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MINIREVIEW

Don't Get Wound Up: Revised Fluoroquinolone Breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa*

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ABSTRACT Fluoroquinolones remain some of the more commonly prescribed antimicrobial agents in the United States, despite the wide array of reported side effects that are associated with their use. In 2019, the Clinical and Laboratory Standards Institute revised the fluoroquinolone antimicrobial susceptibility testing breakpoints for both *Enterobacteriaceae* and *Pseudomonas aeruginosa*. This breakpoint revision was deemed necessary on the basis of pharmacokinetic and pharmacodynamic analyses suggesting that the previous breakpoints were too high, in addition to the inability of the previous breakpoints to detect low-level resistance to this antibiotic class. In this minireview, we review the published data in support of this revision, as well as the potential challenges that these breakpoint revisions are likely to pose for clinical laboratories.

KEYWORDS breakpoint, CLSI, ciprofloxacin, fluoroquinolone, levofloxacin

Antimicrobial susceptibility testing (AST) is one of the most important functions of a clinical microbiology laboratory. In the United States, as well as in many other countries, Clinical and Laboratory Standards Institute (CLSI) M100 performance standards (1) are widely used for the interpretation of AST results. Regardless of whether testing is performed by disk diffusion (i.e., Kirby-Bauer method) or MIC-based methods, results are compared with established criteria that enable the laboratory to categorize the result as susceptible, intermediate, or resistant to a given antibiotic.

The setting of AST breakpoints is a complex and dynamic process that integrates microbiological, pharmacokinetic (PK)/pharmacodynamic (PD), and clinical outcome data (2, 3). In 2019, the 29th edition of the CLSI M100 supplement features several breakpoint revisions for a number of bacteria (4); however, revision of the fluoroquinolone (FQ) breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa* will arguably affect the largest number of isolates tested in clinical laboratories. A comparison of the previous and revised CLSI FQ breakpoints, as well as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (5), is shown in Table 1. In 2017, EUCAST lowered the FQ breakpoints for several organisms (including Gram-positive bacteria). It is important to note that the revision of the CLSI FQ breakpoints was limited solely to *Enterobacteriaceae* and *P. aeruginosa* and other bacteria were not included in this change. In addition, there are currently no CLSI-approved breakpoints for moxifloxacin for either *Enterobacteriaceae* or *P. aeruginosa*. In this minireview, we review the evidence for the necessity of this FQ breakpoint revision, as well as the issues this is likely to pose for clinical microbiology laboratories.

REVIEW OF FLUOROQUINOLONES

FQs are a class of broad-spectrum antibiotics that inhibit bacterial DNA synthesis by interfering with the enzymes DNA gyrase and topoisomerase IV. Although the individ-

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TABLE 1 Comparison of ciprofloxacin and levofloxacin MIC breakpoints

Gram-negative organism and FQ	MIC breakpoints (μg/ml)[a]		
	2019 CLSI breakpoints (4)	2018 CLSI breakpoints (48)	EUCAST breakpoints (5)
Enterobacteriaceae			
Ciprofloxacin	S, ≤0.25; I, 0.5; R, ≥1	S, ≤1; I, 2; R, ≥4	S, ≤0.25; R, >0.5
Levofloxacin	S, ≤0.5; I, 1; R, ≥2	S, ≤2; I, 4; R, ≥8	S, ≤0.5; R, >1
P. aeruginosa			
Ciprofloxacin	S, ≤0.5; I, 1; R, ≥2	S, ≤1; I, 2; R, ≥4	S, ≤0.5; R, >0.5
Levofloxacin	S, ≤1; I, 2; R, ≥4	S, ≤2; I, 4; R, ≥8	S, ≤1; R, >1

[a]S, susceptible; I, intermediate; R, resistant.

ual spectrum of activity varies somewhat by agent, many FQs are active against numerous Gram-positive, Gram-negative, and other bacterial pathogens, such as *Mycoplasma pneumoniae* and *Legionella pneumophila*. FQs demonstrate high bioavailability, wide distribution, and good tissue penetration, with a high tissue-to-serum drug concentration ratio (6). As a result, they are approved in the United States for the treatment of a wide variety of acute and chronic bacterial infections, ranging from lower respiratory tract infections to travelers' diarrhea. Consequently, FQs are one of the most commonly prescribed classes of antibiotics, with ~30 million oral prescriptions for FQs being dispensed in the United States in 2014 (7). Use of FQs has increased dramatically over time, with a reported 245.5% increase in Medicaid payments for quinolone agents between 1991 and 2015 (7, 8).

