Colistin Breakpoints for *Pseudomonas aeruginosa* and *Acinetobacter* spp.

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1 Foreword

The Clinical and Laboratory Standards institute (CLSI) is a not-for-profit membership organization that brings together the varied perspectives and expertise of the worldwide laboratory community for the advancement of a common cause: to foster excellence in laboratory medicine by developing and implementing medical laboratory standards and guidelines that help laboratories fulfill their responsibilities with efficiency, effectiveness, and global applicability.

Using the CLSI voluntary consensus process, the Subcommittee on Antimicrobial Susceptibility Testing develops standards that promote accurate antimicrobial susceptibility testing and appropriate reporting. The subcommittee reviews data from various sources and studies (eg, *in vitro*, pharmacokinetic-pharmacodynamic [PK-PD], and clinical studies) to establish antimicrobial susceptibility test methods, breakpoints, and quality control (QC) ranges.

The details of the necessary and recommended data for selecting appropriate breakpoints and QC ranges, and how the data are presented for evaluation, are described in CLSI document M23.[1] CLSI antibacterial breakpoints are provided in CLSI documents M100[2] and M45.[3]

Over time, a microorganism's susceptibility to an antimicrobial agent may decrease, resulting in a lack of clinical efficacy and/or safety. In addition, microbiological methods, QC parameters, and the manner in which breakpoints are established may be refined to ensure more accurate results. Because of these types of changes, CLSI continually monitors and updates information in its documents. Although CLSI standards and guidelines are developed using the most current information available at the time, the field of science and medicine is always changing; therefore, standards and guidelines should always be used in conjunction with clinical judgment, current knowledge, and clinically relevant laboratory test results to guide patient treatment. For more information, visit www.clsi.org.

This CLSI rationale document is based on CLSI agenda items submitted by the CLSI-EUCAST Joint Colistin Ad Hoc Working Group.

2 Introduction

Colistin (polymyxin E) is a member of the polymyxin group of antimicrobial agents. The polymyxins are composed of large amphipathic cyclic lipopeptides that are positively charged at physiological pH.[4] Their mode of action is through electrostatic interaction with the lipopolysaccharide (LPS) component of the gram-negative cell wall. This interaction leads to a competitive displacement of the divalent cations that normally stabilize the LPS. This disruption of the outer membrane integrity leads to cytoplasmic leakage and cell death.[4,5] The polymyxins, including colistin, are active against most gram-negative bacilli, including *Enterobacteriaceae* (excluding *Proteae* and *Serratia* spp.), *Pseudomonas aeruginosa,* and *Acinetobacter* spp. *Neisseria* spp., *Brucella* spp., and *Burkholderia* spp. are intrinsically resistant to the polymyxins. Polymyxin resistance is primarily the result of modification of the polymyxin LPS target. A transmissible form of resistance, mediated by plasmid-borne *mcr* genes, has been described among the *Enterobacteriaceae*.[5] There is complete cross-resistance between the polymyxins. For current and past colistin breakpoints, see Tables 1 and 2, respectively.

Colistin is approved by the US Food and Drug Administration (FDA) for the treatment of acute or chronic infections due to susceptible strains of gram-negative bacilli, particularly those caused by susceptible strains of *P. aeruginosa*.[6] In practice, colistin use is typically relegated to salvage therapy for infections caused by multidrug-resistant (MDR) *P. aeruginosa, Acinetobacter baumannii,* or carbapenem-resistant *Enterobacteriaceae*. In these scenarios, colistin is primarily used as part of combination therapy. Inhaled formulations of colistin are also available. **NOTE:** The breakpoints in this document do not apply to inhaled use.

