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Quality Control Guidelines for Disk Diffusion and Broth Microdilution Antimicrobial Susceptibility Tests with Seven Drugs for Veterinary Applications

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This multicenter study proposes antimicrobial susceptibility (MIC and disk diffusion methods) quality control (QC) parameters for seven compounds utilized in veterinary health. Alexomycin, apramycin, tiamulin, tilmicosin, and tylosin were tested by broth microdilution against various National Committee for Clinical Laboratory Standards (NCCLS)-recommended QC organisms (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853). In addition, disk diffusion zone diameter QC limits were determined for apramycin, enrofloxacin, and premafloxacin by using *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923. The results from five or six participating laboratories produced ≥99.0% of MICs and ≥95.0% of the zone diameters within suggested guidelines. The NCCLS Subcommittee for Veterinary Antimicrobial Susceptibility Testing has recently approved these ranges for publication in the next M31 document.

Quality control (QC) parameters aid microbiology laboratories in monitoring MICs of antimicrobial agents as well as the performance of these agents in disk diffusion susceptibility tests (10). The National Committee for Clinical Laboratory Standards (NCCLS) has recently established performance standards for these tests when bacteria isolated in veterinary practice are being handled (9). The NCCLS Subcommittee for Veterinary Antimicrobial Susceptibility Testing has made great strides in improving the quality of laboratory testing of veterinary antimicrobials. Key to this process has been the establishment and maintenance of QC limits, allowing veterinary microbiology laboratories to produce results with the assurance that their respective procedures are in control (4, 9). The controlled multilaboratory study described here was designed to obtain MICs and/or disk diffusion ranges to establish preliminary standards for the following veterinary antimicrobial agents: alexomycin (3), apramycin (2), enrofloxacin (4, 13), premafloxacin (4, 8), tiamulin (1), tilmicosin (12), and tylosin (5). By determining and analyzing the MICs and disk diffusion ranges of the aforementioned antimicrobial agents, this study attempted to establish QC parameters that would be practical for routine laboratory use.

The NCCLS approved guideline M37-A (9) was used to design QC trials performed in 1996 and 1997 to establish MIC and/or disk ranges for the following antimicrobial agents (manufacturer): alexomycin and premafloxacin (Pharmacia and Upjohn, Kalamazoo, Mich.); apramycin, tilmicosin, and tylosin (ELANCO, Indianapolis, Ind.); enrofloxacin (Bayer, Shawnee Mission, Kans.); and tiamulin (Fermenta Animal Health Co., Kansas City, Mo.). Apramycin, enrofloxacin, and premafloxacin disk lots were manufactured by Difco Laboratories (Detroit, Mich.) or Becton Dickinson Microbiology Systems (BBL) (Cockeysville, Md.). The following six laboratories participated in one or both evaluations: University of Texas, Houston; The Cleveland Clinic Foundation, Cleveland, Ohio; University of Massachusetts Medical Center, Worcester; University of Iowa College of Medicine, Iowa City; AccuMed International, Inc., Westlake, Ohio; and Washington University, St. Louis, Mo. Six base lots of cation-adjusted Mueller-Hinton broth and agar from four manufacturers (BBL, Difco Laboratories, Accumedia, and Oxoid [Basingstoke, England]) were used to prepare the microdilution trays and agar plates (PML Microbiologicals, Wilsonville, Oreg.). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and 25923, *Enterococcus faecalis* ATCC 29212, and *Streptococcus pneumoniae* ATCC 49619 were used for testing. The MIC QC trial design consisted of each laboratory, over a period of at least 3 days, testing 5 replicates of each QC strain with a medium common to all laboratories and 20 replicates with a lot unique to each participant, for a total of 25 MIC determinations per antimicrobial agent per QC strain per laboratory. For the disk QC trial, 10 replicates of each American Type Culture Collection (ATCC) strain (20 if only one disk lot was available) were tested by each laboratory on its unique lot for a total of 30 of 60 zone determinations per disk lot per QC strain. These MIC and disk testing methods conformed to NCCLS M31-P procedures. The statistical methods used were found in the NCCLS document (6) and include data from at least five qualifying laboratories.

