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.

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.
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. baumannii; 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 septicemia have been documented worldwide [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 substitution 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 -80°C in 80% / 20% (v/v) glycerol in LB medium in our repertoire, were retrieved as fresh cultures onto Mac Conkey agar with incubation at 37°C 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 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'-AAATCTGCTCGTGTGGTGG-3' | 52°C for 30s, 36 cycles | 343 bp |
|                | 5'-GCCATACTACAGCCTACCC-3' | | |
| parC           | 5'-AAGCCCTACAGCGCCTGATT-3' | 60°C for 60s, 36 cycles | 327 bp |
|                | 5 '-AAAGTTATCCTTGCCATTCGCT-3' | | |

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. baumanniiMDR-ZJ06, A. baumanniiAB030 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 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.
Fig. 1. Electropherogram of \textit{parC} and \textit{gyrA} detected from \textit{A. baumannii} related to fluoroquinolone resistance.

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

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

Fig. 2. The partial sequence chromatogram of a. \textit{parC} and b. \textit{gyrA} genes amplified using genomic DNA as the template isolated from \textit{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.

REFERENCES

1. Shrivastava S, Shrivastava P, Ramasamy J. World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics [Internet]. Journal of Medical Society. 2018;32:76. Available:http://dx.doi.org/10.4103/jms.jms_25_17

2. Wertheim H. Hospital acquired infections and antibiotic drug resistance in a Vietnamese hospital [Internet]. Available:http://dx.doi.org/10.1186/isrctn0853695

3. Brito D von D de, de Brito D von D, Oliveira EJ, Steffen Abdallah VO, da Costa Darini AL, Gontijo Filho PP. An outbreak of Acinetobacter baumannii septicemia in a neonatal intensive care unit of a University Hospital in Brazil [Internet]. Brazilian Journal of Infectious Diseases. 2005;9:301–9. Available:http://dx.doi.org/10.1590/s1413-86702005000400006

4. Smiline ASG, Vijayashree JP, Paramasivam A. Molecular characterization of plasmid-encoded blaTEM, blaSHV and blaCTX-M among extended spectrum β-lactamases [ESBLs] producing Acinetobacter baumannii [Internet]. British Journal of Biomedical Science. 2018;75:200–2. Available:http://dx.doi.org/10.1080/09674845.2018.1492207

5. Girija SAS, Jayaseelan VP, Arumugam P. Prevalence of VIM- and GIM-producing Acinetobacter baumannii from patients with severe urinary tract infection [Internet]. Acta Microbiologica et Immunologica Hungarica. 2018;65:539–50. Available:http://dx.doi.org/10.1556/030.65.2018.038

6. Vila J, Ruiz J, Goni P, de Anta TJ. Quinolone-resistance mutations in the topoisomerase IV parC gene of Acinetobacter baumannii [Internet]. Journal of Antimicrobial Chemotherapy. 1997;39:757–62. Available:http://dx.doi.org/10.1093/jac/39.6.757

7. Drlica K, Malik M, Kerns RJ, Zhao X. Quinolone-mediated bacterial death [Internet]. Antimicrobial Agents and Chemotherapy. 2008;52:385–92. Available:http://dx.doi.org/10.1128/aac.01617-06

8. Ribera A, Jurado A, Ruiz J, Marco F, Del Valle O, Mensa J, et al. In vitro activity of clinafloxacin in comparison with other quinolones against Stenotrophomonas maltophilia clinical isolates in the presence and absence of reserpine [Internet]. Diagnostic Microbiology and Infectious Disease. 2002;42:123–8. Available:http://dx.doi.org/10.1016/s0732-8893(01)00335-2

9. Liu YH, Kuo SC, Lee YT, Chang ICY, Yang SP, Chen TL, et al. Amino acid substitutions of quinolone resistance determining regions in GyrA and ParC associated with quinolone resistance in Acinetobacter baumannii and Acinetobacter genomic species 13TU [Internet]. Journal of Microbiology, Immunology and Infection. 2012;45:108–12. Available:http://dx.doi.org/10.1016/j.jmii.2011.09.001