Table 1. Current CLSI Colistin Breakpoints*

Organism Group	S	SDD	I	R
P. aeruginosa	≤2 μg/mL	-	-	≥4 μg/mL
Acinetobacter spp.	≤2 μg/mL	-	-	≥4 μg/mL

* Last reviewed June 2016; first published in CLSI document M100, 27th ed.
Abbreviations: I, intermediate; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

Table 2. Historical CLSI Colistin Breakpoints Replaced by Current Colistin Breakpoints*

Organism Group	S	SDD	I	R
P. aeruginosa	≤2 μg/mL	-	4	≥8 μg/mL

* Last published in CLSI document M100, 26th ed.
Abbreviations: I, intermediate; R, resistant; S, susceptible; SDD, susceptible-dose dependent.

3 Standard Dosages and Pharmacokinetic Data

Table 3. Current FDA Dosing Recommendations According to Creatinine Clearance[6]

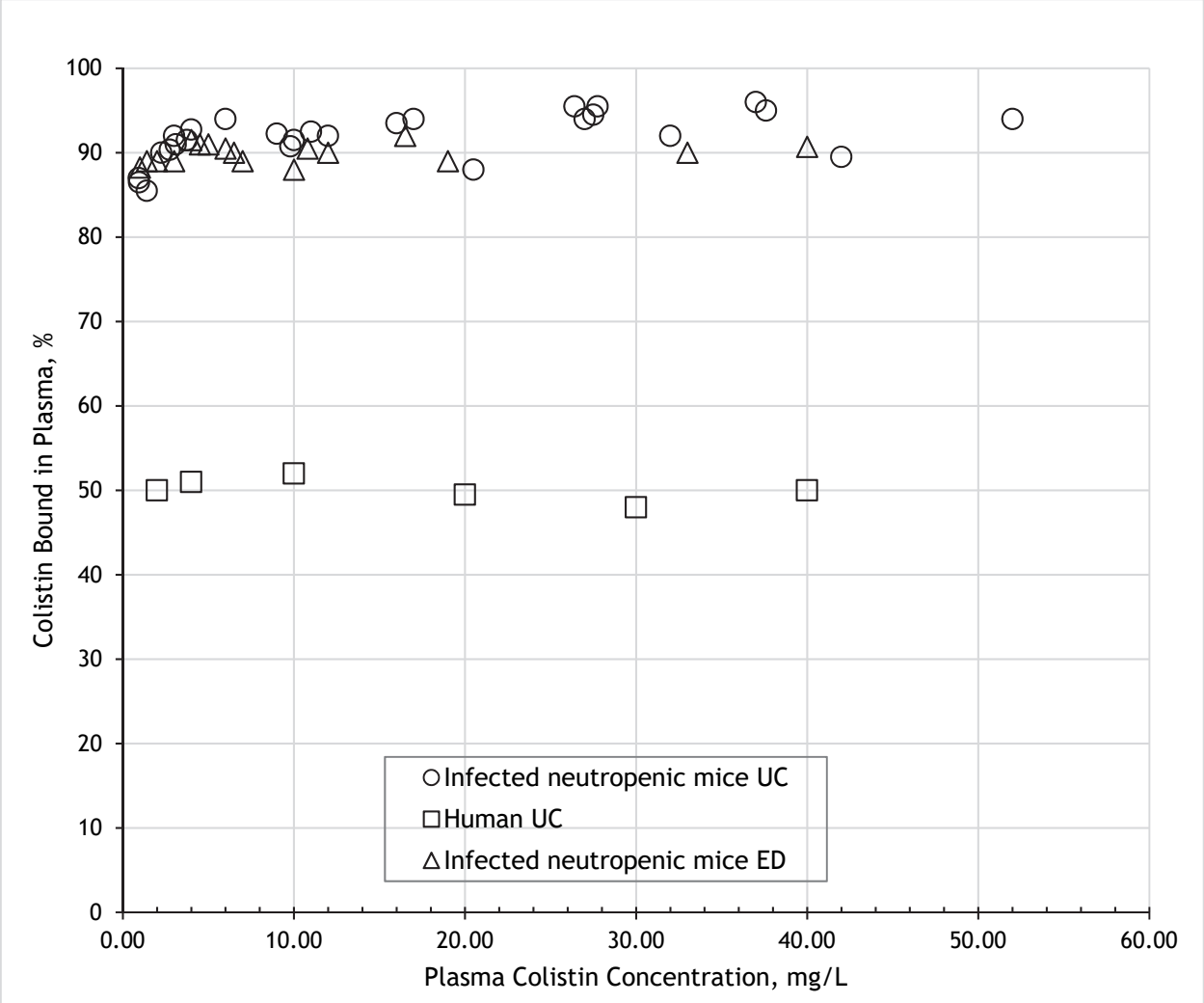
Renal Function Group, mL/minute	Daily Dose,* mg/kg
≥80	2.5-5
50 - <80	2.5-3.8
30 - <50	2.5
10 - <30	1

