Microbes and Infectious Diseases 2023; 4(2): 590-600

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Mutations in *gyrA* and *parC* genes in fluoroquinolone-resistant *Acinetobacter baumannii* that causes hospital acquired infection

Marwa S. Taha [1], Sarah M. Shoeib [*2], Marwa A. Abdelwahab [1]

1- Department of Medical Microbiology and Immunology, Faculty of Medicine, Tanta University, Egypt

2- Department of Clinical Pathology, Faculty of Medicine, Tanta University, Egypt

ARTICLE INFO

Article history:

Received 25 December 2022

Received in revised form 24 January 2023

Accepted 27 January 2023

Keywords:

Acinetobacter baumannii

fluoroquinolone resistance

GyrA

ParC

PCR-restriction fragment length polymorphism

ABSTRACT

Background: *Acinetobacter baumannii*, are involved in hospital- acquired infections and are increasingly developing resistance to fluoroquinolones, such as ciprofloxacin. The most common method of fluoroquinolone resistance is alteration in genes that encode DNA gyrase (*gyrA*) and topoisomerase IV (*parC*).**Methods**: We sought to isolate fluoroquinolone- resistant *A. baumannii* and search for changes in *gyrA* (Ser83Leu) and *parC* (Ser80Leu) loci by polymerase chain reaction-restriction fragment length polymorphism (PCR- RFLP).**Results:** We found that all 68 *A. baumannii* isolates that were part of this research were multidrug resistant and harbored *gyrA* and *parC* loci. Most isolates had ciprofloxacin minimal inhibitory concentrations of >128 µg/mL (50.8%). Mutations in *gyrA* were the most prevalent (47.45%), followed by *parC* mutations (33.9%) and combined mutations in both genes (23.7%).**Conclusions**: Single mutations in either *gyrA* (Ser83Leu) or *parC* (Ser80Leu) genes may be attributed to fluroquinolone resistance in *A. baumannii*.

Introduction

Acinetobacter species can be found in various areas of the environment, including land, fresh water, and food, as well as in the healthcare setting, such as ventilation systems, moisturizers, catheters, and other surgical equipment [1].

Of these species, *Acinetobacter baumannii (A. baumannii)* is the most abundant bacterium found in human samples, followed by *Acinetobacter luffy*, *Acinetobacter pittii*, *Acinetobacter nosocomialis*, *Acinetobacter haemolyticus*, and *Acinetobacter johnsonii* [2].

Prolonged stays in intensive care, intubation, surgical procedures, and the wide-ranging prescription of antibiotics in hospitals are all potential reasons for the appearance and spread of *Acinetobacter* species that do not respond to the majority of existing antibiotics [3,4]. Today, *A. baumannii* has emerged as the Gram-negative bacterium that is most difficult to manage and treat in hospitals [5].

Ciprofloxacin is a fluoroquinolone medication that is effective against Gram-negative bacteria; however, many Gram-negative bacteria, such as *A. baumannii*, are developing resistance to these antibiotics [6, 7].

Resistance mechanisms to fluoroquinolones are classified into three categories: Mutations in

DOI: 10.21608/MID.2023.183097.1435

* *Corresponding author:* Sarah M. Shoeib

E-mail address: sara.shoeib@med.tanta.edu.eg

gyrase and topoisomerase IV which are examples of target-mediated resistance, plasmid-mediated resistance and chromosome-mediated resistance [8].

Bacterial DNA gyrase and DNA topoisomerase IV are enzymes essential for genetic material production in bacteria. Fluoroquinolones limit the formation of DNA secondary structures in the cell, thereby halting DNA synthesis and, as a result, cell proliferation [9,10].

DNA gyrase enzyme is made up of two GyrA and two GyrB subunits, and topoisomerase IV enzyme is made up of two ParC and two ParE subunits. The most common method of fluoroquinolone resistance is alteration in the genes that encode these subunits [11]. Most of these modifications are the result of mutations in the quinolone resistance determining regions (QRDRs) of the *gyrA* and *parC* genomes; the most frequently described mutations are at Ser-83 in *gyrA* and at Ser-80 in *parC*. Genetic alterations in the *gyrB* (Ser83Leu) and *parE* (Ser80Leu) loci, on the other hand, are not involved in fluoroquinolone resistance [12]**.**

The epidemiological features and clinical significance of *Acinetobacter* species are now better understood as a result of the development of many molecular approaches [13]**.** DNA profiling is an accurate tool for detecting genetic alterations, but it is expensive, takes a lot of time, and labor intensive in screening for several clinical isolates [14]**.** The restriction fragment length polymorphism (RFLP) method of polymerase chain reaction (PCR) is a rapid and reproducible alternative approach for identifying mutations linked to fluoroquinolone resistance in *A. baumannii* [15]**.**

The aim of this study was to detect *gyrA* (Ser83Leu) and *parC* (Ser80Leu) gene mutations by PCR-RFLP among fluoroquinolone- resistant *A. baumannii* isolates in patients with hospital acquired infections.

This work focused on the simultaneous occurrence of *gyrA* (Ser83Leu) and *parC* (Ser80Leu) gene mutations in *A. baumannii* isolates because they are the most frequently described mutations within the QRDRs [16].