Table 1 provides a summary of broth microdilution test results for *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853. Frequency distributions of the MIC endpoints were recorded (Table 1) for each drug-organism pair for the unique medium lots; the common lot data was used to assess interlaboratory variations of technologist interpretation only. In most instances, the drugs established clear modal values by using clinically relevant MIC dilution scales. Generally, the modal MIC $\pm$ 1-$\log_2$ dilution range was calculated so that QC limits could be determined; these ranges are boldface in Table 1. The data revealed that all five organisms had nearly all (minimum of 95%) of their MIC determinations within their proposed QC ranges (9).

The MIC results for *S. aureus* ATCC 29213 were very consistent for all antimicrobials tested. The MICs attained a

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TABLE 1. MICs of multicenter (five or six sites) collaborative studies to establish QC strain ranges for selected antimicrobial agents[a]

QC strain and antimicrobial agent	No. of occurrences at the following MIC (μg/ml)[b]															% in range
	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64	
S. aureus ATCC 29213																
Alexomycin	0	1	**37**	**50**	**12**	0	0	0	0	0	0	0			0	99.0
Apramycin	0	0	0	0	0	0	0	0	**29**	**53**	**18**	0			0	100.0
Tiamulin					0	0	**12**	**83**	**25**	0	0	0			0	100.0
Tilmicosin								0	**16**	**96**	**8**	0	0	0	0	100.0
Tylosin		0	0	0	0	0	**0**	**65**	**35**	0	0	0	0		0	100.0
E. faecalis ATCC 29212																
Alexomycin	0	**11**	**80**	**29**	0	0	0	0	0	0	0	0			0	100.0
Apramycin	0	0	0	0	0	0	0	0	0	0	0	**10**			**110**	100.0
Tilmicosin								0	0	0	0	**9**	**35**	**76**	0	100.0
Tylosin		0	0	0	0	0	**0**	**57**	**54**	**9**	0	0	0		0	100.0
S. pneumoniae ATCC 49619																
Tiamulin					0	0	**8**	**47**	**51**	**14**	0	0			0	100.0
E. coli ATCC 25922																
Alexomycin	0	0	0	0	0	0	0	0	0	0	0	**2**			**98**	100.0
Apramycin	0	0	0	0	0	0	0	0	**0**	**41**	**59**	**0**			0	100.0
Tilmicosin								0	0	0	0	0	0	**6**	**114**	100.0
Tylosin		0	0	0	0	0	0	0	0	0	0	0	0		**100**	100.0
P. aeruginosa ATCC 27853																
Alexomycin	0	0	0	0	0	0	0	0	0	0	0	0			**100**	100.0
Apramycin	0	0	0	0	0	0	0	0	**0**	**50**	**50**	**0**			0	100.0
Tilmicosin								0	0	0	0	0	0	0	**120**	100.0
Tylosin		0	0	0	0	0	0	0	0	0	0	0	0		**100**	100.0

[a] Only results from the five to six unique medium lots are listed.
[b] Boldface type indicates proposed MIC ranges.

100.0% distribution within the ranges proposed for apramycin, tiamulin, tilmicosin, and tylosin. All tylosin MICs occurred at either 1 or 2 μg/ml, and a mode was evident at 1 μg/ml (proposed range, 0.5 to 2 μg/ml). The *E. faecalis* ATCC 29212 results for apramycin, tilmicosin, and tylosin were all within their proposed 3- or 4-$\log_2$ dilution ranges (Table 1). Apramycin and tilmicosin produced modal MICs that were at the extreme upper limit of their practical test dilution schedules. *S. pneumoniae* ATCC 49619 results for tiamulin were all within the proposed 4-$\log_2$ dilution range, dictated by 82% of all reported results occurring at either 1 or 2 μg/ml (broad mode).

