Introduction:

Acinetobacter baumannii has appeared as one of the most significant pathogens involved in health care associated infections in recent decades. The species Acinetobacter baumannii is frequently involved in an intensive care settings where it is an etiology of severe infections such as ventilator-associated pneumonia, bacteremia, urinary tract infections,
meningitis and wound infections \cite{1,2}. It affects mainly the severely immune-compromised, and is typically selected by prior antimicrobial therapy \cite{3}.

Currently, one of the most concerns in medicine is enhancement of antimicrobials resistance of bacterial pathogens. This truth is correlated with higher mortality and morbidity rates, extended hospital stays and increased treatment-related costs \cite{4-6}. Such negative modes have also been noticed in Acinetobacter spp. strains.

Carbapenems are considered substantial antimicrobial agents for healing infections due to multidrug-resistant \textit{Acinetobacter} spp. However, several reports have been shown the emergence of resistance to these drugs, with increasing frequency, among \textit{Acinetobacter} spp. clinical isolates \cite{7,8}.

Among various mechanisms may award carbapenem resistance in \textit{Acinetobacter} spp., production of carbapenemases is considered the most important one, most often by those belonging to Ambler’s class D,these enzymes are called carbapenem-hydrolyzing class D betalactamasases (CHDLs), and less frequently by metallo-betalactamaes (MBLs) \cite{9}.

There are four main OXAtype carbapenemases subgroups correlated with \textit{A. baumannii}. OXAs emerge such weak hydrolysis of carbapenems that they should not allow the development of resistance; however, they are sometimes coupled with insertion elements that can develop carbapenemase expression \cite{10,11}. Insertion sequences (IS) are the smallest and the most generous transposable elements with the ability to independent transposition in microbial genomes. They may lead to several changes in the genetic materials of the microbes such as insertion mutations, genome rearrangements; and increase the dissemination of resistance and virulence determinants among species \cite{12-14}. \textit{ISAbal} is flanked by 15-bp short inverted repeat sequences, and is bound by 9-bp short direct repeats that correspond to target site duplications likely generated upon transposition \cite{15,16}.

Several promoters-containing IS elements play a role in the expression of downstream genes of antimicrobial resistance \cite{17}. Earlier studies identified ISABA-1 neighboring to a \textit{β}-lactamase resistance gene (ampC) in \textit{A. baumannii} \cite{18,19}, and it has been shown that transcription of ampC was dependent on promoter sequences within the element \cite{19}, also several authors were reported that increase carbapenem hydrolysis rates may arise due to the acquisition of the \textit{ISAbal} elements upstream of the naturally existing OXAtype carbapenemase \textit{(blaOXA-51-like)} in addition to acquired \textit{(blaOXA-23, blaOXA-58)} encoding gene.\cite{20,21}. Mugnier et al. \cite{22} demonstrated that \textit{ISAbal} and the composite transposon \textit{Tn2006} were have the ability to transposition in \textit{E. coli} strains, also the capability of \textit{ISAbal} to mobilize gene of an antimicrobial resistance.

**Materials and Methods:**

**Bacterial isolates:**

Seventy \textit{Acinetobacter baumannii} isolates were collected from clinical specimens (42 from sputa and 28 from blood) of inpatients in Baghdad Teaching hospital and Martyr Gazi Al-Hariry hospital between September 2012 and March 2013. All strains were identified using non-fermenting bacteria identification cards by Vitek-2 compact system (bioMerieux, France)

**Antimicrobial susceptibility testing:**

The disk diffusion method was used to assess susceptibility to the following antimicrobial agents: Amikacin (30µg/disc); gentamicin (10µg/disc); amoxicillin/clavulanic acid (20/10µg/disc); cefotaxime (30µg/disc); cefazidime (30µg/disc); cefepime (30µg/disc); aztreonam (30µg/disc); imipenem (10µg/disc); meropenem (10µg/disc); ciproflo-xacin (5µg/disc); and sulfam-
ethoxazole / trimethoprim (23.75/1.25 µg/disc) (Himedia, India).

