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COMMENTARY

Fluoroquinolone Resistance in *Salmonella* and the Utility of Perfloxacin Disk Diffusion

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Fluoroquinolone resistance is a serious and increasingly common problem in *Salmonella*. Two companion studies in this issue of the *Journal of Clinical Microbiology* (E. Deak, R. Skov, J. A. Hindler, and R. M. Humphries, J Clin Microbiol 53:3405–3410, 2015, http://dx.doi.org/10.1128/JCM.01393-15; R. Skov, E. Matuschek, M. Sjölund-Karlsson, J. Åhman, A. Petersen, M. Stegger, M. Torpdahl, and G. Kahlmeter, J Clin Microbiol 53:3411–3417, 2015, http://dx.doi.org/10.1128/JCM.01287-15) provide data to support the use of perfloxacin disk diffusion as a convenient and inexpensive surrogate laboratory method to detect fluoroquinolone resistance in *Salmonella* when the direct measurement of fluoroquinolone MICs is not feasible. Recently updated CLSI and EUCAST susceptibility breakpoints will help to optimize clinical outcomes and reduce the likelihood of emergent resistance.

Fluoroquinolone resistance in *Salmonella*. Since 1987, when ciprofloxacin was approved for clinical use in the United States, fluoroquinolones (FQs) have been widely prescribed for a diverse range of infections, including bacterial enteritis and typhoid fever. A broad antibacterial spectrum, favorable safety profile, and excellent oral absorption have contributed to the popularity of these agents (1, 2). However, marked increases in FQ resistance in a variety of bacterial species and clinical settings were observed within a decade of the introduction of these agents (3), and *Salmonella* infections have typified this trend.

In view of rising rates of resistance to ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol among both typhoidal and nontyphoidal *Salmonella* isolates (4, 5), FQ initially seemed to provide an ideal therapeutic alternative to treat serious *Salmonella* infections (6). However, FQ-resistant *Salmonella* strains were detected soon thereafter (7, 8). By 1997, 60% of *Salmonella enterica* serotype Typhi and Paratyphi isolates in some parts of India had ciprofloxacin MICs of ≥2 μg/ml (9). To a lesser extent, the prevalence of FQ resistance in nontyphoidal *Salmonella* isolates throughout the world has also been rising (10). Since the U.S. National Antimicrobial Resistance Monitoring System (NARMS) began tracking ciprofloxacin susceptibility in 1996, the percentage of *Salmonella* isolates that are nonsusceptible to ciprofloxacin has increased from <0.5% up to 3.5% (11), while in the EUCAST (European Committee on Antimicrobial Susceptibility Testing) database, 6.0% of *Salmonella* isolates are nonsusceptible to ciprofloxacin (12).

Nalidixic acid, a nonfluorinated quinolone, was initially used by clinical laboratories as a surrogate agent to detect *Salmonella* with FQ resistance due to target site (*gyrA* and *parC*) mutations in the quinolone resistance-determining region (QRDR) (13). However, the situation became more complex with the discovery of plasmid-mediated quinolone resistance (PMQR) (reviewed in reference 14), which includes various *qnr* variants, *oqxAB*, *qepA* and *aac*(6′)-*Ib-cr* genetic determinants (15). Strains with PMQR may be difficult to detect because the resulting MIC elevations are typically more modest than those associated with QRDR mutations and do not confer resistance to nalidixic acid (16).

Low-level FQ resistance is associated with poorer clinical outcomes. Although PK-PD (pharmacokinetic-pharmacodynamic) studies have suggested a ciprofloxacin susceptibility breakpoint of 0.12 μg/ml (17, 18), *Salmonella* isolates with ciprofloxacin MICs of ≤1.0 μg/ml have long been considered to be susceptible. However, patients with infections caused by *Salmonella* with low-level FQ MIC elevations (0.125 to 1.0 μg/ml) have been found to have a greater likelihood of FQ treatment failure, delayed resolution of fever, and mortality (17, 19–21). In recognition of the clinical significance of low-level FQ resistance, CLSI (Clinical and Laboratory Standards Institute) and EUCAST lowered their susceptibility breakpoints for *Salmonella* in recent years (16). However, not all clinical laboratories have the ability to determine MICs, and levofloxacin or ofloxacin disk diffusion assays fail to provide a clean separation of susceptible and nonsusceptible *Salmonella* strains with defined resistance mechanisms (22).

FQ treatment of bacteria with low-level FQ resistance may promote the development of high-level resistance. In addition to conferring a greater likelihood of suboptimal responses to treatment, there is concern that PMQR may facilitate the development of higher-level FQ resistance following FQ exposure. PMQR genes raise the "mutant prevention concentration" of FQ for *Salmonella* (23, 24) and other bacteria (25, 26). This refers to the concentration of an antimicrobial agent required to prevent the emergence of drug-resistant subpopulations *in vitro* (27). Such *in vitro* studies suggest that bacteria with low-level FQ resistance may more readily give rise to mutants with high-level resistance following FQ treatment. In fact, *Salmonella* isolates resistant to FQ, third-gen-

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TABLE 1 Recommended breakpoints for detection of FQ resistance in *Salmonella* (adapted from reference 34)

Agent	Breakpoint[a]			Source(s) of breakpoint criteria
	S	I	R	
Ciprofloxacin (MIC [μg/ml])	≤0.06	0.12–0.5	≥1.0	CLSI
	≤0.06		≥0.12	EUCAST
Levofloxacin/ofloxacin (MIC [μg/ml])	≤0.12	0.25–1.0	≥2.0	CLSI
Perfloxacin (disk diffusion[b]) (mm)	≥24		≤23	CLSI, EUCAST

[a] S, susceptible; I, intermediate; R, resistant.
[b] Using a 5-μg perfloxacin disk.

eration cephalosporins, and azithromycin have now been reported (28), creating a major therapeutic challenge.

