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Detection of *gyr*A and *par*C Genes in Clinical *Acinetobacter Baumannii* Isolates

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ABSTRACT

100 isolates of *Acinetobacter baumannii* were collected from different clinical sources including (blood, sputum and burns) from hospitals in Baghdad - Iraq. In order to investigate its resistance to fluoroquinolones. MIC assay for ciprofloxacin was performed using E-test, and PCR assay for *gryA* and *parC* genes. The results of the MIC showed that *A. baumannii* was sensitive to ciprofloxacin at concentration=<4 µg/ml. As for the PCR assay, the prevalence of *gyr*A gene in 40 of the isolates was 40%, while the *par*C gene in 16 of the isolates was 16%. This research shed light on the rapid spread of fluoroquinolone resistance that included both ciprofloxacin and levofloxacin among *A.baumannii* bacteria isolated from clinical sources.

KEYWORDS: *Acinetobacter baumannii*; PCR; *gyr*A; *par*C; fuoroquinolone resistance.

الخلاصة

تم جمع 100 عزلة من بكتريا *Acinetobacter baumannii* من مصادر سريرية مختلفة شملت (الدم، القشع والحروق) من مستشفيات بغداد– العراق. وذلك للتحري عن مقاومتها لمضادات fluoroquinolones. تم اجراء اختبار MIC لمضاد ciprofloxacin بأستخدام E-test, و فحص PCR لجينات gry A و *parC*. اظهرت نتائج MIC تحسس بكتريا *A. baumannii* لمضاد ciprofloxacin عند التركيز= < 4 مايكرو غرام / مل. اما فحص PCR اظهر انتشار جين *gyr* A في 40 من العزلات% 40 بينما جين *par* C في 16 من العزلات 16%. سلط هذا البحث الضوء على الانتشار السريع لمقاومة fluoroquinolone التي شملت كلا من مضاد ciprofloxacin و levofloxacin بين بكتريا *A.baumannii* المعزولة من المصادر السريرية.

INTRODUCTION

Fluoroquinolones are chemically synthesized counterparts of quinolones which are now considered the major key categories of drugs that treat a variety of pathogenic bacteria, including *Acinetobacter baumannii* infection [1]. Quinolone resistance in pathogenic bacterial organisms such as *A. baumannii*, is caused by mutations in the quinolone resistance determining regions (QRDRs) caused by point mutations of both of *gyr*A and *par*C genes [2], this causes structure alterations in DNA gyrase besides the topoisomerase IV, lowering their sensitivity toward fluoroquinolones. Through using plasmid-mediated efflux pumps, these organisms potentially survive quinolones like levofloxacin and ciprofloxacin [3, 4], occur inside plasmids that encodes the aminoglycoside ribosomal methylase *rmt*B. Expanding incidence of resistance among bacterial strains of *A. baumannii* has been described abroad; even so, few specific descriptions of quinolone resistance and its explanation have previously reported in Iraq. Before the 1970s, *A. baumannii* was a nasty opportunist that had quietly emerged as a serious bacterium. *A. baumannii* is a multidrug-resistant (MDR) pathogen which might infect civil hospitals by infecting wounded army soldiers who have been transported from warzones [3].

The important concern of these microbes is their opportunity to re acquire antibiotic resistant genes, resulting in multidrug resistance MDR[4,5]. Misu se of antibiotic inside of hospital services causes the formation and spread of MDR by many Acinetobacter ssp., particularly the widespread use of extended-spectrum cephalosporins and quinolons [6, 7]. Additionally, multi drug resistance (MDR) *A. baumannii* is a powerful bacterium that generates nosocomial infections, which have a significant incidence and fatality rate in hospitals [8]. The goal of the current research is

to detect the emerging of fluoroquinolone resistance in clinical isolates of *A. baumannii* isolated from different sources from some hospitals in Bagdad, Iraq.