Material and Methods

Patients

For this cross-sectional study, we used samples from 430 patients in the Departments of Pediatrics, Chest Medicine, and Internal Medicine and the intensive care unit of Tanta University hospital. Hospital-acquired infections (infections that developed after 48 hours of admission) had been diagnosed in all patients. Samples (wound 153, burn 112, tracheal aspirate 61, sputum 52, urine 39 and bed sores 13) were collected from June 2020 to June 2021. The demographic characteristics of the patients are listed in **table (1)**. Inclusion criteria included clinical signs of hospital-acquired infections and poor responses to antibiotics treatment.

Ethical consideration

Tanta University's Faculty of Medicine's Research Ethics Committee approved the protocol for the study (approval code 35796/9/22).

Bacterial isolation

Samples taken from admitted patients, including those from sputum, endotracheal tubes, urine, infected wounds, and bed sores were transported as soon as possible to the laboratory of the Microbiology Department for further processing. First, the samples were coded, then cultured on MacConkey and blood agar media (Oxoid, Basingstoke, UK), after 24 hours incubation at 37 °C, Gram –stained smears were prepared from isolated colonies and then were microscopically examined. Colonies were identified as *Acinetobacter* by routine microbiological methods, and identification of the genus and species were confirmed by the VITEK2 system (bioMérieux).

Antibiotic susceptibility

The Kirby disc diffusion method was used to conduct the antimicrobial susceptibility test on Mueller-Hinton agar (Oxoid, UK) in accordance with Clinical and Laboratory Standards Institute (CLSI) standards [17]. We performed the antibiogram test with amikacin (30 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), piperacillin/tazobactam (100 µg/10 µg), imipenem (10 µg), ampicillin/sulbactam (10 µg/10 µg), meropenem (10 µg), cefotaxime (30 µg), cefepime (30 µg), and ceftriaxone (30 µg) (Oxoid, UK). As a quality check, we used the standard strain of *Escherichia coli* (ATCC 25,92; Mast Group Ltd., Merseyside, UK). Multidrug-resistant *A. baumannii* isolates were those that were resistant to at least one compound from three or more antimicrobial groups.

Minimal inhibitory concentrations (MICs) for ciprofloxacin

In accordance with the manufacturer's instructions, we assessed the MIC of ciprofloxacin in *A. baumannii* isolates by using the agar diffusion method with E-test (bioMérieux, France) on Mueller-

Hinton agar medium. Tested ciprofloxacin concentrations varied from 0.002 to 32 µg/mL. *A. baumannii* bacterial suspension equal to 0.5 McF arland standard was used to inoculate Mueller-Hinton agar plates. Next, ciprofloxacin ETEST strip s were inserted on each plate. After overnight incubation at 37ºC, the interpretive MIC susceptibility breakpoints of ciprofloxacin against *A. baumannii* according to CLSI standards were used to read the MICs and interpret the results: MICs of 1 µg/mL indicated susceptibility to a drug, MICs of 2 µg/mL indicated intermediate susceptibility, and MICs of 4 µg/mL indicated resistance.

Molecular study

DNA extraction

A mini kit from Qiagen (Hilden, Germany) was used to extract the DNA from the isolated bacteria in accordance with the kit's instructions. Then the extraction was stored in sterile 1-ml tubes at minus 20 degrees Celsius until the next step.

PCR amplification

We prepared 20 µl of PCR reaction mixtures; each reaction contained 2 µl of template DNA, 1 µl of each primer, the used primers were summarized in(table **2**) [18] and 10 µl of DreamTaq Green PCR Master Mix (2X) (K1081, Thermo Fisher, USA), then the volume was completed to 20 µl by adding 6 µl of nuclease free water. The Creacon thermal cycler (Holland, Inc) was used and programmed as follows: initial denaturation at 95°C for 5 minutes, then 30 cycles of denaturation at 95°C for 30 seconds, annealing at 47°C for 30 seconds for *parC* and 53°C for *gyrA*, and extension at 72°C for 45 seconds, followed by a final extension at 72°C for 7 minutes. The amplified products were evaluated with 2 % agarose gel electrophoresis and photographed with ultraviolet illumination.

Detection of genetic changes in *gyrA* (Ser83Leu) and *parC* (Ser80Leu) loci by PCR RFLP

The amplification products were subjected to restriction analysis with the HinfI restriction enzyme (provided by Thermo SCIENTIFIC® cat no. # ER0801). The reaction mixture contained 2 µl of HinfI enzyme, 10 µl of PCR products, 2 µl of 10X Buffer Tango, and 18 µl of Nuclease free water. The restriction digested DNA fragments were examined with 2 % agarose gel electrophoresis and imaged under ultraviolet light. To size the products, we used 100-bp DNA ladder (Thermo Scientific, Waltham, MA, USA).

The band size of *gyrA* gene was 343 bp, after HinfI enzyme digestion**,** the existence of two bands, one 291 bp and one 52 bp in size, suggested that the QRDR of the *gyrA* locus was unchanged.

The band size of parC gene is 327bp, after HinfI enzyme digestion**,** the existence of two bands, one 206 and one 121 bp in size, suggested that the QRDR of the *gyrA* locus was unchanged.

Table 1. Characteristics of patients infected by isolated *Acinetobacter baumannii*.

Characteristics		**N =68**
Age (years)	Range	1.5-75
	Mean (± standard deviation)	33.46 (±19.4)
Gender	Male	44 (64.7%)
	Female	24 (35.3%)
Sample type	Wound (153)	28 (41.2%)
	Burn (112)	21(30.9%)
	Tracheal aspirate (61)	9 (13.2%)
	Sputum (52)	6 (8.8%)
	Urine (39)	3 (4.4%)
	Bed sores (13)	1(1.5%)

Table 2. Primer sequences and the expected size of the products of PCR.