Despite high rates of use, there are significant adverse effects associated with the use of FQs. These have led the U.S. Food and Drug Administration (FDA) to issue a series of black box warnings regarding the potential for disabling and potentially irreversible serious adverse effects, including tendonitis and tendon rupture, as well as exacerbation of muscle weakness in patients with myasthenia gravis (9, 10). More recently, adverse neurological side effects have been noted among elderly patients receiving high dosages and patients concurrently using nonsteroidal anti-inflammatory medications (9). Oral and injectable FQs available for use in the United States also carry warnings regarding QT interval prolongation and torsades de pointe, in addition to hypoglycemia or hyperglycemia in elderly or diabetic patients. Finally, use of FQs is associated with increased risk for the development of *Clostridioides difficile* (*Clostridium difficile*) infection (9). In the case of urinary tract infections (UTIs), historically one of the more common reasons for FQ prescriptions, it is now recommended that FQs be reserved for use in patients for whom no alternative treatment option is available (11).

In addition to the adverse effects associated with the use of FQs, the emergence of resistance to FQs is a major public health concern. Increased use of FQs for the treatment of infections caused by *Enterobacteriaceae* and *P. aeruginosa* has led to reductions in the rates of susceptibility to these agents over time. Between the early 1990s and the year 2000, rates of ciprofloxacin susceptibility decreased from 89% to 76% for aerobic Gram-negative bacilli isolated from intensive care unit patients, with susceptibility rates of only 68% being observed for *P. aeruginosa* by 2000 (12). Similar findings have been noted in large-scale surveillance studies, with ciprofloxacin resistance having increased from 3.7% to 17.8% among *Enterobacteriaceae* strains and from 11.9% to 17.3% among *P. aeruginosa* strains between 1999 and 2008 (13). Rates of resistance to FQs among *Enterobacteriaceae* strains have been further negatively affected by the global spread of multidrug-resistant *Escherichia coli* sequence type 131 (ST131) (14).

Importantly, when specific efforts have been taken to reduce consumption of FQs, rates of susceptibility have been shown to increase (15). This observation, combined with the previously discussed clinical complications associated with FQ use, makes limiting FQ use an important target for antimicrobial stewardship efforts. Notably, issues with increasing FQ resistance among Gram-negative organisms extend beyond

Enterobacteriaceae and *P. aeruginosa*; the CDC National Antimicrobial Resistance Monitoring System (NARMS) surveillance of *Campylobacter* species found an increase in the rate of ciprofloxacin resistance from 13% in 1997 to almost 25% in 2011 (16).

The mechanisms of resistance to FQs are varied and can be chromosomal and/or plasmid mediated in nature (reviewed in references 17 and 18). Briefly, the most common mechanism leading to FQ resistance is mutation within the quinolone resistance-determining regions (QRDRs), the genes that encode the drug targets themselves. Other chromosomal mutations that affect susceptibility to FQs include reductions in permeability (e.g., porin mutation) and increased efflux. The presence of plasmid-mediated quinolone resistance (PMQR) was first discovered in the 1990s, and several distinct mechanisms have since been described. So-called Qnr proteins bind to the target proteins gyrase and topoisomerase IV, protecting them from inhibition by FQs. Plasmid-mediated enzymatic inactivation due to the aminoglycoside acetyltransferase AAC(6′)-Ib can be difficult to detect in the laboratory, conferring MIC increases of only 3- to 4-fold. In contrast, plasmid-mediated efflux (e.g., OqxAB or QepA) typically leads to larger reductions in susceptibility (16- to 32-fold MIC increases) and can be more readily detected by AST.

