REVIEW ARTICLE

J Bacteriol Virol. Vol 52. No 2. June 2022; 52(2): 41-53
https://doi.org/10.4167/jbv.2022.52.2.041
pISSN 1598-2467 / eISSN 2093-0429

JBV

Focused Commentary; About Revision of CLSI Antimicrobial Breakpoints, 2018-2021

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Received : June 13, 2022
Revised : June 22, 2022
Accepted : June 30, 2022

No potential conflict of interest relevant to this article was reported.

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In recent years, antimicrobial resistance has been on the rise and infectious disease specialists and other clinicians face several challenges when treating patients with antimicrobial resistant infections. Clinical and Laboratory Standards Institute (CLSI) annually publishes guidelines for antimicrobial resistance tests, including revised antimicrobial breakpoints, because of the rise in antimicrobial resistance and changes to nomenclature owing to advances in whole genome sequencing technology. However, many laboratories use historical guidelines and do not follow the revised breakpoints. Moreover, the current breakpoints have limitations. This study evaluated the changes in antimicrobial breakpoints over the last 4 years, from 2018 to 2021. Here, I describe the microbiological and clinical background of the CLSI breakpoint revision and evaluate the advantages and limitations of new breakpoints with a review of large study data. In addition, I reviewed problems associated with each antimicrobial breakpoint and made suggestions for how they might be improved, for example, increasing or decreasing the minimum inhibitory concentration (MIC) or zone diameter, deleting or adding an S, I, or R category, introducing new concepts (such as susceptible-dose dependent (SDD)), and requesting more evaluation methods. Conclusions; CLSI annually publishes guidelines for antimicrobial resistance tests. I reviewed problems associated with each antimicrobial breakpoint for last 4 years, and made suggestions for how they might be improved.

Key Words: CLSI, Antimicrobial, Breakpoints

INTRODUCTION

Antimicrobial resistance is a major concern in the medical field. A breakpoint is a chosen concentration (μg/ml) of an antibiotic which defines whether a species of bacteria is susceptible or resistant to the antibiotic. The Clinical and Laboratory Standards Institute (CLSI) publishes revised antimicrobial breakpoints annually. Although the current breakpoints reduce the very major and major errors, they still have limitations despite being established through complicated processes, such as microbiological techniques, pK/pD, and clinical studies. Here, I reviewed the microbiological background and clinical significance of the revised CLSI antimicrobial guidelines for the last four years, from 2018 to 2021, and evaluated the limitations of the revisions in depth.

Gram positive: Daptomycin to *Enterococci*, ceftaroline to MRSA, oxacillin to *Staphylococcus spp.* other than *S. aureus* and *S. lugdunensis*, Dalbavancin, Lefamulin
Gram negative: Azithromycin to *Neisseria gonorrheae*, azithromycin to *Shigella spp.*, colistin, β-lactam combination agents, and cefiderocol to CRE and CRPA, ciprofloxacin and levofloxacin to *Enterobacterales* other than *Salmonella spp.* and *P. aeruginosa*.

In this study, the point and counterpoint of the revision are discussed and amendments are suggested to the current minimum inhibitory concentration (MIC) and disk diffusion breakpoints.

MATERIALS AND METHODS

Daptomycin To *Enterococci*

A breakpoint is a chosen concentration (mg/L or μg/mL) of an antibiotic which defines whether a species of bacteria is susceptible or resistant to the antibiotic. If the MIC is less than or equal to the susceptibility breakpoint, the bacteria is considered susceptible to the antibiotic.

In 2018, the breakpoint parameter in the 28th CLSI edition was susceptible only breakpoint, S ≤ 4 μg/ml for *E. faecium*. In 2019, the CLSI revised the daptomycin breakpoint for *Enterococcal* spp. twice.

The susceptibility breakpoints were changed to S ≤ 1 μg/mL, SDD breakpoint = 2-4 μg/mL, and R ≥ 8 μg/ml for *E. faecium,* and in the second revision, the changes included SDD ≤ 4 μg/mL and R ≥ 8 μg/ml for *E. faecium* and S ≤ 2 μg/mL, I = 4 μg/mL, and R ≥ 8 μg/mL for *E. spp*. other than *E. faecium* (1) (Table 1).

This double revision was made because daptomycin resistance had shown an increase in *Enterococci* and many clinical failures with an MIC ≤ 4 μg/mL were reported (2).

SDD is a newly introduced breakpoint parameter and indicates that a higher dose should be used than that in the S category (1). There were several reasons that the CLSI introduced SDD as a parameter when making these revisions. Firstly, 90% of the pK/pD target attainment was achieved with an 8 mg/kg daptomycin dose in isolates with MICs of 2-4 μg/mL, although the target attainment was low with a 6 mg/kg daptomycin dose. In the approach of pK/pD to antibiotic therapy, pharmacokinetics (pK) is concerned with the time course of antimicrobial concentrations in the body while pharmacodynamics (pD) is concerned with the relationship between those concentrations and the antimicrobial effect.

