Re-evaluation of Recommended Cefditoren MIC Quality Control Ranges for Four ATCC Strains

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ABSTRACT

Background: Cefditoren is a novel orally administered aminothiazolyl cephalosporin with a broad spectrum of activity. This study will re-evaluate the MIC QC ranges currently recommended by the NCCLS.

Methods: Seven laboratories participated in a M23 study design recommended by the NCCLS to evaluate the current MIC QC ranges for cefditoren. Three Mueller-Hinton (MH) broth lots were tested for *Escherichia coli* (EC) ATCC 25922 and *Staphylococcus aureus* (SA) ATCC 29213. Four HTM and lysed horse blood supplemented MH lots were tested for *Haemophilus influenzae* (HI) ATCC 49247 and *Streptococcus pneumoniae* (SP) ATCC 49619, respectively. Each site tested the QC strains over 10 days generating 30 or 40 MIC results per QC strain at each center for a total of 210 to 280 MIC values. Cefpodoxime was used as comparative control agent and daily colony counts ensured proper inoculum concentrations.

Results: Previous studies recommended broad (4-dilution) QC ranges for EC ATCC 25922, SA ATCC 29213 and SP ATCC 49619 and several investigations over three years ultimately decided upon a 3-dilution range for HI ATCC 49427. This study observed identical modal MIC values for the EC, HI, and SP among all centers and tight ranges (2-4 dilutions) for all QC except HI ATCC 49247 (6 dilutions). The cefpodoxime QC results for all centers (420 values) showed 99.5% were within recommended NCCLS ranges. Colony counts yielded 8 x 10^4 to 8.8 x 10^5 CFU/ml.

Conclusions: Using these results, we recommend the following MIC ranges: EC ATCC 25922 (0.12 - 1 µg/ml), SA ATCC 29213 (0.25 - 1 µg/ml), HI ATCC 49247 (0.06 - 0.25 µg/ml) and SP ATCC 49619 (0.016 - 0.06 µg/ml). Using these modified ranges, all of the centers' results, except for HI ATCC 49247 (94.3%) were within ranges. This study establishes that currently recommended ranges for EC ATCC 25922 and HI ATCC 49247 are appropriate. However, further testing may support narrowing of the 4 dilution ranges for SA ATCC 29213 and SP ATCC 49619 to a three dilution range.

INTRODUCTION

Cefditoren (formerly ME1206) is a broad-spectrum orally administered cephalosporin pivoxil ester that has documented activity against major pathogens causing community-acquired respiratory tract infections.[1,2,3,4,5,10,11] Many *Streptococcus pneumoniae* isolates having resistances to other orally active cephems such as cefaclor, loracarbef, cefixime, ceftibuten, cefprozil and cefuroxime axetil, are susceptible to cefditoren at MIC values of ≤ 0.5 µg/ml.[3,4,5] Similarly the MIC_{90} results for cefditoren when tested against *Haemophilus influenzae* and *Moraxella catarrhalis* have remained at 0.016 to 0.5 µg/ml.[3,4,5] The microbiological profile of cefditoren suggests clinical potential for the treatment of respiratory tract and uncomplicated skin and skin structure infections.

To enable accurate *in vitro* susceptibility testing of cefditoren by clinical microbiology laboratories or by antimicrobial surveillance systems, quality control (QC) guidelines are urgently needed, generated by methods recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[8] Several studies of this type have included cefditoren [NCCLS Minutes of the Subcommittee on Antimicrobial Susceptibility Testing, 1999 and 2000][12] dating from 1998. Inconsistent results were detected among these studies for results obtained from the QC strain *H. influenzae* ATCC 49247. Because of concerns about these published guidelines[7,12] another QC study of broth microdilution tests was conducted in 2001 following the NCCLS M23-A2.[8]

MATERIALS & METHODS

Seven independent laboratories tested American type culture collection (ATCC) strains *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 against cefditoren and cefpodoxime (control). Participants used reference broth microdilution methods and performance standards or guidelines.[7,8,9] The study design also adhered to NCCLS QC testing parameters using approved guideline M23-A2. Organisms were tested daily for ten days using three Mueller-Hinton lots for *S. aureus* and *E. coli;* four lots of Mueller-Hinton with 3.5% lysed horse blood for *S. pneumoniae*; and four lots of Haemophilus Test Media for *H. influenzae*. Panels were manufactured by TREK Diagnostic Systems, Inc. (Westlake, OH) using media lots provided by Difco, BBL and Criterion. The resulting tests provided a total of 210 to 280 MIC test values. Internal QC was performed at each laboratory using cefpodoxime as a control agent and one or two media lots. This resulted in a total of 420 MIC QC results for cefpodoxime tested against the four strains, with 99.5% of results within NCCLS recommended ranges.[9] The participants also performed colony counts daily on one of the QC organisms and included at least two colony counts of each tested strain during the ten-day test period. The range of all participants colony counts was 8.0 x 10^4 to 8.8 x 10^5 CFU/ml with an average of 2.9 x 10^5 CFU/ml.

