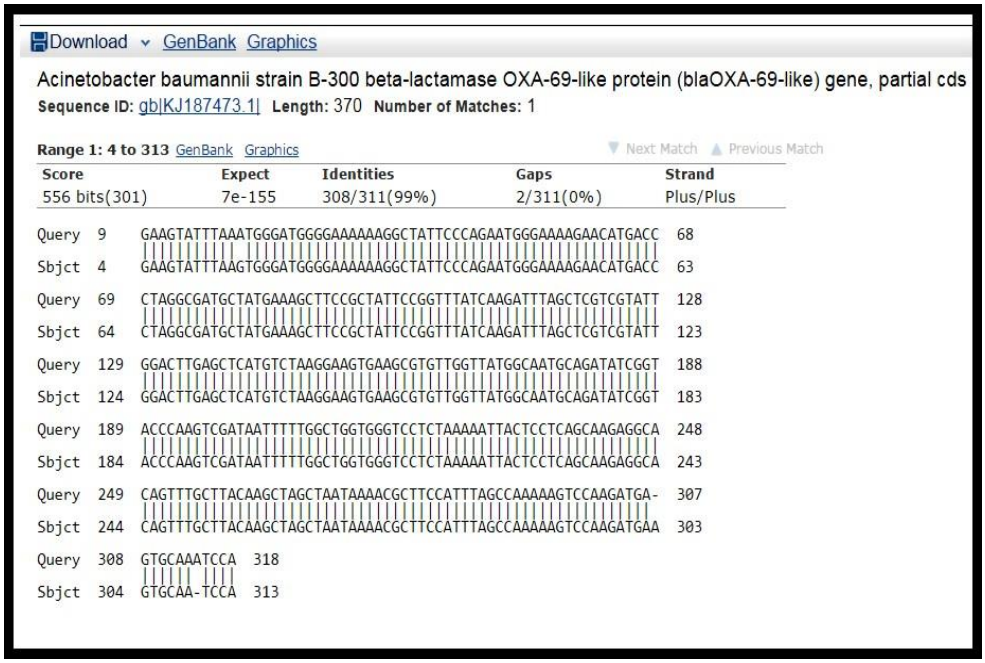

Figure 2: Alignment of *bla*OXA51 gene sequence of the local isolate *A.baumannii* K1 with the *bla*OXA69 gene (accession number KJ187473).

Figure 3, showed the phylogenetic relationships (by using MEGA6 software) among 5 *A.baumannii* isolates obtained in this study (KKG1,KKG2,KKG3,KKG4 and KKG5) and other strains of *A. baumannii* based on partial nucleotide sequence of *bla*OXA51gene identity were placed in the same clusters. All of our isolates were placed in the two clusters, KKG4 and KKG5 in one cluster and the rest isolates in the other. The clusters also included *A. baumannii* species reported from elsewhere.