Secondly, the clinical outcome improved with an 8 mg/kg dose and the MIC was not tightly correlated with the LiaFSR mutation in isolates with MICs of 1-4 μg/mL (2). Resistance to daptomycin in *E. faecium* is mediated by activation of the LiaFSR membrane stress response pathway. Many studies have focused on improving the performance of antimicrobial tests by introducing the concept of SDD to daptomycin breakpoints (1, 2). According to recent trends, pK/pD analysis is significantly influential in setting up breakpoints and improvement of pK/pD analysis permits many changes in the revision of antimicrobial breakpoints, including introducing SDD to daptomycin breakpoints.

Table 1. CLSI Daptomycin breakpoint

	2018	2019 (1st)†	2019 (2nd)‡
E.faecium	S≤ 4¥	S≤ 1, SDD 2-4, R≥ 8	SDD≤ 4, R≥ 8
E.spp	S≤ 4	S≤ 1, SDD 2-4, R≥ 8	S≤ 2, I 4, R≥ 8

Abbreviations; CLSI: clinical laboratory standards institute, S: susceptible, I: intermediate, SDD: susceptible dose dependent
† : first revision
‡ : second revision
¥ ; unit, MIC μg/mL

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The recent development of whole-genome sequencing has enabled the detection of LiaFSR mutations, which could help determine new daptomycin breakpoints.

There are also several arguments which could be used against the introduction of SDD. First, regarding the introduction of SDD, daptomycin false resistance should be considered because falsely high MICs may be observed using the E-test or Microscan (3). False resistance observed by the E-test or Microscan may have led to the first and second revisions of the daptomycin breakpoints. Secondly, bacterial regrowth was observed following growth reduction using an 8-12 mg/kg dose of daptomycin, according to one study (4). Third, systematic studies on the clinical safety and therapeutic effects of high-dose daptomycin are rare (2). Regarding the issues of false resistance and regrowth, the daptomycin breakpoint should be reevaluated in isolates, including problematic isolates, and a review of studies on clinical safety and therapeutic effects with doses of 8-12 mg/L is important.

The second revision in 2019 was followed in rapid succession after the first. The reason for the second revision is that the wild-type was bisected because of the presence of the LiaFSR mutation in isolates with MIC ≤ 1 μg/mL (5). There are two key reasons for the decision to make this second revision. First, the new breakpoint was the same as that of the M 100. 28th ed., (S ≤ 4) except changing from S to SDD, so the adoption of a new breakpoint is useful for cAST (commercial antimicrobial susceptibility tests) without validation because the 28th breakpoint is cleared by the FDA (5).

Second, introducing the SDD category without S category reduced the risk of daptomycin underdosing. Third, the new breakpoint differentiated *E. faecium* from *other* enterococcal species. There is one key argument against this second revision: the S breakpoint MIC ≤ 1 μg/mL should not be disregarded since the presence of the LiaFSR mutation is not tightly correlated with clinical failure (6). Therefore, a large clinical study of the correlation between LiaFSR mutations and clinical outcomes is required. Laboratories should consider the points and counterpoints of the first and second revisions of the daptomycin breakpoints while adopting breakpoints for the interpretation of antimicrobial tests.

Ceftaroline to MRSA

In 2019, the MIC breakpoint of MRSA was revised to S ≤ 1 μg/mL, SDD 2-4 μg/mL, and R ≥ 8 μg/mL, from 2018s parameters of S ≤ 1 μg/mL, I 2 μg/mL, and R ≥ 4 μg/mL (7) (Table 2).

Although MRSA is resistant to β-lactams owing to the absence of affinity to penicillin-binding protein PBP2a, it is susceptible to ceftaroline, which also has a lower mortality rate than comparators such as vancomycin or daptomycin (8).

