Investigation of parC Gene Among Clinical of Acinetobacter Baumannii Using PCR and DNA Sequencing

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Abstract

The current study aimed to isolate and identify Acinetobacter baumannii by conventional and confirmatory methods using the API 20 E system, and to evaluate the sensitivity of bacterial isolates against a number of antibiotics. In addition to examining some resistance genes and DNA sequencing. 10 clinical samples were obtained from different sources, most of which were burn patients from patients in Kirkuk city hospitals under the supervision of physicians. specialists. Bacteria participating in the study were diagnosed based on cultural characteristics, biochemical tests, and confirmatory diagnosis using the API 20 E system, an antibiotic susceptibility test was conducted with 12 antibiotics based on the Kirby-Bauer disk diffusion method. At the molecular level some resistance genes have been detected. Also, the DNA sequences were studied by the Korean company Macrogen.

The results were subjected to statistical analysis to determine the genetic dimension using (MEGA-X) software. All samples were collected at the following rates: 5 isolates from burn swabs, 3 isolates from wound swabs, 1 isolate from sputum and 1 from bacteremia. The results of the antibiotic sensitivity test showed that bacterial isolates in the study were completely resistant to most antibiotics by 100%, While isolates were sensitive to the antibiotic Imipenem amounted to 40%

At the molecular level of this study, the results of the study of the fluoroquinolone resistance gene conferred by parC mutations showed that the percentage of isolates carrying these genes was 100%. The study performed DNA sequence analysis of parC gene isolated samples when matched against global samples registered in the NCBI GenBank, isolates showed to be 100% identical, Quinolone group.

Keyword: *Acinitobacter baumanii; Antibiotic Susceptibility; genetic dimension.*

INTRODUCTION

The genus Acinetobacter is an important opportunistic human pathogen. Acinetobacter baumannii is the most prevalent clinical species worldwide (peleg et al., 2008) and is the second largest factors that cause a common disease among gram- negative bacteria (Luna. & Aruj., 2007). A. Baumannii has also been classified as pathogens and anti-microbial resistance (ESKAPE) (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) (Priyadharshini et al.,2021). this bacterial infection usually occurs in patients

with significant contact with the healthcare system or hospitalized patients (Munoz-Price, & Weinstein, 2008) A. baumannii is responsible for a very wide range of infections including Burns inflammation , wound inflammation , urinary tract inflammation , meningitis , and pneumonia . (He, et.al., 2015) It has become a major threat to health, as it causes of nosocomial infections such as bacteremia and ventilator-associated pneumonia (Hua et al., 2021). A. baumannii has been linked to many anti-microbial that are not only limited to Extended-Spectrum β-Lactamase,but have been reports of the development of resistance to Fluoroquinolones, which represent a therapeutic for this bacteria (Valentine, et al., 2008). Fluoroquinolones mainly work on bacterial DNA topoisomerases enzymes class I (DNA gyrase) and class II. These enzymes are very important for DNA replication In several bacteria. Fluoroquinolones inhibit the supercoiling action in bacterial cells mediated by both enzymes and lead to impairment of DNA replication and thus inhibit bacterial cell division (Zaki, et al., 2018). Changing the target site of an antibiotic is a key mechanism of bacterial resistance(Eghbali et al.,2016) The main mechanism for the resistance of fluorocinolones is mutations that occur to genes that encoding the DNA subunits gyrase gyrB, gyrA, and topoisomerase IV parE and parC(Elshahat et al.,2021). The mutations mainly involve quinolone resistance determining regions (QRDRs). from the gyrA gene and its homologous region of the parC gene. On the other hand, mutations in the regions of gyrB and parE genes are of lesser significance (Elshahat et al.,2021).

Materials and methods of work

Sample collection

A total of 10 clinical specimens from Different Sources of burns, wounds, bacteremia and sputum were collected during five months from hospitals in Kirkuk city. Burns specimens were 5 isolates, wounds specimens were 3 isolates, while bacteremia and sputum specimens were One isolates for each One.

Identification of Acinetobacter baumannii isolates

The samples were cultured on MacConkey agar media and blood agar media by the streaked method. The MacConkey Agar media was incubated aerobically at a temperature of 37 ° C for a period of 18 to 24 hours. Isolates were identified depending on the shape, color and size of the colonies, after a number of phenotypic diagnostic tests were conducted. The biochemistry of the bacteria involved in the study biochemical tests sach as catalase test, oxidase test, urease test, and IMVIC test, (Indol, Methyle red, Voges-proskauer and Citrate) Assertiveness was diagnosed using the API 20E System (BioMérieux, France). (Abid et al., 2021a).

