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Diagnosis of Gens gyrA and parC in *Acinetobacter baumannii* Resistant to the Quinoloin in Baghdad.

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ABSTRACT

During the year 2017, 500 clinical samples were collected from different hospitals in Baghdad. Sixty-four isolates of the *Acinetobacterbaumannii* bacteria were obtained from patients with wounds and burns .Bacteria have been identified by various bacteriological methods. All isolates were subjected to (Kibry-Bauer) sensitivity test for a group of antibiotic . All isolates showed high resistance to all antibiotics used, as well as high resistance of Ciprofloxicin (62.5%) and Norfloxicin(71.87%). GyrA and parC gene were isolated from bacterial isolates by migration on the gel electrophoresis .Squencing showed the emergence of different mutations for isolates, confirming that themutations in both genes increase the resistance of Quinolion. During the study, both genes were directly proportional to the amount of mutations in isolates.
Keywords: Ciprofloxicin, Norfloxicin, *Acinetobacterbaumannii*

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INTRODUCTION

Acinetobacterbaumannii as gram negative bacteria called coccobacillus because it is spherical in the stasionary phase and is short bacillus in the logarithmic phase, non glucose-fermenting ,flagellate are absent so is considerd non –motile nonfastidious , No spores formed ,oxidase negative, catalase –positive (Peteg*et al*. 2008;Hsieh *et al* .2013)

Acinetobacterbaumannii opportunistic pathogen because ability to spread and survive in hospital environment (machines and surfaces) and in particular intensive care units due to its a cquire resistance determinants agents (Towner, 2009).

It has a great ability to develop resistance to new antibiotic and it resistance to UV radiation, detergents dehydration and chemical sanitizer (Acosta *eta*l ;2011) in the recent year Acinetobacterbaumannii consider is a sign of global epidemiology change of infection (Joly,2005). Fluoroquinolones are broad-spectrum bactericidal operators utilized to treat different bacterial diseases. Fluoroquinolones are developing as practical choices for treating A. baumannii diseases, but the clinical rate of fluoroquinolone resistance proceeds to increase. (Hujer*et al*., 2009; Chopra *et al* .,2010)

Resistance regularly includes chromosomal mutation in the quinolone resistance determining regions (QRDRs) of either one or both of the DNA gyrase or topoisomerase IV (parC)genes that speak to the essential and auxiliary intracellular targets for this lesson of anti-microbials(Spence and Towner,2003; Vila *et al* .,1995)The presence of resistance for Quinolion genes in *A.baumannii* in Iraq has not been verified.

Aim of study

Detection both gyrA and parC gene with their resistance variation and association between them in *Acinetobacterbaumannii*

MATERIAL AND METHODS

Specimen's collection

During the period between April 2017 and the end of October 2017 500 sample were collected. Sample included: urine, burns were collected from different hospitals in Baghdad (the Medical city ,Al-kindi, Al-Yarmuk ,Central Public Health Laboratory and Al-Karama)by swabs sterilized containers.

Identification of *Acinetobacterbaumannii*

The collected samples were streaked directly MacConkey agar and on blood agar, incubated for 24 hrs at 37ºC. non lactose fermenting colonies on MacConkey agar and The non hemolytic opaque creamy colonies on blood agar.

Microscopical examination

All isolation was pigmented with gram stain to study cell shape and color by light microscope

Biochemical test

All isolates were conducted by Biochemical test according to Forbes et al. (2007) include: Catalase test, Oxidase test, Urea test, Citrate test, Hemolysine production, Indole test, Growth at 44˚C.

Identification by (API) 20E system

Api20 E system used to diagnose the isolated bacteria accurately throught diagnose genes and species by using 20 biochemical test this system include strip content 20 biochemical test. this strip put in plastic case with a cover with a little tap water to provide sufficient moisture for incubation.

Antibiotic susceptibility test (Kirby –Bauer)

Disc diffusion method was used to examine the sensitivity of the bacteria Acinetobacterbaumannii by 13 different antibiotic table (1)

Escherichia coli ATCC-25922 were used as quality reference strains in susceptibility determination

Table 1: Diameter interpretive standards of inhibition zone according to CLSI,(2017).