Impact of revised FQ breakpoints. The new FQ breakpoints for *Salmonella* have not been greeted with universal enthusiasm. In one Indian study, revised CLSI breakpoints lowered the percentage of FQ-susceptible *S.* Typhi isolates from 95% to 3% (29). Another Indian paper reported that 20% of 50 *Salmonella* isolates were ciprofloxacin resistant and 70% were ciprofloxacin intermediate based on the new breakpoints (30). The latter authors advocated the continuing use of ciprofloxacin in this setting, stating that "Blindly following western guidelines for a disease which is highly endemic in the subcontinent will spell the death knell of a cheap and effective drug." However, this is an inadvisable approach that will only expedite the development of high-level resistance and is likely to produce inferior clinical outcomes. In a report from Bangalore, India, only 3 out of 50 (6%) *S.* Typhi or *S.* Paratyphi A isolates were susceptible to ciprofloxacin; 38 out of 50 (76%) of these isolates were resistant to nalidixic acid, indicating the presence of a QRDR mutation, and only 7 (14%) were ciprofloxacin intermediate and nalidixic acid susceptible (31). Such data suggest that FQs have already been lost as a useful therapeutic option for many patients with enteric fever in parts of India, as well as Bangladesh (32) and Pakistan (33).

Perfloxacin disk diffusion. The studies of Deak et al. (34) and Skov et al. (35) show that perfloxacin provides better separation of ciprofloxacin-susceptible and -nonsusceptible strains than other disk diffusion substrates, including ciprofloxacin itself. Some false resistance may be encountered (34), although Skov et al. did not observe this problem, and at least one of the false-resistant strains of Deak et al. was actually found to contain a QRDR mutation. The use of perfloxacin as a surrogate for ciprofloxacin may be particularly useful in resource-limited settings, but unfortunately, perfloxacin disks are not commercially available in the United States at this time. Laboratories that elect to use the perfloxacin disk diffusion method should note that there may be differences among disk manufacturers. Skov et al. found Bio-Rad disks to contain higher concentrations of perfloxacin than the other disks tested, along with larger zones of inhibition (35).

Laboratories using the perfloxacin disk diffusion method should be aware that the presence of inner colonies suggests resistance (34). Laboratories able to directly measure FQ MICs are likely to find MICs to be the most straightforward approach for determining FQ susceptibility in *Salmonella* (Table 1). If broth microdilution is not available, the ciprofloxacin Etest is reported to show 89.6% categorical agreement with the reference broth microdilution method, with only minor errors (36).

An important limitation of the perfloxacin disk diffusion method is that it cannot detect the presence of the *aac*(6′)-*Ib-cr* PMQR determinant, as this mechanism is specific for FQ possessing a piperazinyl secondary amine (ciprofloxacin and norfloxacin) (15). This is a minor concern in North America and Europe at this time, as the prevalence of *aac*(6′)-*Ib-cr* in *Salmonella* in these regions is quite low. Of 1,215 *Salmonella* isolates from 13 European countries screened for PMQR, only 3 strains with *aac*(6′)-*Ib-cr* were found (37). Of 19,010 nontyphoidal *Salmonella* isolates from humans in the United States from 1996 to 2006, only one isolate harboring *aac*(6′)-*Ib-cr* was detected out of the 283 isolates with a ciprofloxacin MIC of ≥0.25 μg/ml, and this isolate was obtained from a child with a history of travel to China (38). Out of 2,165 *Salmonella* human isolates obtained in the United States in 2007, only 6 of the 51 isolates (11.8%) with a ciprofloxacin MIC of ≥0.25 μg/ml were found to carry *aac*(6′)-*Ib-cr* (39). Nevertheless, it is worth noting that *aac*(6′)-*Ib-cr* appears to be more common among other members of the family *Enterobacteriaceae* in the United States and Canada (40, 41). In addition, the *aac*(6′)-*Ib-cr* determinant seems to be more common among *Salmonella* from China, with some surveys reporting its presence in 30 to 40% of nontyphoidal *Salmonella* isolates from chickens and humans (42, 43). The future spread of *aac*(6′)-*Ib-cr* warrants careful monitoring.

Concluding remarks. It is worth reflecting on how we got to this point. As Roberts and colleagues have observed, "Despite over 70 years of clinical antibiotic use, bacteria continue to out-perform clinicians" (44). FQ once had many characteristics of a "dream drug"—an oral antibiotic that could be used against many common infections (1). However, the widespread use and misuse of these agents, including for the treatment of infections caused by bacteria that were only marginally susceptible, has been predictably followed by the emergence of resistance. In 1984, a survey of *Enterobacteriaceae* revealed only 5 out of 4,039 strains that were resistant to ciprofloxacin (MIC > 1 μg/ml), but by 2000, 5% of *Escherichia coli* strains were resistant, and by 2008, >30% were resistant (45). Although *Salmonella* breakpoints have been adjusted, PK-PD analyses suggest that the FQ breakpoints for *Enterobacteriaceae* other than *Salmonella* are still too high (46). Should they be the next to fall?

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