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Interpretive Criteria and Quality Control Limits for Ceftibuten Disk Susceptibility Tests

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In vitro studies were undertaken to evaluate susceptibility tests with 30-μg ceftibuten disks. The following interpretive criteria were proposed: ≤17 mm for resistance (MIC, ≥32 μg/ml) and ≥21 mm for susceptibility (MIC, ≤8.0 μg/ml). A multilaboratory quality control study led to the conclusion that *Escherichia coli* ATCC 25922 should provide zones 29 to 35 mm in diameter.

Ceftibuten (7432-S; SCH39720) is an orally administered cephalosporin that is currently being evaluated in clinical trials. It is not inactivated by bacterial β-lactamases (4) and is very active against common respiratory tract pathogens (3) as well as members of the family *Enterobacteriaceae*. Unlike many other oral cephalosporins, ceftibuten is not a prodrug ester but is readily adsorbed into the bloodstream without structural modification. Following a 200-mg dose, peak levels in serum of 11.6 μg/ml have been reported (M. Kakashima, M. Iida, T. Yoshida, T. Kitagawa, T. Oguma, and H. Ishii, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 591, 1986). The serum half-life of ceftibuten is 1.5 to 2.1 h, and protein binding is approximately 67%. On the basis of such preliminary pharmacokinetic information, a susceptible MIC breakpoint of ≤8.0 μg/ml would seem to be appropriate for ceftibuten; an MIC breakpoint of ≤4.0 μg/ml could be proposed as a more conservative definition of the susceptible category. The alternative breakpoint might be necessary if dosing schedules are changed or if clinical experience indicates the need for a change from the ≤8.0-μg/ml breakpoint. The clinical efficacy of ceftibuten in treating pneumococcal infections is especially important, since pneumococci frequently require 4.0 or 8.0 μg/ml for inhibition (2, 3).

In 1988 Jones and Barry (2) published the results of preliminary trials with disks that were produced by the investigators. Subsequent work demonstrated that ceftibuten disks lost potency during the drying process, and methods were then developed to permit commercial manufacturers of disks to prepare fully potent 30-μg disks. The disks that were used in the earlier study (2) probably contained less than 30 μg of active ceftibuten; consequently, we undertook another evaluation, this time using 30-μg ceftibuten disks from BBL Microbiology Systems (Cockeysville, Md.). A separate multilaboratory study was also undertaken to establish quality control limits for ceftibuten disk tests. The study protocol outlined by Gavan et al. (1) was followed for this phase. Three different lots of commercially manufactured 30-μg ceftibuten disks from two manufacturers were tested on each of 50 separate test plates in each of six different participating laboratories. Each participant used a different lot of Mueller-Hinton agar as well as a control lot of agar that was common to all investigators. *Escherichia coli* ATCC 25922 was the only control strain that consistently gave zones of inhibition with 30-μg ceftibuten disks. A single 30-μg cephalothin disk was also tested for control purposes.

For establishing interpretive criteria, 537 bacterial isolates were tested by broth microdilution methods (6) and by disk diffusion tests (7). Ceftibuten was obtained from Schering Corp. (Bloomfield, N.J.) and dissolved in dimethyl sulfoxide. Further dilutions were prepared in cation-supplemented Mueller-Hinton broth (50 mg of calcium and 25 mg of magnesium per liter) and dispensed into microdilution trays which were stored at −40°C or colder. The inocula were adjusted to give approximately 5×10^5 CFU/ml, and MICs were recorded after 16 to 18 h at 35°C. For testing fastidious species, the medium was supplemented with 2 to 3% lysed horse blood, as specified by the National Committee for Clinical Laboratory Standards (6, 7). For *Haemophilus influenzae* isolates, disk diffusion and agar dilution tests were performed with the haemophilus test medium of Jorgensen et al. (5) as recommended by the National Committee for Clinical Laboratory Standards (7).