Id	Antibiotic	Code	Diameter of inhibition zone (mm)		
			Susceptible	Intermediate	Resistant
1	Piperacillin	PI	≥21	18-20	≤17
2	Ampicillin –sulbactam	AMS	≥15	12-14	≤ 11
3	Ceftazidime	CAZ	≥18	15-17	≤14
4	Cefepime	FEP	≥18	15-17	≤14
5	Doripenem	DOR	≥18	15-17	≤14
6	Meropenem	MEM	≥18	15-17	≤14
7	Colistin	CL	≥18	15-17	≤14
8	Gentamicin	GM	≥15	13-14	≤12
9	Netilmicin	NET	≥17	15-16	≤14
10	Doxycycline	Dy	≥13	10-12	9≤
11	Ciprofloxicn	Cip	≥21	12-14	≤11
12	Norfloxcin	Nor	≥21	12-14	≤11
13	Trimethoprim/Sulfamethoxazole	SXT	≥16	11-15	≤ 10

Detection gene

Gene(gyrA,parC) detection by Polymerase chain reaction(PCR)DNA was extracted from 34 Acinetobacterbaumannii clinical isolation commercial purification system (Maxime PCR PreMix kit (i-Taq) 20µlrxn) .Discard the flow-through and Collection Tube(2)(3)(4) altogether.

Table 2: Sequences of primers used for conventional PCR to detect *Acinetobacterbaumannii*

Gene name	Primer	Sequence	Tm (C˚)	GC%	Product size	Refernces
GyrA	F	5'-AAATCTGCCCGTGTCGTTGGT- 3'	59.6	52.4	344base pair	Park et al., 2011
	R	5'-GCCATACCTACGGCGATACC-3'	57.5	60.0		
ParC	F	5'-ATGAGCGAGCTAGGCTTAAA- 3'	53.7	45.0	300base pair	Park et al., 2011
	R	5'-TTAAGTTGTCCTTGCCATTCA-3'	52.3	38.1		

Table 3: The Components of the Maxime PCR PreMix kit (i-Taq)

No	Material	Concetration
1	i-Taq DNA Polymerase	5U/µl
2	DNTPs	2.5mM
3	Reaction buffer (10X)	1X
4	Gel loading buffer	1X

Table 4: Mixture of the specific interaction for diagnosis gene

No	Components	Concentration
1	Taq PCR PreMix	5µl
2	Forward primer	10 picomols/µl

3	Reverse primer	10 picomols/µl
4	DNA	10µl
5	Distill water	8 µl
6	Final volume	25µl

The optimal condition has identified for (Initial denaturation and annealing) after a work several experiments to gain for this condition, the temperature has changed through the work of (Gradient PCR) for all samples to select the optimal condition, and also changed the concentration for DNA template between (1.5-2µl) where is considered these two factors from important factors in primer annealing with complement (5)(6).

Table 5: The optimum condition of detection gyrA gene

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min.	1 cycle
2-	Denaturation -2	95°C	45 sec	35 cycle
3-	Annealing	52°C	45 sec	
4-	Extension-1	72°C	45 sec	
5-	Extension -2	72°C	7 min.	1 cycle

Table 6: The optimum condition of detection parCgene

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min.	1 cycle
2-	Denaturation -2	95°C	45 sec	35 cycle
3-	Annealing	52°C	45 sec	
4-	Extension-1	72°C	45 sec	
5-	Extension -2	72°C	7 min.	1 cycle

Sequencing and Sequence Alignment

The PCR products were separated on a 2% agarose gel electrophoresis and visualized by exposure to ultra violate light (302 nm) after ethidium bromide or Red Stain staining. Sequencing of gene was performed by national instrumentation center for environmental manage. (nicem) onlineat(http://nicem.snu.ac.kr/main/?en_skin=index.html),biotechnology lab, machine is DNA sequencer 3730XL, Applied Biosystem), Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.g.

Statistical Analysis

Bivariate Pearson's correlations and Chi-square were undertaken to determine the relationships between antibiotic groups (Sensitive, Intermediate and Resistant) and the correlations between tested genes. Statistical significance was defined as $p \leq 0.05$ and statistical significances were carried out using GraphPad Prism version 6 (GraphPad Software Inc., La Jolla, CA).