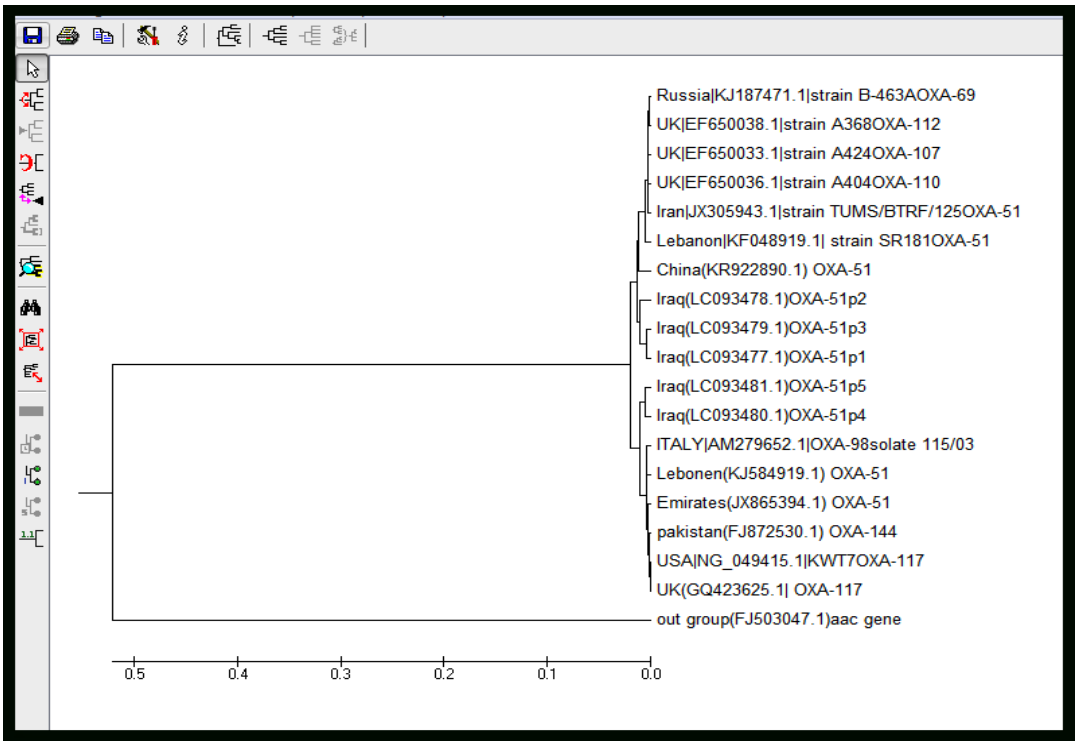

Figure 3: Phylogenetic relationships based on partial nucleotide sequence of the *bla*OXA51 genes of *A.baumannii* local isolates (KKG1 to KKG5). Cluster analysis was based upon the UPGMA (Unweighted Pair Group Method with Arithmetic mean) method. *A.baumannii* strain HZ01 based on gene aac as out group.

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The phylogenetic relationships analysis of the two local isolates KKG4 and KKG5 which had many differences in their nucleotides, demonstrated a high similarity (99 %) with many isolates especially those isolated from ICU and nosocomial infections in Middle East countries, such as Lebanon and Emirates and high identity with the variants *bla*OXA144, *bla*OXA117 and *bla*OXA98. Other three isolates KKG1, KKG2 and KKG3 placed in another cluster, were closely related to the reference strain TUM/ BTRF 125 from Iran, isolates from Asian countries and other variants of *bla*OXA51 such as *bla*OXA69, *bla*OXA107, *bla*OXA110 and *bla*OXA112. Turton *et al*., (2007) [12] indicated presence of the amino acid similarities between the OXA-51-like enzymes which consist of closely related groups, the clusters were surrounding with OXA-66, OXA-69 and OXA-98 and these groups also contained the same or closely related *bla*OXA-51-like gene. Enzymes of the OXA-69 cluster are common, particularly in Eastern Europe, OXA-69 weakly hydrolyses both Imipenem and Meropenem, while OXA-51 weakly hydrolyses Imipenem only [13]. Sequence variations in the OXA-51-like enzymes may be contributed to increase resistance by changing the spectrum of these enzymes. *bla*OXA- 51-like genes sequence analysis demonstrated that regions of these genes are preferentially altered as a result of the selective pressure of antibiotic usage [14].

In *bla*OXA-23-like genes sequencing and by using nucleotide BLAST (BLASTn) from NCBI database of *bla*oxa23 sequences of the local isolates, it was found there are no differences in the nucleotides of our queries and the subjects. The results of multiple sequence alignment revealed very high identity (more than 99 %) of *bla*OXA23 gene of local isolates with the most global isolates and strains specially with the isolates from Asian countries such as Iran, China and Japan as the alignment in Figure 4, which demonstrated 99 % similarity of our sequence query with the subject of *bla*OXA23 gene of *A.baumannii* isolated from burn patient in Iran.

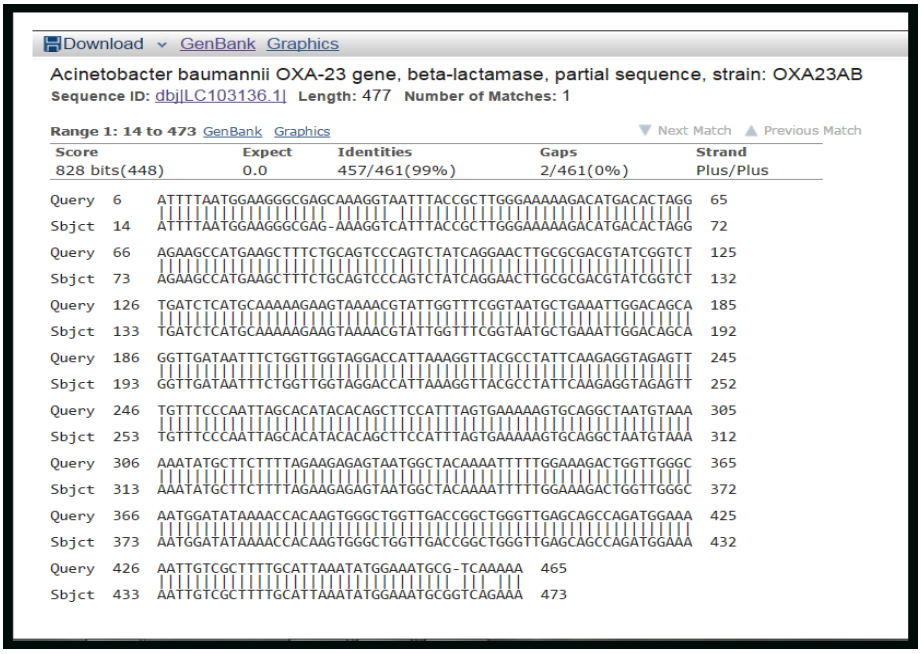

Figure 4: Alignment of *A. baumannii bla*oxa23 gene sequence from the local isolate KKG2 with reference strain *A.baumannii* OXA23AB available in GenBank.