MATERIALS AND METHODS

Bacterial collection and identification

Non-repeat 100 bacterial isolates of A. *baumannii* were isolated from different hospitals in Baghdad from January to April 2022. The clinical bacteria were included 50 isolates from blood, 30 isolates from urine, 10 isolates from sputum, 10 isolates from burns swab. The isolates were identified using CHROMagar media which specific for Gram negative bacteria. After the identification by chrome media, the isolates were stored by deep freez at -20°C using 20% of glycerol in tryptic soy broth.

Antibiotic susceptibility

The diffusion discs test was used to evaluate antibacterial sensitivity to various antibiotics, included cefepime, cefotaxime, ciprofloxacin, levofloxacin, amoxicillin/clavulanic acid, meropenem, and amikacin according to the Clinical Laboratory Standards Institute (CLSI). Furthermore, minimum inhibitory concentration (MIC) of ciprofloxacin was measured by E-test (BioMerieux, France). The bacterial strain Escherichia coli ATCC 25922 was used to control the quality negative stander.

Procedure of PCR technique

The DNA extraction was done utilizing the boiling process to dismiss genomic DNA of the bacterial isolates. The PCR expansion were done to detect the prevalent genes included gyr A and of *par*C. By additional with usage of inner regulated gene *usp*A, both of *gyr*A and *par*C primers were used for detecting the wild type and with their mutations. Table 1 shows the primers and their sequences. For amplification the reaction, 50µl of the mixture contained 1µg of the template DNA, 1µM of each of the primers, 200 µM of dNTPs, 1X buffer, 1.5 mM $MgCl_2$ and 1.5U of Taq DNA polymerase. The essential circumstances were used for the multiplication: pre-denaturation at 94°C for 5 min, 30 cycles of amplification (94°C, 1 min, 55°C, 1 min, 72°C, 2 min) and a final extension at 72°C for 10 min. Under UV illumination, generated products from PCR toward genes were seen on a 1.5 % agarose gel including ethidium bromide [9].

Table 1. List of primers sequences genes.

Primers sequences genes	Sequences	Product size (bp)	Reference
*gyr*A	5'TACACCGGTCAACATTGAGG-3 5'TTAATGATTGCCGCCGTCGG-3'	647	[9]
*par*C	5'AAACCTGTTCAGCGCCGCATT-3' 5'-GTGGTGCCGTTAAGCAAA-3'	395	[9]

Statistical analysis

The data were performed and analyzed using SPSS and IBM SPSS. The one-way evaluation of similarity was used to compare the classes under study. The statistical analysis was reported as percentages. The chi-square statistic was used to compare percentages. P-values less than 0.05 were deemed significant.

RESULTS AND DISCUSSIONS

The present research inclusive a number of 100 *A. baumannii* isolates which all were collected from different hospitals in Baghdad, Iraq then identified as *A. baumannii* based on colony morphology on CHROMagar™ Acinetobacter medium as shown in Figure 1.

The diffusion discs test was used to evaluate antibacterial sensitivity on all *A. baumannii* isolates. Results exhibited highest level resistance of *A. baumannii* was towards cefotaxime 100 (100%) while the least resistance ability of *A. baumannii* was towards both of colistin and amikacin 10 (10%) as clarified in Figure 2. The MICs of *A. baumannii* toward the ciprofloxacin antibiotic had expressively greater resistance pattern >4 µg/ mL in *A. baumannii.* Furthermore, the prevalence of *gyr*A and of *par* C genes in this study showed that the frequent common gene was in a total 40 isolates (40%) for *gyr*A gene, while a number of 16 (16%) for *par*C gene and 10 (10%) for both genes as illustrated in Figure 3,4 and 5.