RATIONALE FOR FLUOROQUINOLONE BREAKPOINT CHANGES

Breakpoints and resistance detection. There is evidence that the pre-2019 CLSI ciprofloxacin and levofloxacin breakpoints were too high to detect low-level FQ resistance among *Enterobacteriaceae* and *P. aeruginosa* strains. Llanes et al. showed that, among *P. aeruginosa* strains overexpressing efflux pumps, 85.9% ($n = 85$) tested susceptible to ciprofloxacin using the pre-2019 CLSI susceptible breakpoint of ≤ 1 μg/ml (19). Similarly, a study of *E. coli* strains with reduced FQ susceptibility colonizing the intestinal tracts of hospitalized patients ($n = 353$) found that nearly 40% of isolates tested would not have been characterized as having reduced susceptibility to FQs using the pre-2019 breakpoints, despite having genotypically confirmed resistance mutations (20). The majority of those isolates (85%) harbored ≤ 2 mutations within the *gyrA* gene. In addition, PMQR genes usually confer low-level quinolone resistance that may test as susceptible with current CLSI breakpoints.

Low-level FQ resistance, which in many cases is undetectable with the previous breakpoints, can serve as a first step in the development of higher-level resistance. Specifically, organisms harboring these low-level resistance mutations may promote the selection of mutants with additional resistance mechanisms that can lead to higher-level quinolone resistance (18). In *P. aeruginosa*, overproduction of efflux pumps allowed for the development of higher-level resistance to FQs *in vitro* (19). Critically, low-level resistance may facilitate selection of high-level FQ resistance *in vivo* (21), which has been described for several members of the *Enterobacteriaceae* family as well as *P. aeruginosa* (22–24). Thus, pre-2019 breakpoints may be insufficient for detecting low-level resistance, putting patients at risk for potential treatment failure.

Overview of the pharmacokinetics and pharmacodynamics of fluoroquinolones. The term PK refers to the movement of a drug within the body. With respect to antimicrobial agents, the term PD refers to the relationship between the drug concentration and the observed effect on the target microorganism. FQs exhibit concentration-dependent killing, with postantibiotic effects against most Gram-negative bacteria ranging from 1 to 4 h. Determination of the PK/PD index that most closely correlates with successful patient outcomes (i.e., clinical and/or microbiological success) has been evaluated in *in vitro* models (25, 26), animal models (27, 28), and clinical studies (29–32). In both neutropenic murine pneumonia and thigh models, the ratio of the area under the concentration-time curve over 24 h at steady state to the MIC (AUC/MIC) had the strongest correlation with microbiological eradication, compared with the ratio of the peak concentration to the MIC, and thus was determined to be the better PK/PD index (27, 28, 33). In simple terms, AUC/MIC is the measure of total drug exposure relative to MIC over a specified time period, and achieving a certain threshold is linked to clinical response. Because FQs

are ~30% protein bound and only free or unbound drug is active, the free fraction of the drug may be used in calculations of the area under the concentration-time curve (AUC), yielding the AUC for the free fraction of the drug (*f*AUC) (26, 33).

PK/PD data for a drug can be used to run a Monte Carlo simulation (MCS) to computationally model an estimate of the likelihood of a given drug dose attaining a predefined PK/PD index (26). The probability of target attainment (PTA) can be modeled by MCS for different MIC values against a variety of pathogens. Advancements in the field of PK/PD modeling have led to an increasingly central role for MCS in the setting of clinical breakpoints. However, breakpoints for many antibiotics were established prior to the widespread availability and acceptability of these modeling methods and instead were based on historic clinical data and MIC distribution data. This ability to define a PK/PD index has led to a reevaluation of CLSI and EUCAST breakpoints for several antibiotics. In the 2017 USCAST document (34), both nonclinical (animal studies) and clinical PK/PD data were used to define the FQ breakpoints. In the following sections, we review the key clinical PK and PD data for FQs in *Enterobacteriaceae* and *P. aeruginosa*, which were a major impetus for revision of the FQ breakpoints for these organisms by the CLSI.

