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Frequency of Chromosomally Encoded *gyrA* and *parC* Genetic Determinants of Fluoroquinolone Resistance in *A. baumannii*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors are equally contributed for the study design, methodology and data collection. All authors read and approved the final manuscript.

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ABSTRACT

Fluoroquinolones are administered as routine drugs of choice for treating complicated urinary tract infections caused by multidrug resistant *Acinetobacter baumannii* strains. It is now a world-wide issue that *gyr* and *par* induced quinolone resistance as one of the major drug resistance mechanisms. This investigation is thus aimed to assess the prevalence of quinolone resistance and to characterize the *gyrA* and *parC* producing strains of *A. baumannii*. Genomic DNA from 50 fluoroquinolone resistant *A. baumannii* were screened for *gyrA* and *parC* by PCR for the genetic relatedness with fluoroquinolone resistance, with sequencing of the representative strains. All the strains were positive for *gyrA*(100%) and 82% (n=41) for *parC*. Presence of *parC* was observed in 56.09% (n=23*)* ciprofloxacin resistant *A. baumannii with* 43.90% (n=18) in levofloxacin resistant *A.*

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baumannii. The findings of the present study showed the prevalence of fluoroquinolone resistance among *A. baumannii* in urinary tract infections and the frequency of *gyrA* and *parC* in inducing the resistance.

Keywords: A. baumanni; MDR-Ab; fluoroquinolones; gyrA; parC.

1. INTRODUCTION

Acinetobacter baumannii is considered as an ubiquitous gram negative non-fermentative bacilli documented as one among the six nosocomial pathogens by WHO [1]. Severe systemic complications associated with *A. baumannii*, among hospitalized patients in intensive care units manifesting various infections like pneumonia, urinary tract infections, endocarditis, post-operative wound infections and septicaemia have been documented world-wide [2,3]. Emergence of *A. baumannii* as a multi-drug resistant (MDR) strain worsens the treatment strategy and further control. Our earlier reports have documented the multi-drug resistance of *A. baumannii* against beta lactams and carbapenems which are induced by plasmid mediated genetic determinants [4,5]. The high level of resistance exhibited by *A. baumannii* against the routine antibiotics of choice such as cephalosporins, trimethoprim-sulfamethoxazole and carbapenem group of drugs in the recent decade had led to the implementation of fluoroquinolones as an empiric treatment especially for urinary tract infections [6].

Fluoroquinolone treatments for *A. baumannii* associated treatment encompass the administration of ciprofloxacin and levofloxacin, that targets the vital enzymes DNA gyrase and topoisomerase IV which are involved in bacterial viability. DNA damage and bacterial cell death is induced by fluoroquinolones by the formation of a topoisomerase-quinolone-DNA complex that has the ability to break the double stranded DNA blocking the DNA replication [7]. These enzymes are encoded by *gyrA, gyrB, parC* and *parR* respectively. In recent years, resistance to quinolones is slowly emerging and alarming due to the stepwise initial mutations in the *gyrA* and *parC* [8]. Single amino acid substation at the *Ser83Leu* of gyrA and an additional amino-acid substitution at *Ser80Leu* of *parC* is documented as the underlying mechanism in the chromosomally encoded quinolone genetic determinants in *A. baumannii* [9].

Periodical monitoring of the quinolone resistant strains will aid in the control of the spread of the resistant strains of *A. baumannii* in a developing country like India. Thus, this study is aimed at the molecular detection of *gyrA* and *parC* mediated quinolone resistance among the clinical isolates of *A. baumannii* with further comparative genomic assessments of the sequenced amplicons of the resistant determinants.

2. METHODS

2.1 Extraction of Genomic DNA

50 fluoroquinolone resistant strains (27 ciprofloxacin resistant and 23 levofloxacin resistant) of *A. baumannii* maintained at -80ºC in 80% / 20% (v/v) glycerol in LB medium in our repertoire, were retrieved as fresh cultures onto Mac Conkey agar with incubation at 37ºC for 24 hrs. Extraction of chromosomal DNA was achieved using the Qiagen DNA extraction kit in accordance with the manufacturer's instructions. Genomic DNA was stored in -20°C until further use.