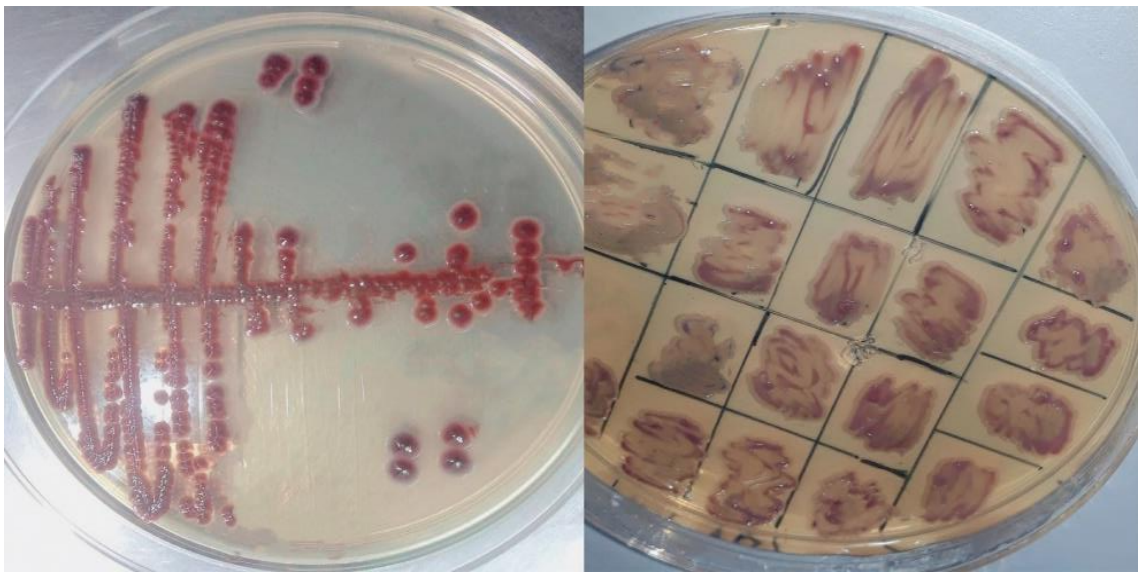

Figure 1. Identification of *Acinetobacter baumannii* on CHROMagar™ *Acinetobacter* medium

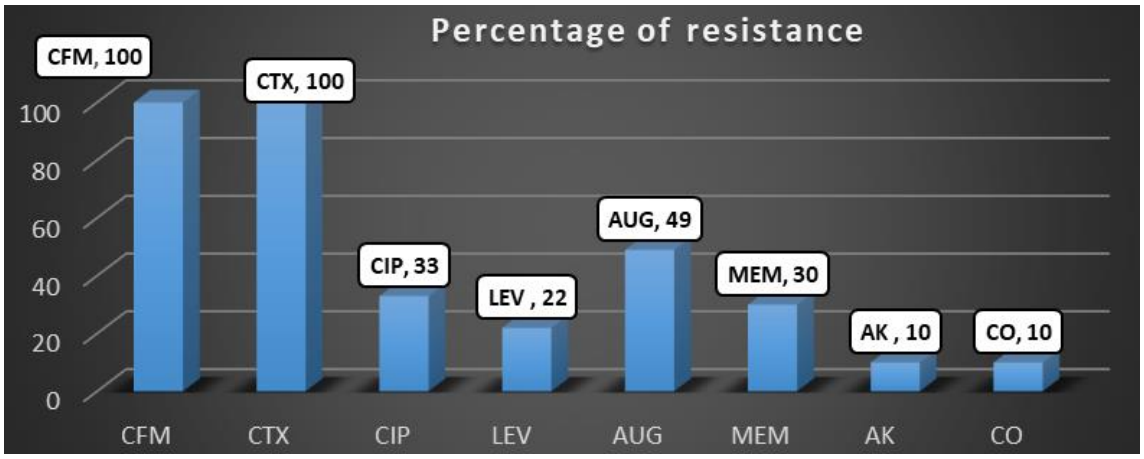

Figure 2. Antibiotic resistance percentages of *Acinetobacter baumannii* isolates.

