GyrA mutations in Fluoroquinolone Resistant Clostridium difficile PCR 027

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To the Editor: Clostridium difficile is the most common cause of bacterial diarrhea in hospitalized patients (1). Antimicrobial drug therapy is the most important risk factor associated with the acquisition of C. difficile, and several antimicrobial agents including clindamycin, amoxicillin, and cephalosporins have been particularly associated with C. difficile infection (2). Acquisition of resistance to clindamycin is considered a mechanism whereby clonal strains emerge and predominate in healthcare environments (3). Historically, fluoroquinolone antimicrobial agents were considered low risk for C. difficile-associated-disease; however, recent studies indicate a shift in the risk associated with their use (4). Furthermore, recent outbreaks in Canada and the United States have been associated with fluoroquinolone exposure (4).

Recently, several C. difficile outbreaks due to PCR ribotype 027 (PCR-027) and associated with increased disease severity and death have been reported worldwide (4). This strain type contains the genes for binary toxin and has an 18-bp deletion and a frameshift mutation in tcdC hypothesized to result in deregulated expression of toxins A and B. These strains produce 16× more toxin A and 23× more toxin B in vitro than toxinotype 0 strains (5). These isolates demonstrate universal high-level resistance to fluoroquinolones in contrast to that of PCR 027 isolates collected before 2001 (4).

We report the mechanism of fluoroquinolone resistance in a cluster (n = 5) of Irish PCR-027 C. difficile isolates that were characterized by using toxinotyping and 16–23S ribotyping. Amplification with PCR and sequencing was used to identify the binary toxin gene (cdtB) and an 18-bp deletion and a frameshift mutation at position 117 in the tcdC gene. Antimicrobial susceptibility to 5 fluoroquinolone antimicrobial drugs was determined with E-tests (AB-Biodisk, Solna, Sweden). The quinolone-resistance–determining region (QRDR) of gyrA and gyrB was amplified by PCR and characterized. The nucleotide sequence data for partial sequences of the gyrA gene were submitted to GenBank and assigned accession nos. DQ821481, DQ821482, DQ821483, and DQ821484.

PCR ribotyping profiles identified 1 cluster of C. difficile PCR-027 with clinical isolates that showed indistinguishable profiles to the control 027 strain. PCR identified the edIB, an 18-bp deletion, and a frameshift mutation at position 117 in the tcdC gene in all 5 isolates. These strains were universally resistant to the fluoroquinolones tested (ofloxacin, ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin, respectively, MIC >32 μg/mL [Table]). Control isolates were susceptible to moxifloxacin and gatifloxacin (MICs 0.3, 0.2 μg/mL, respectively); however, these strains had reduced susceptibility to levofloxacin (MIC 3 μg/mL) and were resistant to ciprofloxacin and ofloxacin (Table). Sequence analysis determined that all 5 PCR-027 isolates had a single transition mutation (C to T), resulting in the amino acid substitution Thr-82-Ile in gyrA (Table). No amino acid substitutions were found in the QRDR of gyrB (data not shown).

Mutations in the active site or the QRDR of DNA gyrase and topoisomerase IV have been associated with increased resistance to fluoroquinolones in several bacteria (6). This report identifies for the first time a mutation in gyrA that is associated with high-level resistance to fluoroquinolones in C. difficile PCR-027. In Escherichia coli, amino acid substitutions that occur at Ser-83 in gyrA have been associated with fluoroquinolone resistance (6). Thr-82 in C. difficile corresponds to Ser-83 in E. coli. Thr-8-Ile amino acid substitutions corresponding to Ser-83 have been associated with fluoroquinolone resistance in several bacteria, including Pseudomonas aeruginosa, Enterobacter aerogenes, Campylobacter jejuni, and C. difficile (6). Ackermann et al. described 2 mutations in gyrA that resulted in an amino acid substitution corresponding to codon 83 in E. coli. Thirteen of the 18 C. difficile isolates had the Thr-82-Ile substitution, and 1 strain had a Thr-82-Val substitution (7). Dridi et al. described this Thr-82-Ile GyrA substitution in 6 resistant C. difficile strains corresponding to 3 serogroups, H1, A9, and IC (8).

Early studies investigating fluoroquinolone antimicrobial agents suggested that most C. difficile isolates were susceptible to these drugs.
Antimicrobial drug resistance to this class has increased with fluoroquinolone use, and currently these drugs remain the most frequently prescribed antimicrobial agents in the United States and Europe. Acquired resistance to the newer fluoroquinolone antimicrobial agents is not restricted to ribotype PCR-027, although different amino acid substitutions in the QDR of \textit{gyrA} and \textit{gyrB} have been described (7–9). Wilcox et al. have described high-level fluoroquinolone resistance in PCR ribotype-001, an endemic strain type found in several healthcare settings in the United Kingdom (10). We have previously described the emergence of a fluoroquinolone-resistant toxin A–, toxin B–positive strain in Dublin (9).

We report a mutation in \textit{gyrA} associated with fluoroquinolone resistance in \textit{C. difficile} PCR-027. Antimicrobial drug resistance in \textit{C. difficile} isolates must be monitored because the emergence of universal fluoroquinolone resistance in different \textit{C. difficile} strain types may be a factor promoting outbreaks in hospitals. As exposure to several different fluoroquinolone antimicrobial drugs have been independently associated with \textit{C. difficile}–associated-disease, restricted use of all fluoroquinolones, rather than changing from 1 quinolone to another, may be a necessary step toward preventing and controlling \textit{C. difficile} outbreaks.

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References


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