SDD is also included in the ceftaroline breakpoint parameters because it is necessary in the pk/pD analysis which showed that 600 mg ceftaroline at MIC 4 μg/mL for 8 hours was well tolerated in MRSA patients (9). However, there are many limitations. First, even though the revised breakpoint reduced the major error by 81% (8, 9), the revised breakpoint still reported low-level ceftaroline resistance, including 44% non-susceptible isolates in the Asia-Pacific region (8, 9). Second, when adopting a new breakpoint, heteroresistance is observed in vancomycin-intermediate *S. aureus* (VISA), daptomycin non-susceptible, or linezolid non-susceptible strains. Third, clonal lineages, such as CC5 and ST22, are associated with the risk of clonal transfer of low-level resistant strains (8, 9). Fourth, the wild type was found to display an MIC of 4 μg/mL, suggesting that a new breakpoint could not differentiate the wild type from the non-wild type (9). Fifth, disk diffusion or E-test has low categorical agreement with reference broth microdilution, and agencies such as CLSI and EUCAST show

Table 2. CLSI Ceftaoline breakpoint

	2018	2019
MRSA	S≤ 1, I 2-4, R≥ 4^{+}	S≤ 1, SDD 2-4, R≥ 8

Abbreviations; MRSA: methicillin resistant *Staphyloccus aureus*
+ ; unit, MIC μg/mL

different ceftaroline breakpoints (9). Finally, clinical data of isolates with MIC ≥ 2 μg/mL are very rare, and SDD cannot be FDA-approved. Considering the continuous geographical reporting of low-level ceftaroline resistance with occasional clonal spread and the many problems in introducing the current ceftaroline breakpoint, the CLSI should reevaluate the ceftaroline breakpoint by microbiological, pK/pD, clinical, and whole genome sequencing studies on a large cohort including low-level ceftaroline-resistant isolates with MIC ≥ 2 μg/mL and challenging isolates near the breakpoint. Whole genome sequencing studies are needed to detect PBP2 mutations, including isolates near breakpoints. It is important to confirm the association of the PBP2 mutation with clinical outcomes and ensure that the current breakpoint is appropriate.

Dalbavancin

In 2018, a dalbavancin breakpoint was added to the CLSI guidelines (Table 3). Telavancin, dalbavancin, and oritavancin are synthetic lipoglycopeptides and vancomycin derivatives (10). Vancomycin is used to treat MRSA infections; however, vancomycin non-susceptible strains (hVISA, VISA, and VRSA) have emerged owing to vancomycin selective pressure. Telavancin, dalbavancin, and oritavancin have more potent bactericidal effects than their comparators and show very low MICs (10). However, they have limited activity against VISA or VRSA strains of MRSA and the VanA type of VRE. Among vancomycin non-susceptible *S. aureus*, telavancin and dalbavancin are susceptible to hVISA but resistant to VRSA and discrepantly susceptible to VISA. Oritavancin is susceptible to hVISA, VISA, and VRSA. (11). Current telavancin or dalbavancin breakpoint studies do not include VISA strains, and we suggest telavancin and dalbavancin *S. aureus* breakpoint reevaluation in isolates including VISA strains. Among vancomycin non-susceptible *Enterococci,* telavancin and dalbavancin are susceptible to VanB-type VRE *E. faecium*. However, *E. faecium* was not susceptible to VanA-type VRE. Oritavancin shows activity to both the VanA and VanB types (12). Therefore, CLSI suggests reporting only VSE but not VRE for telavancin, dalbavancin, and oritavancin breakpoints in *Enterococci (*1).

In addition, between agencies, such as the CLSI and FDA, the antimicrobial breakpoint is different; for example, dalbavancin breakpoints are reported as S ≤ 0.25 μg/mL and S ≤ 0.12 μg/mL, respectively, showing the possibility of errors between MIC 0.12-0.25 μg/mL. Dalbavancin breakpoint reevaluation in isolates with MICs between 0.12-0.25 μg/mL is therefore needed. Moreover, the addition of oritavancin disk diffusion breakpoints to the 2022 CLSI guidelines should be considered because oritavancin disk diffusion is well correlated with reference broth microdilution (13).

Oxacillin to *Staphylococcus spp.* other than *S. aureus* and *S. lugdunensis*

In 2021, the oxacillin MIC breakpoint in *Staphylococcus spp.* other than *S. aureus* and *S. lugdunensis* (S. spp.) was revised by the CLSI (14), with the MIC revised to S ≤ 0.5 and R ≥ 1 from S ≤ 0.25 μg/mL and R ≥ 0.5 μg/mL in 2020 (Table 4).

Table 3. CLSI Telavancin, Dalbavancin, Oritavancin breakpoint

	MRSA	*Enterococcus spp.*	Streptococcus, β-hemolytic	Streptococcus viridans
Telavancin	S≤ 0.12[+]	S≤ 0.25	S≤ 0.12	S≤ 0.06
Dalbavancin	S≤ 0.25	S≤ 0.25	S≤ 0.25	S≤ 0.25
Oritavancin	S≤ 0.12	S≤ 0.25	S≤ 0.25	S≤ 0.25

Abbreviations; MRSA: methicillin resistant *Staphylococcus aureus*
+ ; unit, MIC μg/mL

Table 4. CLSI Oxacillin breakpoint

	2020	2021
S.spp other than *S. aureus, S. lugdunensis*	S≤ 0.25, R≥ 0.5[+]	S≤ 0.5, R≥ 1