2.2 PCR Amplification of gyrA and parC

PCR reaction mixture [15 µl] was prepared by adding 7.8 µl of 2x master mix [Taraka, Japan] in 5.6 µl of double distilled water with 0.31 µl of 100 pmol/ml concentration of the specific F'primer and R'primer [Eurofins Genomic India Pvt Ltd, Bangalore] of gyrA and parC genes. 1 µl of the DNA was added to the master mix and the amplification was performed with the PCR conditions as given in Table 1. PCR amplification was carried out in Eppendorfthermocycler, Germany. The resulting PCR amplicons were examined in 1% agarose gel electrophoresis containing ethidium bromide and was visualized by gel documentation system. The 100 bp DNA ladder was used to assess the PCR amplicon size.

2.3 Sequencing of the Genetic Determinants for Amplicon Confirmation

The amplicon product of gyrA and parCwere bi-directionally sequenced using Big-Dye terminator cycle sequencing kit and 3730XL Genetic

Table 1. PCR primers and conditions for *gyrA* and *parC* detection in *A. baumannii*

Gene of target	Primers	PCR conditions	Amplicon size
gyrA	5'-AAATCTGCTCGTGTCGTTGG-3' 5'-GCCATACCTACAGCAA TACC-3'	52°C for 30s, 36 cycles	343 bp
parC	5'-AAGCCCGTACAGCGCCGTATT-3' 5'-AAAGTTATCTTGCCATTCGCT-3'	60°C for 60s 36 cycles	327 bp

Analyzer. Sequences from forward and reverse primers were aligned using Bio-Edit Sequence Alignment Editor v7.2.5 which were subjected to BLAST (Basic Local Alignment Search Tool) for nucleotide similarity search. The sequences were aligned by ClustalW software version 1.83 for DNA multiple sequence alignment using default parameters. The parC sequences of *A. baumannii*MDR-ZJ06, *A. baumannii*AB030 and for gyrA sequences A.oleivorans DR1 were used as templates.

3. RESULTS AND DISCUSSION

Molecular characterization of *parC* and *gyrA* genes showed PCR positivity of 82% and 100% *(n=50)* in the tested strains (Figs. 1&2). Figs. 2 and 3 depicts the partial sequences and multiple sequence alignment of the *parC* and *gyrA* genetic determinants of resistance. In association with the resistance, presence of *parC* was observed in 56.09% (n=23) ciprofloxacin resistant *A. baumannii* with 43.90% *(n=18)* in levofloxacin resistant *A. baumannii*.

A. baumannii are associated with a wide range of nosocomial infections encompassing meningitis, septicaemia, pneumonia, skin and wound infections, urinary tract infections and are considered as a major challenge in the patient health care [10]. In recent years, it is imperative to note the administration of fluoroquinolones, for multi-drug resistant strains of *A. baumannii*, as they are broad-spectrum bactericidal agents [11]. However, it is alarming to glimpse at the emergence of fluoroquinolone resistance encompassing various mechanisms such as efflux pumps (Ade ABC & AdeM), plasmid and genomic resistant determinants such as *gyr* and *par* etc. [12].

In the present investigation, we have selected a total of 50 isolates as fluoroquinolone resistant strains that showed 100% positivity for *gyrA* PCR. This result correlates with the earlier study conducted in Iran documenting 90% positivity for the *gyrA* mediated ciprofloxacin resistant strains of *A. baumannii* [13]. In addition, *gyrA* mediated ciprofloxacin resistance is high when compared to the efflux pump mediated resistance which is considered as a secondary mechanism in inducing ciprofloxacin resistance among *A. baumannii* [14]. This documents the high-level resistance against fluoroquinolones mediated by *gyrA* and *parC* which is normally associated with a double mutation rather than multiple mutations recorded in other gram negative bacilli such as *E.coli* [15,16].

In the present study, we characterized the *gyrA* and *parC* from genomic DNA rather than plasmid DNA as most of the studies portrayed the fluoroquinolone resistance was mainly due to the chromosomal mutations in the quinolone determining regions (QRDRs) representing the intracellular targets for fluoroquinolones [17]. We also observed *parC* to be present in 82% of the strains which correlates with the earlier study that has documented a 100% presence in the ciprofloxacin resistant *A. baumannii* [18]. This might be associated with the mutations in *parC* playing a vital role in inducing fluoroquinolone resistance with a MIC >32µg/mL [6]. High levels of fluoroquinolone resistance documented in this study and in the earlier studies have also added the co-existence of efflux pumps to achieve these high levels of fluoroquinolone resistance [19].

