SHORT COMMUNICATION

Detection of quinolone-resistance mutations of parC gene in clinical isolates of Acinetobacter baumannii in Iran

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Background: The purpose of this study was to screen of parC gene mutations in clinical isolates of *Acinetobacter baumannii* from intensive care units (ICUs) of Alzahra Hospital, Isfahan, Iran. **Materials and Methods:** Seventy isolates of *A. baumannii* between March 2011 and June 2012 were studied. Susceptibility test was established by E-test method. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and sequencing was performed for detection of parC gene mutation. **Results:** 77.1% of specimens were highly resistant. Mutation at position 80 in parC was observed in 93% of isolates. **Conclusion:** High proportion of *A. baumannii* isolates had a mutation in *parC* that can play an important role in increased incidence of these isolates.

Key words: A. baumannii, multidrug resistant, parC gene

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INTRODUCTION

Acinetobacter baumannii is an omnipresent nonfermentative gram negative microorganism that frequently colonizes in the skin and upper respiratory tract of hospitalized patients.[1,2] In two recent decades multiple antibiotic-resistant strains of this microorganism have been implicated in nosocomial infections occurring in intensive care units (ICUs) worldwide.[3-5] Acinetobacter spp may lead to various types of infections include pneumonia, urinary tract infection, endocarditis, surgical site infection, meningitis, military injuries, and septicemia.[1,3,6] *A. baumannii* clinical isolates has increased worldwide especially in some Asian countries and it is classified as a difficult nosocomial infection to treat and control.[7,8] In the United States and Europe, *A. baumannii* accounts for 2.5-10% of all infections caused by gram negative bacteria seen in ICUs.[9,10] There is little information about antibiotic resistance of Acinetobacter spp in hospitals of Iran. Reported *A. baumannii* prevalence in only two studies on ICU patients in our country was 3.75 and 22.4%, respectively.[11,12] It is increasingly recognized as an important cause of community-acquired pneumonia, with a high mortality rate of 40-64%.[10,13-15]

Multidrug resistant prevalence of Acinetobacter spp. has been rising and the choice of treatment with usually used antibiotics, including lactams, aminoglycosides, chloramphenicol, tetracycline, and rifampin has become limited.[10,16] Quinolones have been utilizes for the treatment of Acinetobacter spp even compared with broad-spectrum cephalosporins and aminoglycosides, until a high rate of resistance to quinolones was detected recently.[3] The mechanism in gram negative bacilli is mutations in target enzymes including deoxyribonucleic acid (DNA) gyrase (encoded by gyrA and gyrB) and topoisomerase IV (encoded by parC and parE). The complex of topoisomerase-quinolone-DNA produces double-stranded breaks in DNA and blocks progress of the DNA replication enzyme complex. Finally it leads to bacterial DNA damage and bacterial cell death.[17] Resistance to quinolones in *A. baumannii* is interfered initially by stepwise selection of mutations in the drug targets *gyrA* and *parC*.[18] In *A. baumannii*, rapid resistance to ciprofloxacin and nalidixic acid is associated with the chromosomal mutations in gyrA and parC.[19] Single amino acid substitution in GyrA (Ser83Leu) is associated with high level resistance to ciprofloxacin and nalidixic acid. An additional amino acid substitution in ParC, mostly Ser80Leu, is associated with higher resistance in *A. baumannii*.[19-21] Scanty data are available on prevalence of quinolone-resistance mutations of parC gene of *A. baumannii* in Iran. The purpose of this study was to screen *of* parC gene mutations in clinical isolates of *A. baumannii* in our country.

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MATERIALS AND METHODS

Bacterial isolates

This is a cross-sectional study carried out in medical and surgical ICUs of Alzahra Hospital, Isfahan, Iran. The study was performed on 70 strains of *A. baumannii* isolates from different patients of five ICUs between March 2011 and June 2012. All expected Acinetobacter isolates, which were nonhemolytic, oxidase-negative, non-lactose fermentative, and gram-negative diplococci, were identified as *A. baumannii* by using the conventional biochemical tests and growth potential at 37 and 44°C.[22]

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests was carried out on all isolates of *A. baumannii* using the Kirby-Bauer Disk Diffusion Agar method according to Clinical Laboratory Standard Institute (CLSI) guidelines for 10 antibiotics [Table 1]. Susceptibility against ciprofloxacin and levofloxacin were established by epsilometer (E-test) method recommended by CLSI (Liofilchem, Italy). The breakpoints proposed by CLSI were used for ciprofloxacin (susceptible 1 mg/mL; resistant 4 mg/mL) and levofloxacin (susceptible 2 mg/mL; resistant 8 mg/mL). The interpretive criteria used were those established in CLSI standard *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.[23]

Polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism

Oligonucleotide primers, 5′-AAACCTGTTCAGCGCCGCATT-3′ and 5′-AAAGTTGTCTTGCCATTCACT-3′ were used for Ser80 mutation within parC. Bacterial DNA PCR isolated as previously described.[1] The PCR product was digested with *Hin*fI under conditions recommended by the manufacturer.[1] Both strands of amplified DNA specimens that had not shown this mutation were sequenced using forward parC primer. DNA sequences obtained were initially aligned with known sequences by using the BLASTX option (at the National Center for Biotechnology Information (NCBI) website) to generate amino acid alignment within the quinolone resistance determining regions (QRDRs). Sequence comparisons were made to the wild-type *A. baumannii* parC (GenBank accession no. X95819) QRDRs.[10]

RESULTS

Seventy isolates of *A. baumannii* from ICUs were studied (respiratory specimens, n = 23; urine samples, n = 16; blood specimens, n = 11; tracheal aspirates specimens, n = 5; cerebrospinal fluid (CSF) specimens, n = 5; and injuries specimens n = 10). Antimicrobial resistance rates of different antibiotics for *A. baumannii* isolated from the ICU of Alzahra Hospital are shown in Table 1. In disk diffusion method gentamicin, levofloxacin and ciprofloxacin (100%) and meropenem and ampicillin/sulbactam (90%) resistant antimicrobial agents against *A. baumannii* strains. We could find no relation between antibiotic therapy and mutation in parC gene (P = 0.68).

Of the 70 isolates, 68 (97.1%) were multidrug resistant. 77.1% (54/70) were resistant to imipenem, amikacin, and ampicillin-sulbactam; thus classifying these isolates as highly resistant. In our study, respiratory and CSF specimens showed the highest and lowest resistance respectively [Figure 1]. The minimal inhibitory concentrations (MICs) of ciprofloxacin were still high, but MICs of levofloxacin were nearly intermediate breakpoint in 23 isolates [Table 2]. Mutation of parC in *A. baumannii*, a fragment of the parC gene including the QRDR then *parC* was analyzed by PCR in 70 clinical isolates [Figure 2]. After digestion by restriction enzyme, the PCR products of *parC* amplified from wild type strain generated two fragment of 206 and 121 bp; whereas, Ser80 to Leu mutant strain remained 327 bp. Mutation at position 80 in parC was observed in 65 (93%) isolates. Sequencing results of five *A. baumannii* clinical isolates were similar to each other, also our results didn't reveal any changes in amino acid sequences.

Table 1: Antimicrobial resistance rates of different antibiotics for A. baumannii isolates from the ICU of Alzahra Hospital

Antibiotic class	Antibiotic	Sensitive	Intermediate (%)	Resistant (%)	CI 95%
Aminoglycosides	Amikacin	0	2 (2.9)	68 (97.1)	93.14–101.7
	Gentamicin	0	0	70 (100)	–
Carbapenems	Imipenem	0	5 (7.2)	65 (92.8)	89.38–98.6
	Meropenem	0	7 (10)	63 (90)	82.29–95.31
Cephems	Ceftazidime	0	1 (1.5)	69 (98.5)	95.73–101.37
	Cefepime	0	3 (4.3)	67 (95.7)	90.86–100.49
Quinolones	Ciprofloxacin	0	0	70 (100)	–
	Levofloxacin	0	0	70 (100)	–
Penicillin + inhibitor	Piperacillin–tazobactam	0	1 (1.5)	69 (98.5)	95.73–101.37
	Ampicillin–sulbactam	0	7 (10)	63 (90)	82.29–95.31

CI = Confidence interval

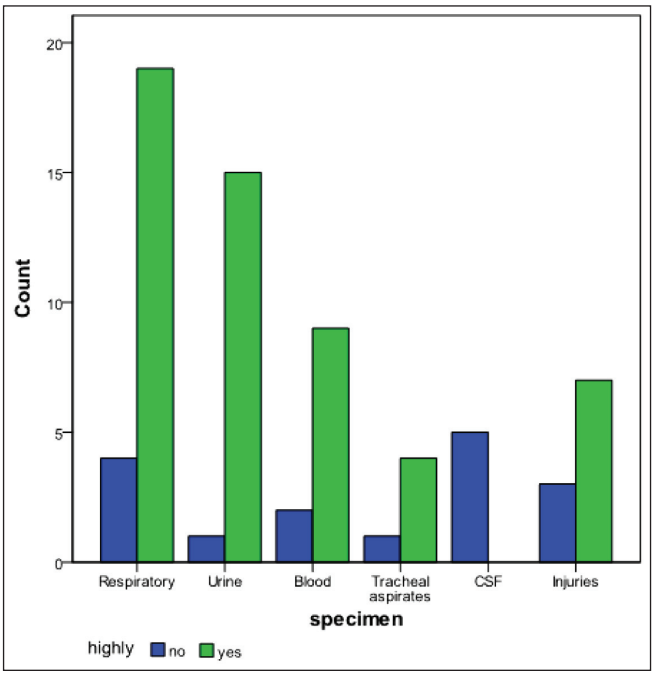

Figure 1: Prevalence of highly resistance *A. baumannii* isolates from the intensive care unit (ICU) of Alzahra Hospital