10. Singla P, Sikka R, Deep A, Seema S, Chaudhary U. Pattern of antimicrobial resistance in clinical isolates of Acinetobacter species at a tertiary level health care facility in Northern India [Internet]. Journal of Evolution of medical and Dental Sciences. 2013;2:159–65. Available:http://dx.doi.org/10.14260/jemds/237

11. Chopra S, Torres-Ortiz M, Hokama L, Madrid P, Tanga M, Mortelmans K, et al. Repurposing FDA-approved drugs to combat drug-resistant Acinetobacter baumannii [Internet]. Journal of Antimicrobial Chemotherapy. 2010;65:2598–601.
12. Hujer KM, Hujer AM, Endimiani A, Thomson JM, Adams MD, Goglin K, et al. Rapid determination of quinolone resistance in Acinetobacter spp [Internet]. Journal of Clinical Microbiology. 2009;47: 1436–42. Available:http://dx.doi.org/10.1128/jcm.02380-08

13. Stewart BA, Johnson AP, Woodford N. Relationship between mutations in parC and gyrA of clinical isolates of Streptococcus pneumoniae and resistance to ciprofloxacin and grepafloxacin [Internet]. Journal of Medical Microbiology. 1999;48:1103–6. Available:http://dx.doi.org/10.1099/00222615-12-1103

14. Khosravi AD, Mohammadian A. Efflux MexAB-Mediated resistance in multidrug and pan-drug resistant strains of Pseudomonas aeruginosa isolated from patients with burn and wound infections [Internet]. Jundishapur Journal of Natural Pharmaceutical Products. 2016;11. Available:http://dx.doi.org/10.17795/jjnp-25352

15. Fàbrega A, Madurga S, Giralt E, Vila J. Mechanism of action of and resistance to quinolones [Internet]. Microbial Biotechnology. 2009;2:40–61. Available:http://dx.doi.org/10.1111/j.1751-7915.2008.00063.x

16. Lee JK, Lee YS, Park YK, Kim BS. Mutations in the gyrA and parC genes in ciprofloxacin-resistant clinical isolates of Acinetobacter baumannii in Korea [Internet]. Microbiology and Immunology. 2005;49:647–53. Available:http://dx.doi.org/10.1111/j.1348-0421.2005.tb03643.x

17. Vila J, Ruiz J, Goni P, Marcos A, de Anta TJ. Mutation in the gyrA gene of quinolone-resistant clinical isolates of Acinetobacter baumannii [Internet]. Antimicrobial Agents and Chemotherapy. 1995;39:1201–3. Available:http://dx.doi.org/10.1128/aac.39.5.1201

18. Nowroozi J, Sepahi AA, Kamarpshiti LT, Razavipour R, Mazhar F. Evaluation of ciprofloxacin (gyrA, parc genes) and tetracycline (tetB gene) resistance in nosocomial Acinetobacter baumannii infections [Internet]. Jundishapur Journal of Microbiology. 2014;7. Available:http://dx.doi.org/10.5812/jjm.8976

19. Valentine SC, Contreras D, Tan S, Real LJ, Chu S, Xu HH. Phenotypic and molecular characterization of acinetobacter baumannii clinical isolates from nosocomial outbreaks in Los Angeles County, California [Internet]. Journal of Clinical Microbiology. 2008;46:2499–507. Available:http://dx.doi.org/10.1128/jcm.00036-08

20. Odsbu I, Khedkar S, Khedkar U, Nerkar S, Tamhankar A, Lundborg CS. High proportions of multidrug-resistant acinetobacter spp. isolates in a district in Western India: A four-year antibiotic susceptibility study of clinical isolates [Internet]. International Journal of Environmental Research and Public Health. 2018;15:153. Available:http://dx.doi.org/10.3390/ijerph15010153

21. Park S, Lee KM, Yoo YS, Yoo JS, Yoo JI, Kim HS, et al. Alterations of gyrA, gyrB, and parC and activity of efflux pump in fluoroquinolone-resistant Acinetobacter baumannii [Internet]. Osong Public Health and Research Perspectives. 2011;2:164–70. Available:http://dx.doi.org/10.1016/j.phrp.2011.11.040

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