***Bla*OXA24 gene**

As in *bla*OXA23 gene, very high similarity (more than 99 %) was recorded for *bla*OXA24 gene with the most of global isolates especially with the Asian strains such as Iran, China and Thailand as the phylogenetic relationships showed in Figure 5.

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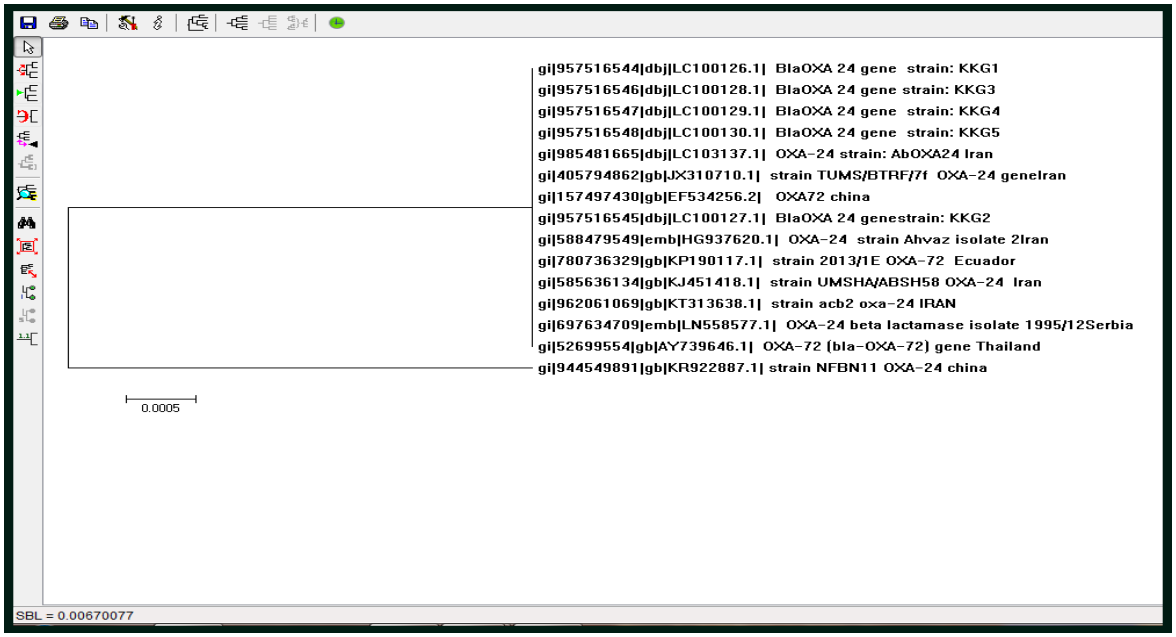

Figure 5: Phylogenetic relationships based on partial nucleotide sequence of the *bla*OXA24 genes of *A.baumannii* local isolates (KKG1 to KKG5).

The results indicated to presence of the variant *bla*OXA72 in the local isolates in our hospitals as one of the important variant of this gene (Figure 6).

