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Proposed MIC Quality Control Guidelines for National Committee for Clinical Laboratory Standards Susceptibility Tests Using Seven Veterinary Antimicrobial Agents: Ceftiofur, Enrofloxacin, Florfenicol, Penicillin G-Novobiocin, Pirlimycin, Premafloxacin, and Spectinomycin

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The present multicenter study proposes broth microdilution quality control (QC) ranges for the antimicrobial agents ceftiofur, enrofloxacin, florfenicol, penicillin G-novobiocin, pirlimycin, premafloxacin, and spectinomycin, which are used in veterinary practice. Six separate laboratories tested replicates of National Committee for Clinical Laboratory Standards (NCCLS)-recommended QC organisms (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212) on medium lots both common and unique to all laboratories. The proposed ranges were within 3 or 4 $\log_2$ dilution steps of the modal MICs for all organism-antimicrobial agent pairs, depending on their MIC distributions. With ≥94.7% of all MIC results being within the proposed QC ranges, all combinations tested comply with NCCLS guidelines and all have been accepted by the NCCLS subcommittee developing susceptibility testing procedures for veterinary laboratories.

Performance standards for quantitative in vitro dilution antimicrobial susceptibility testing of aerobic bacteria isolated from humans have been published by the National Committee for Clinical Laboratory Standards (NCCLS) for more than two decades (4). Similar standards have recently been proposed by NCCLS for bacteria isolated in veterinary practice (6). In these standards, antimicrobial agents used for animals are listed by the species against which they are used and by indications. Criteria for the interpretation of susceptibility are based on species-specific pharmacokinetic data when they are available and are correlated to therapeutic outcomes. When such information is not available for older compounds, human breakpoint criteria have initially been applied (6).

Quality control (QC) limits for monitoring the MICs of these marketed veterinary antimicrobial agents for animal pathogens must be evaluated (8), and MICs of new or investigational compounds must be established to ensure the precision and accuracy of the susceptibility test procedure. The multicenter study described here proposes QC ranges of the MICs of antimicrobial agents used in veterinary practice by using NCCLS (4,6)-recommended reference strains from the American Type Culture Collection (ATCC), e.g., *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212. Interpretive criteria and antimicrobial activities have previously been proposed or established for the following antimicrobial agents: ceftiofur, a cephalosporin (7); enrofloxacin and premafloxacin (10), both fluoroquinolones; florfenicol, a drug related to chloramphenicol (9); penicillin G-novobiocin (3); pirlimycin, a clindamycin derivative (2); and spectinomycin (11).

The antimicrobial agents were obtained from Sigma Chemical Company (St. Louis, Mo.) or Pharmacia-Upjohn (Kalamazoo, Mich.). Six lots of cation-adjusted Mueller-Hinton broth base from three manufacturers (Becton Dickinson Microbiology Systems, Cockeysville, Md.; Difco Laboratories, Detroit, Mich.; and Oxoid, Basingstoke, England) were used to prepare the microdilution trays at PML Microbiologics (Tualatin, Oreg.). NCCLS approved guideline M23-A (5) was used to establish QC parameters. Six separate laboratories (University of Texas, Houston; The Cleveland Clinic Foundation, Cleveland, Ohio; University of Massachusetts Medical Center, Worcester; University of Iowa College of Medicine, Iowa City; University of California Medical Center, Los Angeles; and Department of Veterans Affairs Medical Center, Iowa City, Iowa) participated in the evaluation. Over a period of at least 3 days, each laboratory tested 5 replicates of each QC organism with a lot of medium common to all laboratories and 20 replicates with a lot of medium unique to that participating laboratory, for a total of 25 MIC determinations for each organism-antimicrobial agent pair. The inoculum was standardized to 5×10^5 CFU/ml. Incubation was performed at 35°C for 16 to 18 h in an ambient air incubator. The statistical calculations described by Barry et al. (1) were used to assign the proposed QC ranges of the MICs. This usually was the modal MIC ± 1 $\log_2$ dilution step or, less commonly, a 4-dilution-step range in cases in which the modal MIC fell between two dilution increments.