RESULTS

- Table 1 lists the distributions of all cefditoren MIC values reported by the seven participant laboratories. A total of 980 MICs were recorded, derived from ≥ 3 medium lots.[8]
- Clear modal values were determined for *S. pneumoniae* ATCC 49619 (0.03 µg/ml; 80.0% of responses), *H. influenzae* ATCC 49247 (0.12 µg/ml; 81.4% of responses) and *S. aureus* ATCC 29213 (0.5 µg/ml; 62.9% of responses). For *E. coli* ATCC 25922, 116 (55.2%) and 88 (41.9%) of the cefditoren MIC values were encountered at 0.25 and 0.5 µg/ml, respectively. This so-called "broad-modal" result required a proposed four-dilution QC range of 0.12 to 1 µg/ml, while all other organisms had a three log_2 dilution range recommendation (Table 1).

Table 1. Distribution of Cefditoren MICs from All Seven Participating Laboratories in the QC Protocol [NCCLS, 2000]

	Occurrences at Each MIC for			
Cefditoren MIC (µg/ml)	*S. pneumoniae* ATCC 49619	*H. influenzae* ATCC 49247	*E. coli* ATCC 25922	*S. aureus* ATCC 29213
0.008		11		
0.016	0[a]	1		
0.03	224[a]	4		
0.06	56[a]	35[b]		
0.12		228[b]	3[a]	
0.25		1[a]	116[a]	2[a]
0.5			88[b]	132[b]
1			3[a]	76[a]
Total	280	280	210	210

[a] Proposed QC range (includes 94.3 - 100.0% of participant results).

- Only 94.3% of reported *H. influenzae* MIC results were found within the proposed QC range (0.06 - 0.25 µg/ml) with all aberrant MICs falling below 0.06 µg/ml (Tables 1 and 2). All but one of the "out-of-range" MICs were recorded at two medical centers. No apparent cause was determined since these results occurred using different medium lots, and each of the involved laboratories had a mode identical to all other participants. With a distinct mode at 0.12 µg/ml (81.4% of results), a range of three log_2 dilutions was suggested (0.06 to 0.25 µg/ml) regardless of the proportion of results (5.7%) occurring outside of the proposed MIC QC limits. This range conforms to that recently published by the NCCLS and validates the correction of earlier proposed MIC QC ranges for *H. influenzae* ATCC 49247.[7,9,12]

Table 2. Cefditoren MIC Distributions Among Seven Laboratories Participating in the M23-A2 [NCCLS 2001a] Design QC Protocol Using *H. influenzae* ATCC 49247 (280 results overall)

Cefditoren	Laboratory Code (occurrences)								
MIC (µg/ml)	A	B	C	D	E	F	G	Total	(%)
0.008				8	3			11	(3.9)
0.016				1				1	(0.4)
0.03				1	2		1	4	(1.4)
0.06	5			15	10	4	1	35	(12.5)[a]
0.12	35	40	40	15	25	35	38	228	(81.4)[a]
0.25						1		1	(0.4)[a]

[a] Proposed range includes 0.06 - 0.25 µg/ml (94.3% of reported values).

- Other proposed QC ranges mimic closely those suggested earlier[12] or found in the NCCLS tables.[9] However, these multi-center trial results suggest wider four log_2 dilution ranges for *S. aureus* ATCC 27212 (0.25 to 2 µg/ml) and *S. pneumoniae* ATCC 49619 (0.016 to 0.12 µg/ml) may not be required on the grounds of 1) well established modal values; and 2) 100.0% of participant MICs reported were within the proposed narrower range.
- These cefditoren QC ranges should be closely monitored after the release of the compound into clinical use and after the production of commercial MIC reagent lots for routine laboratory use.
- Table 3 lists the evolution of cefditoren MIC QC range guidelines and the results from this seven-center investigation.

Table 3. Proposed Cefditoren QC Ranges from a Seven-Laboratory Sample in 2001 Using Four Control Strains; Also Recommended Ranges per Barry and Brown (CMI-1 through-3) Are Listed for Comparison

	Proposed MIC Range (µg/ml)			
Organism	This Study	CMI-1	CMI-2	CMI-3
E. coli (ATCC 25922)	0.12-1	0.12-1	-	-
S. aureus (ATCC 29213)	0.25-1	0.25-2	-	-
H. influenzae (ATCC 49247)	0.06-0.25	0.016-0.12[a]	0.06-0.5[b]	0.06-0.25[c]
S. pneumoniae (ATCC 49619)	0.016-0.06	0.016-0.12	-	-

[a] 1998 study by M23-A2 design at 10 sites resulting in a NCCLS [2000b] approved four-dilution range.
[b] 1999 study by a non-M23-A2 design [NCCLS, 2001a] at 30 sites.
[c] 2000 study of nine sites leading to a NCCLS [2001b] revised three-dilution range.

CONCLUSIONS

- Validation of currently advocated NCCLS cefditoren QC ranges was achieved and a suggestion made that more strict or narrow ranges might be appropriate for two of the recommended QC organisms.[9]
- When cefditoren becomes available clinically, its potency should be followed in comparison to existing oral β-lactams using reference-quality quantitative methods and the cited QC guidelines. Through this practice of continued vigilance for evolving resistances, the role of cefditoren shall be defined for the therapy of community-acquired infections especially those cases caused by β-lactam non-susceptible pneumococci.

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