* Colistin base activity.
Abbreviation: FDA, US Food and Drug Administration.

PK data from a multinational, multicenter study focusing on the PK-PD properties of colistin (administered intravenously as colistin methanesulfonate [CMS]) in critically ill patients with multiresistant gram-negative infections were analyzed.[7] PK data for 162 patients not receiving renal replacement therapy, with a broad range of creatinine clearances (minimum = 5.6 mL/minute, maximum = 211.2 mL/minute) were reviewed. The apparent clearance of formed colistin also ranged widely (minimum = 1.85 L/h,

maximum = 41.3 L/h). With the physician-selected daily doses of CMS, the steady-state average concentrations (Css,avg) of formed colistin ranged from 0.24 to 9.81 mg/L (median = 2.2 mg/L).

Protein binding was determined by two independent methods: ultracentrifugation and rapid equilibrium dialysis in polytetrafluoroethylene cells. Protein binding of colistin was concentration independent over the observable ranges of concentration found in mice and humans (see Figure 1). The average unbound fraction of colistin for the healthy human (QC) plasma samples was 0.49 ± 0.03. For plasma of neutropenic infected mice, the average (± standard deviation [SD]) percentage bound for all colistin concentrations presented in Figure 1 was 92.9% ± 3.3% when binding was measured by ultracentrifugation, and 90.4% ± 1.1% by equilibrium dialysis. The average of the two methods was 91.6%. Thus, the average unbound fraction for colistin in plasma of neutropenic infected mice was 0.084.



Abbreviations: ED, equilibrium dialysis; UC, ultracentrifugation.

Figure 1. Protein Binding of Colistin in Infected Neutropenic Mice (by UC and ED) and in Normal Human Plasma (by UC)

Protein binding was also determined in plasma from 66 critically ill patients who were receiving CMS intravenously for the treatment of infection caused by an MDR gram-negative organism. Binding was determined by UC, and samples of healthy human plasma were included in each of the 11 UC runs in which the binding in the patient samples was measured. Table 4 provides a summary of the results.[8]