RESULTS AND DISCUSSION

Isolation and Identification of *Acinetobacterbaumannii*

A total of 500 clinical specimens of burns and wounds from the same hospitals in Baghdad (the Medical city, Al-kindi, Al-Yarmuk, Central Public Health Laboratory and Al-Karama) at the same period were collected from May to November 2017. Total isolates of Acinetobacterbaumannii sixty four isolates were 12.8% of them diagnosted in different ways.

Identification

All specimens were cultured on blood agar and MacConkey agar plates. On MacConkey agar, the colonies appeared smooth ,pale ,non fermented lactose suger while On blood agar appeaed colonies developing gray- non blood analyzer for being non productive of enzyme hemolacin. *A.baumannii*have the potential to grow at a temperature between(44)C˚, this characteristic of the distinction between *Acinetobacterbaumanni* and the rest of the species which cannot grow that degree of heat (Feizabadi*et a*l ., 2008; Peymani*et al*., 2012).The results of the microscopic examination showed that the isolated bacterial cell small in the form coccobacilli that are organized individually or in pairs and appeared as Gram-negative.All isolates showed positive results for citrate utilization test and catalase test, while the isolates gave negative results to indole production test, oxidase test, urease production test and motility test. Kligler iron agar developed an alkaline slant, no change bottom,not producte for H2S.Biochemical examination for the diagnosis of Acinetobacterbaumannii using Api-20 system.

The present study appearance highest resistance to Piperacillin(84.3%) Ampicillin_sulbactam(53.12%), Ceftazidime(90.62%), Cefepime(56.25%), Doripenem(93.62%), Meropenem(81.25%), Colistin(78.12%), Gentamicin(78.12%), Netilmicin(46.87%), Doxycycline(DY), Ciprofloxcin(62.5%), Norfloxcin(71.87%), Trimethoprim/Sulfamethoxazole (71.87%) Statistacally there was high significant difference (** p≤0.01) It is notice able that the resistance is generally higher for all Antibiotics type and this supports the fact that *Acinetobacterbaumannii* possesses Multi-Drug resistance. Also through the study it is clear that the bacteria are resistant to high direction quinoloin As the ratio of resistance to Ciprofloxicn(62.5%),Norfloxcin(71.87%),

Table 7: Percentage of antiobiotic susceptibility rate of 64 Acinetobacterbaumannii clinical isolate against 13 antimicrobial agents

Antibiotic	Resistant	Intermediate	Sensitive	Chi-square χ2	*P* value
PI	54(84.3%)	10(15.6%)	0	77.6**	<0.0001
AMS	34(53.12%)	10(15.6%)	20(31.25%)	13.66**	0.0011
CAZ	58(90.62%)	4(6.25%)	2(3.12%)	94.87**	<0.0001
FEP	36(56.25%)	10(15.6%)	18(28.12%)	16.68**	0.0002
DOR	60(93.75%)	0	4(6.25%)	105.8**	<0.0001
CL	50(78.12%)	0	14(21.8%)	52.53**	<0.0001
GM	50(78.12%)	0	14(21.8%)	52.53**	<0.0001
DY	26(40.62%)	8(12.5%)	30(46.87%)	12.8**	0.0017
CIP	40(62.5%)	4(6.25%)	20(31.25%)	30.56**	<0.0001
NOR	46(71.87%)	8(12.5%)	10(15.6%)	43.02**	<0.0001
SXT	46(71.87%)	2(3.12%)	16(25%)	47.49**	<0.0001
NET	30(46.87%)	10(15.6%)	24(37.5%)	9.86**	0.0072
MEM	52(81.25%)	0	12(18.75%)	69.68**	<0.0001

Piperacillin(PI),Ampicillin_sulbactam(AMS),Ceftazidime(CAZ),Cefepime(FEP),Doripenem(DOR),Meropenem(MEM),Colistin(CL),Gentamicin(GM),Netilmicin(NET),Doxycycline(DY)Ciprofloxicn(CIP),Norfloxcin(NOR),Trimethoprim/Sulfmethoxazole (SXT). P value considered significant at * p≤0.05 and ** p≤0.01

In the local study done by Ghaima(2016) , illustrated that the resistance rates of antibiotics as follows Ciprofloxicn(80.2%).While they were(81.3%)Meropenem, moreover there was multiple resistance to most antibiotics, as the antibiotic type used in present study so this result support present result.

