AKADÉMIAI KIADÓ

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ORIGINAL ARTICLE

Fluoroquinolone resistance conferred by *gyrA*, *parC* mutations, and AbaQ efflux pump among *Acinetobacter baumannii* clinical isolates causing ventilator-associated pneumonia

NANCY M. ATTIA[1] and AMIRA ELBARADEI[2,3]*

[1] Microbiology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt

[2] Department of Microbiology and Immunology, Faculty of Pharmacy and Drug Manufacturing, Pharos University in Alexandria, Alexandria, Egypt

[3] Alexandria University Hospital, Alexandria University, Alexandria, Egypt

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ABSTRACT

Acinetobacter baumannii has emerged as an important nosocomial pathogen due to its ability to survive in hospital settings and its antimicrobial resistance. It is one of the key pathogens in ventilator-associated pneumonia (VAP). The aim of this study was to characterize the mechanisms of quinolone resistance among *A. baumannii* isolates causing VAP and to investigate the presence of the novel *abaQ* gene among them. Quinolone-resistant *A. baumannii* isolates causing VAP were collected over a period of 4 months. Mutations within *gyrA* and *parC* were analyzed and the presence of *qnrA*, *qnrB*, *qnrS*, and *abaQ* was investigated genotypically. Twenty-one *A. baumannii* isolates were collected, most of them (76.2%) were extensively drug-resistant (XDR) and only one isolate (4.8%) was pandrug-resistant (PDR). All isolates showed high level of resistance to ciprofloxacin, while *qnrA*, *qnrB* and *qnrS* were absent among our isolates. This is the first report of *A. baumannii* isolates co-harboring Ser81Leu in *gyrA* and Ser84Leu in *parC* together with the novel *abaQ* gene. Interestingly, a new mutation in *gyrA* quinolone resistance-determining region Arg89Cys was detected among two of our isolates. The emergence of XDR and PDR isolates among *A. baumannii* causing VAP is an alarming threat.

KEYWORDS

quinolone resistance, *gyrA*, *parC*, *abaQ*, *Acinetobacter baumannii*, MALDI-TOF MS

INTRODUCTION

Acinetobacter baumannii, is a Gram-negative, aerobic, non-lactose fermenting bacteria, which was deemed a low-category pathogen. However, it has emerged as a significant nosocomial pathogen, causing bloodstream, respiratory tract, and urinary tract infections [1]. In addition, *A. baumannii* has become a momentous cause of ventilator-associated pneumonia (VAP) worldwide, which is a frequent nosocomial infection among critically ill patients to which high rates of morbidity and mortality have been linked [1, 2].

More attention has been drawn to *A. baumannii* due to its growing antimicrobial resistance. *A. baumannii* shows intrinsic resistance to different antibacterial agents such as aminopenicillins, cephalosporins of the first and second generation, and chloramphenicol [3]. According to the 2016 guidelines of the American Thoracic Society and Infectious Diseases Society of America (ATS-IDSA) [4], quinolones are among the proposed empiric options for clinically suspected VAP. However, quinolones as well as other antibiotics are increasingly

* Corresponding author:
Amira ElBaradei
Department of Microbiology and Immunology, Faculty of Pharmacy and Drug Manufacturing, Pharos University in Alexandria, Canal El Mahmoudia Street, beside Green Plaza Complex, Alexandria, Egypt;
Phone: +20 3 3877032/033; +20 01 205233907; Fax: +20 3 3877149;
E-mail: amiraelbaradei@gmail.com

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rendered inactive due to various acquired resistance mechanisms by *A. baumannii* [5]. Hence, *A. baumannii* causing VAP are often therapeutically challenging and are therefore associated with high mortality [6].

Quinolone resistance has progressively spread worldwide. Resistance to quinolones is mainly mediated by mutations in the quinolone resistance-determining region (QRDR) of the DNA gyrase gene (*gyrA*) and/or topoisomerase IV gene (*parC*). These substitutions are primarily focused in the amino terminal domains of GyrA and ParC, which refer to residues 67 to 106 and residues 63 to 102 located in *Escherichia coli*, respectively [7].

Another mechanism for quinolone resistance is the protection of the DNA by the inhibition of quinolone binding to DNA gyrase and topoisomerase. This is mediated via plasmid-mediated quinolone-resistance (PMQR) determinants, which include *qnrA*, *qnrB*, and *qnrS* [8].

Recently, a novel efflux pump has been described in *A. baumannii*. It is a putative major facilitator superfamily (MFS) transporter, which is encoded by *abaQ* gene. This efflux pump is the first to mediate quinolone resistance among *A. baumannii* isolates. Moreover, it plays an important role in surface-associated motility and in virulence [9].

Our aim was to characterize the mechanisms of quinolone resistance and to investigate the presence of the novel *abaQ* gene among *A. baumannii* clinical isolates causing VAP.

MATERIALS AND METHODS

Strains

A. baumannii isolates resistant to both ciprofloxacin (CIP) and levofloxacin (LEV) were collected from clinical samples of VAP submitted to the Microbiology Department, Medical Research Institute, Alexandria University. These isolates were collected and included in this study over a period of 4 months (starting from September 2016 to the end of December 2016).