Ciprofloxacin PK/PD data. PD data for intravenous (i.v.) ciprofloxacin were ascertained as part of a phase III clinical efficacy study among critically ill patients, with the majority of data being obtained from patients with lower respiratory tract infections (29). The study included patients infected with *P. aeruginosa* ($n = 25$) or other aerobic Gram-negative rods ($n = 36$). Compared with a total serum drug concentration AUC/MIC of <125, patients achieving an AUC/MIC of ≥ 125 were found to have statistically significantly higher rates of successful microbiological eradication (26% versus 86%; $P < 0.005$) and clinical cure (42% versus 81%; $P < 0.005$). Furthermore, patients with AUC/MIC values of <125 had longer times to bacterial eradication (median, 32 days), compared with patients with AUC/MIC values of 125 to 250 (median, 6.6 days).

It is important to note that there are data suggesting that AUC/MIC values of >250 may be associated with better microbiological or clinical outcomes. In one study (31), the authors observed a significantly shorter time to bacterial eradication for AUC/MIC values of ≥ 250, compared to AUC/MIC values of 125 to 250 (1.9 days versus 6.6 days; $P < 0.005$). Similarly, a 27.8-fold increase risk of ciprofloxacin failure was reported for patients with *Enterobacteriaceae* bloodstream infections (BSIs) when the AUC/MIC was less than 250 (observed cure rates of 91% and 28.6%, respectively). However, the authors did not observe a statistically significant difference in cure rates for AUC/MIC values of ≥ 250 versus 125 specifically, which they attributed to the distribution of MICs for their isolates being lower (MIC_{50}, 0.023 mg/liter; MIC_{90}, 0.25 mg/liter), resulting in higher AUC/MIC values being included, compared to the study by Forrest et al. (29). Based on available data, a total drug AUC/MIC of ≥ 125 is now the widely accepted PD target for ciprofloxacin and was the value used by the CLSI as the foundation for the ciprofloxacin breakpoint revision.

Several MCS analyses examining the optimal PK/PD breakpoint for ciprofloxacin based on different dosing regimens have been published. At standard ciprofloxacin doses (400 mg i.v. every 12 h), a PTA of $>90\%$ for AUC/MIC of ≥ 125 was observed for isolates with MICs of 0.25 μg/ml. However, the PTA substantially decreased as the MICs increased to 0.5 μg/ml (PTA of 50%) and 1 μg/ml (PTA of 0%) for Gram-negative pathogens (29). The PTA remained 0% for isolates with MICs of 1 μg/ml even with high-dose ciprofloxacin (i.e., 400 mg i.v. every 8 hours) (32). Importantly, the PTA was low ($<10\%$) when MICs increased above 0.25 μg/ml, irrespective of the dosage, for the higher PD threshold of AUC/MIC of ≥ 250 (31).

For *P. aeruginosa* BSIs, a PD model of the relationship between ciprofloxacin *f*AUC/MIC and the probability of cure (POC) was used to determine by MCS the predicted relative effectiveness of ciprofloxacin dosage regimens (30). The PTA was determined using goals of *f*AUC/MIC of >86 or total AUC/MIC of >123. Even with high-dose ciprofloxacin, *P. aeruginosa* isolates with MICs of 1 μg/ml were unlikely to be

effectively treated, with a PTA of 0% and a POC of 40%. Differences between PTA and POC outcomes at the higher MICs are likely due to the fact that clinical cures can occur even when target attainment is not achieved. The authors noted that combination therapy with ciprofloxacin is typically used for invasive pseudomonal infections, which also might have affected the POC rates in this study. Thus, although the PTA might have been suboptimal for MICs of 0.5 μg/ml, the clinical breakpoint was recommended to be 0.5 μg/ml. It is important to note that the 2019 CLSI *P. aeruginosa* ciprofloxacin breakpoints are based on a dosage regimen of 400 mg every 8 h.

In summary, ciprofloxacin breakpoints of $\leq$0.25 μg/ml for *Enterobacteriaceae* and $\leq$0.5 μg/ml for *P. aeruginosa* are generally supported by clinical PK/PD data. The revised breakpoints for ciprofloxacin and levofloxacin in the CLSI M100 29th supplement now have specific doses listed for both *Enterobacteriaceae* and *P. aeruginosa* (4). Although there are some indications that even lower breakpoints may potentially be warranted from a PK/PD standpoint, additional studies (e.g., clinical outcome and modeling studies) are needed before breakpoints can be lowered further.