To establish the QC ranges of the MICs of the drugs, a total of 150 individual test results from six laboratories were used. However, the results from one laboratory were observed to

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TABLE 1. MICs of seven antimicrobial agents for veterinary use from a multicenter collaborative study to establish QC ranges for four strains

QC strain and antimicrobial agent	No. of occurrences at the following MIC (μg/ml)																		
	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
E. coli ATCC 25922																				
Ceftiofur					0	0	0	[29	115	6][a]	0	0	0	0	0	0				
Enrofloxacin		0	[24	123	3]	0	0	0	0	0	0	0	0							
Florfenicol								0	0	0	[0	93	55]	2[c]						
Penicillin G-novobiocin[b]		0	0	0	0	0	0	0	0	0	0	0	[1	149[c]]						
Pirlimycin			0	0	0	0	0	0	0	0	0	0	0	0	[150[c]]					
Premafloxacin	0	0	[16	57	72	5]	0	0	0	0	0	0	0							
Spectinomycin									0	0	0	0	[0	62	84	4]	0	0	0	
P. aeruginosa ATCC 27853																				
Ceftiofur					0	0	0	0	0	0	0	0	0	[2	104	43]	1[c]			
Enrofloxacin		0	0	0	0	0	0	0	3	[30	90	27]	0							
Florfenicol								0	0	0	0	0	0	0[c]	[150[c]]					
Penicillin G-novobiocin		0	0	0	0	0	0	0	0	0	0	0	0	[150[c]]						
Pirlimycin			0	0	0	0	0	0	0	0	0	0	0	0	[150[c]]					
Premafloxacin	0	0	0	0	0	0	0	0	1	[8	97	41]	3							
Spectinomycin									0	0	0	0	0	0	0	0	0	[55	92	3[c]]
E. faecalis ATCC 29212																				
Ceftiofur[d]					0	0	0	0	6	[41	74	4]	0	0	0	0				
Enrofloxacin		0	0	0	0	0	[0	68	64	18]	0	0	0							
Florfenicol								0	0	0	[20	120	2]	8						
Penicillin G-novobiocin		0	0	0	0	0	0	[0	74	71	5]	0	0							
Pirlimycin			0	0	0	0	0	0	0	1	[11	108	30]	0						
Premafloxacin	0	0	[0	89	57]	4	0	0	0	0	0	0	0							
Spectinomycin									0	0	0	0	0	0	0	[4	141	5]	0	
S. aureus ATCC 29213																				
Ceftiofur					0	0	0	[30	92	28]	0	0	0	0	0	0				
Enrofloxacin		0	0	0	[0	108	38]	4	0	0	0	0	0							
Florfenicol								0	0	0	[8	114	22]	6						
Penicillin G-novobiocin		0	0	[30	105	14]	1	0	0	0	0	0	0							
Pirlimycin			0	0	0	0	0	[2	135	13]	0	0	0	0						
Premafloxacin	[8	107	34]	1	0	0	0	0	0	0	0	0	0							
Spectinomycin									0	0	0	0	0	0	0	[2	96	50]	2	

[a] Brackets indicate the proposed QC ranges of MICs.
[b] Penicillin G-novobiocin was tested at a 1:2 ratio, respectively. Only the penicillin G MIC results are provided.
[c] These datum points indicate the number of MIC occurrences above the tested range. The upper limit of this drug's MIC QC range could not be determined because of the clinically relevant ranges selected.
[d] One laboratory's data were omitted because of technical variation of the common lot of broth microdilution trays with this QC organism.

have significant variation compared with the results from the other locations for one antimicrobial agent (ceftiofur versus *E. faecalis*). The omission of this laboratory's data from data for both the common and unique medium lots resulted in 125 total determinations for the cited organism-antimicrobial agent pair. All proposed ranges in these analyses were in compliance with the NCCLS guideline (5) for statistics derived from a minimum of five laboratories.

The MICs of all antimicrobial agents tested for *E. coli* ATCC 25922 were highly consistent (Table 1). The results for the majority of drugs (five) established clear modal values by using a clinically relevant MIC dilution scale. The commonly applied method of establishing QC ranges (1) by selecting the modal MIC $\pm$ 1 $\log_2$ dilution step was used. The upper limits of the MIC ranges of penicillin G-novobiocin and pirlimycin for *E. coli* could not be determined because of the ranges selected (0.004/0.008 to 8/16 μg/ml and 0.008 to 16 μg/ml, respectively). Premafloxacin and spectinomycin were assigned a 4-dilution QC range, as recommended by Barry et al. (1), centered around their bimodal distributions. Only florfenicol had two outlier values beyond the proposed range, resulting in 98.7% of the MICs being within the QC range (≥95% generally acceptable).