Among the 50 isolates, 27 were characterized as ciprofloxacin resistant and 23 were as levofloxacin resistant by performing standard Kirby Bauer disc diffusion method as per CLSI guidelines (unpublished data). In a developing country like India, high levels of fluoroquinolone resistance have been documented by many reports based on the antibiotic susceptibility tests ranging from 65% to 83% [20]. Thus, the selection of the resistant strains for fluoroquinolone resistance was performed as per standards for further screening of the *gyrA* and *parC* genetic determinants.

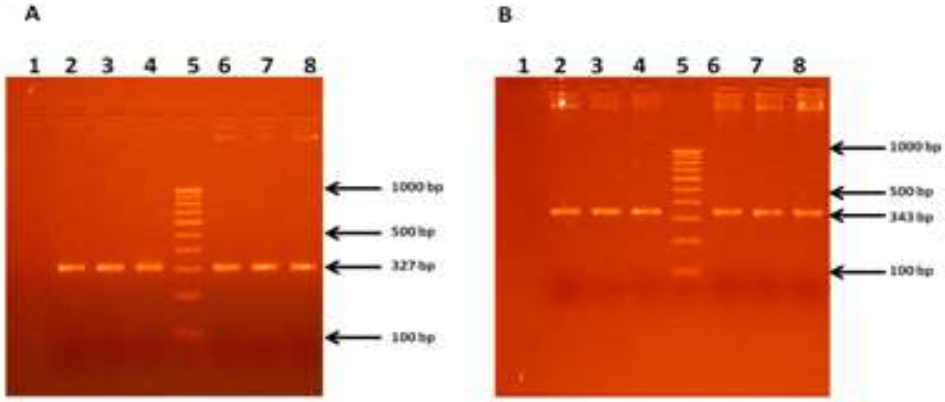

Figure A: Lane 1: Negative Control; 2,3,4,6,7,8 PCR amplified product of *parC* gene (327 bp); Lane 5: 100 bp DNA ladder.

Figure B: Lane 1: Negative Control; 2,3,4,6,7,8 PCR amplified product of *gyrA* gene (343 bp); Lane 5: 100 bp DNA ladder.

Fig. 1. Electropherogram of *parC* and *gyrA* detected from *A. baumannii* related to fluoroquinolone resistance

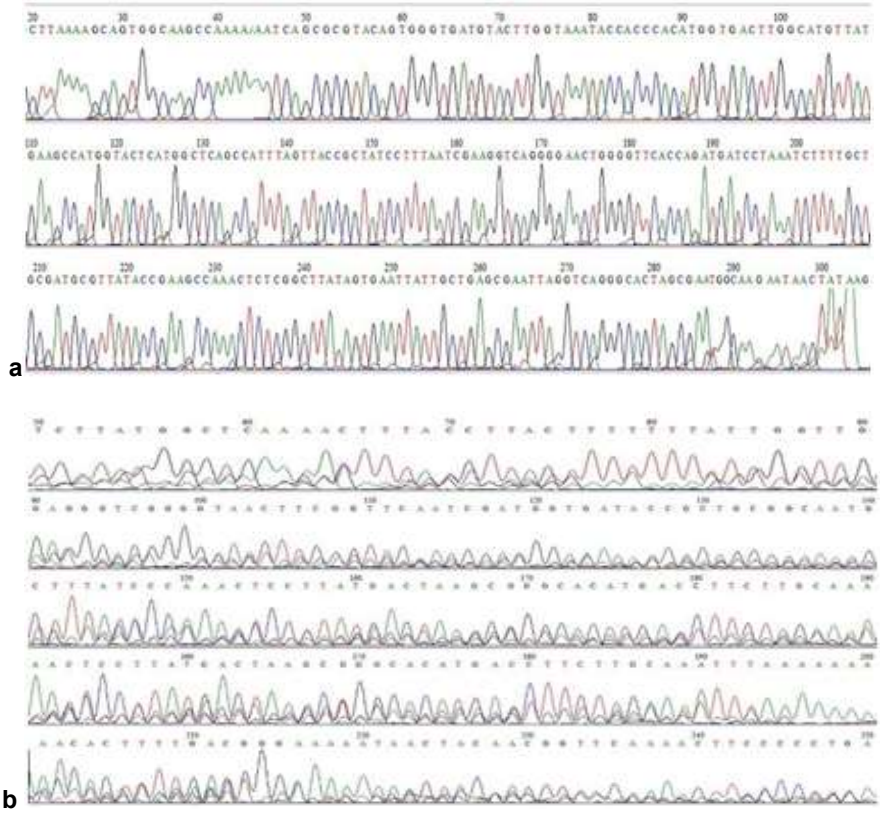

Fig. 2. The partial sequence chromatogram of a. *parC* and b. *gyrA* genes amplified using genomic DNA as the template isolated from *A. baumannii*