Antibiotic Sensitivity Test:

Anti-biotic susceptibility test was performed for 12 different anti-biotics, Kirby-Bauer disc diffusion method was used. These anti-biotics included Amoxicillin, Ampicillin, Piperacillin, Chloramphenicol, Cefotaxime, Amoxicillin-Clavulanic acid, Amikacin, Trimethoprim-Sulphamethoxazole, Ceftazidime, Levofloxacin, Tetracycline, Imipenem, most of which are commonly used in hospitals in Iraq to treat various infections. The results were compared with the National Committee for Clinical Laboratory Standards. (CLSI., 2018) Each isolate was interpreted as resistant, sensitive, or intermediate-resistant by comparison with standard inhibition zones. (Abed et al., 2021 B).

Genomic DNA Extraction

DNA was extracted from all 10 A. baumannii clinical isolates. after bacterial isolates were grown on brain heard infusion broth and incubated at 37 Cº for18- 24 hours. By using a commercial purification system (Presto™

Mini gDNA Bacteria Kit from the company - Geneaid, Taiwan) this simple and effective method is used to extract DNA easily, with high accuracy and in less time and is used for Gram-negative and Gram-positive bacteria. Genomic DNA has been extracted based on the bacterial.

Detection of ParC gene:

Gene (ParC) detection by (PCR) polymerase chain reaction as shown in Tables (1), (2), (3)

Table(1)Primer sequences of parC gene of A.baumannii and size of primer used in PCR reaction.

Gene name	primer	Sequence	Zise base pair
ParC	F	5-AAGCCCGTACAGCGCCGTATT-3	327bp
	R	5- AAAGTTATCTTGCCATTCGCT-3	

Table(2)A combination of the specific interaction for diagnosis of ParC gene

NO	Material	Concetration
1	PCR Master mix	12.5 µl
2	F-primer	1 µl
3	R-primer	1 µl
4	DNA template	3 µl
5	ddH_2O	7.5 µl
total size		25 µl

Table(3)PCR steps to detect the ParC gene

NO	Phase	Time	Temperature	NO. of cycle
1	Initial Denaturation	4 min.	95 C̊	1 cycle
2	Denaturation	30sec	95 C̊	35 cycle
3	Annealing	45sec	58 C̊	
4	Extension	45sec	72 C̊	
5	Final Extension	5 min.	72 C̊	1 cycle

Electrophoresis process

Electrophoresis was used for detection of PCR products and DNA (2 gram of agarose for detection of PCR products and 1 gram of detection of extracted DNA) is added to 100 mL of 1X SB buffer using a 100-1500 bp DNA ladder (Bioneer, Korea) at 75 V for 1 hour which was visualized with help of safe red dye and documented by UV (Sambrook and Russell 2001).

Results and discussion

The bacteria involved in the study were diagnosed based on cultural characteristics, biochemical tests, and confirmatory diagnosis using the API 20 E system. The results of the study showed that 10 isolates belonging to the type of Acinetobacter baumannii were obtained from all samples collected at the following rates: 5 isolates from burn swabs, 3 isolates from wound swabs, 1 isolate of sputum and 1 of bacteremia out of 150 samples from Different sources, Isolates were diagnosed according to on conventional examinations such as microscopic examination all the examined isolates appeared as gram-negative coccobacilli and sometimes they organized as diplococci, cultural characteristics, and biochemical tests as in Table (4)

Table 4: Results of biochemical tests.

Test	**Result**
lactose fermentation	(-ve)
Gram stain	(-ve)
Catalase test	(+ve)
Citrate utilization	(+ve)
Growth at 44 °C	(+ve)
Hemolysis	(-ve)
Methyl red test	(-ve)
Indole production test	(-ve)
Oxidase test	(-ve)
Urease test	(-ve)
Motility test	(-ve)
Vogus-proskauer test	(-ve)
Sugars utilization in TSI Agar	(k/k)
Growth on blood agar	(+ve)
H_2S gas production	(-ve)