Table 1 summarizes the results of the two types of tests. The majority of the members of the family *Enterobacteriaceae* were susceptible to ceftibuten; median MICs for different species ranged from ≤0.6 to 4.0 μg/ml. Only *Enterobacter cloacae*, *Serratia marcescens*, and *Morganella morganii* provided strains that were not susceptible (MICs, >8.0 μg/ml). Ceftibuten was essentially inactive against *Pseudomonas aeruginosa* as well as the gram-positive bacilli, enterococci, and *Streptococcus bovis*. Staphylococci were also resistant to ceftibuten (data not shown). *Branhamella catarrhalis* isolates were susceptible, as were *Streptococcus pyogenes* isolates and penicillin-susceptible strains of *Streptococcus pneumoniae*. Penicillin-resistant pneumococci were relatively resistant to ceftibuten, as has been demonstrated previously (2, 3). *H. influenzae* isolates were all susceptible to ceftibuten (MIC, ≤8.0 μg/ml; zones, ≥23 mm). β-Lactamase-producing and -nonproducing strains of *H. influenzae* were both very susceptible to ceftibuten (MIC, ≤0.12 μg/ml; zones, ≥35 mm), but strains with chromosomally mediated ampicillin resistance (β-lactamase negative) tended to be less susceptible to ceftibuten (median MIC, 4.0

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TABLE 1. Ceftibuten susceptibility test results with 537 microorganisms that were used for evaluating 30-μg ceftibuten disk tests

Microorganism[a] (no. tested)	MIC (μg/ml)		Zone diam (mm)	
	Range	Median	Range	Median
Citrobacter diversus (10)	≤0.06	≤0.06	30–37	34
C. freundii (25)	≤0.06–8.0	2.0	21–31	24
Escherichia coli (20)	≤0.06–8.0	0.5	24–34	29
Klebsiella spp. (21)[b]	≤0.06–0.5	≤0.06	27–35	32
Enterobacter aerogenes (28)	0.25–8.0	1.0	6–30	25
E. agglomerans (11)	≤0.06–8.0	1.0	22–31	26
E. cloacae (29)[c]	≤0.06–>32	4.0	6–32	23
Serratia spp. (27)[d]	0.12–>32	0.5	15–33	29
Proteus spp. (30)[e]	≤0.06–0.25	≤0.06	28–38	35
Morganella morganii (10)	≤0.06–32	0.5	17–31	30
Providencia spp. (23)[f]	≤0.06–0.12	≤0.06	31–39	36
Salmonella enteritidis (10)	≤0.06–0.25	≤0.06	24–42	33
Shigella spp. (12)[g]	0.12–0.5	0.25	26–33	29
Yersinia enterocolitica (10)	0.12–8.0	1.0	27–38	32
Pseudomonas aeruginosa (25)	>32	>32	6–17	6
Other gram-negative bacilli (17)[h]	8.0–>32	>32	6–30	15
Branhamella catarrhalis (30)	0.25–16	0.25	27–38	31
Gram-positive bacilli (30)[i]	>32	>32	6	6
Enterococcus spp. (30)[j]	>32	>32	6–16	6
Streptococcus bovis (10)	>32	>32	8–14	12
S. agalactiae (20)	16–>32	32	15–18	16
S. pyogenes (20)	0.25–2.0	0.5	23–37	25
S. pneumoniae				
Penicillin susceptible (20)	2.0–8.0	4.0	23–28	25
Penicillin resistant (10)	16–>32	>32	6–24	13
Haemophilus influenzae[k]				
β-Lactamase negative Amp[s] (19)	≤0.03–0.12	≤0.03	36–44	39
β-Lactamase negative Amp[r] (19)	0.25–8.0	4.0	23–39	30
β-Lactamase positive Amp[r] (21)	0.06–0.12	0.06	35–40	38