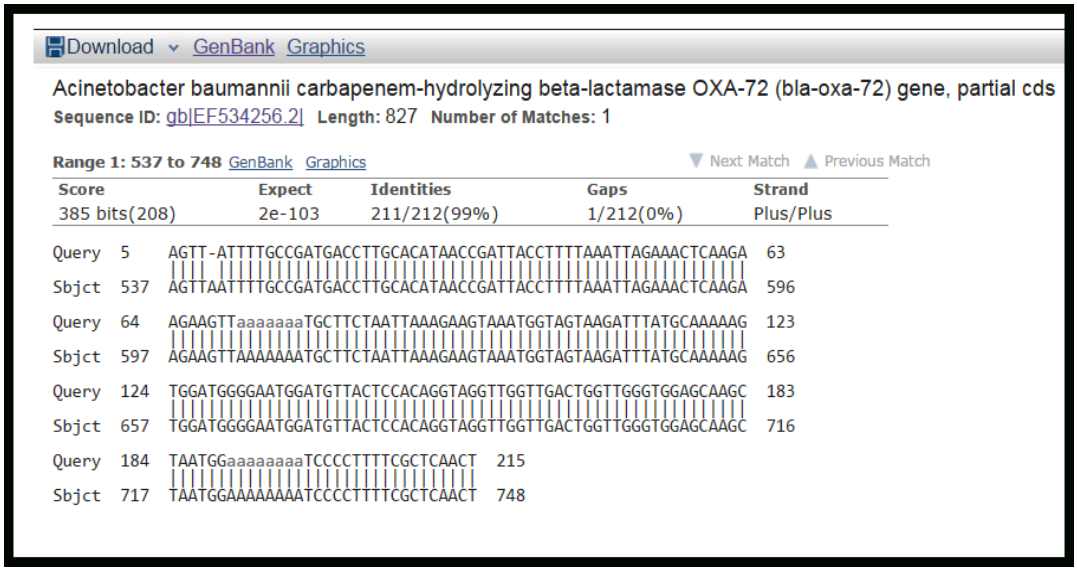

Figure 6: Alignment of *A. baumannii bla*OXA24 gene sequence of the local isolate KKG3 with *bla*OXA-72 of reference strain *A.baumannii* isolated from Chinese hospitals available in GenBank.

Many studies reported the role of *bla*OXA72 in carbapenemes resistance and outbreaks in the hospitals, Kuo *et al.* (2013) [15] found that *bla*OXA72 was plasmid-borne in *A.baumannii* isolated from Taiwanese hospitals and this gene contributed directly to Imipenem resistance. Also it was demonstrated that *bla*OXA72 gene caused an outbreak in one of the Croatian hospitals and this gene may be led to rising of carbapenemes resistance [16]. *bla*OXA-72 was first discovered in Thailand and then spread rapidly to other countries of Asia and Europe [17].

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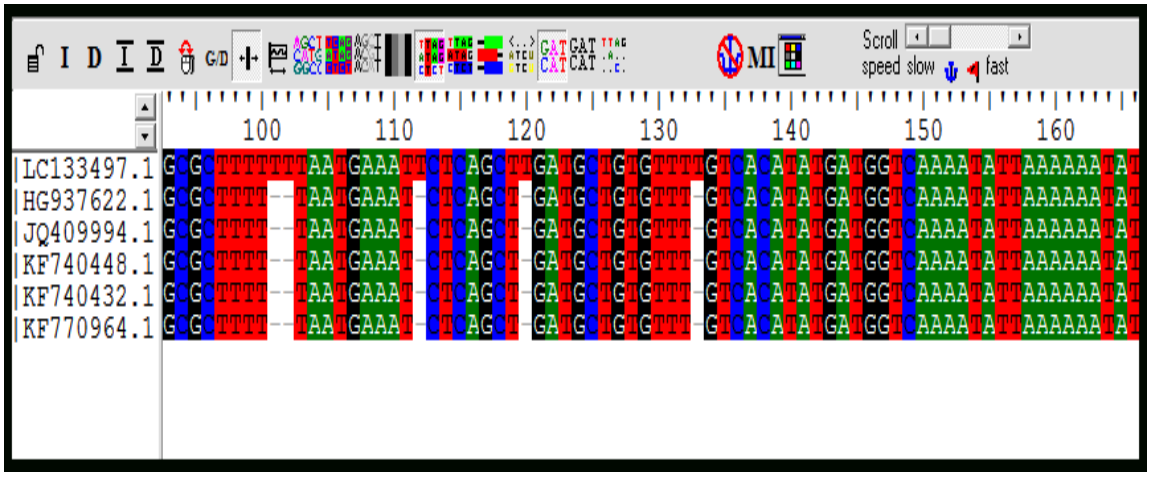

Figure 7: Multiple sequence alignment of *bla*OXA58 from local isolate KKG5 (LC133497) with some reference strains as the accession number at the start of each strain available in GenBank.

The results of multiple sequence alignment of the local *bla*OXA58 gene sequence (accession number LC133497) with some of reference strains available in GenBank by using BioEdit software revealed the presence of some insertion mutations in the positions 83, 84, 94, 102 and 115 as shown in Figure 7.

PCR assay and sequence analysis of *bla*OXA-58 genes and its IS transposase genes presented in carbapenems resistant isolates revealed sequence heterogeneity [18]. The study conducted in Greece showed the importance of *bla*OXA-58 gene in carbapenem resistant *A.baumannii* isolates and this gene possessed variations in the sequence, also it was found that this gene was not always plasmid-located (Poirel *et al*., 2006)

CONCLUSION

Our results showed that Sequencing analysis and phylogenetic relationships demonstrated the presence of many variants such as *bla*OXA69, *bla*OXA98, *bla*OXA107, *bla*OXA110 for *bla*OXA51 and the variant *bla*OXA72 for the gene *bla*OXA24, and very high identity with genes isolated from Asian isolates as for the gene *bla*OXA23.

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