Target gene	**Primer sequence (5′-3′)**	**Expected size, bp**
GyrA	Forward AAATCTGCCCGTGTCGTTGGT Reverse GCCATACCTACGGCGATACC	343
ParC	Forward AAACCTGTTCAGCGCCGCATT Reverse AAAGTTGTCTTGCCATTCACT	327

Results

Patient characteristics and distribution of *A. baumannii* isolates from clinical samples

A. baumannii was recovered from 68 of 430 samples taken from patients. The 68 nonduplicate isolates of *A. baumannii* were recovered from diverse departments over a 1-year period. The ages and genders of the patients infected with isolated *A. baumannii* and the sample types are listed in **(table 1).**

Antimicrobial susceptibility pattern of *A. baumannii* isolates

All 68 *A. baumannii* isolates that were evaluated in this investigation were multidrug resistant as they did not respond to piperacillin - tazobactam, ampicillin - sulbactam, trimethoprim - sulfamethoxazole, ceftriaxone, cefotaxime, or ceftazidime. 59 (86.7%) were resistant to amikacin; 66 (97%), to cefepime; 60 (91.1%), to meropenem; 61 (89.7 %), to ciprofloxacin; 61 (89.7%), to levofloxacin; and 62 (91.1%), to Imipenem (**Table 3).**

Minimal inhibitory concentration for ciprofloxacin

Out of 68 *A. baumanni* isolated, 59 was resistant for ciprofloxacin & showed MICs of >4 g/mL. Most isolates (50.8%) had MICs of > 128 g/mL **(*Table 4*).**

PCR was used to confirm the presence of *gyrA* and *ParC* alleles

gyrA and parC gene domains were successfully amplified in the included A. baumannii isolates **(Figures 1 and 2).**

HinfI restriction analysis detects genetic changes in the *gyrA* (Ser83Leu) and *parC* (Ser80Leu) loci

Variation in the *gyrA* (Ser83Leu) and *parC* (Ser80Leu) alleles are indicated by failure of digestion of the PCR products **(Figures 3 and 4).** The QRDR of both *gyrA* (Ser83Leu) and *parC* (Ser80Leu) showed a single mutation in isolates with ciprofloxacin MICs of 16, 32, 64, 128, and >128 μg/mL, whereas isolates with MIC of >128 μg/mL had double mutations in QRDRs of both genes. Mutations of *gyrA*(Ser83Leu) were the most prevalent mutations (47.45%), followed by *parC* (Ser80Leu) mutations (33.9%), and the combination of mutations in both genes (23.7%). After HinfI enzyme digestion**,** the existence of two bands, one 291 bp and one 52 bp in size, suggested that the QRDR of the *gyrA* locus was unchanged, as demonstrated in nine isolates with MICs of 4 μg/mL, seven isolates with MICs of 8 μg/mL, one isolate with a MIC of 32 μg/mL, one isolate with a MIC of 64 μg/mL, and four isolates with MICs of 128 μg/mL, and nine isolates with MICs of >128 μg/mL. The QRDR of the *parC* locus was also intact, as evidenced by the presence of two bands, 206 and 121 bp in size, after HinfI enzyme digestion, and as demonstrated in nine isolates with MICs of 4 μg/mL, seven isolates with MICs of 8 μg/mL, one isolate with a MIC of 16 μg/mL, two isolates with MICs of 32 μg/mL, two isolates with MICs of 64 μg/mL, four isolates with MICs of 128 μg/mL and 14 isolates with MICs of >128 μg/mL **(Table 5) and (figure 3 and 4).**

Table 3. Antibiotics resistance among isolates of *Acinetobacter baumannii*

Antimicrobial agent	Disc content	Rate of resistance among isolates
Ampicillin-sulbactam	(10 μg/10 μg)	100%
Amikacin	(30 μg)	86.7%
Ciprofloxacin	(5 μg)	89.7%
Ceftazidime	(30 μg)	100%
Cefotaxime	(30 μg)	100%
Cefepime	(30 μg)	97%
Ceftriaxone	(30 μg)	100%
Imipenem	(10 μg)	91.1%
Levofloxacin	(5 μg)	89.7%
Meropenem	(10 μg)	91.1%
piperacillin-tazobactam	(100 μg/10 μg)	100%
Trimethoprim-sulfamethoxazole	(1.25/23.75μg)	100%

Table 4. Antibiotics resistance by MIC among isolates of *Acinetobacter baumannii*

	MIC (µg/mL)													
	4		**8**		**16**		**32**		**64**		**128**		**>128**	
***A.baumannii* isolate**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
	9	16.3	7	3.11	1	1.6	2	3.2	3	4.9	6	9.8	31	50.8

MIC: minimal inhibitory concentration.

Table 5: Distribution of *gyrA* and *parC* mutations among resistant *Acinetobacter baumannii* in relation to MIC.

Characteristics	**MIC (µg/mL)**							**Total**
	4	8	16	32	64	128	>128	
A.baumannii isolate	No.	No.	No.	No.	No.	No.	No.	No.
	9	7	1	2	3	6	31	59
gyrA mutation	-ve (9)	-ve (7)	+ve (1) -ve (0)	+ve (1) -ve (1)	+ve (2) -ve (1)	+ve (2) -ve (4)	+ve (22) -ve (9)	+ve (28) (47.45)
parC mutation	-ve (9)	-ve (7)	-ve (1)	-ve (2)	+ve (1) -ve (2)	+ve (2) -ve (4)	+ve (17) -ve (14)	+ve (20) (33.9%)
Combined mutations	-ve	-ve	-ve	-ve	-ve	-ve	+ve (14) -ve (17)	+ve (14) (23.7%)

MIC, minimal inhibitory concentration.