Abbreviations; S. spp: Staphylococcal species
+ ; unit, MIC μg/mL

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In 2018, the original term coagulase-negative *Staphylococci* (CNS) was changed to 'S. spp. other than *S. aureus, S. lugdunensis* (S. spp.) according to matrix-assisted laser desorption ionization-time of flight mass spectrometry and whole-genome sequencing, which could differentiate between S. spp. and *S. aureus/ S. lugdunensis*, S. spp. display mecA heterogeneity between species, and mecA-positive opportunistic S. spp. strains are clinically important. MecA-mediated oxacillin resistance in S. spp. could not be detected accurately with the CLSI CNS breakpoint, so the term has been changed and the breakpoints were determined as S ≤ 0.25 μg/mL and R ≥ 0.5 μg/mL in 2020. However, even according to 2020 guidelines, 2.1% of very major errors and 7.1% of major errors were found in mecA-mediated S. spp., exceeding the permission criteria, so adapting the breakpoint to be in the permission criteria allowed the criteria to be revised to S ≤ 0.5 μg/ml and R ≥ 1 μg/mL in 2021 (14). The oxacillin MIC and disk diffusion method have reliable performance in S. spp, however when the oxacillin MIC exceeded 0.5 μg/mL S. spp. were somewhat less correlated with mecA, and, the revised oxacillin MIC breakpoint is correlated well with disk diffusion, benefiting from the ease of the disk diffusion method in laboratories. However, the cefoxitin MIC and disk diffusion methods showed poor performance, suggesting that they cannot be used as a surrogate for mecA. Therefore, in 2021, the CLSI reported that the cefoxitin test is not acceptable because of the high major error in S. spp. (14). In one study, the cefoxitin disk diffusion test led to misidentification of oxacillin-resistant S. spp. as mecA-negative *S. aureus* (6). The limitation of the new breakpoint in S. spp. is that a species-specific breakpoint is not possible, and a single MIC breakpoint may produce highly significant errors in some species. Moreover, most S. spp. were poorly correlated with mecA when the oxacillin MIC exceeded 0.5 μg/mL, therefore the MIC breakpoint of oxacillin should be reevaluated in isolates including those with MIC ≥ 0.5 μg/mL. (15). In addition, isolates with MIC values near 0.5 μg/mL or 1 μg/mL should be retested with mecA PCR or PBP2a (16). Species-specific breakpoints are not possible because of the presence of too many staphylococcal species, therefore, laboratories should cautiously interpret the antimicrobial tests for *S.* spp. with the current breakpoints to reduce major errors in some species.

Lefamulin

In 2021, the lefamulin MIC and disk diffusion breakpoint were added to the CLSI, following the same FDA breakpoint (14) (Table 5). The MIC breakpoint was S ≤ 0.25 μg/mL in *Staphylococci*, ≤ 0.5 μg/mL in *S. pneumoniae*, and ≤ 2 μg/mL in *H. influenza* and *H. parainfluenza*. The disk diffusion breakpoint was S ≥ 23 mm in *Staphylococci*, S ≥ 17 mm in *S. pneumoniae*, and S ≥ 17 mm in *H. influenza* and *H. parainfluenza*. Lefamulin was approved by the FDA in 2019 as an antimicrobial pleuromutilin to treat community-acquired bacterial pneumonia by inhibiting the growth of most respiratory pathogens. This drug acts by binding to bacterial ribosomes, inhibiting ribosomal activity, and preventing protein synthesis (17). The MIC of lefamulin, a drug which displays good pK/pD activity, is 2-3 times lower than that of the comparators vancomycin and linezolid (18) and has a 100% bactericidal effect on penicillin-, macrolide-, tetracycline-, and fluoroquinolone-resistant *S. pneumoniae* and MRSA (MIC of 0.25 μg/mL), suggesting that it could be used as an empiric treatment for community-acquired bacterial pneumonia (18). The study of lefamulin is very limited because it was only approved in 2021; however, isolates with MICs greater than 0.25 μg/mL were observed in 0.3% of S. aureus isolates. These isolates showed expression of *vga*, an efflux pump gene in *S. aureus*, and *ISA(E)*, another efflux pump gene in *S.*

Table 5. CLSI Lefamulin breakpoint

	MIC	Disk diffusion
Staphyloocci	S≤ 0.25†	S≥ 23 ‡
S.pneumoniae	S≤ 0.5	S≥ 17
H. influenza	S≤ 2	S≥ 17
H. parainfluenza	S≤ 2	S≥ 17

Abbreviations; MIC: minimal inhibitory concentration
† ; unit, MIC μg/mL
‡ ; unit, disk diffusion, mm

Table 6. CLSI Azithromycin Breakpoint to *N. gonorrheae*

	2016	2019	2021
N. gonorrheae	Wild-type ≤ 1†	S≤ 1 (MIC)‡	S ≥ 30 (Disk diffusion)¥

† ; unit, μg/mL
‡ ; unit, μg/mL
¥ ; unit, disk diffusion, mm

pneumoniae, by whole genome sequencing. With increased MICs, monitoring of this horizontally transferable gene and breakpoint reevaluation in isolates, including isolates with MICs greater than 0.25 μg/mL, are needed. The clinical association with this transferable gene is also needed to be evaluated on a larger cohort.