While the isolate using in Jabur study (2014)from patient at Hilla hospital showed a resistance rates as (30%)for Ciprofloxacin ,(100%) for Tetracycline and (40%) for Piperacillin with Gentamicin ,the results were not supportive of our results Because showing a low resistance to the Ciprofloxacin . Our results were also consistent with the results of (Al-Khafaji,2006) indicating that the resistance to the Ciprofloxacin high reached (100%). Differences between studies may due to differences in patient immunity, hospital differences, or geographical area.

The comparison of the local studies and the current study shows that Acinetobacter was resistant to most antibiotics, which include aminoglycosides, fluoroquinolones, carbapenems. The increase in resistance is due to several reasons, including the misuse of antibiotics. The development of virulence factor through the continuous mutations of genes and the transmission of resistant mutant genes, Evolution of resistance.

There are many regional studies on the resistance of Acinetobacter to antibioticsto a study conducted in the Al- Hospital at King Hussein Medical Centre in Amman , for 116 isolates that *Acinetobacterbaumannii* resistant to most antibiotics, The resistance rates for quinolones (94.8%), ceftriaxone, cefotaxime and ticarcillin (100%), piperacillin (98.3%) and, Ampicillin/sulbactam (89.7%), trimethoprim/sulfamethoxazole (75.9%) gentamicin (87.9%) but lower for colistin (1.7%) and minocycline (26.7%)51 (Batarseh*et al*.,2016)results were consistent with our results that the resistance ratio is high towards quinolones.

Results of a hospital in Turkey study agree with present study which reported that all isolates were tested for susceptibility to 14 antimicrobials. The high resistante to ciprofloxacin (87.54%), to piperacillin (90.03%), to ceftazidime (80.78%), to meropenem (78.29%) tocefepime (81.13%), to ampicillin-sulbactam (79.35%), to gentamicin (34.16%) and to trimethoprim/sulfamethoxazole (81.13%) were observed. The lowest resistance rates was for cefotaxime (3.55%)(Cick*etal* .,2013).

In a study conducted in University Teaching Hospital, Osogbo, Nigeria, the Acinetobacter isolates showed 100% resistance to both ciprofloxacin and amikacin and 90.9% to both ceftriaxone and ceftazidime, while resistance to the other antibiotics used in this study were: piperacillin (81.8%), meropenem (63.6%) , gentamycin (72.2%)55 (Odewale et al ., 2016)also, its results were identical to our results.

Genomic DNA extraction

4-6:-Detection of resistance Quinolone genes (gyrA,parC)by Polymerase Chain Reaction (PCR)

In order to detect the presence of Quinolone resistant genes (ciprofloxacin,Norfloxacin) and determination the spread of each gene among A. baumannii clinical isolates, uniplex polymerase chain reaction (PCR) for each DNA extracted sample have been used. The PCR reaction included 34 isolates for detection the sets (gyrA,parC) genes. The PCR product has been confirmed by analyzing of the bands on the electrophoresis of the gel . showed in Figures (1)(2).