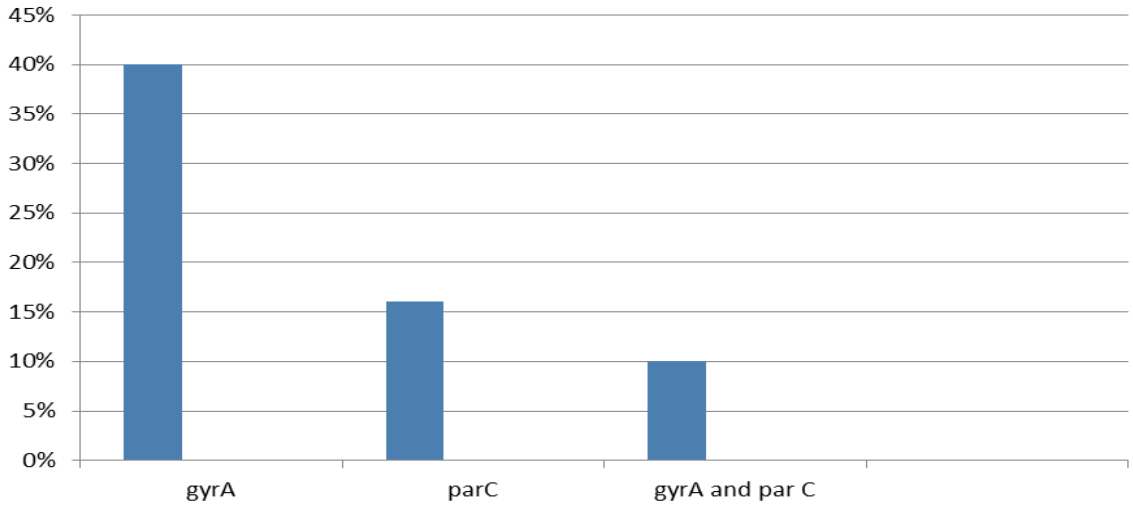

Figure 3. The prevalence of *gyr* A and of *par* C genes in *Acinetobacter baumannii* isolates

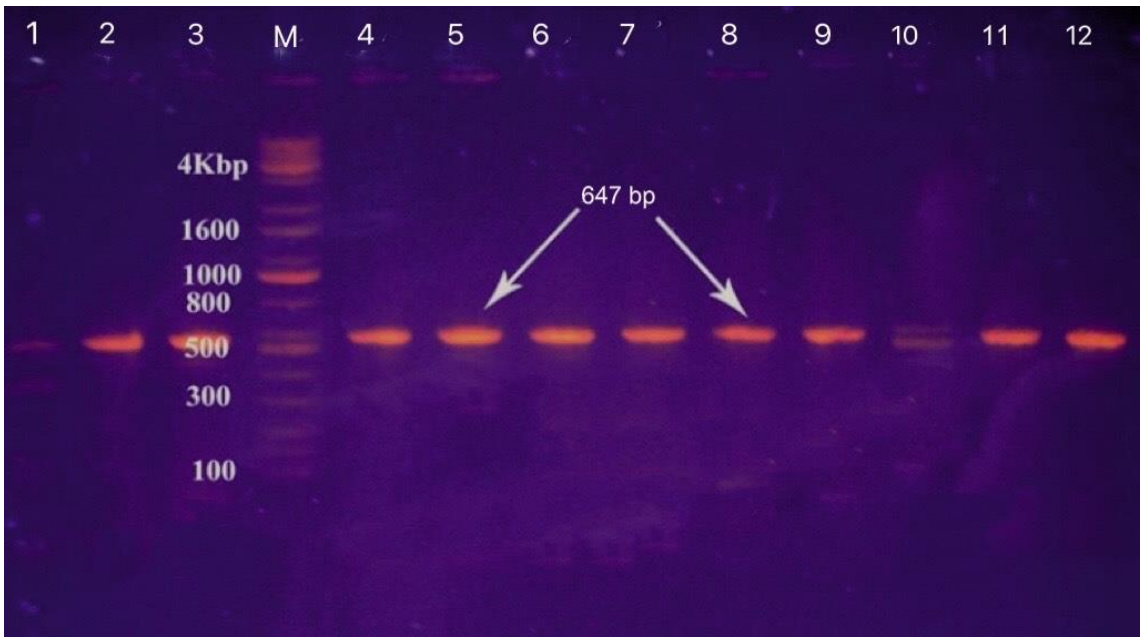

Figure 4. Gel electrophoresis of *gyr*A gene (647 bp)