Azithromycin to *Neisseria gonorrheae.*

In 2016, CLSI reported the epidemiological cut-off (ECV) of azithromycin as wild type ≤ 1 μg/mL and non-wild type > 2 μg/mL in *N. gonorrheae* to detect azithromycin resistance.

In 2019, the CLSI reported true susceptible-only breakpoints as S ≤ 1 μg/mL according to continuous azithromycin resistance (1) (Table 6). In 2021, a disk diffusion breakpoint was added with S ≥ 30 mm (14). The WHO global gonococcal antimicrobial surveillance program (WHO-GASP) recently reported globally increased azithromycin resistance in *N. gonorrheae*. However, despite breakpoint revision, discrepant MICs are reported in isolates with MICs of 1 μg/mL or 2 μg/mL. Different MIC breakpoints between agencies are another issue, with EUCAST R > 0.5 μg/mL and CLSI R > 2 μg/mL, suggesting that it is necessary to reevaluate MIC breakpoints including isolates with MICs greater than 0.5 μg/mL (19). Even at an MIC ≤ 1 μg/mL, *mtr* (multiple transferable resistance) efflux pump gene mutations are occasionally found (20). Low-level azithromycin resistance with the *mtr* mutation could lead to high-level resistance, requiring monitoring in the case of a low MIC.

Here, we suggest deleting the S breakpoint in *N. gonorrheae*, such as with daptomycin. Instead, we recommend the introduction of the SDD concept because the therapeutic effect was observed at a 2 g azithromycin dose and treatment failures were occasionally found with MICs greater than 0.5 μg/mL (19, 20). In particular, because we do not know the therapeutic effect in the MIC range of 2-16 μg/mL, and clonal spreads with strains with *mtr* gene mutations are occasionally found, we should determine the intermediate and resistant breakpoints among isolates with MICs of 2-16 μg/mL.

According to the 2022 CLSI guidelines, the addition of intermediate and resistant azithromycin breakpoints for *N. gonorrhea* is required. The disk diffusion method poorly correlates with agar dilution (14, 21, 22); therefore, the azithromycin disk diffusion breakpoint should be reevaluated for isolates near the breakpoint.

Azithromycin to *Shigella spp.*

In 2019, CLSI reported an ECV to detect azithromycin resistance in Shigella species. The ECV MIC breakpoint was as follows: wild type ≤ 8 μg/mL and non-wild type ≥ 16 μg/mL in *S. flexneri* and wild type ≤ 16 μg/mL and non-wild type ≥ 32 μg/mL in *S. sonnei* (1). The ECV disk diffusion breakpoint was wild type ≥ 16 mm and non-wild type ≤ 15 mm in *S. flexneri*. In 2021, the true MIC and disk diffusion criteria were reported, with MIC breakpoints of S ≤ 8 μg/mL, I = 16 μg/mL, and R ≥ 32 μg/mL and disk diffusion breakpoints of S ≥ 16 mm, I = 11-15 mm, and R ≤ 10 mm in *Shigella* spp. (Table 7).

Azithromycin is used to treat fluoroquinolone-resistant *Shigella* species; however, the prevalence of azithromycin-resistant *Shigella* species has recently been on the rise (23). In one study, more than 40% of *S. flexneri* were azithromycin non-wild type and most resistance was transmissible by plasmid-mediated *mph(A)*, suggesting the possibility of clonal spread (23).

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Therefore, the CLSI revised the azithromycin breakpoint for *Shigella* spp. in 2021. Because an *S. flexneri* strain with an ECV MIC of 16 μg/mL occasionally showed *mph(A)*, the 2021 MIC breakpoint changed to an MIC of 16 μg/mL as an intermediate. Because zone diameters of 14 or 15 mm occasionally do not have the *mph(A)* gene, which does not agree with the ECV criteria (24), the 2021 disk diffusion criteria were changed to the range of zone diameters of 11-15 mm as intermediate.

