GyrA mutations in Fluoroquinolone Resistant Clostridium difficile PCR 027

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gyrA Mutations in Fluoroquinolone-
resistant Clostridium difficile
PCR-027

To the Editor: Clostridium difficile is the most common cause of bacte-
rial 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 cepha-
losporins have been particularly associated with C. difficile infection
(2). Acquisition of resistance to clindamycin is considered 1 mechanism
whereby clonal strains emerge and predominate in healthcare environ-
ments (3). Historically, fluoroquinolone antimicrobial agents were
considered low risk for C. difficile–
associated disease; however, recent
studies indicate a shift in the risk asso-
ciated with their use (4). Furthermore,
recent outbreaks in Canada and the
United States have been associated
with fluoroquinolone exposure (4).

Recently, several C. difficile out-
breaks 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 toxi-
type 0 strains (5). These isolates
demonstrate universal high-level
resistance to fluoroquinolones in con-
trast to that of PCR 027 isolates col-
clected before 2001 (4).

We report the mechanism of fluo-
roquinolone resistance in a cluster (n =
5) of Irish PCR-027 C. difficile iso-
lates that were characterized by using
loxokinotyping and 16–23S ribotyping.
Amplification with PCR and sequenc-
ing was used to identify the binary
toxin gene (cdtB) and an 18-bp dele-
tion and a frameshift mutation at posi-
tion 117 in the tcdC gene. Anti-
microbial susceptibility to 5 fluoro-
quinolone antimicrobial drugs was
determined with E-tests (AB-Biodisk,
Solna, Sweden). The quinolone-resis-
tance–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 indistin-
guishable profiles to the control 027
strain. PCR identified the cdtB, an 18-
bp deletion, and a frameshift mutation
at position 117 in the tcdC gene in all
5 isolates. These strains were univer-
sally resistant to the fluoroquinolones
tested (ofloxacin, ciprofloxacin, levo-
floxacin, moxifloxacin, and gatiflo-
xacin, respectively, MIC >32 µg/mL
[Table]). Control isolates were suscep-
tible 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 cipr-
ofloxacin and oflo-xacin (Table).

Sequence analysis determined that all
5 PCR-027 isolates had a single transi-
tion mutation (C to T), resulting in the
amino acid substitution Thr-82-Ile in
gyrA (Table). No amino acid substitu-
tions were found in the QRDR of gyrB
(data not shown).

Mutations in the active site or the
QRDR of DNA gyrase and topoiso-
merase IV have been associated with
increased resistance to fluoro-
quinolones in several bacteria (6). This
report identifies for the first time a
mutation in gyrA that is associated
with high-level resistance to fluoro-
quinolones in C. difficile PCR-027. In
Escherichia coli, amino acid substitu-
tions 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-
to-Ile amino acid substitutions corre-
sponding to Ser-83 have been associ-
ated with fluoroquinolone resistance
in several bacteria, including
Pseudomonas aeruginosa, Entero-
bacter aerogenes, Campylobacter jejuni, and C. difficile (6). Ackermann
et al. described 2 mutations in gyrA
that resulted in an amino acid substitu-
tion corresponding to codon 83 in E.
coli. Thirteen of the 18 C. difficile iso-
lates had the Thr-82-Ile substitution,
and 1 strain had a Thr-82-Val substitu-
tion (7). Dridi et al. described this Thr-
82-Ile GyrA substitution in 6 resistant
C. difficile strains corresponding to 3
serogroups, H1, A9, and 1C (8).

Early studies investigating fluo-
roquinolone antimicrobial agents
suggested that most C. difficile iso-
lates 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 QRDR of gyrA and 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 gyrA associated with fluoroquinolone resistance in C. difficile PCR-027. Antimicrobial drug resistance in C. difficile isolates must be monitored because the emergence of universal fluoroquinolone resistance in different 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 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 C. difficile outbreaks.

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