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In Vivo Selection of Moxifloxacin-Resistant *Clostridium difficile*

Clostridium difficile infection (CDI) was described in 1978 and soon became recognized as the main complication of antimicrobial use. In fact, one of the major risk factors associated with this infection is the use of antimicrobial agents (1). Clindamycin was the first antibiotic to be related to an increase in the incidence of CDI followed by parenteral cephalosporins, especially cefotaxime and ceftriaxone. More recently, there has been a strong association between fluoroquinolone use and CDI, which is also reflected in the fact that the epidemic strain NAP1 (ribotype 027) is resistant to all fluoroquinolones (11).

We report the first documented case of *in vivo* selection of moxifloxacin resistance in *C. difficile* causing infection in a patient who had previously received fluoroquinolones.

The patient was a 78-year-old male with multimorbidity admitted for hip dislocation. At the time of admission, the patient already had diarrheal stools and developed recurrent pseudomembranous colitis during his hospital stay. A toxigenic *C. difficile* strain (isolate 1) was detected, by C. Diff Quick Check Complete dual-antigen enzyme-inmunoassay (D-EIA) (TechLab, Blacksburg, VA) and culture on selective CLO medium (bioMérieux, Marcy l'Etoile, France). MICs were determined by Etest (bioMérieux, Marcy l'Etoile, France), yielding a wild-type phenotype, including susceptibility to metronidazole, vancomycin, and moxifloxacin and resistance to other fluoroquinolones (Table 1). The patient had received ciprofloxacin and rifampin 2 months before admission, followed by clindamycin and rifampin for an ankle infection. After the first episode of CDI, which was treated with metronidazole for 12 days, the patient also had a respiratory tract infection that was treated with levofloxacin for 2 weeks. Subsequently, a moxifloxacin-resistant (MIC $>$ 32 μg/ml) *C. difficile* (isolate 2) was isolated and was responsible for two new recurrent episodes of CDI in the patient. The levofloxacin MIC had also increased significantly in isolate 2.

Fluoroquinolone resistance mechanisms were investigated through PCR amplification and sequencing of *gyrA* and *gyrB* (6). While wild-type sequences were obtained in isolate 1, isolate 2 showed the Thr82Ile substitution in GyrA, the most frequently described mutation in fluoroquinolone-resistant *C. difficile*, including the hypervirulent clone NAP1 (ribotype 027) (4, 5, 14, 15, 16). Moxifloxacin-susceptible and -resistant isolates showed an identical pattern in ribotyping analysis (2). Although we were not able to assign the strain to a known ribotype, perhaps due to the lack of enough control strains (ribotypes 001, 014, 027, and 078), it was identified as clone ST-122 through multilocus sequence typing (MLST) (8). This clone belongs to clade 2, the same clade as sequence type 1 (ST-1) (ribotype 027), but to our knowledge, so far it has not been related to resistance to fluoroquinolones.

Moxifloxacin resistance in *C. difficile* has increased dramatically in recent years, and this resistance is frequently associated with resistance to other fluoroquinolones, in particular to levofloxacin, showing high-level resistance (9, 14). Spigaglia et al. (15) demonstrated that moxifloxacin and levofloxacin are able to rapidly select *C. difficile* mutants *in vitro* that are highly resistant to fluoroquinolones, showing amino acid substitutions in GyrA and/or GyrB. Moreover, our work confirms that levofloxacin is able to select Thr82Ile GyrA mutants *in vivo*, conferring resistance also to new fluoroquinolones, such as moxifloxacin. Therefore, we must consider the possibility that the use of older fluoroquinolones may allow the emergence of *C. difficile* bacteria that are also resistant to newer fluoroquinolones and that this phenomenon probably promotes wide dissemination of this pathogen (3, 7, 10, 11, 12, 13).

TABLE 1 MICs of the antibiotics tested for the three *C. difficile* isolates

Isolate	Date of isolation (day/mo/yr)	MIC (μg/ml)[a]						
		MTR	VAN	CLI	ERY	CIP	MOX	LEV
1	20/04/2011	0.125	0.5	3	1	>32	1	16
2	13/05/2011	0.064	0.5	4	2	>32	>32	>32
3	07/06/2011	0.064	0.5	2	1	>32	>32	>32

[a] MTR, metronidazole; VAN, vancomycin; CLI, clindamycin; ERY, erythromycin; CIP, ciprofloxacin; MOX, moxifloxacin; LEV, levofloxacin.

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