Levofloxacin PK/PD data. In contrast to ciprofloxacin, data evaluating the levofloxacin PK/PD relationship to clinical outcomes in Gram-negative infections are limited. In one key study of nosocomial pneumonia patients treated with levofloxacin at 750 mg i.v. daily, achieving a total drug AUC/MIC of 87 to 110 led to a 4-fold higher rate of microbiological eradication, although a link with clinical outcomes was not found (26). The authors concluded that this was a reasonable PD target for *P. aeruginosa* as long as a second antibiotic agent was added to the therapy. In a small study of 14 critically ill patients with ventilator-associated pneumonia (35), a levofloxacin dose of 500 mg every 12 h for 24 h, followed by 500 mg every 24 h, was effective when the MIC was less than 0.72 μg/ml, using a PK/PD target of total drug AUC/MIC of 100 to 125. However, the infecting pathogens in that study were varied, with 3 patients infected with *P. aeruginosa* and 8 patients infected with other Gram-negative pathogens. In contrast, a study of 134 patients with Gram-positive or Gram-negative pathogens isolated from respiratory, skin, or urinary tract sources found a peak/MIC ratio of 12.2:1 and not AUC/MIC to be statistically linked to clinical and microbiological outcomes (36, 37). Peak/MIC ratio and AUC/MIC were highly correlated, however, leading to a calculated AUC/MIC of 110.

Frei et al. (32) evaluated aerobic Gram-negative rods by MCS analysis and found that high-dose levofloxacin (750 mg i.v. every 24 h) had a 0% PTA for AUC/MIC of $\geq$125 when MICs are $\geq$1 μg/ml, which was the pre-2019 CLSI breakpoint for *P. aeruginosa*. In the USCAST document on quinolone susceptibility test interpretation criteria (38), the PTA for levofloxacin at 750 mg i.v. every 24 h at a *f*AUC/MIC of $>$72 was 93.7% for a MIC of 0.5 μg/ml and 61.5% for a MIC of 1 μg/ml, for both *Enterobacteriaceae* and *P. aeruginosa*. The dosage used by the CLSI for the levofloxacin breakpoint determination was 750 mg i.v. or orally every 24 h.

Clinical outcome data. As mentioned previously, many of the data leading to the revision of these breakpoints were based on PK/PD analyses. Overall, there are only a handful of studies assessing the impact of ciprofloxacin and levofloxacin MICs on clinical outcomes for infections caused by *Enterobacteriaceae* or *P. aeruginosa*. In a cohort of 312 patients with BSIs treated with levofloxacin, there was no significant difference in all-cause mortality rates among patients with so-called intermediate (0.5 μg/ml) and higher (1 to 2 μg/ml) levofloxacin MICs, compared to patients whose isolates had lower MIC values ($\leq$0.5 μg/ml) (39). *E. coli*, *Klebsiella* spp., and *Pseudomonas* accounted for 86% of the isolates in that study. Nevertheless, the authors observed a statistically significant increase in the length of stay of 5.67 days for patients whose isolates had levofloxacin MICs of 1 or 2 μg/ml, compared to patients whose isolates had MICs of $\leq$0.25 μg/ml. Importantly, the dose of levofloxacin used in that study (500 mg every 24 h) was lower than the dose of 750 mg every 24 h used for the breakpoint revision.

Beyond BSIs, Rattanaumpawan and colleagues examined the impact on clinical outcomes in complicated UTIs (cUTIs), looking specifically at a more granular lower MIC range (40). Patients in that study were adult female patients with FQ-susceptible UTIs caused by *E. coli*. The authors compared outcomes for patients whose isolates had MIC values of ≤0.12 μg/ml versus 0.25 to 2 μg/ml, and they observed treatment failure rates of 0.8% and 6.9%, respectively. However, that was a retrospective analysis and a variety of FQs and dosages were used in the study, making it difficult to extrapolate the findings to the 2019 CLSI FQ breakpoints.