Large clinical studies on a cohort including isolates with MIC 16 μg/mL and disk diffusion zone diameter 14 or 15 mm are required, and the association of the *mph(A)* gene and clinical outcomes should be evaluated with whole genome sequencing. The 2021 azithromycin MIC and disk diffusion breakpoints are breakpoints that do not differentiate between species. In 2022, species-specific MIC criteria were required because *S. flexneri* has a lower MIC than *S. sonnei*.

Colistin to *P. aeruginosa* and *Enterobacterales*

Colistin is used for CRPA and CRE despite its toxicity. In 2017, the colistin MIC breakpoint of *P. aeruginosa* was revised to S ≤ 2 μg/mL and R ≥ 4 μg/mL from S ≤ 2 μg/mL, I = 4 μg/mL, and R ≥ 8 μg/mL (25).

In 2020, the breakpoint was changed to I ≤ 2 μg/mL and R ≥ 4 μg/mL because the MIC of 2 μg/mL bisected the wild type by pK/pD analysis (26) (Table 8). The reasons of colistin breakpoint revision in *P. aeruginosa* are the following: firstly, colistin methanesulfonate sodium, a multicomponent colistin, has a higher MIC than colistin when hydrolyzed. Secondly, a high error rate is observed in disk diffusion, E-test, or agar dilution because colistin has a low diffusion capacity in agar owing to its high molecular weight. Third, heteroresistance is observed, and colistin can bind to microplates (27). Many studies have focused on improving the antimicrobial testing performance of *P. aeruginosa* with revised colistin breakpoints (26).

For *Enterobacterales,* in 2017, the ECV was set up as wild type ≤ 2 μg/mL and non-wild type ≥ 4 μg/mL, not to be clinically used (25). In 2020, the true breakpoint was reported as I ≤ 2 μg/mL and R ≥ 4 μg/mL (Table 8), because the CRE isolates were continuously shown to be on the increase and the plasmid-mediated *mcr-1* gene was detected by whole genome sequencing. In another study, whole-genome sequencing detected the *mcr-1* gene in isolates with MIC ≤ 2 μg/mL; therefore, breakpoint I ≤ 2 μg/mL should be changed to I ≤ 1 μg/mL (28).

Recently, the MgrB chromosomal or *mcr-1* plasmid-mediated colistin resistance mechanism in *Enterobacterales* has been reported; therefore, careful monitoring of these resistant strains in laboratories is required, and we should confirm whether the current breakpoint is appropriate.

Table 7. CLSI Azithromycin breakpoint to *Shigella spp.*

	ECV, 2019		2021	
	MIC[+]	Disk diffusion[‡]	MIC[+]	Disk diffusion
S. flexneri	Wild≤ 8, Non-wild ≥ 16	Wild≥ 16, Non-wild ≤ 15	S ≤ 8, I 16, R≥ 32	S ≥ 16, I 11-15, R≤ 10
S. sonnei	Wild≤ 16, Non-wild ≥ 32		S ≤ 8, I 16, R≥ 32	S ≥ 16, I 11-15, R≤ 10

Abbreviations; ECV: epidemiological cut off, wild: wild type, Non-wild: non-wild type
+ ; unit, MIC μg/mL
‡ ; unit, disk diffusion, mm

Table 8. CLSI Colistin breakpoint

	2016	2017	2020
P. aeruginosa	S ≤ 2, I =4, R ≥ 8[+]	S≤ 2, R≥ 4	I≤ 2, R ≥ 4
Enterobacterales		Wild-type ≤ 2, Non-wild type ≥ 4	I≤ 2, R ≥ 4

+ ; unit, MIC μg/mL

Table 9. CLSI Cefiderocol breakpoint

Enterobacterales	S≤ 4, I 8, R≥ 16[†]
P. aeruginisa	S≤ 4, I 8, R≥ 16
A. baumannii	S≤ 4, I 8, R≥ 16
S. maltophilia	S≤ 4, I 8, R≥ 16

† ; unit, MIC μg/mL

As a resistance detection method, colistin agar test or colistin broth elution test is an alternative to broth microdilution (1). However, *Acinetobacter baumannii* strains tested using these methods result in very large errors, indicating that only the broth microdilution method should be used in A. baumannii.

Cefiderocol to *Enterobacterales* and *P. aeruginosa*

Following FDA approval of cefiderocol in 2018, CLSI added the cefiderocol MIC breakpoint to *Enterobacterales, P. aeruginosa, Acinetobacter* spp. and *S. maltophilia* as S ≤ 4 μg/mL, I = 8 μg/mL, and R ≥ 16 μg/mL (29). In 2019, a disk-diffusion breakpoint was added (1) (Table 9). Cefiderocol is a cephalosporin siderophore that chelates ferric iron and is transferred to the periplasmic space *via* the membrane iron transport system, where the cephalosporin component binds to PBP3, thereby inhibiting peptidoglycan synthesis. Cefiderocol is highly active, with an MIC ≤ 2 μg/mL against most meropenem-resistant *Enterobacterales* and *P. aeruginosa* strains, showing a more potent effect than cefepime (30). Cefiderocol is more useful in CRE strains than β-lactam combination agents or colistin because β-lactam combination agents have low bactericidal effects in metallo-β-lactamase-producing strains, however colistin has high toxicity despite this effect.

