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Quality Control Guidelines for Testing Gram-Negative Control Strains with Polymyxin B and Colistin (Polymyxin E) by Standardized Methods

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An eight-laboratory study addressed the urgent need for quality control (QC) ranges for susceptibility determination when testing colistin (polymyxin E) and polymyxin B, two polycationic peptide antimicrobial agents, against multidrug-resistant gram-negative bacilli. For *Escherichia coli* ATCC 25922l, the QC ranges were as follows: for colistin, 0.25 to 1 μg/ml (11 to 17 mm), and for polymyxin B, 0.25 to 2 μg/ml (13 to 19 mm). For *Pseudomonas aeruginosa* ATCC 27853, the QC ranges were as follows: for colistin, 0.25 to 2 μg/ml (11 to 17 mm), and for polymyxin B, 0.25 to 2 μg/ml (14 to 18 mm). More than 97% of all reported QC results were within these proposed ranges.

The polymyxin class antimicrobial agents (colistin or polymyxin E and polymyxin B) are polycationic peptides that were originally synthesized from *Bacillus polymyxus* (1, 13). The mechanism of action for the polymyxins has been determined to be secondary to surfactant-like properties that produce enhanced permeability of the bacterial cytoplasmic membrane, leading to bacterial death (1, 13, 15). These agents were first described more than five decades ago and were initially applied to therapy for gram-negative bacillary infections before the discovery of other broad-spectrum agents, such as the aminoglycosides, carboxypenicillins, and cephalosporins (14). Toxicity issues (12) and the emergence of alternative antimicrobial regimens resulted in the elimination of colistin and polymyxin B from National Committee for Clinical Laboratory Standards (NCCLS) interpretive category and quality control (QC) tables in the early 1980s, although polymyxin B has continued to be widely used in topical over-the-counter, triple-antibiotic ointment (neomycin-polymyxin B-bacitracin) preparations (6, 7). Recently the occurrence of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. in several nations in epidemic proportions has necessitated the reconsideration of polymyxin therapies (2, 4, 5, 12, 14) with the subsequent need for accurate susceptibility testing by reference and standardized methods (3, 9, 10). Contemporary updates on polymyxin pharmacokinetics and pharmacodynamics have also been published (5). This report describes results from a multilaboratory trial designed to establish colistin and polymyxin B QC ranges for disk diffusion and the broth microdilution MIC method (9, 10), using a study design published in the NCCLS M23A2 document (11).

An eight laboratory QC study group was organized for the development of MIC and disk diffusion QC guidelines for the polymyxins. The QC group consisted of laboratories at the Centers for Disease Control and Prevention (Atlanta, Ga.), University of Alberta (Edmonton, Alberta, Canada), The Cleveland Clinic Foundation (Cleveland, Ohio); University of Texas Medical Center (Houston, Tex.), University of Rochester Medical Center (Rochester, N.Y.), Denver Health Medical Center (Denver, Colo.), University of Washington (Seattle, Wash.), and JMI Laboratories (North Liberty, Iowa). Each laboratory followed a protocol based on the NCCLS M23-A2 document as well as procedural details found in the M2-A8 and M7-A6 test methods (9, 10).

The MIC study utilized frozen-form, reference broth microdilution panels prepared by the Centers for Disease Control and Prevention (lot AC-6). The panels contained four lots of cation-adjusted Mueller-Hinton broth (Difco, Detroit, Mich. [two lots; no. 2198184 and no. 0325004], Oxoid, Hampshire, United Kingdom [one lot; no. 258631], and BBL, Sparks, Md. [one lot; no. 2218968]). MICs of colistin were tested by using reagent grade colistin sulfate, and both polymyxins were obtained from Sigma Chemical Co. (St. Louis, Mo.). Each laboratory tested *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, generating 320 MIC QC results for each drug and organism. Gentamicin (*E. coli* only) and tetracycline were used as MIC control agents. Colony counts of the initial inoculum were performed from the broth microdilution trays by subculturing in a quantitative manner on drug-free solid medium. Counts ranged from 1.6×10^5 to 8.0×10^5 CFU/ml and averaged 5.0×10^5 CFU/ml for all participating laboratories (target inoculum, 5.0×10^5 CFU/ml). All control MIC results were within those ranges published in NCCLS standard M100-S15 (11).