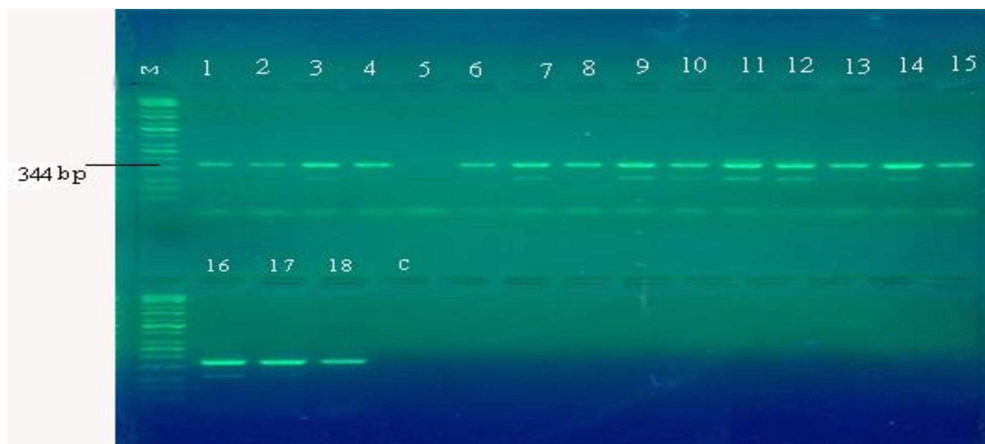

Fig 1: PCR product the gyrAgene,the band size 344 bp. The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours.M: DNA ladder (100);Lane1-17:Acinetobacter baumanniiisolates;Lane C: Negative control

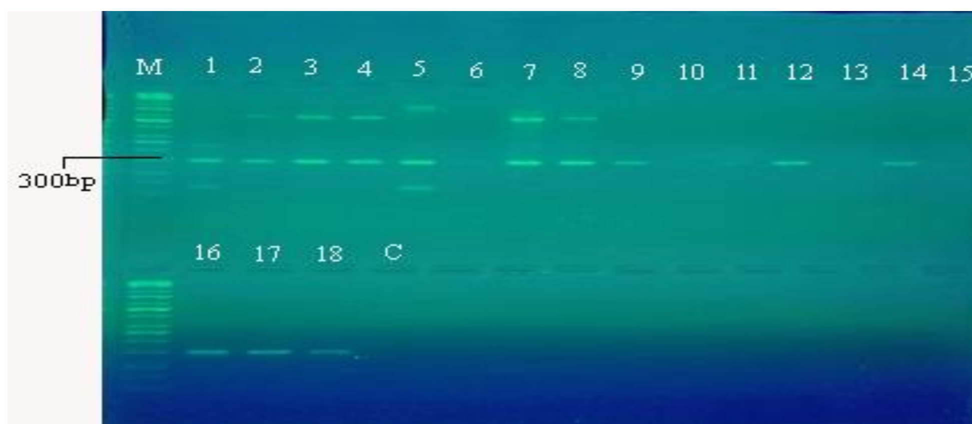

Fig 2: PCR product the parCgene,the band size 300bp. The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100));Lane1-17:Acinetobacter baumanniiisolates; Lane C: Negative control.

Present gene for multidrug resistance of Acinetobacterbaumannii

The presence or absence of these gene give information about the resistance of Quinolone as it affects the increase in resistance show in table (9).

Table 8: Multidrug resistance genes (gyrA, parC) in 34isolates from different patients

Gene	MDR for A.baumannii isolates		Chi-square- X^2	P- value
	No(%)of positive isolates	**No(%)of Negative isolates**		
Gyr A	**14(41.17)**	**20(58.82)**	**1.059 NS**	**0.3035**
ParC	**14(41.17)**	**20(58.82)**	**1.059 NS**	**0.3035**

P value considered significant at * p≤0.05 and ** p≤0.01

This study was conducted on the group of quinoloin as an important group for the treatment of *A.baumannii* bacteria, which has recently become a Multidrug resistance. Sensitivity tests, as well asMIC, showed that the isolates of *A.baumannii* were resistant to a high percentage of the quinoloin that cross a specific group to destroy the cell's DNA .On this basis, the genes studied the resistance of DNA to the bacteria, as well as efflux pump genes, which are one of the most effective ways to help bacteria resist. The interaction of the PCR reaction shows the presence of the genes that are resistant to the quinoloin in the34 isolates of the *A.baumannii*. The molecular test of the PCR gives more accurate results for the identification and diagnosis of bacteria compared to the culture media, as well as the biochemical tests and confirmatory tests

In our study, 14 isolates out of a total of 34 isolates of the *A.baumannii* showed the presence of the gyrA, parC gene and 20 isolates which did not show the gene, which is one of the main genes that help the bacteria to resist the quinolion . The results showed that there are no significant between the presence and absence of the gene figure (1, 2).