The 2019 cefiderocol breakpoint is investigational and will be approved in 2021; currently, the FDA or EUCAST criteria should be used until cefiderocol approval is obtained (31-33). The limitations of cefiderocol breakpoints are numerous. First, the disk diffusion breakpoint of cefiderocol does not have an intermediate category, producing very major and major errors. Second, *A. baumannii* strains show difficulty in interpreting the broth microdilution method, because of the trailing endpoint phenomenon, and in the disk diffusion method, because of the growth of pinpoint colonies in the inner zone. Third, there is no FDA criteria of *A.* spp. other than *A. baumannii* and *S. maltophilia* (34, 35). Fourth, cefiderocol-resistant *P. aeruginosa* strains are occasionally found, suggesting the need for breakpoint re-evaluation in *P. aeruginosa* strains (36). Lastly, species-specific breakpoints are not possible because of the presence of several species. As a resistance detection method, the cefiderocol antimicrobial test uses iron-depleted cation-adjusted Müller-Hinton broth; therefore, alternative cefiderocol methods, such as colistin broth elution tests, are required (37).

Once the investigational cefiderocol criteria are approved by the CLSI in 2021, performance reevaluation of disk diffusion against reference broth microdilution and determination of the intermediate zone are required. Moreover, species-specific criteria should be considered in the 2022 guidelines to reduce major and very major errors. Owing to the poor performance and testing difficulties as well as the absence of established guidelines, the cefiderocol breakpoint of *P. aeruginosa*, *Acinetobacter* spp., and *S. maltophilia* should be reevaluated.

β-Lactam combination agents to *Enterobacterales* and *P. aeruginosa*

In 2018, the MIC breakpoints of ceftazidime/avibactam and ceftolozane/tazobactam against *Enterobacterales* and *P. aeruginosa* were reported by CLSI (29). In 2019, the MIC and disk diffusion breakpoints of meropenem/vaborbactam against *Enterobacterales* and *P. aeruginosa* were reported (1). In 2021, the MIC and disk diffusion breakpoints of imipenem/relebactam were reported (14) (Table 10). Amoxicillin/clavulanic acid and piperacillin/tazobactam inhibited only TEM (Temoniera) or SHV (sulfhydryl reagent variable), but not ESBL, CRE, or CRPA. Ceftazidime/avibactam,

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Table 10. CLSI β-lactam combinatin agents breakpoint

	Enterobacterales		*P. aeruginosa*	
	MIC†	Disk diffusion‡	MIC†	Disk diffusion‡
Ceftazidime/Avibactam	S≤ 8/4, R≥ 16/4	S≥ 21 R≤ 20	S≤ 8/4 R≥ 16/4	S≥ 21 R≤ 20
Ceftolozane/Tazobactam	S≤ 2/4, I 4/4, R≥ 8/4	S≥ 21, I 18-20, R≤ 17	S≤ 4/4, I 8/4, R≥ 16/4	S≥ 21, I 17-20, R≤ 16
Meropenem/Vaborbactam	S≤ 4/8, I 8/8, R≥ 16/8	S≥ 18, I 15-17, R≤ 14	S≤ 2/4, I 4/4, R≥ 8/4	S≥ 19, I 16-18, R≤ 15
Imipenem/Relebactam	S≤ 1/4, I 2/4, R≥ 4/4	S≥ 25, I 21-24, R≤ 20	S≤ 2/4, I 4/4, R≥ 8/4	S≥ 23, I 20-22, R≤ 19

† ; unit, MIC μg/mL
‡ ; unit, disk diffusion, mm

ceftolozane/tazobactam, meropenem/vaborbactam and imipenem/relebactam inhibit ESBL, CRE or CRPA. Avibactam or vaborbactam reduces the MIC of β-lactam drugs by several fold, showing an effect on ESBL, AmpC, or KPC; however, it has no effect on metallo-β-lactamase, oxa-type β-lactamase, or resistance to porin mutation (38). As an antimicrobial resistance detection method, the disk diffusion method of ceftazidime/avibactam or imipenem/relebactam has low categorical agreement compared to reference broth microdilution in CRE isolates and it overcalls resistance (39-41). Therefore, reevaluation of the disk diffusion method is required. The E-test of imipenem/relebactam categorical agreement was > 90%; however, the E-test showed a one-grade high MIC result. Therefore, isolates with an E-test MIC of 2-4 μg/mL should be retested with broth microdilution to reduce major or minor errors. The resistance to β-lactam combination agents is transferred by conjugation, with the possibility of horizontal transfer of low-level resistance in CRE and CRPA, and the resistance mechanism of β-lactam combination agents should be detected using whole genome sequencing.