Similarly, the disk diffusion tests were performed by the NCCLS M2-A8 method (9), using three lots of Mueller-Hinton agar (BBL [two lots; no. 4014660 and no. 4021062] and Remel, Lenexa, Kan. [one lot; no. 403187]). Two lots of disks were

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TABLE 1. Distribution of QC MIC and zone diameter results among participants in the polymyxin B and colistin (polymyxin E sulfate) study (8)

Method or result	No. of occurrences with QC strain:			
	E. coli ATCC 25922		*P. aeruginosa* ATCC 27853	
	Colistin	Polymyxin B	Colistin	Polymyxin B
Disk diffusion (mm)				
11	1[a]	0	4[a]	0
12	13[a]	0	46[a]	0
13	147[a]	0[a]	148[a]	0
14	154[a]	22[a]	160[a]	4[a]
15	88[a]	209[a]	95[a]	56[a]
16	66[a]	113[a]	25[a]	191[a]
17	11[a]	84[a]	2[a]	146[a]
18	0	38[a]	0	73[a]
19	0	13[a]	0	10
20	0	1	0	0
MIC (μg/ml)				
0.25	71[b]	43[b]	2[b]	0[b]
0.5	188[b]	135[b]	135[b]	100[b]
1	61[b]	135[b]	170[b]	146[b]
2	0	5[b]	13[b]	74[b]
4	0	2	0	0

[a] Proposed range that included 97.9 to 100.0% of reported zone diameters from eight laboratories.
[b] Proposed range of MIC values that included 99.4 to 100.0% of all reported results.

utilized versus each QC strain: colistin (10 μg; BBL lot no. 3119600 and Remel lot no. 281526) and polymyxin B (300 U; BBL lot no. 3209907 and Remel lot no. 290537). Single lots of gentamicin (10 μg) and tetracycline (30 μg) disks were applied as control agents. A total of 720 control zone diameters were generated with zones measured with a caliper, and 99.4% of reported results were within NCCLS QC ranges (11). All out-of-control results were repeated before analysis.

The study followed the NCCLS guidelines (8), with the eight sites producing 320 MICs of each polymyxin agent against the two QC strains. The total number of zone diameters generated for each polymyxin and QC organism was 480. The number of results produced was significantly greater than the minimal criteria specified for each method by the NCCLS (8). Analyses of data to determine MIC range limits were dictated by the NCCLS guideline (8). Selected ranges included 97.9 to 100.0% and 99.4 to 100.0% of participant results for the disk diffusion and broth microdilution tests, respectively. All proposed QC ranges were further optimized to encompass ≥95% of all reported results, as recommended by the NCCLS M23-A2 guideline (8). The results were also tabulated and compared by intra- and interlaboratory analysis to determine potentially unacceptable technical variations occurring at any study site. Different reagent lots were also compared to determine variations among manufacturer's products. No significant variation between laboratories or media lots was observed.

Table 1 lists the distribution of results (zones of inhibition or MICs) for both polymyxin agents tested against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. For the disk diffusion method, 5- or 7-mm zone ranges were calculated by using the median methods (8). The polymyxin B range was proposed at 13 to 19 mm for the *E. coli* QC strain and incorporated 99.8% of results (479 of 480). The disk product package insert suggests a 12- to 16-mm range that only included 71.7% of zones reported by the participants. Prior reports have also questioned the available QC ranges for these polymyxins (3). The colistin range proposed for the QC *E. coli* strain was 11 to 17 mm (100.0% of results in range), also differing from the product package insert recommendations (11 to 15 mm; 84.0% in range). The proposed disk diffusion QC ranges for *P. aeruginosa* ATCC 27853 were 11 to 17 and 14 to 18 mm for colistin and polymyxin B tests, respectively, incorporating 100.0% of results.

For MIC QC ranges of three or four $\log_2$ dilution steps for each polymyxin, modal MICs were 0.5 or 1 μg/ml for each agent. These results contrast with the only published "expected colistin MICs" (not range) of 0.5 to 1 and 2 to 4 μg/ml with testing against *E. coli* and *P. aeruginosa* QC strains, respectively. These strains were found in the NCCLS proposed standard PSM-7 in 1980 (6). Other early NCCLS documents (7) also suggested polymyxin B disk diffusion QC ranges of 7 to 13 mm when testing *Staphylococcus aureus* ATCC 25923; this organism was not tested as part of this protocol or recommended for QC purposes.

These summarized results from a multicenter study (8) provide the initial structured QC ranges for colistin (polymyxin E) and polymyxin B to be considered for inclusion in NCCLS tables (11). All proposed ranges incorporated ≥97.9% of study-generated zone diameters and MICs without significant occurrence of interlaboratory variation or medium quality issues. These QC ranges will allow clinical microbiology laboratories to test these polymyxin agents for possible therapeutic guidance, particularly against multidrug-resistant gram-negative strains. Finally, the NCCLS Subcommittee on Antimicrobial Susceptibility Testing recently approved these QC ranges for publication in 2005, associated with susceptible interpretive criteria of ≤2 μg/ml for the MIC method (10, 11), the preferred test (3). The poor agar diffusion characteristics of polymyxins limit the predictive accuracy of the disk diffusion test (3, 6, 7).

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