Novel mutations in the QRDR region gyrA gene in multidrug-resistance Corynebacterium spp. isolates from intravenous sites

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Abstract The resistance to fluoroquinolones in corynebacteria is due to mutations occurring in the quinolone-resistance-determining region (QRDR) of the gyrA gene encoding the enzyme gyrase A subunit. In recent years we can observe an increasing number of infections caused by multidrug-resistant Corynebacterium striatum, Corynebacterium jeikeium and Corynebacterium urealyticum, including wide range of disorders, such as invasive infections. In this study 14 Corynebacterium spp. isolated from intravenous sites were sequenced and new combinations of mutations in the QRDR of the gyrA gene were found in C. jeikeium and C. urealyticum. Nowadays, no study comparing mutations in this region and the susceptibility to fluoroquinolones in C. jeikeium and C. urealyticum has been described. All the isolates that showed double mutation (position 87 and 91) in the QRDR gyrA gene had high MIC to the fluoroquinolones tested.

Keywords Corynebacterium · Multidrug-resistance · Fluoroquinolones · gyrA mutation · QRDR

The antibiotic resistance among Corynebacterium species has increased, sometimes leading to the use of vancomycin as the drug of choice (Yoo et al. 2015). Some Corynebacterium species belonging to human flora skin such as Corynebacterium striatum, Corynebacterium jeikeium and Corynebacterium urealyticum have expressed multidrug-resistance can cause a wide range of disorders as bacteremia, endocarditis, septicemia and others invasive infections mainly in immunocompromised patients (Bernard 2012).

Resistance to fluoroquinolones has become common in some bacterial pathogens. Analysis of the sequences of the QRDR of the gyrA gene in isolates of C. striatum, Corynebacterium amycolatum, and Corynebacterium macginleyi have shown that resistance to fluoroquinolones is associated with mutations of a
spontaneous nature in this gene and depends on the number of mutations and the type of amino acid that has been substituted (Sierra et al. 2005; Eguchi et al. 2008; Alibi et al. 2017).

In this study, we investigated 14 multidrug-resistant isolates of *C. striatum* (n = 7), *C. jeikeium* (n = 4) and *C. urealyticum* (n = 3) from blood (n = 10) and catheter segments (n = 4), during the period of 48 months (Aug. 2009 – Aug. 2013) of 13 hospitalised patients attended in two hospitals located at the metropolitan area of Rio de Janeiro, RJ, Brazil. All isolates were deposited in the bacteria collection: Coleção de Bactérias do Ambiente e Saúde of Fundação Oswaldo Cruz (CBAS/FIOCRUZ—www.cbas.fiocruz.br).

The identity of isolates was confirmed by 16S rRNA and rpoB genes sequencing (Baio et al. 2013). The antimicrobial susceptibility test by disk-diffusion method according to Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST) document showed that all isolates of *C. jeikeium*, *C. urealyticum* and three isolates of *C. striatum* were resistant to penicillin, gentamicin, clindamycin, erythromycin, rifampin and imipenem. Five isolates of *C. striatum* showed variable susceptible to gentamicin, rifampin and imipenem, but all isolates of corynebacteria were susceptible to linezolid, tetracycline and vancomycin. The minimum inhibitory concentration (MIC) using E-test strips (AB Biodisk, Sweden) for ciprofloxacin, levofloxacin and moxifloxacin was performed according to BrCAST document. Due to absence of levofloxacin breakpoints in this guideline, the interpretation of values was interpreted in accordance to criteria defined by BrCAST for *Staphylococcus* spp. (Alibi et al. 2017).

The sequences of the QRDR of the gyrA gene were compared to that of the quinolone-susceptible type strains *C. striatum* ATCC 6940, *C. jeikeium* ATCC 43734 and *C. urealyticum* DSM 7109 (Table 1). All type strains have codons for Ser-87 and Asp-91 in the QRDR region of the gyrA gene. The MIC for fluoroquinolones were compared to the mutations in QRDR of the gyrA gene. All *C. striatum* isolates showed mutations in the codon 87, only two isolates showed double mutation in codons 87 and 91. Two *C. jeikeium* isolates showed MIC > 32 µg/mL for all fluoroquinolones tested probably due to novel mutations in the QRDR gyrA gene, where in the codon for Ser-87 changed to Ile-87 and the codon Asp-91 to Tyr-91. The three *C. urealyticum* isolates showed double mutation in codons 87 and 91. Two isolates showed mutation of Ser-87 to Tyr-87 and one isolate of Ser-87 to Val-87. The codon Asp-91 was changed to Ala-91 (two isolates) and Tyr-91 (one isolate). All *C. urealyticum* isolates showed MIC > 32 µg/mL to ciprofloxacin, levofloxacin and moxifloxacin. All isolates Corynebacterium spp. with mutations in the codons 87 and 91 had the highest MIC for moxifloxacin.

