Disk Diffusion Method for Erythromycin and Ciprofloxacin Susceptibility Testing of *Campylobacter jejuni* and *Campylobacter coli*

Christiane Gaudreau

Département de Microbiologie médicale et Infectiologie, CHUM-Hôpital Saint-Luc, Montréal, Québec, Canada

Using the disk diffusion method, Lehtopolku et al. (1) reported diameters of 6 mm to 44 to 70 mm for susceptible, as well as resistant, *Campylobacter jejuni* and *C. coli* isolates exposed to seven and six antimicrobial agents, respectively. These results disagree with the erythromycin and ciprofloxacin disk diffusion method standardization by the Clinical and Laboratory Standards Institute (CLSI) (2) and with the other disk diffusion studies for erythromycin and ciprofloxacin (3–5). The CLSI reported that the absence of a zone (6 mm) around the 15- μ g erythromycin disk or around the 5- μ g ciprofloxacin disk indicates resistance to macrolides or ciprofloxacin, respectively, and that a diameter of >6 mm requires a MIC (2). Their erythromycin resistance breakpoint of ≥ 16 mg/liter and their two *Campylobacter* spp. resistant to erythromycin but susceptible to azithromycin (1) disagree with the CLSI recommendations, namely, that the erythromycin resistance breakpoint is ≥ 32 mg/liter and the erythromycin-resistant isolates are resistant to azithromycin (2).

In our studies with *C. jejuni* and *C. coli* (3, 4) and erythromycin, 303 susceptible isolates (MICs, ≤ 4 mg/liter) had a ≥ 15 -mm zone and 57 resistant isolates (MICs, ≥ 64 mg/liter) had no zone around the 15- μ g erythromycin disk. For ciprofloxacin, 223 susceptible *C. jejuni* and *C. coli* isolates (MICs, ≤ 1 mg/liter) had a ≥ 25 -mm zone around the 5- μ g ciprofloxacin disk, except for 1 isolate (21 mm), and had a zone around the 30- μ g nalidixic acid disk and 155 resistant (MICs, ≥ 4 mg/liter) and 2 intermediately resistant (MIC of 2 mg/liter) isolates had zones of 6 to 20 mm around the ciprofloxacin disk and had no zone around the nalidixic acid disk. The nalidixic acid disk diffusion test results of 92 *C. jejuni* isolates at the Laboratoire de Santé Publique du Québec and the erythromycin and nalidixic acid disk diffusion test results of 146 *C. jejuni* isolates at the Hôpital Sacré-Coeur matched our results (3). In our laboratory, disk diffusion testing was repeated for many isolates with matching results even if not reported (3, 4, 6; our unpublished data).

Since our studies were published (3, 4), in two studies done in our laboratory and one or more other laboratories, the *C. jejuni* and *C. coli* erythromycin, nalidixic acid, and ciprofloxacin disk diffusion method was as in agreement and reproducible as it was for susceptible as well as for resistant isolates (0% and $\leq 0.5\%$ of the isolates were intermediately resistant to erythromycin and ciprofloxacin, respectively) compared with the MICs determined by agar dilution, the Etest, or both methods, including 44 erythro-

mycin-resistant isolates (our unpublished data). Overall, in comparison to the agar dilution method, the *C. jejuni* and *C. coli* erythromycin and ciprofloxacin disk diffusion method (3, 4, 6) so far agrees for 100% and 98% of the isolates for erythromycin and ciprofloxacin, respectively. Our results (3, 4, 6) match the CLSI erythromycin and ciprofloxacin disk diffusion method standardization (2): all isolates without a zone (6 mm) around erythromycin or ciprofloxacin disks were resistant to macrolides and ciprofloxacin, respectively.

The agar dilution, microdilution, and/or disk diffusion methods should give reliable results before their use for *C. jejuni* and *C. coli* susceptibility testing.

REFERENCES

1. Lehtopolku M, Kotilainen P, Puukka P, Nakari UM, Siitonen A, Eerola E, Huovinen P, Hakanen AJ. 2012. Inaccuracy of the disk diffusion method compared with the agar dilution method for susceptibility testing of *Campylobacter* spp. J. Clin. Microbiol. 50:52–56.
2. Clinical and Laboratory Standards Institute. 2010. Methods for antimicrobial dilution and disk susceptibility testing for infrequently-isolated or fastidious bacteria: approved guidelines. Publication no. M45-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
3. Gaudreau C, Girouard Y, Ringuette L, Tsimiklis C. 2007. Comparison of disk diffusion and agar dilution methods for erythromycin and ciprofloxacin susceptibility testing of *Campylobacter jejuni* subsp. *jejuni*. Antimicrob. Agents Chemother. 51:1524–1526.
4. Gaudreau C, Girouard Y, Gilbert H, Gagnon J, Bekal S. 2008. Comparison of disk diffusion and agar dilution methods for erythromycin, ciprofloxacin and tetracycline susceptibility testing of *Campylobacter coli* and for tetracycline susceptibility testing of *Campylobacter jejuni* subsp. *jejuni*. Antimicrob. Agents Chemother. 52:4475–4477.
5. Schönberg-Norio D, Hanninen ML, Katila ML, Kaukoranta SS, Koskela M, Eerola E, Uksila J, Pajarre S, Rautelin H. 2006. Activities of telithromycin, erythromycin, fluoroquinolones, and doxycycline against *Campylobacter* strains isolated from Finnish subjects. Antimicrob. Agents Chemother. 50:1086–1088.
6. Gaudreau C, Michaud S. 2003. Cluster of erythromycin- and ciprofloxacin-resistant *Campylobacter jejuni* subsp. *jejuni* from 1999 to 2001 in men who have sex with men, Québec, Canada. Clin. Infect. Dis. 37:131–136.

Address correspondence to christiane.gaudreau.chum@ssss.gouv.qc.ca.

For the author reply, see doi:10.1128/JCM.02575-12.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.
doi:10.1128/JCM.02363-12