(-ve)=Negative, (+ve)=Positive

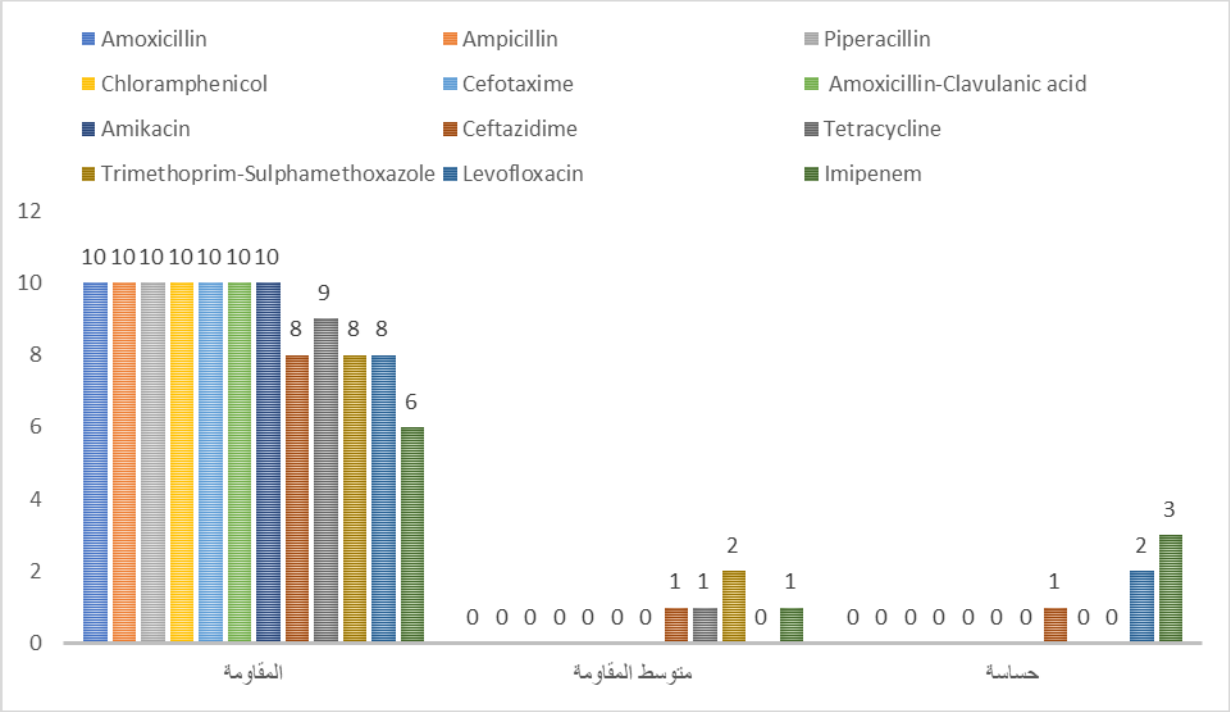
The results of the sensitivity tests of 10 isolated Acinetobacter baumannii bacteria for 12 different antibiotics found in this study showed a clear difference in antibiotic resistance. Results of susceptibility tests of ten Acinetobacter baumannii isolates to 12 different antibiotics found in this study showed a clear difference in antimicrobial resistance. The results indicated that there is a high resistance to antimicrobials used at different rates, as shown in Figure (1), that they are completely resistant to most antibiotics, for example (100%) to amoxicillin, ampicillin, chloramphenicol, cefotaxime, piperacillin, amikacin, and amoxicillin. -Clavulanic acid, and it was resistant to the antibiotic tetracycline by 90%, then levofloxacin, ceftazidime, trimethoprim-sulfamethoxazole, 80%, and finally imipenem 60%. This is consistent with the study (Abed et al, 2022) where their study showed that the lowest resistance to imipenem was 50%.

The higher resistance of A.baumannii to the group of Cephalosporins is due to the fact that it has multiple resistance mechanisms and its production of beta-lactamase enzymes, as well as its ability to change the outer membrane proteins and the flow pumps that work to expel and expel the antibody outside the bacterial cell (Mavroidi et al., 2015) Studies indicate However, the resistance of bacteria to cefotaxime and cefazidime antibiotics may be due to the fact that they have genes encoding the beta-lactamase enzymes that destroy these antibiotics (Villalón et al., 2013), and the reason for the high resistance is due to the production of enzymes Chloramphenicol acyltransferase (Brooks et al., 2016). Pore size increases resistance to some antibiotics such as Chloramphenicol (Vrancianu. et al., 2020) In the past few years, A.baumannii has increased resistance to Imipenem due to excessive use of anti-microbial.