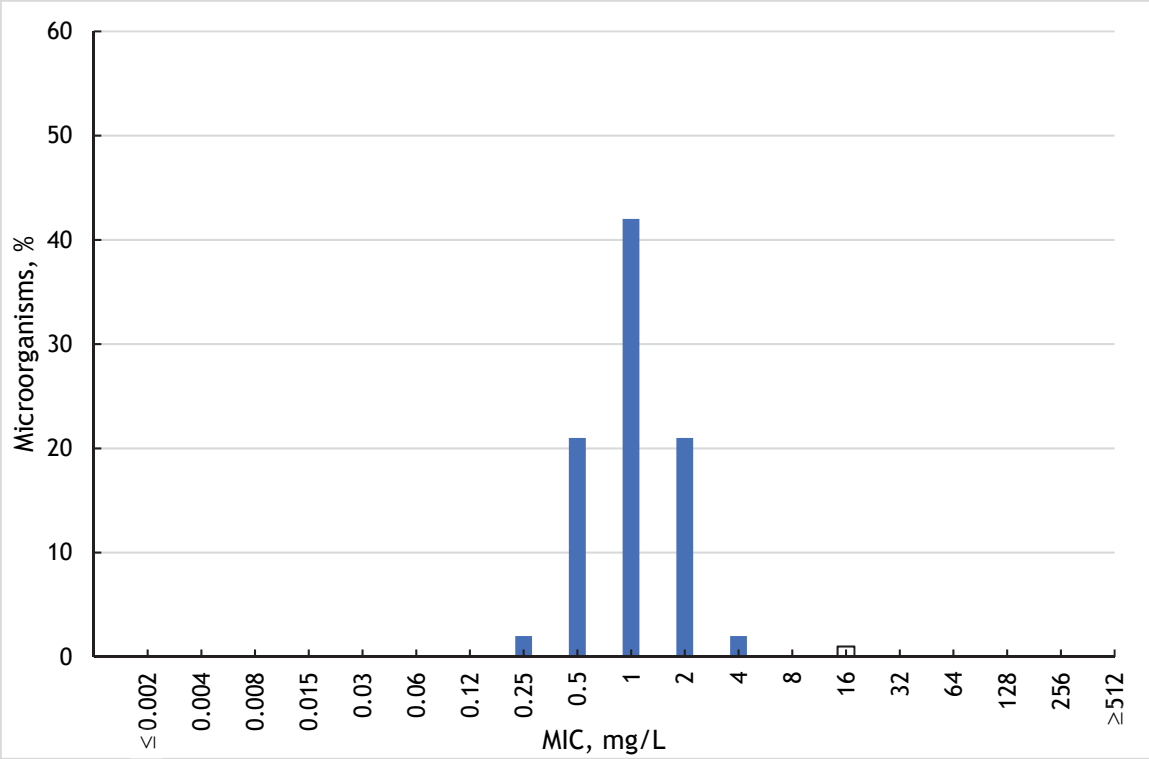
Table 4. Unbound Fraction of Colistin in Plasma of Critically Ill Patients and Healthy Humans
(Reprinted from *Int J Antimicrob Agents*, Vol 35 / No 2, Falagas ME, Rafailidis PI, Ioannidou E, et al., Colistin therapy for microbiologically documented multi-drug resistant gram-negative bacterial infections: a retrospective cohort study of 258 patients, pp. 194-199, © 2010, with permission from Elsevier.)

	Unbound Fraction	
	Critically Ill Patients	Healthy Humans
Number of patients*	66	11
Average	0.49	0.48
SD	0.11	0.06
10th percentile	0.36	0.41
25th percentile	0.42	0.42
50th percentile (median)	0.48	0.47
75th percentile	0.56	0.51
90th percentile	0.63	0.59

* Number of individual critically ill patients or number of ultracentrifugation runs in which those samples were analyzed along with samples of plasma from healthy humans.
Abbreviation: SD, standard deviation.