Table 2: MIC break points of ciprofloxacin and levofloxacin in *A. baumannii* isolates

Antibiotic	MIC break point μg/ml		
	S	I	R
Ciprofloxacin	≤1	2	≥4
	0	0	100% (70)
Levofloxacin	≤2	4	≥8
	0	32.8% (23)	67.2% (46)

MIC = Minimum inhibitory concentration

Statistical Analysis

Statistical analysis was done by Statistical Package for Social Sciences (SPSS) software (version 19, 2010, SPSS Inc, Chicago, IL, USA). Descriptive statistics and chi-square tests were applied to assess association between antibiotic resistances in various clinical specimens. All *P*-values were two-tailed ≤ 0.05 was considered statistically significant.

DISCUSSION

It is widely known that quinolone resistance; amino acid substitutions in gyrA and parC have important functions in gram negative bacilli.[16] The donation of amino acid substitutions in gyrA and parC to quinolone resistance may be variable among different gram negative bacilli. In *Pseudomonas aeruginosa*, the fluoroquinolone resistance is principally because of gyrA mutations with parC mutations being less important.[24] Three or four mutations in both gyrA and parC genes are required for high-level resistance to ciprofloxacin in *E. coli*, but double mutations in gyrA and parC were needed for high level resistance to quinolones in

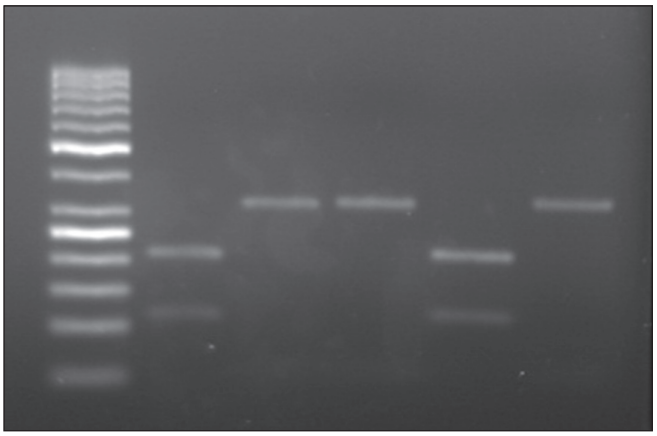

Figure 2: Detection of the mutation in the parC gene of *Acinetobacter baumannii* by restriction (HinfI) fragment length polymorphism analysis of polymerase chain reaction (PCR) products. Deoxyribonucleic acid (DNA) derived from clinical isolates was amplified using primers specific for parC. PCR products were digested with HinfI and separated by 2% agarose gel lane 2, 3, and 5: Nondigested products (327 bp); lane 1 and 4: HinfI-digested product (206 and 121 bp). M = marker

A. baumannii.[19] Our study showed that mutation at position 80 in parC was observed in 93% of isolates in *A. baumannii* in Iran and all of which are resistance to ciprofloxacin and levofloxacin. Sequencing results of five *A. baumannii* clinical isolates had not any changes, also no amino acid sequence changes were observed. It is probably due to mutation of *gyrA* alone, activated efflux pumps or mutation of parE. In a previous similar study that was performed in Korea, no mutation of gyrA and parC was found in six out of 59 clinical isolates in *A. baumannii* which are sensitive to ciprofloxacin and gatifloxacin. Nearly 90% of these isolates are resistant to ciprofloxacin and included at least one mutation with substitution of Leu for Ser80 in parC.[16] Similar to other reports, our results showed high incidence of mutation of parC in *A. baumannii* in Iran; in contrast, low frequency of mutation of *gyrA* and *parC* was detected in *A. baumannii* isolates of Taiwan.[1] It is well approved that mutation of *gyrA* and *parC* play a significant function in drug resistance. A logical interpretation is the mutation outside Ser80 in parC region may be more important in drug resistance in Iran (mutation in 7% of our isolates probable due to mutation of *gyrA* alone, activated efflux pumps or mutation of parE). In addition to mutations in DNA gyrase and topoisomerase IV efflux pump which increases the accumulation of drug may also include in multidrug-resistant isolates.

CONCLUSION

High percentage of MDR *A. baumannii* isolates (97.1%) was found in our country, yet high proportion of these isolates had a mutation in *parC*. Also mutation of parC in our country may play an important role in increased incidence of MDR *A. baumannii* and rapid detection of quinolone-resistance *A. baumannii* isolates can help physicians to justly treat these infections.

AUTHOR'S CONTRIBUTIONS

Farzin Khorvash: Substantial contributions to the conception or design of the work. Hossein Fazeli and Bahareh Vakili: Acquisition of samples, analysis and interpretation of data for the work. Moj Khaleghi and Bahareh Vakili: Drafting the work or revising it critically for important intellectual content. All of the authors for final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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