The MICs for the gram-negative QC strains, such as *E. coli* ATCC 25922, were also consistent in the fact that the results of all antimicrobials fell within the proposed dilution ranges. Alexomycin, tilmicosin, and tylosin were not active against these species (3, 5, 12) and produced MICs at the upper limits of the applied dilution ranges. Apramycin displayed a more even distribution within its proposed 3-$\log_2$ dilution QC range (4 to 16 μg/ml; modes, 4 and 8 μg/ml). The results of all studied drugs for *P. aeruginosa* ATCC 27853 achieved 100.0% of results within the proposed ranges (Table 1), but three drugs showed no activity with MICs of >16 to >32 μg/ml. Apramycin MIC results were equally distributed between 2-$\log_2$ dilutions (4 and 8 μg/ml), and a range of 2 to 16 μg/ml was recommended.

Table 2 summarizes the disk diffusion results for *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923 tested against apramycin, enrofloxacin, and premafloxa-

TABLE 2. Disk diffusion susceptibility for *E. coli*, *P. aeruginosa*, and *S. aureus* QC strains

QC organism	Antimicrobial agent	No. of tests	Zone diameter (mm)		Proposed[a] 95% limits (mm)	% results in range
			Median	Mean		
E. coli ATCC 25922	Apramycin	300	18.0	17.7	15–20	98.5
	Enrofloxacin	304	35.0	35.6	32–40	95.0
	Premafloxacin	300	29.0	29.1	26–34	100.0
P. aeruginosa ATCC 27853	Apramycin	300	15.0	15.4	13–18	100.0
	Enrofloxacin	300	17.0	17.5	15–19	95.7
	Premafloxacin	280	15.0	14.5	11–17	95.5
S. aureus ATCC 25923	Apramycin	300	21.0	20.7	17–24	100.0
	Enrofloxacin	300	29.0	29.3	27–31	97.0
	Premafloxacin	300	32.0	32.0	29–37	100.0

[a] Range proposed containing ≥95% of all participant results.

cin disks. Each laboratory submitted approximately 50 observations per QC strain which were analyzed by individual laboratory and on a cumulative result basis. The tests were performed on both common and unique lots, with the unique lots accounting for two-thirds of the pooled data. The lack of significant inter- and intralaboratory variations illustrates the uniformity of the test performance. The six participating laboratories produced nearly identical mean and mode values for all drug-organism pairs (data not shown). The median zone diameter value of all tests was used to determine the proposed QC ranges. The upper and lower control limits were then determined by taking the cumulative median zone diameters of the five or six laboratories for each of the drug-organism pairs ± one-half of each average millimeter zone range. The NCCLS also suggests that ranges contain 95% of test values. For example, the combination of *E. coli* and apramycin produced a median zone diameter of 18 mm with an average range of 6 mm, resulting in a proposed range of 15 to 21 mm (18 ± 3 mm). However, since no test values occurred at 21 mm, the range was adjusted to include only zone diameters at which test values occurred and also achieve ≥95% of results within the proposed limits (Table 2). Consequently, the actual recommended range for apramycin disks tested against *E. coli* was 15 to 20 mm (98.5% of reported values). For all drug-organism combinations tested, a minimum of 95% of test values was contained within proposed ranges (9, 10). Limits were placed on this study by the QC strains selected. This study tested veterinary compounds against QC strains of human origin because similar strains of animal origin have not been available for use in the national standards. Currently, the NCCLS is in the process of establishing QC strains of animal origin that will be reserved strictly for veterinary use in testing specific fastidious species common to animal infections.

Overall, expanding the number of veterinary antimicrobials that can be reliably tested by broth microdilution and agar disk diffusion methods helps reduce potential interlaboratory variability and improves confidence in results from veterinary laboratories. Most importantly, this study proposes MICs and/or disk diffusion ranges for newer compounds and some older agents in wide clinical use (alexomycin, apramycin, enrofloxacin, premafloxacin, tiamulin, tilmicosin, and tylosin). Accurate methods could assist in the resolution of the controversies of the use of antimicrobials in animal husbandry regarding increasing resistance and threats to humans (1, 2, 8) by providing precise and accurate quantitative methods. The ranges listed here are only proposals and should be considered tentative until a consensus or drug registry agency adopts and/or modifies them for routine clinical use. Recently the NCCLS Subcommittee for Veterinary Antimicrobial Susceptibility Testing approved the listed ranges for publication in the next M31 document (6). These results also confirm and extend previously published QC studies with antimicrobials for animal health (4, 12).

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