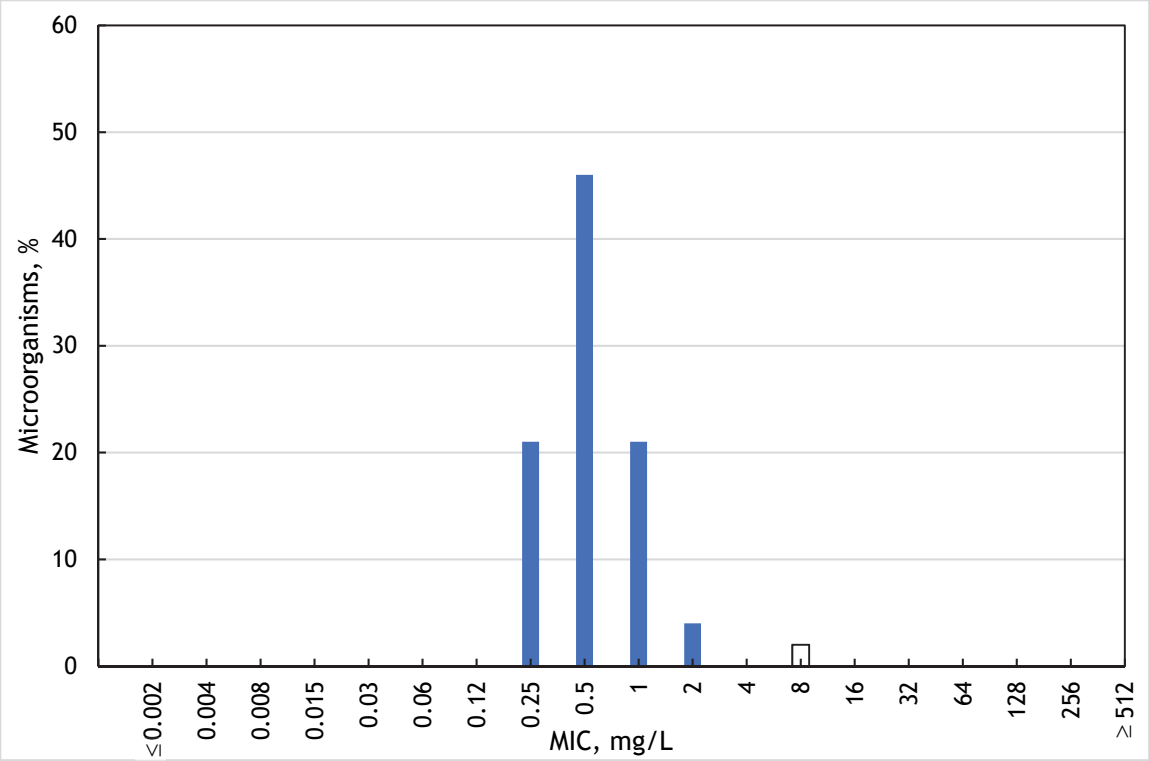
4 Minimal Inhibitory Concentration Distribution Data

Minimal inhibitory concentration (MIC) distribution data from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) databases were reviewed and are presented in Figures 2 and 3.[9]



Abbreviation: MIC, minimal inhibitory concentration.

Figure 2. International MIC Distributions for *P. aeruginosa* and Colistin.[*,9] 4208 observations from eight data sources were used to determine the epidemiological cutoff value of 4 mg/L. The wild-type organism epidemiological cutoff values are ≤ 4 mg/L.
* MIC distributions include collated data from multiple sources, geographical areas, and time periods and can never be used to infer rates of resistance.



Abbreviation: MIC, minimal inhibitory concentration.

Figure 3. International MIC Distributions for *A. baumannii* and Colistin.[*,9] 251 observations from eight data sources were used to determine the epidemiological cutoff value of 2 mg/L. The wild-type organism epidemiological cutoff values are 2 mg/L.

[*] MIC distributions include collated data from multiple sources, geographical areas, and time periods and can never be used to infer rates of resistance.

5 Pharmacodynamic Data

Pharmacodynamic data for colistin and various microorganisms are shown in Table 5.

Table 5. Target Values of Colistin *f*AUC/MIC for Stasis and 1- and 2-log_{10} Kill Against *P. aeruginosa* and *A. baumannii* in Mouse Thigh and Lung Models of Infection[10] (Modified from Cheah SE, Wang J, Nguyen VT, Turnidge JD, Li J, Nation RL, New pharmacokinetic/pharmacodynamic studies of systemically administered colistin against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in mouse thigh and lung infection models: smaller response in lung infection, *J Antimicrob Chemother.*, 2015, Vol 70 / No 12, pp. 3291-3297, by permission of Oxford University Press.)