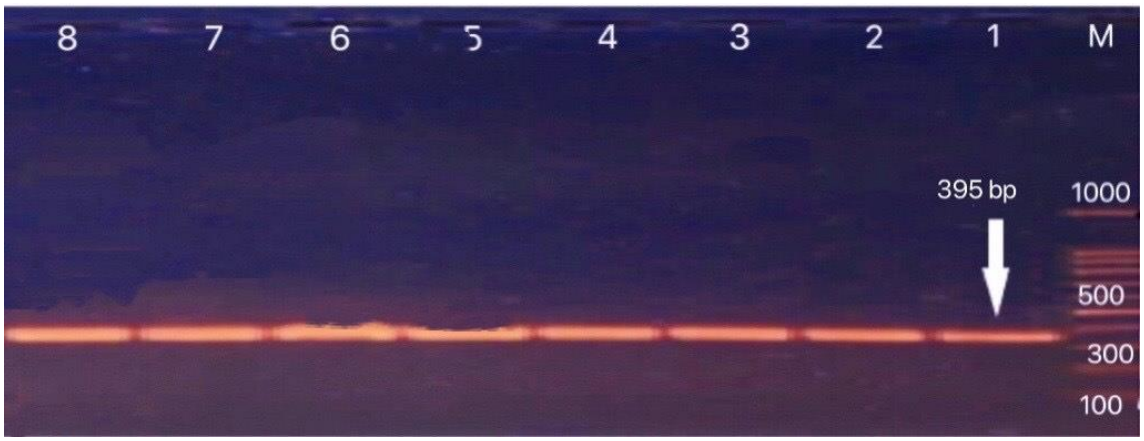

Figure 5. Gel electrophoresis of *par*C gene (395 bp).

For discussion, antibiotic resistance is emerging at an astonishing speed, particularly in impoverished countries. Resistance has an impact on attempts for treating infectious bacteria illnesses in both communities and hospitals. As a result, investigation throughout this subject seems critical for understanding underlying processes as a precursor for decreasing infectious concerns [10, 11]. Bacteria such as *A. baumannii* are urgent concerns since they have the potential to withstand a variety of drugs, including fluoroquinolones [7, 12]. These aggressive multi-drug resistance pathogens provide a daunting task to infection control researchers across the world [13]. Resistance to fluoroquinolones is characterized by abnormal in their specific proteins or enzymes, the existence of this resistance, the development of efflux pumps, and alterations in cell membrane [14, 15, 16]. This research highlighted the rapid spreading of quinolone resistance which included both of ciprofloxacin and levofloxacin among *A. baumannii* of clinical setting. Such results supported prior research that found indicated many gene variation with either *gyrA* or maybe a combination of *gyrA* and *parC* were required to create excellent non-susceptible to subsequent generations of fluoroquinolones, including ciprofloxacin [17]. Inside prior research conducted in the United States, all 58 isolates of gram-negative bacteria were resistant to fluoroquinolones antibiotics contained *gyrA* [17], and roughly 85 % had additional *par* C mutations. The current research demonstrates the development in fluoroquinolone antibiotic resistance across clinical isolates of *A. baumannii* [18]. The resistant *A. baumannii* isolates were

caused by mutations of the *gyrA* gene instead of *parC* gene. The research emphasizes a need of adhering for infectious diseases management recommendations and an antimicrobials strategy.

CONCLUSIONS

Talk about any qualifications important to your MDR *A. baumannii* is regarded as a major danger. This research proved the occurrence of potential resistance for the fluoroquinolone drugs by *A. baumannii* isolates isolated from Iraq. The presence of fluoroquinolone resistance resistant *A. baumannii* bacterial isolates makes it urgent alarming need real attentions for the antibiotic's guidelines and their resistance control possibilities.

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