[a] For simplicity, data obtained with two *Cedecea lapagei* and two *Citrobacter amalonaticus* strains were omitted. All four strains were found susceptible by both methods (MIC, 0.12 to 2.0 μg/ml; zones, 28 to 39 mm).
[b] Includes 16 *K. pneumoniae* and 5 *K. oxytoca* strains.
[c] Includes 25 *E. cloacae*, 2 *E. sakazakii*, and 2 *E. hafnia* strains (the latter two species were found susceptible by both methods).
[d] Includes 25 *S. marcescens* and 2 *S. liquefaciens* strains.
[e] Includes 20 *P. mirabilis* and 10 *P. vulgaris* strains.
[f] Includes 11 *P. stuartii*, 10 *P. rettgeri*, and 2 *P. alcalifaciens* strains.
[g] Includes three *S. boydii*, three *S. dysenteriae*, three *S. flexneri*, and three *S. sonnei* strains.
[h] Includes 11 *Acinetobacter calcoaceticus* subsp. *anitratus*, 2 *Achromobacter xylosoxidans*, 2 *Flavobacterium* sp., and 2 *Aeromonas* sp. strains.
[i] Includes 10 *L. monocytogenes*, 10 *C. jeikeium*, and 10 *Bacillus* sp. strains.
[j] Includes 20 *E. faecalis*, 6 *E. faecium*, 2 *E. durans*, and 2 *E. hirae* strains.
[k] Amp[s], Ampicillin susceptible; Amp[r], ampicillin resistant.

μg/ml; zones, 23 to 39 mm). The latter strains of *H. influenzae* and penicillin-susceptible *S. pneumoniae* isolates would be most markedly affected by a one-dilution decrease in the MIC breakpoint from ≤8.0 to ≤4.0 μg/ml. Such a change in the breakpoint will be justified only if those types of infections fail to respond to ceftibuten. To date, such clinical features have not been observed, and an adjustment of the MIC breakpoint is not now anticipated.

Regression analysis provided a correlation coefficient of 0.81 and a regression formula of $y = 43.7 - 1.8x$ (y = zone diameter in millimeters and x = MIC as $\log_2 + 9$ μg/ml). The calculated zone size correlate for an MIC of ≤8.0 μg/ml was rounded off to ≥21 mm, and the usual 3-mm intermediate range left a resistant category of ≤17 mm. The interpretive criteria that were previously proposed (2) were less appropriate, presumably because of differences in the actual potency of disks that were used in the two studies. A scattergram (Fig. 1) was constructed for all species other than *H. influenzae* to evaluate the calculated zone size criteria. There was only 1 (0.2%) very major discrepancy (*Enterobacter sakazakii*), and there were 20 (4.2%) minor discrepancies. Strains of *S. pneumoniae* for which the MIC was 8.0 μg/ml produced very large zones of inhibition that could not be distinguished from zones that were produced by more susceptible strains. The alternative MIC breakpoint of ≤4.0 μg/ml would cut through that normally distributed population of MICs and would lead to greater minor discrepancy rates. Separate analyses of disk diffusion tests with the 59 *H. influenzae* isolates displayed no interpretive discrepancies, i.e., all zones were ≥23 mm and all MICs were ≤8.0 μg/ml. For ampicillin-resistant β-lactamase-negative strains, zones ranged from 23 to 39 mm and MICs were 0.25 to 8.0 μg/ml. For all other strains, MICs were ≤0.12 μg/ml and zones were ≥35 mm. The former strains are very uncommon in clinical material, and the clinical utility of ceftibuten in treating infections due to such microorganisms is only a matter of conjecture.