The identification of these isolates as *A. baumannii* was confirmed using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; Bruker, Billerica, MA, USA) as well as the presence of $bla_{\text{OXA-51}}$ gene.

Antimicrobial susceptibility testing

Disk diffusion method was used for the antimicrobial susceptibility testing of the collected strains. All antibiotic disks were chosen according to the CLSI recommendations [10]; these were ampicillin/sulbactam, aztreonam (TZP), ceftazidime, cefepime (FEP), cefotaxime (CTX), ceftriaxone (CRO), imipenem (IPM), meropenem (MEM), colistin (CT), gentamicin, tobramycin, amikacin (AK), doxycycline (DO), CIP, LEV, and trimethoprim/sulfamethoxazole. The strains were reported as susceptible, intermediate, or resistant to the previously mentioned agents according to the CLSI M100-S28 breakpoints [10]. These breakpoints were also

Table I. Primers used in this study

Primer	Nucleotide sequence (5′–3′)	Amplicon length in bases	Reference
$bla_{\text{OXA-51}}$ (F)	TAA TGC TTT GAT CGG CCT TG	350	[11]
$bla_{\text{OXA-51}}$ (R)	TGG ATT GCA CTT CAT CTT GG		
gyrA (F)	CGACCGATTGCCATTGAGGA	682	This study
gyrA (R)	CGGTACGGTAGGCATCAACA		
parC (F)	CAGAAAACCGCTCTGTAGCC	862	This study
parC (R)	TCATGATCCGATTCATCACG		
qnrA (F)	AGAGGATTTCTCACGCCAGG	661	[12]
qnrA (R)	TGCCAGGCACAGATCTTGAC		
qnrB (F)	GGMATHGAAATTCGCCACTG	562	[12]
qnrB (R)	TTTGCYGYYCGCCAGTCGAA		
qnrS (F)	GCAAGTTCATTGAACAGGGT	605	[12]
qnrS (R)	TCTAAACCGTCGAGTTCGGCG		
abaQ (F)	GCTGCCAACTGCATAACTGG	490	This study
abaQ (R)	GCTGGCAATGGTTGTTCGTT		

Note: All primers were purchased from Invitrogen by Thermo Fisher Scientific (CA, USA); the PCR Master mix used was MyTaq Red Mix, which was obtained from BioLine (London, UK). F: forward; R: reverse.

used to determine CIP and LEV MIC values, which were obtained using broth microdilution method [10].

Detection of resistance genes

Genotypic detection of *qnrA*, *qnrB*, *qnrS*, and the newly described efflux pump *abaQ* was performed using polymerase chain reaction (PCR). Detection of *gyrA* and *parC* mutations was carried out using PCR followed by sequencing of the amplified products using the chain-termination method. Mutations within the QRDR were determined by BioEdit sequence alignment editor, using the control sequence for *gyrA* (NZ_CZWC01000014.1) and *parC* (NZ_CZWC01000100.1) in *A. baumannii*. Then, alignments using Clustal Omega were applied to determine the position of these mutations in relation to *E. coli gyrA* and *parC* sequences. Primers, used in this study, are listed in (Table I). Some of these primers were designed in this study using Primer3 (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

RESULTS

Twenty-one *A. baumannii* isolates were included in this study. These isolates were collected from different samples obtained from VAP including endotracheal tubes, sputum, and bronchoalveolar lavage samples. Their identification as *A. baumannii* isolates was confirmed by MALDI-TOF MS as well as the presence of $bla_{\text{OXA-51}}$ gene among all of them.

All the isolates resistant to both CIP and LEV were also resistant to TZP, FEP, CTX, CRO, IPM, MEM, and AK. Only one isolate was found CT-resistant. The disk diffusion test results are listed in Table II.

MIC values of CIP revealed that all of the isolates showed high-level resistance to CIP (≥32 μg/ml), whereas MIC values of LEV ranged from 16 to 256 μg/ml. Details of the MIC and the detected mutations in *gyrA* and *parC* values are shown in Table III.

Genotypically, *abaQ* gene was detected among all of our isolates, whereas *qnrA*, *qnrB*, and *qnrS* were absent.

DISCUSSION

VAP is considered one of the most commonly acquired nosocomial infections. It is among the most significant nosocomial infections caused by *A. baumannii* [6]. Moreover, multidrug-resistant (MDR), extensively drug-resistant (XDR), and even pandrug-resistant (PDR) *A. baumannii* isolates are continuously being reported [5, 13]. Among our