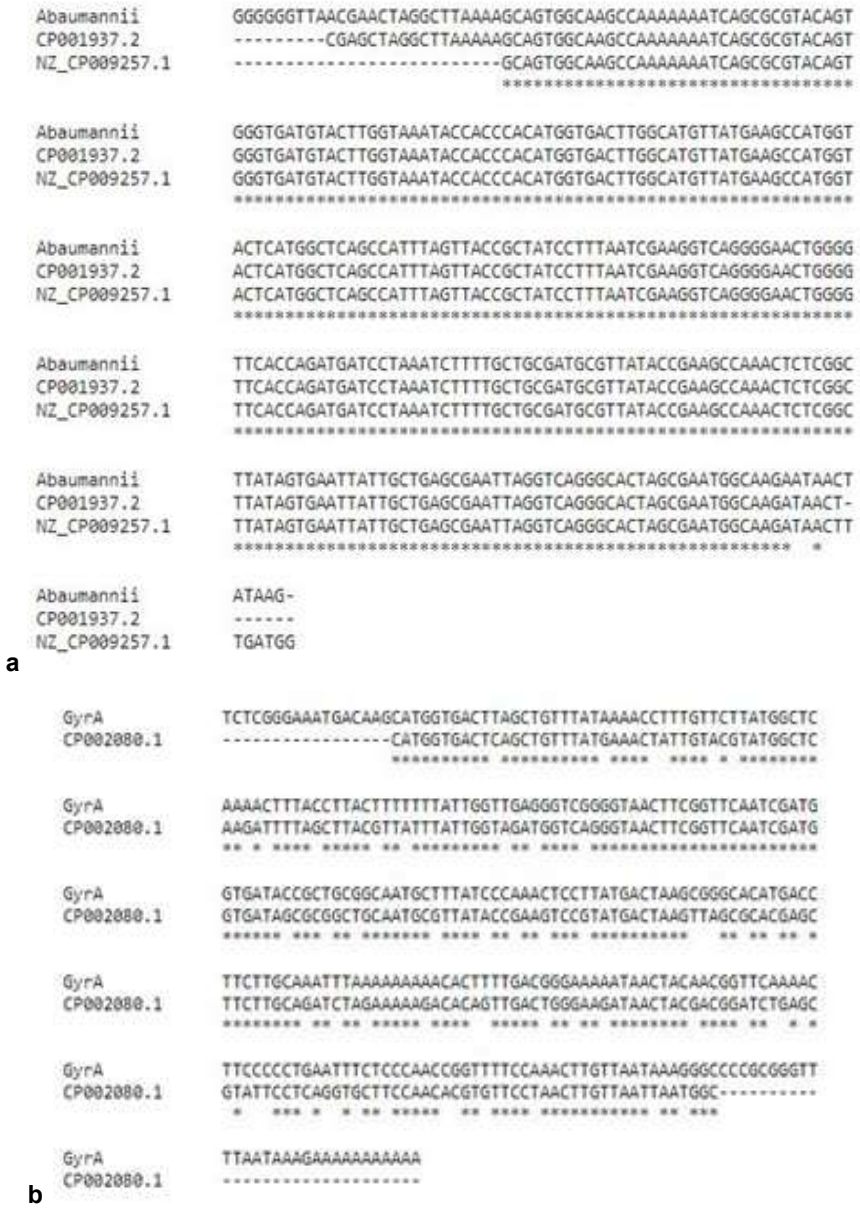

Fig. 3. Multiple sequence alignment of partial (a) *parC* and (b) *gyrA* gene from the present study with a sequence of *A. baumannii* that was available in the database. The deleted regions are depicted as dashes (--), mismatch as gap ( ) and conserved sequences as star (*)

Among the fluoroquinolone resistant genetic determinants screened, co-occurrences of the *gyrA* and *parC* is also not uncommon. 14.6% *(n=6)* of the strains showed the presence of both *gyrA* and *parC*. Earlier studies have also documented the co-occurrences of the genetic determinants mediating fluoroquinolone resistance along with resistance for other groups of antibiotics too resulting in the emergence of multidrug resistant strains of *A. baumannii* [21].

4. CONCLUSION

Present investigation emphasizes the frequency of the *gyrA* and *parC* mediated fluoroquinolone resistance amidst the *A. baumannii* isolates from urinary tract infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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