It should be noted that the paucity of data on the impact of MICs on clinical outcomes for *P. aeruginosa* and most *Enterobacteriaceae* strains stands in contrast to the clear differences in clinical outcomes observed among patients with enteric fever (43). Observations of clinical treatment failure were the driving force that led to a lowering of the breakpoints (susceptible breakpoints of ≤0.06 μg/ml for ciprofloxacin and ≤0.12 μg/ml for levofloxacin) for *Salmonella* spp. (including *Salmonella enterica* subsp. *enterica* serotype Typhi and *Salmonella enterica* subsp. *enterica* serotype Paratyphi). The outcome data were further supported by microbiological and PK/PD data (41–43), and most *Salmonella* isolates with decreased ciprofloxacin susceptibility harbored single mutations in the *gyrA* gene (44). In contrast, the major impetus for revision of the FQ breakpoints for *Enterobacteriaceae* and *P. aeruginosa* has been the availability of PK/PD analyses showing that the pre-2019 FQ susceptibility breakpoints were likely too high.

LABORATORY CHALLENGES WITH IMPLEMENTATION OF THE REVISED BREAKPOINTS

Importantly, there are several impediments to the adoption of revised susceptibility breakpoints by clinical laboratories that use commercial AST systems. First and foremost is the potential lack of regulatory clearance by the FDA for revised breakpoints on the AST systems being used. There are several regulatory factors at play for commercial AST system manufacturers, which historically have led to potential reluctance in seeking clearance for revised breakpoints. Furthermore, FDA clearance of revised breakpoints was hampered by a previous requirement that manufacturers adhere strictly to breakpoints that were often established at the time of drug approval. A detailed discussion of these issues is beyond the scope of this article, but interested readers are referred to the excellent publication on this topic by Humphries et al. (45).

Critically, such issues have proven to be problematic for clinical laboratories wishing to revise outdated breakpoints. The cephalosporin and carbapenem breakpoints were lowered in 2010. However, in a recent survey of acute care hospitals and long-term acute care hospital laboratories in Los Angeles County, 28.1% of laboratories surveyed (36/128 laboratories) continued to use pre-2010 carbapenem breakpoints (46). Although the FDA updated drug labels with the lowered breakpoints several years later, this delay is likely to have led to an under-reporting of resistance (46, 47). The 21st Century Cures Act, which was signed into law in 2017, seeks to address many of the issues that have led to delays in the adoption of revised breakpoints by commercial AST manufacturers. Despite this, clinical laboratories using commercial AST systems are forced to confront a situation in the near term in which the AST devices they are using might not have FDA clearance for the revised breakpoints.

As shown in Table 1, the revised ciprofloxacin and levofloxacin breakpoints are substantially lower than the previous breakpoints for *P. aeruginosa* and *Enterobacteriaceae* (with the exception of *Salmonella* spp.) published in the CLSI M100 28th supplement (48). Breakpoint revisions, particularly those that result in severalfold MIC reductions, can be problematic for clinical laboratories that use automated AST systems, which may not include dilutions in their products that cover the revised breakpoints. According to information obtained from package inserts and manufacturer's brochures, the concentrations of ciprofloxacin and levofloxacin in the panels of the major automated commercial AST systems used in clinical laboratories in the United States (Phoenix, MicroScan, Sensititre, and Vitek 2) appear to be sufficiently low to

TABLE 2 MIC calling ranges on commercial automated AST systems

AST system and FQ	MIC calling range for Gram-negative panels (μg/ml)
Beckman Coulter MicroScan	
Ciprofloxacin	0.5–4[a]
Levofloxacin	0.25–4[a]
BD Phoenix	
Ciprofloxacin	0.25–4
Levofloxacin	0.25–8
bioMérieux Vitek 2	
Ciprofloxacin	0.25–4
Levofloxacin	0.12–8
Thermo Fisher Sensititre	
Ciprofloxacin	0.5–2 or 0.5–4
Levofloxacin	1–8

[a]The ranges for panels with the widest MIC calling ranges are shown. Other panels for the MicroScan system may have different MIC calling ranges.

encompass the revised FQ breakpoints for *Enterobacteriaceae* and *P. aeruginosa* (Table 2). However, clinical laboratories should be aware that individual antibiotic concentrations in specific products for these systems can vary. Thus, laboratories should confirm the reportable MIC ranges for the commercial AST products they use as a first step in determining whether revised FQ breakpoints can potentially be implemented in their laboratories.