Because of the absence of strict measures to combat infection, the spread of resistance breeds among hospitals increased. It has been found to be resistant to other groups of antibiotics such as aminogicosides and quinolones (Abd El-Baky et al., 2020). The reason is also due to the emergence of many multiple resistance mechanisms, and the most common mechanism is its ability to produce enzymes (CHDLs). (Lee.et.al.,2011) Carbapenem hydrolyzing class D β-lactamases that degrade carbapenems Drugs, or subunits of the DNA gyrase gyrA or topoisomerase parC, that inhibit DNA replication are related with high levels of resistance to Quinolones (Mohammed et al., 2021) as well as the possession of efflux pumps that propel the drug out of the cell (Hong.et. al.2013) and may also be due to the presence of genes

carried on conjugative plasmids or jump genes that mediate resistance to anti Quinolines (Al-Hamadani et al., 2014).

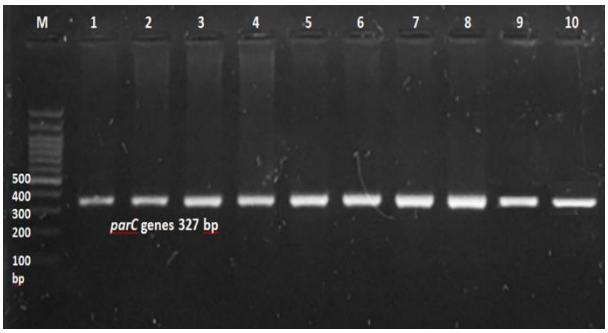
Figure (1) Percentages Isolates of A.baumannii bacteria that are resistant, intermediate and sensitive to antibiotics



Molecular detection of parC gene

The purpose of detect was a search for the presence of resistant gene (Levoploxacin) and to determine the prevalence of gene among the clinical isolated of A. baumannii , polymerase chain reaction (PCR) was used for DNA-extracted samples. The polymerase chain reaction included 10 isolates to detect parC gene. The results of the PCR were assured by transferring it on gel by electrophoresis as shown in figures (2). All isolates from our study of a group of ten isolates of A.baumannii exhibited the existence of parC gene, which is one of the main genes that help bacteria to resist quinolone. This is It agrees with a local study conducted in Baghdad hospitals by researcher Rana Hassan (Hassan, et al., 2019), and also agrees with a Chinese study, which showed that all 16 of its isolates contain the (parC) gene (Zhang, et al., 2017).

Figure (2) Electrophoresis of parC gene



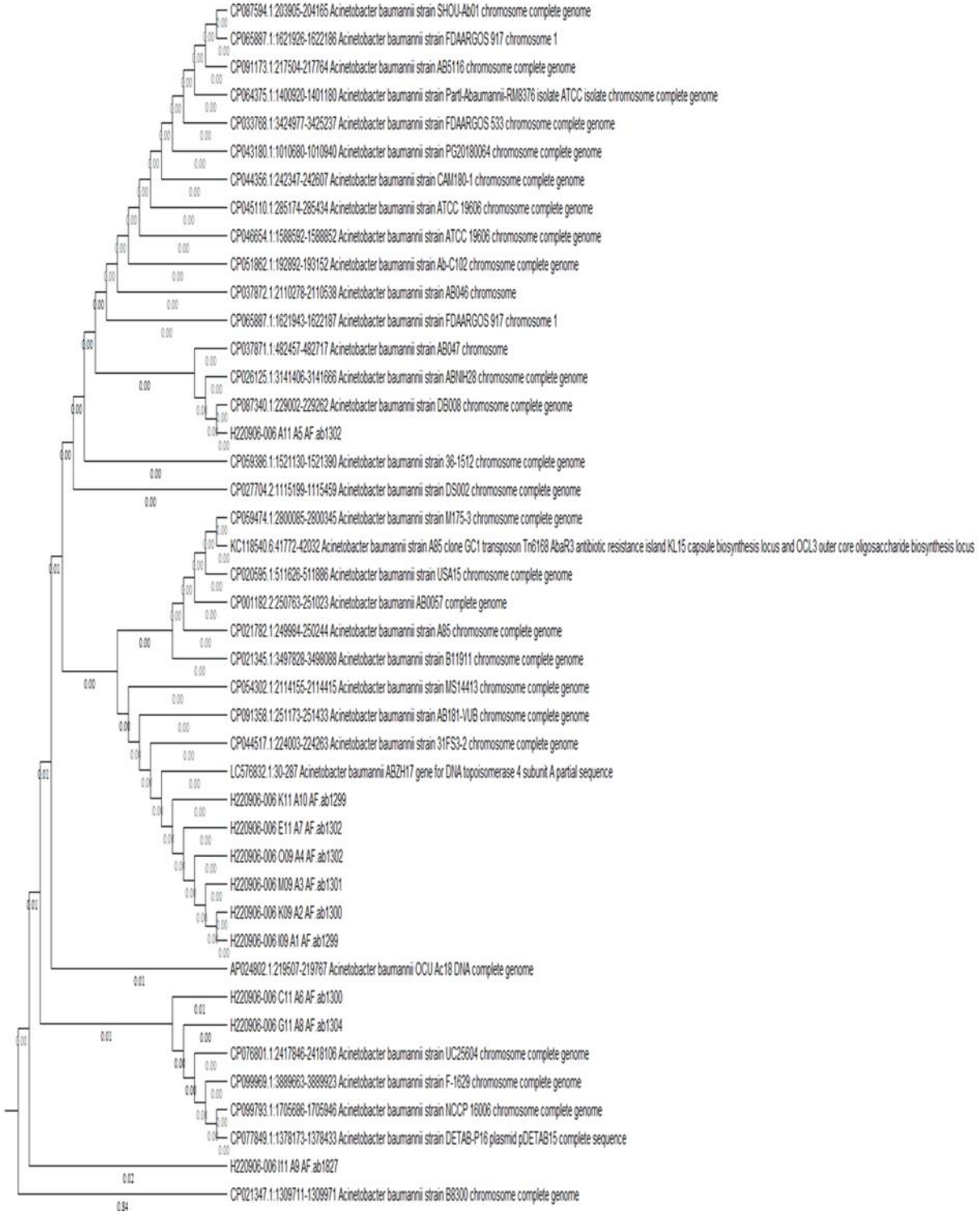
Genetic variance and gene tree analysis between samples and the closest matching ParC gene