Fluoroquinolones have been extensively used in the empirical treatment of urinary tract infections, including in Brazil (Hisano et al. 2015). These drugs accumulate in the organs of the body leading to the selection of spontaneous mutants in large populations that colonize the skin and mucous membranes, such as corynebacteria, which can cause nosocomial bacteremia (Sierra et al. 2005; Alibi et al. 2017). Studies have shown that increasing fluoroquinolones resistance rates in almost all bacterial species have limited empirical antimicrobial treatment options (Dalhoff 2012).

To our knowledge, no study comparing the mutations in the gyrA gene and the susceptibility to fluoroquinolones in *C. urealyticum* and *C. jeikeium* has been described. In summary, we report here the emergence of fluoroquinolone resistance in Corynebacterium species isolated from blood and catheter segments with novel mutations at amino acid positions 87 and 91 in QRDR gyrA gene producing high levels of resistance to ciprofloxacin, levofloxacin and moxifloxacin.
| Species        | Isolates/ CBAS no | GyrA (amino acids) | MIC (µg/mL) | Genbank no |
|---------------|-------------------|--------------------|-------------|------------|
|               |                   | CIP    | LVX    | MXF       | gyrA      | 16S rRNA | rpoB |
| C. striatum   |                   |        |        |           |           |          |      |
| 2130/ CBAS 612 | Tyr   Asp     | 2      | 1.5    | 0.75      | MG010352  | KJ855309 | KR010642 |
| 2296/ CBAS 615 | Phe   Ala     | > 32   | > 32   | > 32      | MG010359  | KJ855313 | KR010636 |
| 2425/ CBAS 620 | Phe   Ala     | > 32   | > 32   | > 32      | MG010366  | KM001911 | KR010631 |
| 2023/ CBAS 618 | Val   Asp     | > 32   | > 32   | 8         | MG010347  | JF342699 | JF342707 |
| 2230/ CBAS 617 | Val   Asp     | > 32   | > 32   | 8         | MG010354  | KJ855311 | KR010641 |
| 2237/ CBAS 616 | Val   Asp     | > 32   | > 32   | 4-6       | MG010355  | KJ855312 | KR010640 |
| 2308/ CBAS 614 | Val   Asp     | > 32   | > 32   | 6         | MG010360  | KJ934785 | KR010635 |
| ATCC 6940     | Ser   Asp     | 0.094  | 0.19   | 0.125     | ACGE01000134 | ACGE01000134 | ACGE01000134 |
| C. jeikium    |                   |        |        |           |           |          |      |
| 2325/ CBAS 677 | Ile   Asp     | > 32   | > 32   | 4-6       | MH513932  | MH510232 | MH513925 |
| 2509/ CBAS 681 | Ile   Asp     | > 32   | > 32   | 4         | MH513935  | MH510235 | MH513928 |
| 2443B/ CBAS 679 | Ile   Tyr    | > 32   | > 32   | > 32      | MH513933  | MH510233 | MH513926 |
| 2444/ CBAS 680 | Ile   Tyr    | > 32   | > 32   | > 32      | MH513934  | MH510234 | MH513927 |
| ATCC 43734     | Ser   Asp     | 0.125  | 0.19   | 0.125     | ACYW01000075 | ACYW01000075 | ACYW01000075 |
| C. urealyticum |                   |        |        |           |           |          |      |
| 2260/ CBAS 675 | Tyr   Ala     | > 32   | > 32   | > 32      | MH513936  | MH510236 | MH513929 |
| 2287B/ CBAS 676 | Tyr   Ala     | > 32   | > 32   | > 32      | MH513937  | MH510237 | MH513930 |
| 2431/ CBAS 678 | Val   Tyr     | > 32   | > 32   | > 32      | MH513938  | MH510238 | MH513931 |
| ATCC 43042     | Ser   Asp     | 0.125  | 0.125  | 0.125     | NC010545  | NC010545 | NC010545 |

*MIC* minimum concentration inhibitory, *CIP* ciprofloxacin, *LVX* levofloxacin, *MXF* moxifloxacin
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Author’s contribution  Conceived o for designed study: VVV and ALMG Performed research: JNR and TBV Analysed data: JNR and PVPB Wrote the paper: JNR, PVPB and VVV.

Compliance with ethical standards

Conflict of interest  All authors report no conflicts of interest to this article.

Ethical approval  This study was developed in compliance with the Brazilian Government’s Ethical Guidelines for research involving human beings (resolution of the National Health Council/Ministry of Health) and approved by the ethical research committee of HUPE/UERJ (CAAE: 01247512.3.0000.5259). The consent to participate was not required because all the investigated isolates were taken as a part of standard care (diagnostic purposes). The samples were not collected for research purposes.

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