Present result agree with previous study conducted in Tehran by (Nowroоz*et a*l .,2014), the results showed that all isolates of the *A. baumannii*resistance to Ciproflоxcin, which are 65 isolates, contain gyrA and parC. Gene which were responsible of the high level of resistance of *A. baumannii* to Ciprofloxacin. Moreover in Tehran researcher isolated 44 isolates, all of which showed the presence of genes gyrA,parC(Adrebili*et al* ., 2015).

Present study agree with a study conducted in Egypt by(Zaki et al ., 2018), concdudedthat most isolates contained mutations for each gene gyrA,parC

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This study in Baghdad goss with global research in the Second Xiangya Hospital of Central South University (Changsha, China)that conducted all sixteen isolates recorded the presence of both gyrA and parC genes which related with resistance to Quinolon (Zhango*et al* .,2017)

In a study conducted in Korea by (Hong *et al*.,2013) , the results showed that all isolates 31 container on mutations per gene gyrA and parC

The increase in resistance to the trend of quinlion is accompanied by an increase in the occurrence of mutations on the chromosomes and therefore we see the occurrence of mutations of genes for the gyrA and parC assisted by mutations that occur in the genes of the Efflux pump(Hong *et al*., 2013)

Sequencing and Sequence Alignment for all genes in this study

The PCR products for all genes were separated on gel electrophoresis and Sequencing of gene was performed by national instrumentation center for environmental management (nicem) online at (http://nicem.snu.ac.kr/main/?en_skin=index.html), biotechnology lab, machine is DNA sequencer 3730XL, Applied Biosystem), Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information(NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit program.

Table 9: Squencing analysis of gyrA gene

No. Of sample	Type of substitution	Location	Nucleotide	Nucleotide change	Amino acid change	Predicted effect	Range of nucleotide	Sequence ID	Score	Expect	Identities	SOURCE
1	-------------------------------------						3827 to 3890	ID: CP024612.1	119	7e-24	100%	*Acinetobacterbaumannii* gyrA
8	-----------------------------------						3837 to 3890	ID: CP024613.1	100	2e-18	100%	
12	---------------------------------------						3837 to 3890	ID: CP024613.1	100	2e-18	100%	
14	--						3843 to 3890	ID: CP024613.1	89.8	6e-15	100%	
24	---						3843 to 3890	ID: CP024613.1	89.8	6e-15	100%	

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No. Of sample	Type of substitution	Location	Nucleotide	Nucleotide change	Amino acid change	Predicted effect	Range of nucleotide	Sequence ID	Score	Expect	Identities	SOURCE
27	--						3836 to 3890	ID: CP024613.1	102	5e-19	100%	
31	--						3841 to 3891	ID: CP024613.1	95.3	1e-16	100%	
7	**Trinsvertion**	3842	**A>C**	**AAG>CAG**	**Lysine> Glutamine**	Missense	3836 to 3890	ID: CP024613.1	97.1	2e-17	99%	*Acinetobacterbaumannii*gyrA
22	**Trinsvertion**	3901	**T>A**	**CGT>CGA**	**Arginine> Arginine**	Nonsense	3845 to 4534	ID: CP018332.1	1192	0.0	98%	*Acinetobacterbaumannii* gyrA
	Trinsvertion	3902	**C>A**	**CCG>ACG**	**Proline> Threonine**	Missense						
	Trinsvertion	3907	**T>A**	**GGT>GGA**	**Glycine > Glycine**	Nonsense						
	Trinsvertion	3916	**T>G**	**ATT>ATG**	**Isoleucine> Methionine**	Missense						
	Trinsvertion	3935	**A>C**	**ACC>CCC**	**Threonine>Proline**	Missense						
	Trinsvertion	3983	**G>T**	**GAA>TAA**	**Glutamic acid> Stop codons**	Missense						
	Trinsvertion	3986	**G>C**	**GCT>CCT**	**Alanine>Proline**	Missense						
	Trinsvertion	4041	**T>A**	**GTC>GAC**	**Valine> Aspartic acid**	Missense						
	Trinsvertion	4043	**A>T**	**AGT>TGT**	**Serine> Cysteine**	Missense						
	Transition	4111	**A>G**	**GAA>GAG**	**Glutamic acid> Glutamic acid**	Nonsense						
	Trinsvertion	4190	**G>C**	**GGT>CGT**	**Glycine > Arginine**	Missense						
	Trinsvertion	4234	**A>C**	**ACT>ACC**	**Threonine> Threonine**	Nonsense						