Model	Species/Strain	Target Value of Colistin *f*AUC/MIC		
		Stasis	1-log_{10} Kill	2-log_{10} Kill
Thigh infection	*P. aeruginosa*			
	ATCC® 27853	9.94	12.4	15.8
	PAO1	6.01	6.53	7.34
	19056	6.41	8.56	11.3
	Mean	7.5	9.2	11.5
	A. baumannii			
	ATCC® 19606	1.47	3.45	9.13
	248-01-C.248	3.91	6.11	7.44
	N-16870.213	9.47	13.9	17.6
	Mean	5.0	78	11.4
Lung infection	*P. aeruginosa*			
	ATCC® 27853	34.1	43.3	51.8
	PAO1	15.2	44.8	-*
	19056	38.6	57.9	105
	Mean	29.3	48.7	78.4
	A. baumannii			
	ATCC® 19606	-†	-†	-†
	248-01-C.248	11.6	20.8	36.8
	N-16870.213	-†	-†	-†

* Unable to determine, because highest bacterial kill was ≈ 1 log_{10}, even at *f*AUC/MIC values resulting from highest tolerated dosage regimens of colistin.

† Unable to determine, because stasis was not achieved, even at *f*AUC/MIC values resulting from highest tolerated dosage regimens of colistin.

Because of the wide variations in creatinine and colistin clearance among patients in the multinational, multicenter study[7] (see Section 3), target attainment estimation using a population PK model and Monte Carlo simulation was not used in this breakpoint assessment. Rather, the patient population studied was divided into six renal function groups (see Table 6).

Table 6. Renal Function Groups

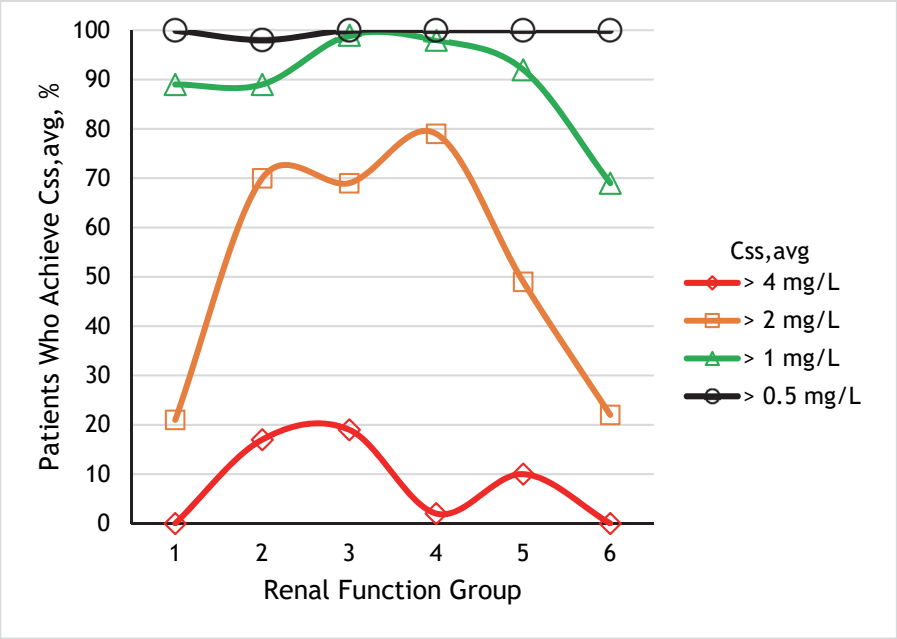
Group	Number of Patients	Creatinine Clearance (Uncorrected) Range, mL/minute
1	27	5.4-26.9
2	27	27.0-40.7
3	27	41.6-57.0
4	27	57.8-76.0
5	27	77.2-117.3
6	27	121.1-211.2

The Css,avg of formed colistin was calculated for each patient based on individual pharmacokinetics, with each patient receiving daily maintenance doses of colistin base activity (see Table 3), as opposed to the physician-selected doses used in the study. A body weight of 70 kg was assumed, and calculations for the two patients with a creatinine clearance < 10 mL/minute were

conducted using a daily dose of colistin base activity of 1 mg/kg. Target attainment rates in each of the six renal function categories were then determined with the following parameters:

- Plasma protein binding in critically ill patients and healthy humans of ≈ 50% (ie, unbound fraction of ≈ 0.5; see Table 4)
- A target *f*AUC$_{24}$/MIC of 12 (the approximate mean 2-log$_{10}$ kill target for *P. aeruginosa* and *A. baumannii* in mouse thigh infection and the approximate highest 1-log$_{10}$ kill target for the three strains of each species in the same infection model (see Table 5) corresponds to a *f*Css,avg/MIC of 0.5. This corresponds to a Css,avg/MIC of 1 in human patients (plasma protein binding ≈ 50% as shown in Figure 4 and Table 7).