If an individual clinical laboratory establishes that the commercial AST panel/product used by the laboratory encompasses antibiotic concentrations sufficiently low to cover the revised breakpoints, then several additional critical steps must be taken prior to implementation of the revised breakpoints. Firstly, laboratories should be aware that interpretations would need to be manually edited to reflect use of the revised breakpoints. Secondly, and more critically, because the breakpoint being used is not cleared by the FDA for the given product, reporting with revised breakpoints is considered an off-label use of an FDA-cleared device. Therefore, the verification required is more complex and broader in scope than for an unmodified FDA-cleared AST device. Laboratories should also be aware that the performance of a cleared system at lower concentrations of antibiotics might not have been fully evaluated by the manufacturer. While there is currently no regulatory requirement for clinical laboratories to adopt a revised breakpoint, use of pre-2019 FQ breakpoints is likely to miss FQ resistance among patient isolates.

Disk diffusion testing. For laboratories that lack the ability to use automated AST systems that incorporate the revised breakpoints or for those that do not wish to validate the revised breakpoints as a modified FDA-cleared device, manual AST (i.e., disk or gradient diffusion testing) may represent the only viable testing option. Furthermore, disk diffusion testing is the primary AST method used in many laboratories worldwide. While manual AST methods require verification prior to implementation, they have the advantage of being FDA cleared and thus do not require as extensive a verification study as a modified FDA-cleared assay or a laboratory-developed test. Therefore, it is important for clinical microbiology laboratories to be aware of any potential issues with performance of disk diffusion testing with the revised FQ breakpoints.

The revised FQ MIC breakpoints were initially approved by the CLSI AST Subcommittee in January 2017 but were not published until 2019. One of the primary reasons for the delay in publication was a paucity of data examining correlations of disk diffusion results with the revised MIC breakpoints. Some of the initial disk diffusion data presented did not meet the CLSI M23 criteria for levofloxacin (49). Furthermore, there was a dearth of data for isolates straddling the revised breakpoints (i.e., MICs of 0.5 to

TABLE 3 Comparison of ciprofloxacin and levofloxacin zone diameter breakpoints for disk diffusion testing

Gram-negative organism and FQ (5-μg disk)	Zone diameter breakpoints (mm)[a]	
	2019 CLSI breakpoints (4)	2018 CLSI breakpoints (48)
Enterobacteriaceae		
Ciprofloxacin	S, ≥26; I, 22–25; R, ≤21	S, ≥21; I, 16–20; R, ≤15
Levofloxacin	S, ≥21; I, 17–20; R, ≤16	S, ≥17; I, 14–16; R, ≤13
P. aeruginosa		
Ciprofloxacin	S, ≥25; I, 19–24; R, ≤18	S, ≥21; I, 16–20; R, ≤15
Levofloxacin	S, ≥22; I, 15–21; R, ≤14	S, ≥17; I, 14–16; R, ≤13

[a]S, susceptible; I, intermediate; R, resistant.

1 μg/ml). Disk diffusion correlation data for several hundred *Enterobacteriaceae* and *P. aeruginosa* isolates were subsequently presented at the June 2018 meeting of the CLSI AST Subcommittee. These data represented a combined data set from the USCAST and the EUCAST. Based on these data, the disk diffusion zone diameter breakpoints shown in Table 3 were approved by the CLSI AST Subcommittee. In brief, there were no very major errors for either ciprofloxacin or levofloxacin for *Enterobacteriaceae*. Ciprofloxacin disk diffusion zone diameter breakpoints were published despite having a minor error rate of 42.3%, which exceeds the CLSI criteria (49). In contrast, the criteria were met for *P. aeruginosa* for both ciprofloxacin and levofloxacin, with lower rates of minor errors than observed for *Enterobacteriaceae*.