Minimum inhibitory concentration (MIC) values of imipenem were determined using agar plate dilution. Two-fold serial dilutions of imipenem were added to molten Mueller-Hinton agar base (Oxoid, England). The resulting plates were inoculated with $10^4$ cfu/spot of bacteria and incubated at 37°C for 24hrs. Escherichia coli ATCC 25922 (Obtained from Central Health Laboratories, Baghdad) was used as control. Antimicrobial susceptibility was counted using break point criteria as defined by the Clinical and Laboratory Standards Institute[23].

**PCR assays and sequencing:**

Genomic DNA was extracted by standard DNA extraction kit (Bioner, Korea). A. baumannii strains were examined for occurrence of ISAb1 sequence by PCR with primers ISAb1/F: CAC GAA TGC AGA AGT TG and ISAb1/R: CGA CGA ATA CTA TGA CAC (Accession No. EU604835) giving rises to a 549 bp fragment. The PCR was performed in a thermo cycler (TECHNE, USA). Reaction mixes contained 20 pmol of each primer, 800µM dNTPs, and 25µ Taq DNA polymerase (Bonier, Korea) in a final volume of 50µl. The amplification conditions were as following: initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 45 s, 56°C for 45 s, 72°C for 3 min and final elongation at 72°C for 5 min[24]. The amplified products were noticed after electrophoresis on a 1% agarose gel with ethidium bromide staining; purified PCR products were then sequenced with the dye termination cycle sequencing technique (Macrogen DNA sequencing, South Korea). Searches and alignments for the nucleotide sequences were carried out with the Blast Program http://www.ncbi.nih.gov/Blast.

### Results and discussion:

Among 70 multi-drug resistant (MDR) A. baumannii, forty five (64.28%) were resistant to all studied antimicrobial agents. The resistance pattern of these MDR isolates were as follow: 100% for cefepim, cefotaxime, and amoxicillin/clavulanic acid; 97.14% for aztreonam; 94.28% for cotrimoxazole; 91.42% for gentamicin; 90% for ceftazidime; 87.14% for ciprofloxacin; 72.85% for amikacin; and 68.57% for imipenem and meropenem (Table-1). Results of antibiogram have been shown that A. baumannii which found in studied hospitals were highly resistant toward antimicrobials used, and this phenomenon may indicate to clinical problematic to conflict this nosocomial pathogen. Previous studies stated that increasing frequencies of MDR A. baumannii among the etiology of nosocomial infections causing a perplexing trouble for clinical treatment of this microorganism [25,26].

Adams et, al.[27] showed that A. baumannii were resistant to several groups of antimicrobials including carbabenems, also A. baumannii with highly resistance to carbapenems and amino glycosides was reported, the mechanisms of these resistance were due to production of both the OXA-23 carbapenemase and the ArmA 16SrRNA methylase respectively[28]. On the other hand, the resistance to floroquinolones was found among Gram-negative bacteria including A. baumannii, and this type of resistance has been shown that correlated with substitutions in guinolone resistance-determining region of DNA gyrase and topoisomerase IV [29,30].

Carbapenem-resistant A. baumannii (48 isolates) were subjected to PCR amplification technique to investigate the existence of ISAb1 sequence. Forty four (91.66%) isolates were given positive results with 549 bp amplified product of ISAb1 sequence (Figure-1). The data of amplified product sequencing was revealed the percentage of identity of ISAb1
sequence (Figure-2) with the sequence references available in http://www.ncbi.nih.gov/Blast site.

Many authors were identified numeral of putative promoters in ISABA-1[19,31]. and it is likely that a various, or more than one, promoter is used in the expression of an adjacent gene. In this respect, based on RTPCR analysis, increased transcription from a promoter located in ISAba-1 was proposed to be accountable for the hyperproduction of AmpC and ceftazidime resistance in A. baumannii [18].

Segal et al.[24] were reported that ISAba1is one of several promotors-containing IS elements that play a role in the expression of genes that encode for antimicrobials resistance, also several studies have been suggested that insertion of ISAba1 upstream of the bla OXA-51-like genes may supply the promoter to increase gene expression potentially offering increased levels of carbapenems resistance [10,32,33].