No. Of sample	Type of substitution	Location	Nucleotide	Nucleotide change	Amino acid change	Predicted effect	Range of nucleotide	Sequence ID	Score	Expect	Identities	SOURCE
	Transition	4251	A>G	CAA>CGA	Glutamine> Arginine	Missense						
	Trinsvertion	4495	T>G	GGT>GGG	Glycine > Glycine	Nonsense						
	Transition	4525	A>G	GAA>GAG	Glutamic acid> Glutamic acid	Nonsense						
13	---						3843 to 3891	ID: CP024613.1	91.6	1e-15	100%	*Acinetobacterbaumannii* gyrA

Table10: Squencing analysis of parC gene

No. Of sample	Type of substitution	Location	Nucleotide	Nucleotide change	Amino acid change	Predicted effect	Range of nucleotide	Sequence ID	Score	Expect	Identities	SOURCE
19	Trinsvertion	212363	A>T	TCA>TCT	Serine> Serine	Nonsense	212357 to 212596	ID: CP024124.1	422	3e-114	98%	
	Transition	212475	G>A	GAA>AAA	Glutamic acid> Lysine	Missense						
	Transition	212582	A>G	TTA>TTG	Leucine>Leucine	Nonsense						
	Transition	212583	G>A	GGT>AGT	Glycine> Serine	Missense						
27	Trinsvertion	**212362**	C>A	TCA>TAA	Serine> Stop codons	**Missense**	212360 to 212600	ID: CP024124.1	435	4e-118	99%	Acinetobacterbaumannii ParC
31	Transition	**212597**	C>T	AGC>AGT	Serine> Serine	**Nonsense**	212359 to 212600	ID: CP024124.1	442	2e-120	99%	Acinetobacterbaumannii ParC
33	Trinsvertion	212357	A>T	AAA>AAT	Lysine> Asparagine	Missense	212355 to 212596	ID: CP024124.1	436	1e-118	98%	Acinetobacterbaumannii ParC

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	Trinsvertion	212362	**C>A**	**TCA>TAA**	**Serine> Stop codons**	Missense						
13	Trinsvertion	187319	G>T	GGG>GGT	Glycine > Glycine	Nonsense	187189 to 187425	ID: CP018332.1	427	6e-116	99%	Acinetobacterbaumannii ParC

Sequencing of PCR products (gyrA, parC)

By analyzing the results of the sequencing of the gyrA gene according to the table (9) using the nucleotide BLAST from the NCBI database for the gyrA gene homogeneity of local isolates, It was found that the isolates(1,8,12,14,24,31,27)did not have any changes in the level of transformation of nucleotide or in amino acids and also the purity ratio is high and the isolates are identical to the NCBI.

The results of the isolation number seventh isolation showed a single mutation of the type of Trinsvertion, Nonsense , in which the amino acid is transformed Lysine>Glutamine. The match ratios were NCBI (99%)

The isolation showed number twenty two showed changes, many mutations between Trinsvertion (12)isolate and Transition(3)isolate and each mutation of the types of Missense and Nonsense .Trinsvertionmutation is more influential than Transition mutation because it is working to change purin to pyrimedine or pyrimidine to purin while Transition mutation is working to changepurin topurin orpyrimidine topyrimidine . Both types of mutations contain two types of mutations, either Missense orNonsense. In the case of the mutation, the amino acid will change and therefore the encoding changes whileNonsense does not change the amino acid but replaces the same amino acid ,for exampleMost of the mutations were of the type Trinsvertion and the little Transition . Most of Trinsvertion is of missense type but little type Nonsense is represented by CGT>CGA converting the amino acid from Arginine to Arginine because the code is analogous as well as the possible amino acids encoded over the code so the resulting mutation is not as effective as the other one GGT>GGA converting Glycine>Glycine,ACT>ACC converting Threonine>Threonine,GGT>GGG ,converting Glycine>Glycine.