Ciprofloxacin and Levofloxacin to *Enterobacterales* and *P. aeruginosa*

In 2019, CLSI revised the MIC and disk diffusion breakpoints of ciprofloxacin and levofloxacin for *P. aeruginosa* and *Enterobacterales* other than *Salmonella* spp. (1) (Table 11). The reason for the 2019 breakpoint revision was that ciprofloxacin and levofloxacin resistance was high, and the 2018 breakpoint could not detect low-level resistance. Ciprofloxacin MIC 0.5-1 μg/mL corresponds to the susceptible category according to the 2018 criteria but intermediate or resistant according to the 2019 criteria. The pK/pD data for ciprofloxacin and levofloxacin do not support the 2019 criteria but do support the 2018 criteria which is setback for the 2019 data, and furthermore clinical data are scarce (42). Moreover, large minor errors were observed in ciprofloxacin disk diffusion in isolates with MICs of 0.5-1 μg/mL (43). Therefore, if an isolate with an MIC of 0.5-1 μg/mL is found, a retest is required, and the ciprofloxacin breakpoint should be reevaluated for isolates including those with an MIC of 0.5-1 μg/mL. In the Microscan panel or Accelerate pheno™ system, dilution is technically difficult for MICs ≤ 1 μg/mL for ciprofloxacin and ≤ 2 μg/ml for levofloxacin (44). As most isolates are in this MIC range, all isolates in this range should be retested manually according to the 2019 guidelines (43). Therefore, we should reevaluate ciprofloxacin and levofloxacin breakpoints in a larger cohort, including isolates near breakpoints, and decide which criteria are appropriate between 2018 and 2019 since pK/pD studies still support the 2018 criteria.

Table 11. CLSI Ciprofloxacin, Levofloxacin breakpoint

	Enterobacterales		*P. aeruginosa*	
	MIC†	Disk diffusion‡	MIC†	Disk diffusion‡
Ciprofloxacin	S≤ 0.25, I 0.5, R≥ 1	S≥ 26, I 22-25, R≤ 21	S≤ 0.5, I 1, R≥ 2	S≥ 25, I 19-24, R≤ 18
Levofloxacin	S≤ 0.5, I 1, R≥ 2	S≥ 21, I 17-20, R≤ 16	S≤ 1, I 2, R≥ 4	S≥ 22, I 15-21, R≤ 14

† ; unit, MIC μg/mL
‡ ; unit, disk diffusion, mm

CLSI revised only the ciprofloxacin and levofloxacin breakpoints and did not include other fluoroquinolone antimicrobials. Because reports of nalidixic acid-susceptible and levofloxacin-intermediate or resistant strains might cause clinical confusion (45), nalidixic acid should be discontinued. In addition, a urine-specific fluoroquinolone breakpoint is required, such as that for cefazolin. Norfloxacin was reinstated in 2020 with the advantage of no periurethral or vaginal damage in UTI after discontinuation owing to toxicity in 2019 (1). However, norfloxacin has minimal clinical efficacy owing to its MIC being higher than that of ciprofloxacin or levofloxacin.

SUMMARY

In this study, I investigated the revised breakpoints of CLSI over the last four years. Considering the background to these revisions, we have outlined the advantages and limitations of these decisions. Owing to increasing antimicrobial resistance and nomenclature changes aided by the development of whole genome sequencing, antimicrobial guidelines are revised annually. I found that even though the current CLSI guidelines reduce the rate of major and very major errors, it is important to regularly re-evaluate the breakpoints of some antimicrobials with particularly challenging isolates.

ABBREVIATIONS

CLSI: clinical laboratory standards institute, MIC: minimum inhibitory concentration, S: susceptible, I: intermediate, R: resistant, SDD: susceptible dose dependent, pK/pD: pharmacokinetic/pharmacodynamic, MRSA: methicillin resistant Staphylococcus aureus, hVISA: hetero-vancomycin intermediate Staphylococcus aureus, VISA: vancomycin intermediate Staphylococcus aureus, VRSA: vancomycin resistant Staphylococcus aureus, VRE: vancomycin resistant Enterococci, CRPA: carbapenem resistant Pseudomonas aeruginosa, CRE: carbapenem resistant Enterobacterales, ESBL: extended spectrum beta-lactamase, KPC: Klebsiella pneumoniae carbapenemase

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