Table 2 presents the results of replicate disk diffusion tests with three lots of 30-μg ceftibuten disks from two commercial manufacturers. The all-laboratory median zone size was 32 mm, and the average range was 6.2 mm. Laboratory E tended to report somewhat smaller zones, and laboratory F tended to report larger zones. When the common lot of Mueller-Hinton agar was used, those two laboratories again reported the same type of slightly skewed ceftibuten zones. When testing *E. coli* ATCC 25922 with 30-μg ceftibuten

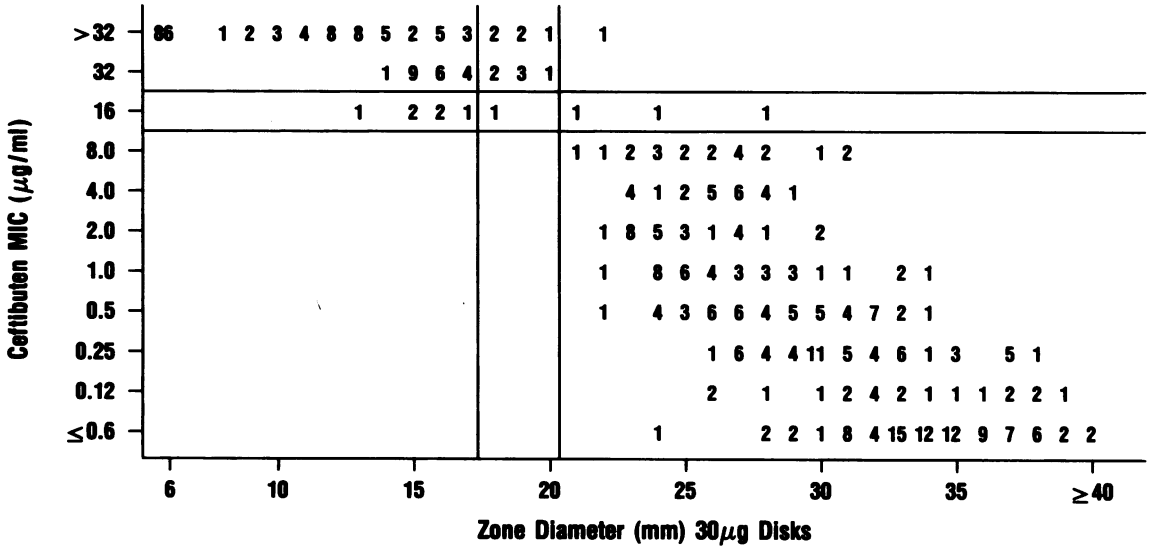

FIG. 1. Scattergram showing distributions of ceftibuten broth microdilution MICs and zone diameters with 30-μg ceftibuten disks. Data for 59 *H. influenzae* isolates are excluded (see text).

disks, we concluded that zones should be 29 to 35 mm (32 mm ± 3 mm). Such limits would have alerted laboratory E workers that they might need to reassess their testing procedure. When cephalothin disks were tested by each participant, laboratory A through D reported zones well within established control limits, but laboratory E reported 5 of 50 zones that were too small (≤16 mm) and laboratory F reported 5 of 50 zones that were too large (≥23 mm). No obvious explanation for these discrepancies could be found.

TABLE 2. Six-laboratory evaluation of susceptibility tests with 30-μg ceftibuten disks versus *E. coli* ATCC 25922[a]

Zone diam (mm)	No. of replicate tests with the indicated zone diam in laboratory:						
	A	B	C	D	E	F	All laboratories
26					7		7
27	2		10		36		48
28	3		12		47		62
29	10		31		31		72
30	54	17	51		18		140
31	31	24	31	5	10	1	102
32	33	45	10	66	1	2	157
33	15	45	5	73		23	161
34	2	19		6		57	84
35						56	56
36						11	11
Median	31	32	30	33	28	34	32

[a] Each of six laboratories reported 150 separate zone diameters; each used a different lot of Mueller-Hinton agar. Similar results were obtained when a single common lot of agar was used by all participants. Spacing between zones designates the proposed 29- to 35-mm control limits.

In summary, 30-μg ceftibuten disks can be used with zone size interpretive criteria of ≤17 and ≥21 mm. Those criteria might also be used for testing *H. influenzae* on haemophilus test medium agar. For quality control purposes, *E. coli* ATCC 25922 should provide zones 29 to 35 mm in diameter.

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