Figure 1. Polymerase chain reaction (PCR) product for the *gyrA* gene. The band size is 343 bp.

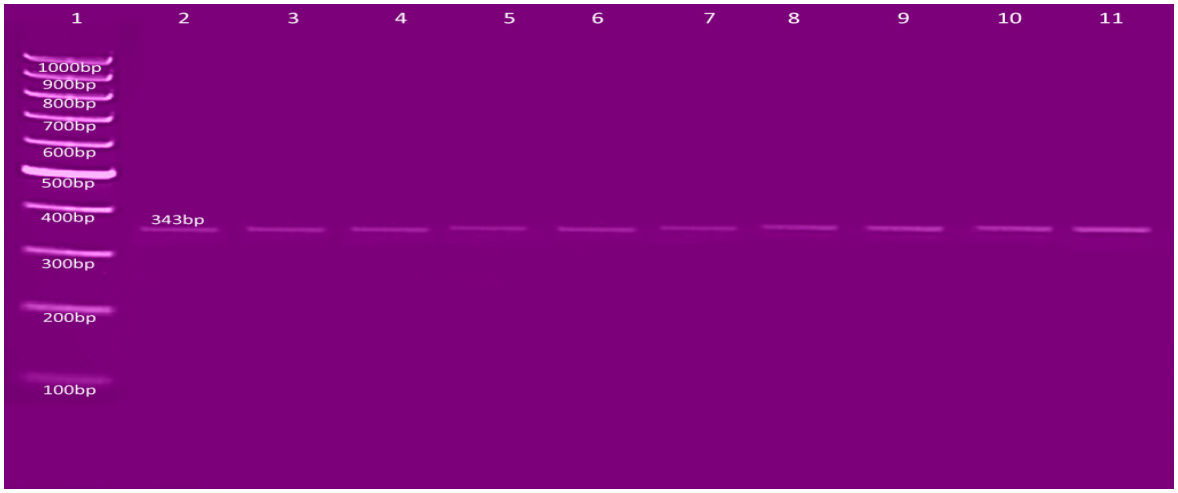


Figure 2. Polymerase chain reaction (PCR) product the *parC* gene. The band size 327bp.

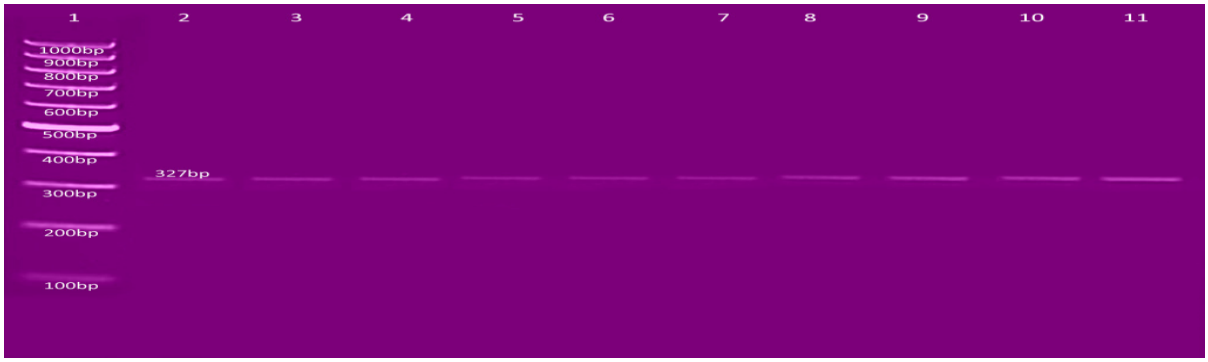


Figure 3. Restriction fragment length polymorphism (RFLP) polymerase chain reaction (PCR) for detection of mutations in *gyrA* genes. Nondigestion of PCR products indicates the presence of mutations in the quinolone resistance-determining region (QRDR) of this gene, whereas digestion of PCR products indicates the absence of such mutations.

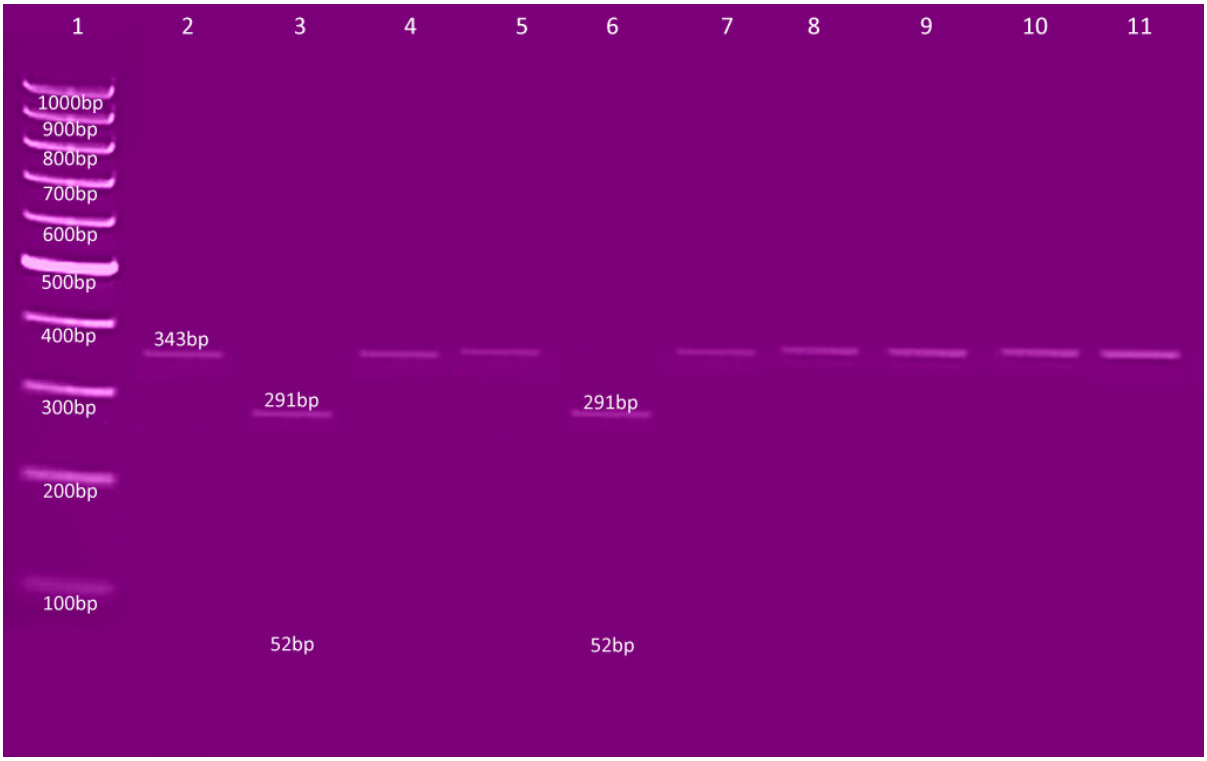
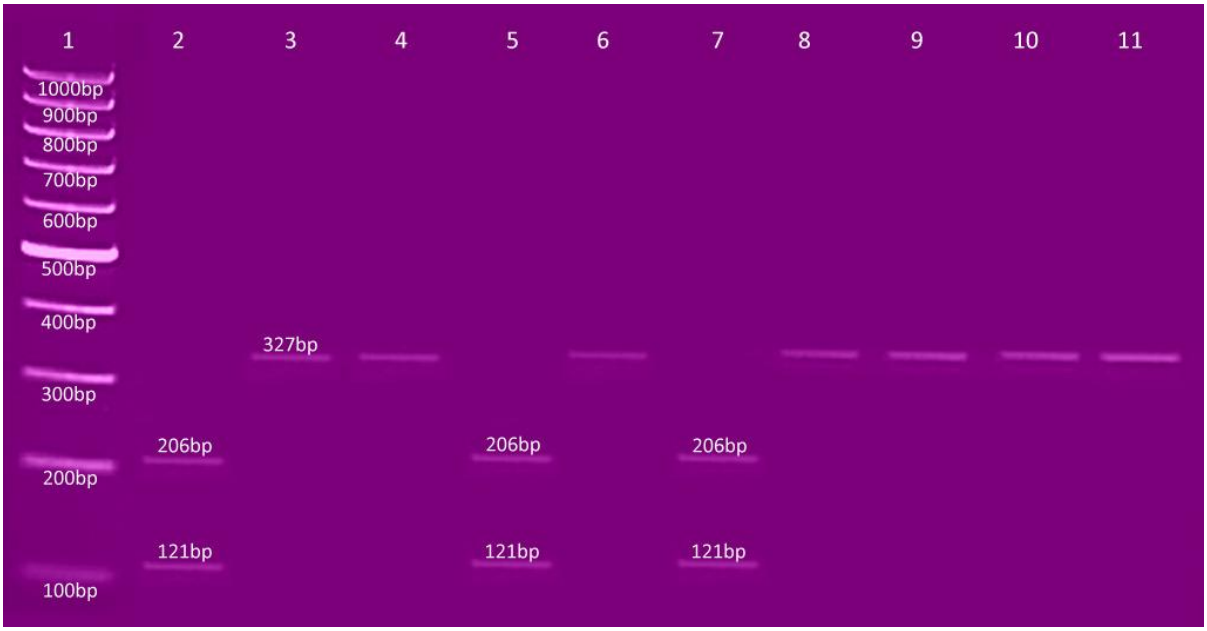


Figure 4. Restriction fragment length polymorphism (RFLP) polymerase chain reaction (PCR) for detection of mutations in *parC* genes. Nondigestion of PCR products indicates the presence of mutations in the quinolone resistance-determining region (QRDR) of this gene, whereas digestion of PCR products indicates the absence of such mutations.



Discussion

As a nosocomial bacterium that causes infections and outbreaks in adults and newborns, *A.baumannii* continues to be the subject of intensive research [19]. Furthermore, due to widespread antibiotic resistance, therapeutic measures are limited; which includes fluoroquinolone [20]. The profiles of antimicrobial resistance vary widely between countries, among hospitals within the same country, and even over time within the same country and the same institution [21]. Therefore, nosocomial infections must be monitored regularly, especially in underdeveloped countries such as Egypt, for antimicrobial susceptibility patterns. The findings will guide the choice for empirical therapy [22]. Our aim was to search for the flouroquinolone-resistant *A.baumannii* organisms that cause health care-associated infections in our tertiary care hospital and to detect of the presence of mutations in *gyrA* (Ser83Leu) and *parC* (Ser80Leu) genes.

In this study, most of the *A.baumannii* isolates were obtained from wounds and the fewest were recovered from bed sore samples 1 (1.4%). These results are in accordance with those of **Zaki et al.** [23], who found that blood cultures (50.35%), urine cultures (30.21%), and wound cultures (60.42%) produced the majority of the *A. baumannii* isolates. Also, **Aljindan, et al.** [24] noticed that wound swabs demonstrated the largest number of *A. baumannii* isolation (140 of 59 strains of *A. baumannii* were isolated from inpatient specimens), whereas nasal swabs, skin swabs and abscess specimens yielded the fewest (1 each).

In contrast to our findings, **Papa et al**. [25] found that 15% of isolates were added from catheters, 17% from blood cultures, and 20% from tracheal aspirates. **Abbo et al.** [26] found that 32% of isolates were recovered from the respiratory specimens, 17% from catheters, 16% from blood, 19% from wounds, and 32% from urine. Furthermore, **Ferreira et al**. [27] noted that samples of sputum and tracheal aspirate were the primary sources of *Acinetobacter* organism. Differences in the types of samples and cases used, the circumstances under which the samples were taken, the various time periods, regional variations in prevalence, and patient situations in general, may account for this inconsistency in these findings [28].

In our study, all isolates were resistant to piperacillin/tazobactam, ampicillin/sulbactam, trimethoprim/sulfamethoxazole, ceftriaxone, cefotaxime, and ceftazidime. Rates of resistance to ciprofloxacin and levofloxacin were the same (89.7 %), as were those to imipenem and meropenem (91.1 %). These findings were consistent with those of **ELshahhat et al.** [29] who reported that 100 % of *A. baumannii* organisms were resistant to ampicillin/sulbactam, amoxicillin/clavulinate, trimethoprim/sulfamethoxazole, cefazolin and cefuroxime, whereas the most effective antibiotics were imipenem (resistance rate of 88%), ciprofloxacin (33%), ceftriaxone (11%), ofloxacin (8 %), cefepime (7%) and amikacin (2%).

Our findings with regard to resistance were consistent with those of **Zaki et al. [**23], who discovered that 100% of *A. baumannii* isolates were resistant to ampicillin, ampicillin/sulbactam & imipenem; rates of resistance to ceftazidime (99.3%) and to cefepime (96.4%) were somewhat lower. This pattern of antibiotics resistance is similar to those reported previously by **Newire et al.** [30] and **Nowroozi et al. [**31]

In contrast to our findings, **Srinivasan et al.** [32] demonstrated that *A. baumannii* was resistant to imipenem, ceftazidime, amikacin, and ciprofloxacin, as well as streptomycin, gentamicin, kanamycin, tetracycline, and nalidixic acid. In addition, **Adams-Haduch et al.** [33] found that 95.9% of bacteria were resistant to ciprofloxacin; 87.7%, to ceftazidime, 40.8%, to cefepime and ampicillin /sulbactam; 18.4%, to imipenem; 22.4%, to meropenem; and 36.7%, to amikacin.

The unchecked widespread use of antibiotics in hospitals and the abuse of empirical treatment without guidance from culture and sensitivity testing are to blame for the high rate of resistance in our study. Furthermore, because most of the isolates were from patients with nosocomial infections, the high rates of drug resistance are understandable. The disparity in sensitivity patterns between the studies mentioned previously may be attributed to various antibiotic policies, the emergence of resistant strains because of indiscriminate antimicrobial therapy, patient's immune status, various infection control strategies, or frequent hospitalization.

Regarding the MIC of ciprofloxacin, 59 of isolates were resistant according to results of the E-test (bioMérieux, Marcy-1'Étoile, France). Thirty-one isolates (50.8%) showed ciprofloxacin MICs of > 128 μg/mL, which indicate high resistance to ciprofloxacin. Our results were consistent with those of **Elshahhat et al.** [29], who reported that of 72 *A. baumannii* isolates resistant to ciprofloxacin, 44 (61.1%) showed ciprofloxacin MICs of > 128 μg/mL. In contrast to our findings **Zaki et al.** [23] found that all isolates of *A. baumannii* that showed resistance in the disc diffusion method also showed resistance in the MIC method.

According to our PCR data, all 68 *A. baumannii* isolates had the *gyrA* and *parC* alleles. PCR RFLP analysis revealed that of the 59 *A. baumanii* isolates, 28 (47.5%) had a mutation at the *gyrA* (Ser83Leu) locus, 20 (33.9%) had a mutation at the *parC* (Ser80Leu) locus, and 14 (23.7%) had mutations at both loci. These results were consistent with those of **Tawfick and El-Borhamy** [21], who discovered single mutations in either *parC* or *gyrA* in 11 isolates and dual changes in the QRDRs of both genes in 38 isolates.

Our findings, however, conflicted with those of **Tantawy et al**. [34], who discovered that in all recovered isolates subjected to PCR RFLP, mutations were present in both *gyrA* (Ser83Leu) and *parC* (Ser80Leu). Also, according to **Zaki et al.** [23] all *A. baumannii* strains resistant to ciprofloxacin possessed *gyrA* (Ser83Leu) or *parC* (Ser80Leu) mutations, or both. Concurrent mutations in both genes were the most common changes (85.5%); 5% of mutations were in either the *gyrA* (Ser83Leu) or *parC* (Ser80Leu) locus [23].

The HinfI enzyme used in the PCR-RFLP approach helps detect mutations only in codon 83 of *gyrA* and codons 80 of *parC*; it cannot help detecting mutations in the Glu-87 codon in *gyrA* and the Glu-84 codon in *parC*. **Kakuta N et al.** [14] developed a mismatched PCR RFLP assay to identify changes in codons 83 and 87 of the *gyrA* locus and codons 80 and 84 of the *parC* locus, which are linked to fluoroquinolone resistance in *A. baumannii;* this technique accurately identified all fluoroquinolone-resistant and fluoroquinolone-susceptible strains of *A. baumannii* and specifically identified important genetic alterations associated with decreased response to fluoroquinolones.

Many studies have demonstrated that concurrent changes in both *gyrA* and *parC* alleles are necessary for *A. baumannii* resistance to ciprofloxacin [31]; however, in our study, of the 59 ciprofloxacin-resistant *A. baumanii* isolates, 14 had single mutations in *gyrA* (Ser83Leu), 10 of which had MICs of ≥ 128 µg/mL, and 6 had single mutations in *parC* (Ser80Leu), 5 of which had MICs of ≥ 128 µg/mL. This finding suggests that single mutations in either *gyrA* or *parC* may play a role in ciprofloxacin resistance. This result was consistent with that of **Elshahat et al.** [29]. **Ardebili et al.** [35] discovered that isolates with combined genetic alterations at *gyrA* and *parC* loci had higher ciprofloxacin resistance than did isolates with changes in only the *gyrA* or *parC* locus. **Zaki et al.** [23] reported that 42.3% of isolates with concurrent mutations had MICs of >128 µg/mL.

Conclusion

The troubling fluoroquinolone resistance observed in *A. baumannii* isolates from Egypt may be related to a single mutation in either *gyrA* (Ser83Leu) or *parC* (Ser80Leu) genes. Because the majority of isolates had MICs of >128 µg/mL, the available therapeutic options were limited. A governmental policy for appropriate prescribing and judicious usage of antimicrobial agents is required. Further investigation of resistance among a larger number of isolates across Egypt is recommended.

Conflicts of interest

None.

Financial disclosure

None

Author's contribution

All authors contributed to this research either through conception and design, material preparation, data collection and analysis, or the first draft of the manuscript. All authors read and approved the final manuscript.

Acknowledgment

None

References

1-**Rebic V, Masic N, Teskeredzic S, Aljicevic M, Abduzaimovic A, Rebic D.** The importance of *Acinetobacter* species in the hospital environment. Med Arch 2018; 72, 325–329.

2-**Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B.** Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges.Clin Microbiol Rev 2017; 30: 409–447.

3-**Singh, H., Thangaraj, P., Chakrabarti, A.** *Acinetobacter baumannii*: A brief account of mechanisms of multidrug resistance and current and future therapeutic management. J Clin Diagn Res 2013; 7: 2602–2605.

4-**Manchanda V, Sanchaita S, Singh NP.** Multidrug resistant *Acinetobacter*. J Glob Infect Dis 2010; 2: 291–304.

5-**Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, et al.** Biology of *Acinetobacter baumannii*: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol 2017; 7*:* 55.

6-**Sharma D, Patel RP, Zaidi STR, Sarker MMR, Lean QY, Ming LC.** Interplay of the quality of ciprofloxacin and antibiotic resistance in developing countries. Front Pharmacol 2017; 8: 546.

7-**Breijyeh Z, Jubeh B, Karaman R.** Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules 2020; 25: 1340.

8-**Aldred KJ, Kerns RJ, Osheroff N.** Mechanism of Quinolone Action and Resistance. Biochemistry 2014; 53(10): 1565–1574.

9-**Redgrave LS, Sutton SB, Webber MA, Piddock LJV.** Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol. 2014; 22: 438-445.

10-**Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BSF.** Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control 2019; 8: 118.

11-**Hooper DC, Jacoby GA.** Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. Cold Spring Harb Perspect Med. 2016; 6: a025320.

12-**Khalil D, Becker CAM, Tardy F.** Alterations in the quinolone resistance-determining regions and fluoroquinolone resistance in clinical isolates and laboratory-derived mutants of Mycoplasma bovis: not all genotypes may be equal. Appl Environ Microbiol 2016; 82: 1060–1068.

13-**Falah F, Shokoohizadeh L, Adabi M.** Molecular identification and genotyping of *Acinetobacter baumannii* isolated from burn patients by PCR and ERIC-PCR. Scars Burn Heal 2019; 5:2059513119831369.

14-**Kakuta N, Nakano R, Nakano A, Suzuki Y, Tanouchi A. Masui T, et al.** A novel mismatched PCR-restriction fragment length polymorphism assay for rapid detection of *gyrA a*nd *parC* mutations associated with fluoroquinolone resistance in *Acinetobacter baumannii*. Ann Lab Med 2020; 40: 27-32.

15-**Taha M, Tartor YH, Abd El-Hamid M, El-Mesalamy MM, EL Sayed A.** PCR-restriction fragment length polymorphism and DNA sequencing for identification of Malassezia species isolated from animals in Egypt. Zagazig Vet J 2020; 48: 128–139.

16- **Valentine SC , Contreras D, Tan S, Real L J, Chu S, Xu HH.** Phenotypic and molecular characterization of Acinetobacter baumannii clinical isolates from nosocomial outbreaks in Los Angeles Country, California. J clinical microbiol 2008; 46(8): 2499-507.

17-**CLSI. Performance Standards for** *Antimicrobial Susceptibility Testing*, 30th ed. CLSI supplement M100, Clinical and Laboratory Standards Institute: Wayne, PA, 2020.

18-**Lee J. K., Lee Y. S., Park Y. K., Kim B. S.** Mutations in the *gyrA* and *parC* genes in ciprofloxacin-resistant clinical isolates of *Acinetobacter baumannii* in Korea. Microbiol Immunol. 2005; 49: 647–653.

19-**Gramatniece A, Silamikelis I, Zahare I.., Urtans V, Zahare I, Dimina E, et al.** Control of *Acinetobacter baumannii* outbreak in the neonatal intensive care unit in Latvia: Whole-genome sequencing powered investigation and closure of the ward. Antimicrob Resist Infect Control 2019;8,:84.

20-**Maragakis LL, Perl TM.** *Acinetobacter baumannii*: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis. 2008; 46: 1254–1263.

21-**Tawfick MM, El-Borhamy MI.** PCR-RFLP-Based detection of mutations in the chromosomal fluoroquinolone targets *gyrA* and *parC* genes of *Acinetobacter baumanii* clinical isolates from a tertiary hospital in Cairo, Egypt. Am J Microb Res 2017; *5*, 37–43.

22-**Boroumand MA, Akhyani H, Sheikhvatan M, Yazdi SH, Saboorian R, Hashemi S, et al.** Evaluation of antimicrobial resistance of *Acinetobacter baumannii* to imipenem, ciporofloxacin and ceftazidime using E test. Iran J Publ Health 2009;38: 130–133.

23-**Zaki MES, Abou ElKheir N, Mofreh M.** Molecular study of quinolone resistance determining regions of *gyrA* gene and *parC* genes in clinical isolated of *Acinetobacter baumannii* resistant to fluoroquinolones. Open Microbiol J 2018; 12:116–122.

24-**Aljindan R, Alsamman K, Elhadi N.** ERIC-PCR genotyping of *Acinetobacter baumannii* isolated from different clinical specimens. Saudi J Med Sci 2018; 6: 13–17.

25-**Papa A., Koulourida V., Souliou E.** Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* in a newly established Greek Hospital. Microb Drug Resist.2009; *15*, 257–260.

26-**Abbo A, Navon-Venezia S, Hammer-Muntz O, Krichali T, Siegman-Igra Y, Carmeli Y.** Multidrug-resistant *Acinetobacter baumannii*. Emerg Infect Dis.2005;1*:*22–29.

27-**Ferreira AE, Marchetti DP, Rosa da Cunha G, Moreira de Oliveira L, Fuentefria DP, Bello AGD, et al.** Molecular characterization of clinical multi resistant isolates of *Acinetobacter* sp. from hospitals in Porto Alegre, state of Rio Grande do Sul, Brazil. Rev Soc Bras Med Trop.2011;44:725–730.

28-**Fahim NAE.** Prevalence and antimicrobial susceptibility profile of multidrug-resistant bacteria among intensive care units patients at Ain Shams University Hospitals in Egypt–a retrospective study. J Egypt Public Health Assoc 2021;96:7.

29-**Elshahat M, Aboelized E, Abd El Rahman R, Nabil M, Yassin A.** Association between mutations in *Gyr A/B* and *Par C/E* genes of *Acinetobacter baumannii* clinical isolates and fluoroquinolones resistance. Microbes Infect Dis. 2021;2: 343–351.

30-**Newire EA, Ahmed SF, House B, Valiente E, Pimentel G.** Detection of new SHV-12, SHV-5 and SHV-2a variants of extended spectrum beta-lactamase in *Klebsiella pneumoniae* in Egypt. Ann Clin Microbiol Antimicrob.2013; 12: 16.

31-**Nowroozi J, Akhavan Sepahi A, Tahmasebinejad Kamarposhti L, Razavipour R, Mazhar F.** Evaluation of ciprofloxacin (*gyrA, parC* genes) and tetracycline (tetB gene) resistance in nosocomial *Acinetobacter baumannii* infections. Jundishapur J Microbiol 2014; *7:*e8976.

32-**Srinivasan VB, Rajamohan G, Pancholi P, Stevenson K., Tadesse D, Patchanee P, et al.** Genetic relatedness and molecular characterization of multidrug resistant *Acinetobacter baumannii* isolated in central Ohio, USA. Ann Clin Microbiol Antimicrob 2009;8: 21.

33-**Adams-Haduch JM, Paterson DL, Sidjabat HE, Pasculle AW, Potoski BA. et al.** Genetic basis of multidrug resistance in *Acinetobacter baumannii* clinical isolates at a tertiary medical center in Pennsylvania. Antimicrob Agents Chemother. 2008; *52*, 3837–3843.

34-**Tantawy EA, El-Sayed HM, Matar H M, El-Azhary BA.** Multi- and Extensive-Drug resistant *Acinetobacter baumannii* in ICUs:

Risk factors, antimicrobial resistance profiles, and co-harboring of *gyrA* and *parC* mutations. Egypt J Med Microbiol. 2020; 29: 109–116.

35-**Ardebili A, Lari AR, Beheshti M, Lari E. R.** Association between mutations in *gyrA* and *parC* genes of *Acinetobacter baumannii* clinical isolates and ciprofloxacin resistance. Iran J Basic Med Sci 201; 18: 623–626.

Taha MS, Shoeib SM, Abdelwahab MA. Mutations in *gyrA and parC* genes in fluoroquinolone- resistant *Acinetobacter baumannii* that causes hospital acquired infection. Microbes Infect Dis 2023; 4(2): 590-600.