The samples from the bacterium A.baumannii were aligned for the ParC gene and the DNA sequence with the corresponding samples registered in the National Center for Biotechnology Information(NCBI), finding the best mathematical pattern, and then finding the hereditary variance between samples and

samples registered globally using the (MEGA-X) program, and the hereditary tree was also analyzed between samples by Unweighted Pair Group Method with Arithmatic Mean(Tree UPGMA) , where the results showed the similarity of the samples under study with Samples registered globally at National Center for Biotechnology Information (NCBI). The rate of conformity for all samples amounted to 100%. From here, we note that our isolates are identical to the global and local isolates registered in (NCBI), and this It indicates the purity of the bacteria isolate and its adaptation to the environment of Iraq. There are several common mutations in different isolates and this indicates adaptation. Where A.baumannii is adapted to live in the Iraqi environment and is genetically adapted to antibiotic resistance, especially quinolones, both in (gyrA, gyrB) and parC genes, which play a major role in resistance by interfering with the mechanism of DNA action.

Many studies have shown that A. baumannii resistance to quinolones has a hereditary basis.. The most significant mutations in gyrA and ParC usually occur in special locations called resistance specific regions. Levofloxacin is a broad spectrum anti-microbil for both Gram negative and Gram positive bacteria. This antibiotic is inhibiting DNA gyrase, topoisomerase II and IV, and the manufacture of enzymes required for bacterial DNA metabolism, thus preventing cell division (Nowroozi, et al., 2014). The phylogenetic tree of ParC is represented in Figure (3), which shows the degree of affinity between the study isolates. The current global and local isolates registered in the NCBI, noting that the affinity percentage ranges between (99-100%). This supports the purity of the isolates isolated from Iraqi hospitals as well as the genetic affinity with global and local isolates. The resistance of A. baumannii to fluoroquinolones is attributed to changes in the structure of topoisomerase IV caused by mutations in the parC genes, which reduces the effect of the drug on the DNA enzyme.

Figure 3: Genetic tree analysis using (MEGA-X) program using (Tree UPGMA) for the parC gene of A.baumannii bacteria



Conclusion:

Finally, the results of this study shed light on the acclimation of A.baumannii to live in the Iraqi environment and adapt genetically to antibiotic resistance. A. baumannii resistance to fluoroquinolones to changes in the structure of topoisomerase IV caused by mutations in parC genes, which the effect of the drug is reduced in DNA enzyme, Especially quinolones, due to the overuse of antibiotics. We suggest observing the growth of the bacteria of interest and their physiological characteristics, and studying the sequences of virulence genes, in addition to display the gene expression of these bacteria.

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