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]

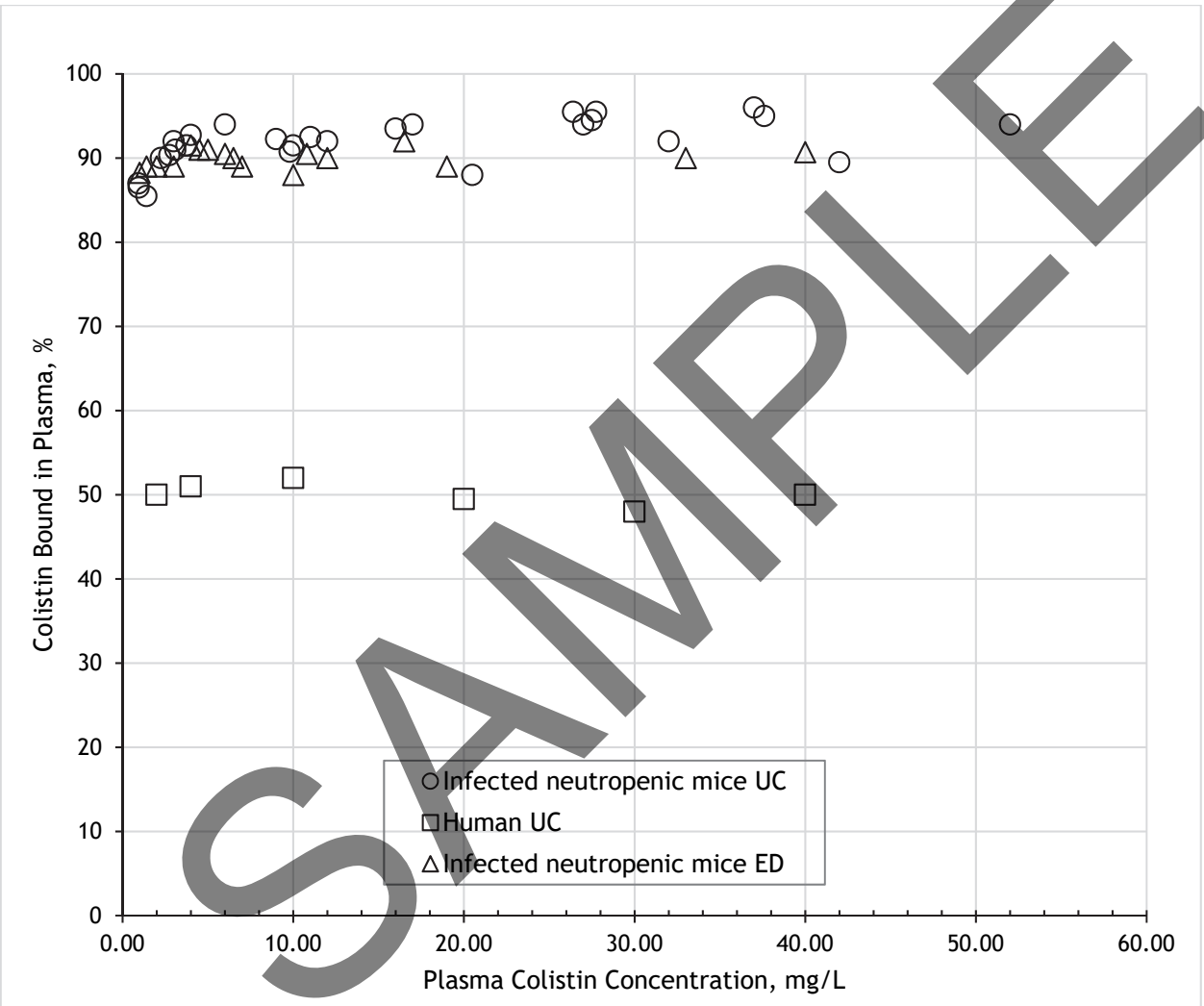
Renal Function Group, mL/minute	Daily Dose,* mg/kg
≥80	2.5-5
50 - <80	2.5-3.8
30 - <50	2.5
10 - <30	1

* 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]