Table II. Susceptibility patterns of the 21 *A. baumannii* isolates

	Resistance		Intermediate		Sensitive	
Antibiotic	***N***	**%**	***N***	**%**	***N***	**%**
Ampicillin/sulbactam	20	95.24	1	4.76	0	0
Piperacillin/tazobactam	21	100	0	0	0	0
Ceftazidime	20	95.24	0	0	1	4.76
Cefepime	21	100	0	0	0	0
Cefotaxime	21	100	0	0	0	0
Ceftriaxone	21	100	0	0	0	0
Imipenem	21	100	0	0	0	0
Meropenem	21	100	0	0	0	0
Colistin	1	4.76	0	0	20	95.24
Gentamicin	19	90.48	1	4.76	1	4.76
Tobramycin	19	90.48	0	0	2	9.52
Amikacin	21	100	0	0	0	0
Doxycycline	6	28.57	5	23.81	10	47.62
Ciprofloxacin	21	100	0	0	0	0
Levofloxacin	21	100	0	0	0	0
Trimethoprim/sulfamethoxazole	18	85.72	2	9.52	1	4.76

Table III. MIC values of ciprofloxacin (CIP) and levofloxacin (LEV) and amino acid substitutions within *gyrA* and *parC* genes for all isolates

MIC of CIP (μg/ml)	MIC of LEV (μg/ml)	*gyrA* Mutation			*parC* Mutation	Number of isolates
		Within QRDR		Outside QRDR		
>256	128	Ser81Leu[a]	–	–	Ser84Leu[b]	8
>256	128	Ser81Leu	Arg89Cys[c]	His43Tyr[d]	Ser84Leu	1
>256	64	Ser81Leu	–	–	Ser84Leu	3
>256	256	Ser81Leu	–	–	Ser84Leu	1
256	64	Ser81Leu	–	–	Ser84Leu	1
256	64	Ser81Leu	–	His43Tyr	Ser84Leu	1
256	32	Ser81Leu	Arg89Cys	His43Tyr	Ser84Leu	1
256	32	Ser81Leu	–	–	Ser84Leu	1
256	16	Ser81Leu	–	–	Ser84Leu	1
128	16	Ser81Leu	–	–	Ser84Leu	2
128	16	Ser81Leu	–	His43Tyr	Ser84Leu	1

Note: MIC: minimum inhibitory concentration; QRDR: quinolone resistance-determining region.
[a]Equivalent to Ser83Leu in *E. coli gyrA.*
[b]Equivalent to Ser80Leu in *E. coli parC.*
[c]Equivalent to Arg91Cys in *E. coli gyrA.*
[d]Equivalent to His45Tyr in *E. coli gyrA.*

21 *A. baumannii* isolates, 1 (4.8%) was PDR, 4 (19%) were MDR, whereas the rest of the isolates (76.2%) were XDR. Huang et al. [6] reported that all the isolates in his retrospective study were MDR. According to Ciginskien et al. [14], the percentages of MDR and XDR *A. baumannii* were (13.3%) and (68.3%), respectively. The continuing occurrence of XDR and PDR *A. baumannii* isolates from VAP is an alarming threat.

All the isolates in this study harbored mutations within the QRDR of *gyrA* and *parC*. The primary mutation (Ser81-Leu in *gyrA*) was present in all the isolates as well as the secondary mutation (Ser84Leu in *parC*). Interestingly, both mutations were found together in 100% of our isolates. According to Ostrer et al. [15], these two mutations are enough to predict CIP and LEV resistance. Furthermore, a new mutation was detected in *gyrA* QRDR in the position 89 (Arg89Cys), which is equivalent to position 91 in *E. coli gyrA*. This substitution resulted in a polar non-charged amino acid (cysteine) instead of the basic amino acid (arginine). Akter et al. [16] reported the critical role of arginine at the position 91 of *E. coli gyrA,* where it stabilizes the drug-protein putative binding pocket through H-bonding interactions with CIP and another interaction with Asp at position 87 [16]. To the best of our knowledge, this mutation has not been reported before in *A. baumannii*. However, a silent mutation in this position has been reported previously in *E. coli* by Conrad et al. [17] and Lehn et al. [18].

In this study, *qnrA*, *qnrB*, and *qnrS* were not detected among our isolates. Several studies found these genes to be absent among their *A. baumannii* isolates [19, 20]. However, Hamed et al. [21] found only one strain-harboring *qnrS* out of his 28 *A. baumannii* isolates, with the absence of *qnrA* and *qnrB* genes.

On the other hand, *abaQ* gene was found among all of our isolates. It encodes the AbaQ efflux pump, which is involved in both pathogenicity and quinolone resistance [9]. Perez-Varela et al. [9] reported that *abaQ* was generally present among *A. baumanii* clinical isolates. However, this is the first study to investigate its presence among *A. baumannii* causing VAP in Egypt.

This study highlights the mechanisms of quinolone resistance among *A. baumannii* causing VAP. Mutations within QRDR are the main mechanism underlying quinolone resistance among our isolates. This is the first study to report a new mutation in the QRDR of *A. baumannii gyrA* (in position 89). This mutation needs further elucidation. On the other hand, *qnrA*, *qnrB*, and *qnrS* genes had no effect on quinolone resistance among our isolates. The significance of the new AbaQ efflux pump encoding gene needs further investigations. The increased XDR *A. baumannii* isolated from VAP represents a huge obstacle to achieving prompt appropriate antimicrobial therapy, which is crucial to enhance clinical outcomes.

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