The MICs of ceftiofur, enrofloxacin, and premafloxacin for *P. aeruginosa* ATCC 27853 produced QC ranges that were within the clinically relevant scale of dilutions, and only one to four MIC determinations were 1 $\log_2$ dilution above or below the proposed limits (Table 1). The modal MICs for all other antimicrobial agents tested were near or above their tested dilution schedules. Thus, upper limits could not be established for these QC organism-drug pairs. The proposed range for spectinomycin may be useful because the MIC mode clearly falls at the upper extreme of the applicable dilution range (61% of occurrences at 512 μg/ml).

The MICs of all of the studied drugs for *E. faecalis* ATCC 29212 were very consistent. Florfenicol had the highest number of occurrences outside its proposed QC range (eight occurrences; 94.7% of the MIC determinations were within the proposed range). All other proposed QC ranges of MICs of the seven drugs contained 95.2 to 100.0% of the generated results. Enrofloxacin and penicillin G-novobiocin MICs were widely

TABLE 2. Proposed QC ranges of MIC when using NCCLS methods[a]

Antimicrobial agent	Proposed QC MIC range (μg/ml) for:			
	E. coli ATCC 25922	*P. aeruginosa* ATCC 27853	*E. faecalis* ATCC 29212	*S. aureus* ATCC 29213
Ceftiofur	0.25–1	16–64	1–4	0.25–1
Enrofloxacin	0.008–0.03	1–4	0.12–1[b]	0.03–0.12
Florfenicol	2–8	>16	2–8	2–8
Penicillin G-novobiocin	8/16–>8/16	>8/16	0.25/0.5–2/4[b]	0.015/0.03–0.06/0.12
Pirlimycin	>16	>16	2–8	0.25–1
Premafloxacin	0.008–0.06[b]	1–4	0.008–0.03	0.002–0.008
Spectinomycin	8–64[b]	256–>512	64–256	64–256

[a] The NCCLS methods have been described previously (6).
[b] Four-dilution range.

distributed (biomodal), indicating the need for a 4-dilution QC range.

The MICs for *S. aureus* ATCC 29213 attained a 100% distribution within the ranges suggested for ceftiofur and pirlimycin, and <2% of the results for premafloxacin, penicillin G-novobiocin, and spectinomycin were beyond the proposed mode ± 1 $\log_2$ dilution range. Outlying MICs of florfenicol and enrofloxacin resulted in 96.0 and 97.3% of the MICs occurring within the proposed QC range, respectively. No requirement for broader ranges was identified.

A summary of the proposed QC ranges for all antimicrobial agent-QC organism pairs tested is provided in Table 2. These data extend the number of antimicrobial agents for veterinary use (seven compounds) that can be tested by the NCCLS broth microdilution method (6). With ≥94.7% of the MIC results within the proposed QC ranges, all tested drug-QC strain combinations comply with NCCLS guidelines (5). Although four organism-drug QC ranges (*E. faecalis* versus ceftiofur and premafloxacin, *E. coli* versus florfenicol, and *P. aeruginosa* versus premafloxacin) exhibited a trend toward one extreme of the proposed MIC limits, we selected a 3-dilution range because of a dominant modal result (≥60%). These ranges have been approved by the NCCLS Subcommittee on Veterinary Susceptibility Testing and will be found in the next NCCLS publication (proposed standard M31-P) (6).

The proposed ranges for the two fluoroquinolones provide important, new information required for the testing of these agents, since fluoroquinolones at these ranges have only recently been applied to infection therapy or prophylaxis in animals. Enrofloxacin is closely related to ciprofloxacin (an enrofloxacin metabolite) and possesses slightly greater activity against gram-positive organisms. Premafloxacin has a remarkable potency against gram-positive QC organisms and was observed to be 16- to 32-fold more active than ciprofloxacin or enrofloxacin. Moreover, the use of these proposed QC ranges should facilitate the accumulation of accurate quantitative susceptibility test data, leading to a greater validity of susceptibility test results from veterinary clinical trials.

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