Bratu et al. [34] also mentioned that there is a correlation between the existence of promoter sequence ISAba1 and the blaOXA-51-like carbapenemase among carbapenem-resistant A. baumannii.

Nowak et al.[35] showed by using PCR analysis the presence of blaOX A-51-like gene and ISAba1 in all carbapenem-resistant A. baumannii isolates in this study, as well as all of these isolates were PCR positive for ISAba1 sequence.

The prevalent of ISABA-1 in acinetobacters containing the element reflects the movability of the element and indicates that transposition events had happened frequently. This proposes plasticity of the acinetobacter genome as transposition of IS elements can cause a different genome rearrangements [24].

On the other hand, our results revealed that four isolates of forty four carbapeneme-resistant isolates were PCR negative for ISAba1(Figure-1), this finding proposes the presence of another mechanisms of resistance to carbapenems such as ESβLs, the alteration of PBPs, changes in porin expression, or efflux of an antibiotic from a cell [36,37].

In conclusion, we have demonstrated the prevalence of A. baumannii with highly resistance to several groups of antimicrobials, also we have identified the insertion sequence ISAba1 among almost all of carbapenems-resistant A.baumannii clinical isolates.

### Table-1: Antimicrobial susceptibility in 70 A. baumannii clinical isolates to 11 antimicrobial agents.

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>R (%)</th>
<th>No.</th>
<th>S (%)</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>72.85</td>
<td>51</td>
<td>27.14</td>
<td>19</td>
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<td>Gentamicin</td>
<td>91.42</td>
<td>64</td>
<td>8.57</td>
<td>6</td>
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<td>Amoxicillin/Clavulanic acid</td>
<td>100</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>100</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>90</td>
<td>63</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Cefepime</td>
<td>100</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>97.14</td>
<td>68</td>
<td>2.85</td>
<td>2</td>
</tr>
<tr>
<td>Imipenem</td>
<td>68.57</td>
<td>48</td>
<td>31.42</td>
<td>22</td>
</tr>
<tr>
<td>Meropenem</td>
<td>68.57</td>
<td>48</td>
<td>31.42</td>
<td>22</td>
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<tr>
<td>Ciprofloxacin</td>
<td>87.14</td>
<td>61</td>
<td>12.85</td>
<td>9</td>
</tr>
<tr>
<td>Sulfamethoxazole/Trimothprim</td>
<td>94.28</td>
<td>66</td>
<td>5.71</td>
<td>4</td>
</tr>
</tbody>
</table>
Figure-1: Representative PCR amplification products of ISAb1 sequence in carbapenems-resistant A. baumannii clinical isolates (549 bp product). Lane M, DNA ladder 100-2000 bp; lanes 1-12, ISAb1-containing A. baumannii; lane 13-14, A. baumannii isolates with PCR negative to ISAb1 sequence; Lane C, Negative control (contain all precursors of PCR mixture without DNA to be amplified). Acinetobacter baumannii strain D46 insertion sequence ISAb1, complete sequence; and AmpC (ampC) gene, complete cds

Sequence ID: gb|KF030679.1| Length: 2739 Number of Matches: 1
Range 1: 837 to 1006    GenBank Graphics

Query-3
CACTGCTCACCAGATAAACTCTCTCTGCTGCAACCATTCAATAACGGTCTTT
ACCAAAA 61

Sbjct-837
CACTGCTCACCAGATAAACTCTCTCTGCTGCAACCATTCAATAACGGTCTTT
TACCAAAA 896

Query-62
ATGGCTATAAAGCGTTGAATCATAGCAATAGCGCATCTTTCGAATCTGAACT
TCCACGT-121

Sbjct-897
ATGGCTATAAAGCGTTGAATCAAAGCAATACGCTCTTTCGTATCTGAATTTC
TACGTT 954

Query-122
AAATTAAGCCTGCTTGGACAGGTATCGCCATCCCACGATATACGATTGC
173

Sbjct-955
TATTAAGCAATGTCCAAAGGATAGGTATCGCTATTCCACGATAAACGATTGC
1006

Figure-2: Sequencing of insertion sequence ISAb1

References:


