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Original Article

Mutation-based fluoroquinolone resistance in carbapenem-resistant *Acinetobacter baumannii* and *Escherichia coli* isolates causing catheter-related bloodstream infections

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ABSTRACT

Objective: We studied the presence of mutations in the chromosomal quinolone resistance-determining regions (QRDRs) of the fluoroquinolone targets *gyrA* and *parC* genes and detected the carbapenem resistance (CR) encoding genes among *Acinetobacter baumannii* and *Escherichia coli* isolates from catheter-related bloodstream infections (CRBSIs).

Methods: The study included 39 non-duplicate isolates of *A. baumannii* (14/39, 35.9%) and *E. coli* (25/39, 64.1%) isolated from 128 confirmed CRBSIs cases. Antimicrobial susceptibility testing was performed, followed by an evaluation of biofilm formation using the tissue culture plate method. The carbapenemase encoding genes were detected by multiplex polymerase chain reaction (PCR). The mutations in QRDRs of *gyrA* and *parC* genes were determined by singleplex PCR amplification followed by DNA sequencing and BlastN analysis in the GenBank database. DNA and the translated amino acid sequences were analyzed using the Mega7 bioinformatics tool.

Results: Multidrug-resistant (MDR) *E. coli* and *A. baumannii* isolates harbored CR encoding genes and combined *gyrA* and *parC* genes mutation. The specific substitutions observed in GyrA were Cys173Arg, Cys174Gly, Asp80Val, Tyr178ASP, Tyr84Gly, Glu85Lys, Ser172Leu, and Asp176Asn, while the specific substitutions observed in the ParC amino acid sequence were point mutation 62 Arg, Phe60Leu, Ils66Val, and Gln76Lys. Point mutation 62Arg was detected in two *A. baumannii* isolates, whereas Ser172Leu mutation was observed in two *E. coli* isolates.

Conclusion: The presence of new single and multiple mutations in QRDR causes the emergence of MDR *E. coli* and *A. baumannii* infections in carbapenem-resistant *Enterobacteriaceae* in Egypt, requiring further investigation in Gram-negative bacteria.

Keywords: *Acinetobacter baumannii,* carbapenem resistance, catheter-related bloodstream infections, *Escherichia coli,* fluoroquinolone resistance

Introduction

Bloodstream infections (BSIs), specifically those caused by multidrug-resistant (MDR) bacterial pathogens, are associated with high morbidity and mortality worldwide due to the difficulty of treating with the available antimicrobial drugs.[1] One of the critical sources of BSIs is the central venous catheter (CVC). Catheter-related BSIs (CRBSIs) are laboratory-confirmed BSIs that develop within 48 h of central line placement and are not related to any infection at another body site.[2] CRBSIs remain significant healthcare-associated infections that can adversely affect patient care, causing a substantial mortality rate.[3,4]

Globally, Gram-negative bacteria (GNB), particularly *Escherichia coli* and *Acinetobacter baumannii*, have recently become prevalent in healthcare settings. Moreover, *E. coli* and *A. baumannii* are identified as leading nosocomial pathogens and among the main causes of BSIs.[5,6] These bacterial species have become increasingly multiple resistant to diverse classes of antimicrobials, particularly the most clinically used ones, including fluoroquinolones (FQs), aminoglycosides, and carbapenems.[7,8] Notably, the carbapenem-resistant *A. baumannii* and *Enterobacteriaceae* members are among the common healthcare-associated pathogens with critical priority according to the WHO global priority list of antimicrobial-resistant bacteria in 2017.[9] Indeed, infections

with carbapenem-resistant *Enterobacteriaceae* (CRE) are a major challenge in healthcare settings and a growing concern worldwide.[8] Moreover, the resistant bacteria to carbapenems, owing to harboring the carbapenemases encoding genes such as bla_{OXA-48}, $bla_{NDM,}$ and bla_{KPC}, were found to be resistant to third-generation cephalosporins and FQs as well.[10]

FQs are bactericidal agents that are used as antimicrobial prophylaxis in immunosuppressed patients and/or primary antibacterial medication.[11] FQs target two homologous enzymes, DNA topoisomerases II (also known as DNA gyrase) and topoisomerases IV, which are essential for supercoiling bacterial DNA.[12] Both enzymes are composed of subunits encoded by *gyrA* and *gyrB* (for DNA gyrase) and *parC* and *parE* (for topoisomerase IV). The development of FQs resistance is a stepwise process resulting from the accumulation of amino acid substitutions (or mutation) in these subunits that are usually correlated with the high levels of FQs resistance.[13] These amino acid substitutions result from mutations in the quinolone resistance determining regions (QRDRs) in both *gyrA* and *parC* genes.[14] On the other hand, plasmid-mediated quinolone resistance occurs due to the plasmid-carried quinolone-resistance genes such as *qyrA*. These genes encode a family of proteins that protect the target enzymes from the action of quinolones.[11]

Surveillance for antimicrobial resistance is crucial to monitor the resistance trends in a developing country like Egypt. Thus, antimicrobial resistance surveillance studies are important to identify emerging MDR bacterial pathogens and resistance mechanisms, in addition to guiding for appropriate selection for empirical antimicrobial therapy and/or support the antimicrobial stewardship programs in healthcare settings.[15] Accordingly, the present study aimed to evaluate the resistance to both carbapenems and FQs among MDR *E. coli* and *A. baumannii* clinical isolates from intensive care unit (ICU) patients suffering from CRBSIs at a tertiary care hospital in Egypt. The study also aimed to investigate the presence of mutations in the chromosomal QRDRs of the fluoroquinolone resistance genes *gyrA* and *parC* and detect the carbapenem resistance (CR) encoding genes bla_{KPC}, bla_{NDM} and bla_{OXA-48}.

Methods

Study patients and clinical samples

In this study, a total of 128 CRBSIs confirmed cases at a tertiary hospital located in 6th October City, Giza, Egypt, during the period from June 2016 to June 2018. All patients included in the study were ICU patients with CVC and acquired BSIs. The blood samples were collected from patients having clinical signs and symptoms of BSIs in a case of new-onset sepsis. Two sets of blood samples were drawn peripherally into BACT/ALERT blood culture bottles, incubated in BACT/ALERT system (BioMerieux, France), and monitored for 5 days. These blood samples were routinely collected and processed by the dedicated team during the medical care of ICU patients having CVC. Positive blood cultures were then recovered by streaking on MacConkey's agar, Blood agar, and Chocolate agar plates. The plates were incubated at 37°C for 18–24 h. Gram stain reaction of the isolates was examined, and the isolates were primarily identified. The microbiological identification of the isolates was then carried out by MALDI-TOF mass spectrometry automated systems.

Determination of antimicrobial susceptibility patterns

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method on Mueller Hinton Agar (MHA) (Oxoid, Hampshire, UK). The results were interpreted as susceptible (S), intermediate (I), or resistant (R), according to Clinical and Laboratory Standards Institute (CLSI) guidelines (30th edition).[16] The antimicrobial discs used in the current study, representing diverse classes of antimicrobials, were Ampicillin/sulbactam (10/10), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), and levofloxacin (5 µg), Piperacillin/tazobactam (30 µg). The isolate was verified MDR when it showed resistance to at least three different antimicrobial classes.[17]

Determination of biofilm formation

According to Ruchi *et al.*,[18] biofilm formation was assayed using the microtiter plate and crystal violet method. A loopful of the bacterial isolate from overnight culture was inoculated into 10 mL of trypticase soy broth containing 1% glucose and incubated overnight at 37°C. Individual wells of sterile 96 well-flat bottom polystyrene tissue culture plates (Greiner Bio-One, Germany) were filled with 200 µl of the bacterial suspension corresponding to 0.5 McFarland. The optical densities (ODs) of stained adherent bacterial films were read using a microtiter plate reader (ThermoFisher Scientific, USA) at 600 nm. The cutoff optical density (ODc) biofilm formation ability was defined as three standard deviations above the mean OD of the negative control. All isolates were classified according to their adherence capabilities into non-adherent, weak, or strong adherent based on the OD value of bacterial biofilms.[18]

The results were interpreted according to the following criteria to classify the different adherent strengths as follows: If the mean of the three repeats OD readings $\leq$ ODc (the mean OD plus three standard deviations of the negative control) = Non-adherent (or non-biofilm producer), $ODc < OD \leq 2 \times ODc$ = weakly adherent (or weak biofilm producer), $2 \times ODc < OD \leq 4 \times ODc$ = moderately adherent (or moderate biofilm producer), and if $4 \times ODc < OD$ = strongly adherent (or strong biofilm producer). *Staphylococcus aureus* ATCC 29213 was used as the positive control for biofilm production.[19]

PCR-based molecular methods

DNA extraction and PCR oligonucleotide primers

For PCR detection of CR genes, total DNA was used as a template in PCR assays. Total DNA was extracted from all tested isolates using the boiling method by heating bacterial cells suspension in sterile distilled water at 100°C for 10 min, followed by removal of cellular debris by centrifugation at 14,000 rpm for 1 min. The supernatant was collected and used as template DNA for PCR amplification. For PCR amplification of QRDRs of *gyrA* and *parC* and DNA sequencing, genomic DNA was extracted from examined isolates using Gene JET Genomic DNA Purification Kit (Thermo Scientific, USA) following the manufacturer's instructions. PCR products were purified for sequencing by QIAquick Gel Extraction Kit (QIAGEN, USA) according to the manufacturer's protocol. The sequences of PCR oligonucleotide primers used in the current study, synthesized by Invitrogen (UK), are listed in Table 1. These primers were examined using NCBI Primer-BLAST, available at NCBI, to ensure specificity (https://www.ncbi.nlm.nih.gov/guide/data-software/).

PCR amplification and DNA sequence analysis of QRDRs of *gyrA* and *parC* genes

In the present study, the QRDRs in both *gyr*A and *par*C genes were detected by PCR in *A. baumannii* and *E. coli* isolates to determine the changes in the structure of DNA gyrase and topoisomerase IV enzymes. The genes *gyrA* and *parC* were analyzed by PCR, followed by DNA sequencing. Out of the sequenced isolates, two quinolone-sensitive isolates were included as a control.

The QRDRs of *gyrA* and *parC* genes were amplified by single-plex PCR. The PCR reaction mixtures were prepared in total volumes of 20 µl. Each reaction contained 2 µl of template DNA, 1 µl of each primer and 10 µL of GoTaq® Green Master 2× Ready Mix (Promega, USA), then the volume was completed to 20 µL by adding 6 µL of nuclease-free water. The PCR amplification program was as follows: Initial denaturation for 5 min at 95°C, then 30 cycles of denaturing at 95°C for 30 s, annealing for 30 s at 47°C for *parC* gene and 53°C for *gyrA* gene, and extension at 72°C for 45 s, followed by a final extension at 72°C for 7 min. The PCR-amplified QRDRs were subjected to DNA sequencing using the technology of Sanger sequencing using Applied Biosystems 3500 Genetic Analyzer at Clinilab, Cairo, Egypt. The obtained DNA sequences and their predicted amino acid sequences were analyzed using online bioinformatics tools, including BLAST analyses tools (blastn and blastp) (http:/www.ncbi.nlm.nih.gov/BLAST/). The multiple sequence alignment tool (ClustalW) and Mega software version 7.0.26 were used.

Multiplex-PCR for detection of carbapenemases encoding genes

The carbapenemases encoding genes bla_{KPC}, $bla_{NDM,}$ and $bla_{OXA\text{-}48}$ were investigated using a multiplex PCR assay previously described by Poirel *et al.*[22]

Statistical analysis

Data are presented as numbers and percentages for categorical variables, and biofilm data are expressed as the mean ± standard deviation (SD).

Results

Identification and frequencies of *A. baumannii* and *E. coli* isolates recovered from different clinical samples

A total of 39 non-duplicate MDR bacterial clinical isolates of *A. baumannii* (14/39, 35.9% isolates) and *E. coli* isolates (25/39, 64.1% isolates) were recovered from the 128 blood samples included in the current study. The bacterial isolates recovered from the blood samples were of other bacterial species that were not targeted in the present study.

Antimicrobial susceptibility profiles of *A. baumannii* and *E. coli* isolates

Overall, there were high resistance levels among *A. baumannii* and *E. coli* isolates to the tested antimicrobial agents. Antimicrobial resistance profiles revealed that all (100%) *A. baumannii* and *E. coli* isolates were resistant to ampicillin, ampicillin/sulbactam, and amoxicillin/clavulanic acid. All *A. baumannii* isolates were resistant to ciprofloxacin and 92.86% were resistant to levofloxacin.

Table 1: Nucleotide sequences of PCR oligonucleotide primers

Target gene	Sequence (5'–3')	PCR amplicon (bp)	Source
gyrA	F: 5' AAATCTGCCCGTGTCGTTGGT 3' R: 5' GCCATACCTACGGCGATACC 3'	343	Rodríguez-Martínez *et al.*[20]
parC	F: 5' AAACCTGTTCAGCGCCGCATT 3' R: 5'AAAGTTGTCTTGCCATTCACT 3'	327	Cattoir *et al.*[21]
$bla_{OXA\text{-}48}$	F: 5' GCGTGGTTAAGGATGAACAC 3' R: 5' CATCAAGTTCAACCCAACCG 3'	438	Poirel *et al.*[22]
bla_{KPC}	F: 5' CGTCTAGTTCTGCTGTCTTG 3' R: 5' CTTGTCATCCTTGTTAGGCG 3'	798	
bla_{NDM}	F: 5' GGTTTGGCGATCTGGTTTTC 3' R: 5' CGGAATGGCTCATCACGATC 3'	621	

PCR: Polymerase chain reaction

E. coli isolates also showed resistance rates to ciprofloxacin and levofloxacin of 76% (19/25) and 36% (9/25), respectively. Regarding carbapenems, 100% (14/14) of *A. baumannii* isolates were resistant to each imipenem and meropenem, while 44% (11/25) and 64% (16/25) of *E. coli* isolates were resistant to imipenem and meropenem, respectively [Table 2].

Biofilm formation ability among isolates

The tissue culture plate method performed to assess the biofilm formation ability among *A. baumannii* quantitatively and *E. coli* isolates revealed that 21.43% (3/14) of *A. baumannii* isolates and 25% (3/12) of *E. coli* isolates are the only microorganisms showed the ability to produce biofilm. All the isolates forming biofilm were described as moderate biofilm formation.

Detection of common carbapenemase encoding genes

In the present study, the most predominant carbapenemase encoding gene among *E. coli* and *A. baumannii* was bla_{NDM}, as it was detected in 44% (4/25) and 50% (7/14), respectively. Collectively, bla_{NDM} was the predominant carbapenemase gene in 46.15% (18/39) followed by bla_{KPC} was detected in 17.95% (7/39) and bla_{OXA48} was detected in 2.26% (1/39).

Mutations in QRDRs of *gyrA* and *parC* genes in FQs-resistant isolates

The QRDRs in both *gyrA* and *parC* genes in *A. baumannii* and *E. coli* isolates were subjected to PCR amplification [Figure 1], followed by DNA sequencing and BLAST analyses. It was found that two isolates showed no mutation in the QRDR, while *A. baumannii* showed combined *gyrA* and *parC* mutations. A combined substitution was observed in all *E. coli* and *A. baumannii* isolates [Figure 2] that showed gene mutations. The specific substitutions observed in GyrA were Cys173Arg, Cys174Gly, Asp80Val, Tyr178ASP, Tyr84Gly, Glu85Lys, Ser172Leu, and Asp176Asn. While the specific substitutions observed in ParC were point mutation 62Arg, Phe60Leu, Ils66Val, and Gln76Lys. Point mutation 62 Arg was observed in two *A. baumannii* isolates, whereas Ser172Leu mutation was observed in two *E. coli* isolates as shown in Table 3.

Discussion

E. coli and *A. baumannii* bacterial species have recently been identified as leading nosocomial pathogens and among the main causes of BSIs.[5,6] According to National Healthcare Safety Network (NHSN) data, *E. coli* and *Acinetobacter spp*. are considered the most common etiologies for CLABSI.[23-25] In addition, mortality rates associated with invasive *A. baumannii* infection are relatively high, especially for carbapenem-resistant cases. The crude mortality for carbapenem-resistant *A. baumannii* infections ranges from 16% to 76%, compared to 5–53% for carbapenem-susceptible infections.[26] Moreover, attributable mortality of 70% has been reported for BSIs due to imipenem-resistant *A. baumannii*, compared with 24.5% for imipenem-susceptible *A. baumannii* in Taiwan.[27] Consequently, the therapeutic options are limited, particularly in critically ill patients. Antimicrobial resistance patterns differ considerably from country to country or among hospitals in the same country and within the same hospital over time.[28,29] Thus, the regular surveillance of nosocomial pathogens for prevalence and antimicrobial resistance outlines is warranted for appropriate empirical antimicrobial therapy. Accordingly, in this study, nosocomial *E. coli* and *A. baumannii* blood isolates from confirmed CRBSI cases were screened for their antimicrobial susceptibility patterns. In addition, FQs-resistant isolates were investigated for the quinolone resistance mechanism through mutations in QRDRs of the chromosomal FQs target genes *gyrA* and *parC*.[30]

Compared to the conventional microbiological identification methods, MALDI-TOF MS showed a precise identification rate of 100% of the target species and reduced the typical turn-around time with no loss of accuracy, providing a fast and accurate method for the identification of these bacteria, particularly in crowded health-care settings.[31,32]

The presence of indwelling devices can cause serious health-care problems, specifically with the production of biofilm that allows bacteria to colonize the indwelling devices and form a shield to protect microbes against antimicrobial agents.[33] Indeed, the previous studies revealed that biofilm formation is associated with the resistance of microorganisms, such as *E. coli*, toward antimicrobial agents, and biofilm formation increases the incidence of healthcare-associated infections, especially in CRBSIs.[34,35] The tissue culture plate method was used in this study to examine whether bacterial isolates

Table 2. Antimicrobial resistance profiles of *A. baumannii* and *E. coli* isolates

Type of antimicrobial	AMP (10μg)	AMC (20/10μg)	SAM (10/10 μg)	TZP (30 μg)	CTX (30 μg)	CRO (30 μg)	CAZ (30 μg)	CN (10 μg)	AK (30 μg)	CIP (5 μg)	LEV (5 μg)	IMP (10 μg)	MEM (10 μg)
E. coli (25)	100 25/25	100 25/25	100 25/25	84 21/25	100 25/25	100 25/25	100 25/25	64 16/25	40 10/25	76 19/25	36 9/25	44 11/25	64 16/25
A. baumannii (25)	100 14/14	100 14/14	100 14/14	85.71 12/14	100 14/14	100 14/14	92.9 13/14	85.71 12/14	71.4 10/14	85.71 12/14	85.71 12/14	92.86 13/14	92.86 13/14

AMP: Ampicillin; AMC: Ampicillin/clavulanic acid; AMC: Ampicillin/sulbactam; TZP: Piperacillin/tazobactam; CTX: Ceftriaxone; CRO: Cefotaxime; CAZ: Ceftazidime; CN: Gentamicin; AK: Amikacin; CIP: Ciprofloxacin; LEV: Levofloxacin; IMP: Imipenem; MEM: Meropenem; *A. baumannii: Acinetobacter baumannii*; *E. coli: Escherichia coli*

Table 3: Mutations in *gyrA* and *parC* in *A. baumannii* and *E. coli:*

Mutations	*A. baumannii*				*E. coli*			
	R1	R2	R5	S3	R1	R2	S3	R4
Quinolone resistance								
Ciprofloxacin	R*	R	R	S	R	R	S	R
Levofloxacin	S*	R	S	S	S	S	S	R
gyrA mutations								
Cys173Arg	Present	__	__	__	__	__	__	__
Cys174 Gly	Present	__	__	__	__	__	__	__
Asp80Val	__	Present	__	__	__	__	__	__
Tyr178ASP	__	Present	__	__	__	__	__	__
Tyr84Gly	__	__	Present	__	__	__	__	__
Glu85Lys	__	__	Present	__	__	__	__	__
Ser172Leu	__	__	__	__	Present	Present		
Val176Asn	__	__	__	__	Present	__	__	__
Asp176Asn	__	__	__	__	__	Present	__	__
His181Tyr	__	__	__	__	__	__	__	Present
His241Tyr	__	__	__	__	__	__	__	Present
Tyr201His	__	__	__	__	__	__	__	Present
parC mutations								
Point mutation 62 Arg	Present		Present					
Phe60Leu	--	Present	__	__				
Ils66Val	--	Present	__	__				
Gln76Lys	--	Present	__	__				

Cys: Cysteine; Arg: Arginine; Gly: Glycine; ASP: Aspartic acid; Val: Valine; Tyr: Tyrosine; Glu: Glutamic acid; Lys: Lysine; Ser; Serine; Asn: Aspragine; His: Histadine; Arg: Arginine; Phe: Phenylalanine; Leu: Leucine; Ils: Isoleucine; Gln: Glutamine; *A. baumannii: Acinetobacter baumannii*; *E. coli: Escherichia coli*

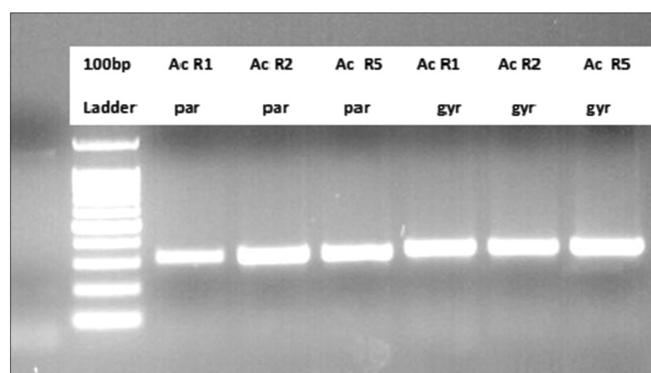

Figure 1: Single plex polymerase chain reaction for amplification of *gyrA* and *parC*

can form biofilm. Based on the results of evaluating biofilm formation ability, six isolates showed a moderate ability to form a biofilm. On the other hand, previous studies showed the higher rates of biofilm production among nosocomial isolates than our rates which could be correlated with the duration of hospitalization and/or prior antibiotic administration.[18,19,36]

The present results agreed with several studies that reported high resistance patterns of GNB to β-lactams in Egypt and worldwide.[27,37-40] On the other hand, our bacterial isolates were 100% sensitive to colistin and polymixin, which appeared to be the most effective antimicrobial agents against *E. coli* and *A. baumannii* isolates. Several studies revealed that FQs are typically used in combination with other antimicrobial agents to treat carbapenem-resistant pathogens.[41-43] Also, the results of the present study agreed with recent Egyptian studies that identified *E. coli* and carbapenem-resistant *Acinetobacter* spp. isolates as MDR organisms resistant to at least one antimicrobial agent in three or more different antimicrobial classes. Therefore, those pathogens have become target pathogens in national Egyptian Antimicrobial resistance in Egypt to decrease the MDR status identified in the clinical settings.[15,17,31,44]

Regarding *E. coli*, all (25/25, 100%) isolates were resistant to each ampicillin, ampicillin/sulbactam, aztreonam, ceftazidime, and ceftriaxone. Similar findings were reported by another previous study from Lahore by Sabir *et al.* (2014), who stated that 100% of the *E. coli* isolates were resistant to penicillin, in addition to 62.6%, 89.50% and 73.80% of isolates were resistant to amoxicillin/clavulanate, cefotaxime, and ceftazidime, respectively.[36] In the present study, *E. coli* isolates showed varied resistance patterns to the other antimicrobial agents; 64% (16/25) and 36% (9/25) of the isolates showed resistance to gentamicin and ciprofloxacin, respectively. Our findings agree with the Mohammadi *et al.* study in which *E. coli* isolates showed resistance to ciprofloxacin, gentamicin,

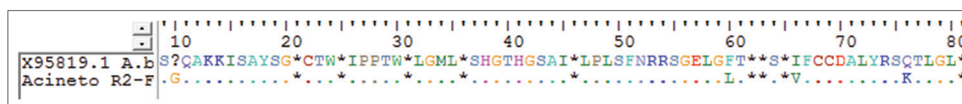

Figure 2: Example of the mutation and substitution of *parC* found in *Acinetobacter baumannii* isolate

piperacillin/tazobactam and amikacin with frequencies of 60%, 31.66%, 33.33%, and 11.66%, respectively.[37]

The current study revealed that the resistance rate among *A. baumannii* isolates was 92.86% to both imipenem and meropenem. This record was in agreement with a previous study from Egypt, which reported 74% resistance to imipenem among *carbapenem-resistance* isolates.[35] Notably, this study's rates of antimicrobial resistance are much higher than previous reports from the same hospital and/or other hospitals in Egypt.[39,40]

There is an increasing rate of resistance to FQs among Gram-negative isolates worldwide.[28] The present study findings agreed with a recent study by Lo *et al.*, who reported resistance frequencies of 70.9% and 65.3% to ciprofloxacin and levofloxacin, respectively.[45] Consistent with our study, Yang *et al.* surveyed 130 hospitals in China and showed that the median resistance rate of *A. baumannii* to FQs was 59.3%, and the median resistance rate of *E. coli* to FQ was 61.67%.[46]

Changes in the structure of the FQs target enzymes DNA gyrase and DNA topoisomerase IV are important mechanisms in conferring resistance to FQs in GNB. In *E. coli*, three or four mutations in both *gyrA* and *parC* genes were found necessary to obtain a high level of resistance to FQs, whilst double mutations at positions 83 (Ser83) of *gyrA* and 80 (Ser80) of *parC* led to a moderate level of resistance to FQs.[28] In the present study, sixteen representative isolates were sequenced then the obtained sequences were subjected to bioinformatic analysis using NCBI BlastN function against the GenBank database to detect the mutation in QRDRs of *gyr*A and *par*C genes. The investigated *A. baumannii* isolates showed combined mutations in both *gyrA* and *parC* encoding genes that explained the high resistance rates among the tested isolates. On the other hand, one isolate of *E. coli* showed a combination of three mutations, and the other three *E. coli* isolates showed only one mutation. Our results agree with Ardebili *et al.*[28] who reported that three or four mutations in the *gyrA* gene are necessary to obtain a high level of resistance to ciprofloxacin in *E. coli*. On the other hand, double mutations of *parC* cause only moderate-level resistance.[28] Our results were also in agreement with recent studies,[47-49] which reported that combined mutation of *parC* and *gyrA* was associated with resistance and suggested that the presence of *gyrA* and *parC* mutations at codon 83 and codon 80 with substitution of serine with leucine in *gyrA* and serine with leucine in *parC* were the most common mutations in *A. baumannii*. In addition, mutation at position 80 in *parC* was observed in 93% of isolates in *A. baumannii* in Iran, and all of which were resistant to ciprofloxacin and levofloxacin.[41] An earlier study by Vila *et al.*[50] reported different types of gene mutations, such as Ala84Pro or Gly81Val, in ciprofloxacin-resistant isolates. However, in the present study, other mutations were detected and associated with a high level of resistance to quinolones recorded in the present study. The specific substitutions observed in *gyrA* were Cys173Arg, Cys174 Gly, Asp80Val, Tyr178ASP, Tyr84Gly, Glu85Lys, Ser172Leu, and Asp176Asn. While the specific substitutions observed in *parC* were point mutation 62 Arg, Phe60Leu, Ils66Val, and Gln76Lys. Point mutation 62Arg was observed in two *A. baumannii* isolates, whereas Ser172Leu mutation was observed in two *E. coli* isolates.

The increased consumption of carbapenems may lead to major selection pressure, which would enrich the preexisting mutants of resistant *A. baumannii* and *E. coli* and result in the development of CR and other antimicrobial agents such as FQs.[51] The prevalence of the bla_{NDM} gene was 46.15% among carbapenem-resistant isolates and represented the predominant carbapenem-resistance encoding gene. The distribution of bla_{NDM} gene in this study was comparable to another Egyptian study that described the bla_{NDM} as the most prevalent carbapenemase resistance encoding gene in a university hospital in Egypt.[52] In the present study, the KPC encoding gene was detected among the tested isolates with a percentage of 17.95%. This is relatively in agreement with other studies that stated KPC carriage by GNB is not the main cause of CR in the Middle East and Egypt.[53,54] Regarding bla_{OXA-48}, only 2.56% of carbapenem-resistant isolates harbored this gene. In agreement with our study, other studies detected only 4.6% and 9.7% of bla_{OXA-48} in Egypt.[52,55] Earlier surveillance study of carbapenem-resistant GNB in a cancer hospital in Egypt, only three isolates harbored bla_{OXA-48}.[55] In contrast to our results, Asem *et al.*[54] reported a higher number of isolates carrying bla_{OXA-48} which may indicate the rapid dissemination of bla_{OXA-48} genes. This marked increase in the rates of antimicrobial resistance and high dissemination of resistance encoding genes and/or mutation could be explained by the lack of a national antimicrobial stewardship program, misuse and overuse of antibiotics in human, animal, and plant care, and the inconsistency of implementation of national infection control guidelines.[15,31,56]

Conclusion

A. baumannii and *E. coli* isolates showed the high frequencies of carriage of carbapenem-resistance encoding genes and the coexisting of several mutations within QRDR regions of the *gyrA* and *parC* genes are expected to contribute to high-level fluoroquinolone resistance among the tested isolates. The

accumulation of triple mutations in the QRDR of the *gyrA* and *parC* genes leads to minimal therapeutic options and calls for further investigation of the mutation in these genes in addition to strict infection control policy and an antimicrobial stewardship program implementation in Egyptian hospitals.

Authors Declaration Statements

Ethics approval and consent to participate

The study protocol was approved by the Research Ethics Committee of Cairo University Medical School in accordance with the Declaration of Helsinki (Ethical approval number: N-13-2020).

Availability of data and material

The data that support the findings of this study are available from the corresponding author on reasonable request.

Competing interests

All the authors declared that there is no conflict of interest. All authors declared that the work is original and does not infringe the copyright or other party's property rights.

All authors have read and approved this submission and have given appropriate credit to everyone who participated in this work.

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Authors Contribution Statement

The following was the contribution, according to the authors: Assoc. Prof. Mahmoud M. Tawfick supervised the practical work and writing and revising of the manuscript. Prof. Abeer Khairy provided: critical feedback and analyzed the practical work. Prof. Amani El-Kholy provided critical feedback, and led the practical work. Dr. Arwa Ramadan conducted the practical work, and the data analysis and wrote the manuscript.

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