Looking in a more granular fashion at *Enterobacteriaceae* isolates that previously would have been considered susceptible to FQ but now would be considered intermediate or resistant by the 2019 FQ breakpoints, high rates of minor errors were observed for ciprofloxacin (54). Of the 58 isolates tested by ciprofloxacin disk diffusion, categorical agreement of 46% with reference broth microdilution was observed, with a 21.1% major error rate and a 46.6% minor error rate, using the 2019 CLSI disk diffusion breakpoints. Categorical agreement of 72.4% was observed for levofloxacin disk diffusion, with a minor error rate of 27.6%. However, when an error-rate-bound method was used to account for the isolates being enriched for MICs outside the wild-type MIC distribution, the minor error rate for levofloxacin achieved CLSI criteria (i.e., <40%) but the minor error rate for ciprofloxacin remained beyond acceptable CLSI limits at 49%. Categorical agreement was 81% for ciprofloxacin and 65.5% for levofloxacin for gradient diffusion testing (Etest). Minor error rates of 19% and 34.5% were observed for ciprofloxacin and levofloxacin, respectively, with the majority of the errors occurring due to Etest results being read 1 dilution above the MIC determined by the reference method. Thus, clinical laboratories that choose to use disk diffusion or gradient diffusion testing methods should be aware that both methods have a tendency to overcall resistance.

OTHER CONSIDERATIONS FOR URINARY TRACT INFECTIONS

Breakpoints are typically determined on the basis of achievable serum levels for a given antibiotic. Based on data from the SENTRY Antimicrobial Surveillance Program for isolates collected in the United States between 2011 and 2013, it is estimated that the revised breakpoints would result in 4% of *Enterobacteriaceae* isolates and 10% of *P. aeruginosa* isolates that were previously considered susceptible being considered resistant with the revised breakpoints (R. M. Humphries, personal communication). However, FQs are highly concentrated in the urine; approximately 80% of levofloxacin is excreted in urine, with peak concentrations that are 60 to 90 times those found in plasma (50). Levofloxacin displays significantly higher urine concentrations than ciprofloxacin, with a peak concentration of 406 μg/ml, compared to 286 μg/ml for ciprofloxacin, within 6 h following oral drug administration (51). Thus, the concentrations of both antibiotics in the urine vastly exceed those found in plasma and/or serum.

The high concentrations achievable in the urine raise the possibility that clinical success could potentially occur despite an organism testing resistant to FQs. Surprisingly, there are relatively few studies that have specifically addressed this question. In one such study, a ≥90% probability of bacterial eradication with treatment with 500 mg levofloxacin every 24 h was observed for patients with cUTIs in which the infecting isolate had a MIC of ≤4 μg/ml (52). The clinical efficacy of levofloxacin (750 mg every 24 h), compared with ceftolozane-tazobactam, was evaluated for cUTIs (including pyelonephritis) (53). When the clinical outcomes for the 370 patients with *Enterobacteriaceae* infections treated with levofloxacin were stratified according to MIC, the authors observed cure rates of 90 to 100% for isolates with MICs of ≤4 μg/ml. Importantly, microbiological eradication rates were similar to those for patients treated with imipenem. Even with MICs of 32 μg/ml, an 84.6% clinical cure rate (11/13 cases) was observed, although only 4 of 13 patients achieved microbiological eradication. The clinical significance of this latter finding is unclear.

In recent years, urine-specific breakpoints for *Escherichia coli*, *Klebsiella* spp., and *Proteus mirabilis* were established for cefazolin. Use of the revised breakpoints for FQ is likely to reduce the number of patients who will receive FQs for treatment of cUTIs. The use of FQs is associated with significant side effects (as described above) and this, coupled with increased resistance, is likely to further drive the use of broad-spectrum antibiotics. It is possible that, with the availability of more data on UTIs, urine-specific FQ breakpoints may be established in the future.

CONCLUSIONS

Reevaluation and subsequent revision of the CLSI FQ breakpoints for *Enterobacteriaceae* and *P. aeruginosa* strains were driven primarily by PK/PD data. The susceptibility breakpoints are now harmonized with those of EUCAST. Whether these revised breakpoints are too liberal or too conservative with respect to clinical outcomes remains to be determined. In the interim, clinical laboratories are faced with the challenge of performing laboratory verifications of these breakpoints in a landscape in which no commercial AST systems are currently cleared for the 2019 breakpoints. Will these revised FQ breakpoints be readily and rapidly adopted by clinical laboratories? That remains to be seen.

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