In terms of Transition mutations, there were two parts that were influential Nonsense for GAA>GAG converting Glutamic acid > Glutamic acid and GAA>GCG Glutamic acid > Glutamic,while Missense represented for CAA>CGA Glutamine >Arginine .It is noticeable that the amount of variability is very large, as well as the area of change from 3845 to 4845. This indicates that it is the isolation of the severity of the resistance and it is possible to be a new strain.The match ratios were NCBI (98%).

The Sequencing resultsparC gene conducted all isolates contained different mutations table(10). The isolation number19 showed that they contained three mutations ofTransition type and one mutation of Trinsvertion type As well as the isolation number 27 of the event occurred to mutations of the type of Trinsvertion and the impact of the type of Missense as it led to transformation Serine to stop codons .

In isolation number thirty one, there is one mutation of Transition(Nonsense) type, while isolating number 33 contained two mutation, both of which are Trinsvertion(Missense). The ratio of homogeneity between all isolates and NCBI ranged from (98-99%).It is noted that there are two common isolates between gyrA and parC gene, which is isolation numbersevsnty two, as well as isolating number 31,

Since it showed that both isolates in the gyrA gene did not change and that the isolating number thirty one was the type ofTransition and included a mutation of Nonsense type. Number sevsnty two isolates contained a single mutation, Trinsvertion type of mutation and included Missensemutation in parC gene, almost the results are similar, especially for isolation number thirty one Isolation number sevsnty two has a single mutation and this suggests that there is a relationship between gyrA and parC gene in antibiotic resistance..

From present study conduded that both gyrA and parC contained different point mutation, this result agree with previons study conducted in Iran, emphasized all isolating (19) isolates of the A.baumannii contained mutations. There was a single mutation on the position Ser-83 of the gyrA gene as well as a mutation of the parC gene at the position Ser-80 , indicating that the mutations that occur to the gene are necessary to change the phenotype that resistant to Ciprofloxcin(Maleki*et al*., 2014)

In another study carried out in Egypt resulting that all the isolates resistant to the Ciprofloxcin , which recorded MIC High, contained mutations of the gene gyrA and parC in codon 83and 80(Zaki*et al*.,2018).

Present isolates showed point mutation that agree with results ofPark et al.,(2011) revealed 56 isolates of the *A. baumannii* showed through the results of a mutation of the gyrA gene represented by Ser83 to Leu for all isolates, while there was a single of a mutation 53 isolates for A.baumannii represented Ser80 to Trp ,Ser 80 to Leu ,Glu84 to Lys 87 .

Ardebili*et al*.,(2015) explained that, The 44 and 4 isolates of *A. baumannii* exhibited full and intermediate-resistant to ciprofloxacin, respectively. Overall, in another study the 42 isolates with double mutations of gyrA and parC genes showed a higher level of ciprofloxacin resistance than the 3 isolates with single mutations of gyrA or parC. He also explained that in *A. baumannii*, topoisomerase IVis a target on quinolones then mutations at residues Ser80 or Glu84 regarding parC make contributions after lowered fluoroquino-lone susceptibility (Vila *et al*.,1995)16.Although parC mutations usually alongside including mutations among gyrA are wanted according to acquire a high-level resistance according to quinolones (Valentine et al.,2008)4 two clinical isolates in our study had mutations in parC without gyrA, suggesting that parC might not only be a secondary target for quinolones but is really as important as gyrA to cause a decreased susceptibility to fluoroquinolones in *A. baumannii* (Ardebili*et al*., 2015).

Present study in the table (7)showed aresistance to antibiotic (Ciprofloxcin and Norfloxcin)the same isolates showed highly variation region table(9,10) with in gene gyrA and parC may related with resistance to quinolone .Present study represent first Iraqi study about all these genes in *Acinetobacterbaumannii* from our result .Acinetobacterbaumannii may becom more adapted to Iraqi environment with more virulence and mor resist to different antibiotic used as medication for Iraqi patients.

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