Thus, with these parameters, the target attainment rate at each MIC is equivalent to the target attainment rate for Css,avg (ie, for total colistin in plasma).



*Daily dose is adjusted according to the 2013 FDA-approved product label.
Abbreviation: FDA, US Food and Drug Administration.

Figure 4. Probability of Colistin Target Attainment*,[11]

Table 7. Percentage Target Attainment for Colistin*,[11]

Renal Function Group	Creatinine Clearance Range	Css,avg (Summary Stats)		Percentage Attainment			
		Average	SD	Css,avg > 4	Css,avg > 2	Css,avg > 1	Css,avg > 0.5
1	5.4-26.9	1.53	0.56	0.0%	22.2%	88.9%	100.0%
2	27.0–40.7	2.69	1.30	14.8%	70.4%	88.9%	96.3%
3	41.6–57.0	2.97	1.52	18.5%	66.7%	100.0%	100.0%
4	57.8–76.0	2.80	0.94	3.7%	77.8%	96.3%	100.0%
5	77.2–117.3	2.25	1.35	11.1%	48.1%	92.6%	100.0%
6	121.1–211.2	1.50	0.93	0.0%	22.2%	59.3%	100.0%

*Daily dose is adjusted according to the 2013 FDA-approved product label.
Abbreviation: SD, standard deviation.

6 Clinical Efficacy

No clinical efficacy data are available.

7 Committee Rationale for the Breakpoint

At the time of breakpoint selection, it was noted that PD target attainment rates using maximum recommended doses were not optimal in patients with normal or supranormal renal function. As such, breakpoints need to be accompanied with language warning of this issue and providing advice about management: "Colistin (methanesulfonate) should generally be administered with a loading dose and at the maximum recommended doses, in combination with other agents."

8 Final Table Entry

Tables 8 and 9 include the final table entries from CLSI document M100.[2]

Table 8. Excerpt From CLSI document M100[2] Table 2B-1, Zone Diameter and MIC Breakpoints for *Pseudomonas aeruginosa*

Test/Report Group	Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	
O	Colistin	≤2	-	≥4	**(16)** Colistin (methanesulfonate) should generally be administered with a loading dose and at the maximum recommended doses, in combination with other agents. **(17)** The only approved MIC method for testing is broth microdilution. Disk diffusion and gradient diffusion methods should not be performed.

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.

Table 9. Excerpt From CLSI document M100[2] Table 2B-2, Zone Diameter and MIC Breakpoints for *Acinetobacter* spp.

Test/Report Group	Antimicrobial Agent	Interpretive Categories and MIC Breakpoints, µg/mL			Comments
		S	I	R	
O	Colistin	≤2	-	≥4	**(5)** Colistin (methanesulfonate) should generally be given with a loading dose and at maximum recommended doses and used in combination with other agents. **(6)** Applies to *A. baumannii* complex only. **(7)** The only approved MIC method for testing is broth microdilution. Disk diffusion and gradient diffusion methods should not be performed.

Abbreviations: I, intermediate; MIC, minimal inhibitory concentration; R, resistant; S, susceptible.

9 Voting Record

Approved in January 2016 (8 approved, 2 opposed, 0 abstained, 1 absent).

10 References

1 CLSI. *Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters.* 5th ed. CLSI guideline M23. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.* 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.

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The findings, recommendations, and conclusions in this rationale document are those of the authors and have not been reviewed through the CLSI consensus process. They do not necessarily reflect the views of any single individual or organization.

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