Cefuroxime, an *in vitro* Comparison with Six Other Cephalosporins

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Jones *et al.* (1978) have previously determined the *in vitro* activity of a new parenteral cephalosporin, cefuroxime, against 5887 bacterial isolates and compared its spectrum of activity to that of the family representative, cephalothin. In the present report, cefuroxime is compared directly with 6 other parenteral cephalosporins: cefamandole, cefazolin, cefoxitin, cephaloridine, cephradine and cephalothin. Recent clinical isolates were obtained from the collaborating laboratories and were studied by the first 2 authors at the Center for Disease Control and at the Sacramento Medical Center. A microdilution technique was used for comparing the activity of the 7 cephalosporins. Additional studies were carried out to compare the bactericidal activities of the 7 drugs, and to determine the effect of varying the inoculum density and incubation time.

Materials and Methods

A total of 397 bacterial isolates was provided by the participating laboratories for this study. Included were 35 *Staphylococcus aureus* (10 methicillin resistant), 18 *Staphylococcus epidermidis*, 11 *Streptococcus fæcalis*, 5 *Streptococcus bovis*, 3 *Streptococcus durans*, 22 *Streptococcus pyogenes*, 23 *Streptococcus pneumoniæ*, 27 *Neisseria gonorrhœæ*, 27 *Neisseria meningitidis*, 29 *Hæmophilus influenzæ* (10 ampicillin resistant), 25 *Escherichia coli*, 6 *Citrobacter freundii*, 6 *Citrobacter diversus*, 25 *Klebsiella pneumoniæ*, 10 *Enterobacter cloacæ*, 10 *Enterobacter ærogenes*, 4 *Enterobacter agglomerans*, 16 *Serratia marcescens*, 1 *Serratia rubidea*, 25 *Proteus mirabilis*, 7 *Proteus vulgaris*, 9 *Proteus morganii*, 9 *Proteus rettgeri*, 1 *Providencia sp.*, 3 *Edwardsiella tarda*, 8 *Shigella dysenteria*, 1 *Salmonella sp.*, 5 *Salmonella typhi*, 6 *Aeromonas hydrophilia*, 10 *Pseudomonas æruginosa*, 5 *Pseudomonas cepacia* and 5 *Pseudomonas maltophilia*.

One hundred and thirty-three of these isolates were tested both at the Center for Disease Control and at Sacramento Medical Center, thus affording the opportunity to compare data obtained with slightly different techniques.

Minimal Inhibitory Concentrations (MICs) were determined by the microdilution broth technique. Mueller-Hinton broth was commercially dispensed in plastic trays (Micro Media Systems, Campbell, California) and sufficient trays were provided for the entire study. The trays were stored at −60°C for as long as 8 weeks with no evidence of drug inactivation, as indicated by end-points with appropriate control organisms. The trays were inoculated with disposable inoculators said to deliver approximately 5 μl to each well. The MIC was recorded as the lowest concentration totally inhibiting bacterial growth after 18–24 h of incubation at 35°C in a forced air incubator. Occasionally, visible growth occurred in concentrations 2 or 3 wells above the MIC (the skipped-tube phenomenon). Limited experiments were undertaken to determine whether the bacterial growth in the higher concentrations could be reproduced upon retesting. It could not. The MICs defined above were most reproducible and thus skipped tubes were overlooked when recording end-points.

The two laboratories differed somewhat in the method used to standardize the inoculum density. At the Center for Disease Control (CDC), a logarithmic phase broth culture was diluted to match the turbidity of a MacFarland 0.5 turbidity standard. The suspension was then further diluted 1:50 in sterile water (with 0.02% Tween 80) and dispensed with a disposable inoculator (approximately 5 μl per well). Consequently, the inoculum finally achieved should be about 1×10^5 colony forming units (CFU)/ml. At the Sacramento Medical Center (SMC) the test organisms were inoculated into 0.5 ml of brain heart infusion broth (BHI) and allowed to incubate 5–6 h at 35°C. These small volume broth cultures were then diluted 1:100 in water (with 0.02% Tween 80) and 5 μl inoculated into the trays. Consequently, the inoculum should be about 5×10^5 CFU/ml.

For testing *S. pyogenes* and *S. pneumoniæ*, the inoculum was standardized in Mueller-Hinton broth with 5% lysed rabbit blood and 0.1 ml of the adjusted cell suspension was added to each well, to give a final concentration of about 1×10^5 CFU/ml. Because of the large volume of inoculum, the final concentration in each well was reduced by 50% (i.e. 0.03–16 μg/ml).

The MICs for *H. influenzæ* were determined by suspending colonies directly into Mueller-Hinton broth with 10% peptic digest and 2% Isovitalex, adjusted to match a MacFarland 0.5 turbidity

Table 1

Microdilution quality control data from 2 independent laboratories[a] testing the 'Seattle' strains of *S. aureus* and *E. coli* against 7 cephalosporins

		MICs (μg/ml) minimum-maximum[b] and (mode)	
Antibiotic	*Lab*[a]	S. aureus	E. coli
Cefuroxime	CDC	0.5 –1.0 (0.5)	2.0 –4.0 (4.0)
	SMC	0.5 –1.0 (0.5)	2.0 –4.0 (4.0)
Cefamandole	CDC	0.25 (0.25)	0.25–0.5 (0.5)
	SMC	0.25–0.5 (0.25)	0.25–0.5 (0.5)
Cefazolin	CDC	0.25 (0.25)	1.0 –2.0 (1.0)
	SMC	0.25–0.5 (0.25)	1.0 –2.0 (1.0)
Cefoxitin	CDC	2.0 (2.0)	2.0 –4.0 (2.0)
	SMC	2.0 –4.0 (2.0)	2.0 –8.0 (4.0)
Cephaloridine	CDC	≤0.06 (≤0.06)	2.0 –4.0 (2.0)
	SMC	≤0.06 (≤0.06)	2.0 –4.0 (4.0)
Cephradine	CDC	1.0 –2.0 (2.0)	8.0 (8.0)
	SMC	2.0 (2.0)	8.0 –16 (8.0)
Cephalothin	CDC	0.12–0.25 (0.12)	4.0 –8.0 (8.0)
	SMC	0.12–0.5 (0.25)	8.0 –16 (8.0)

[a]: 9 separate tests performed in Atlanta, Georgia (CDC) and 17 separate tests performed in Sacramento, California (SMC), each laboratory using slightly different methods for standardizing the inoculum density (see Table 2)

[b]: Only 1 value is noted when all MICs were the same (i.e. minimum = maximum)

Table 2

Direct comparison of microdilution MICs performed in 2 independent laboratories using slightly different methods for standardizing the inoculum[a]

	Differences in MICs (2-fold dilutions)[a]					
Antibiotic	*−2*	*−1*	*−0*	*+1*	*+2*	*> +2*
Cefuroxime	1[b]	6	60	25	2	6
Cefamandole	2	6	50	28	7	7
Cefazolin		3	59	28	4	6
Cefoxitin	1	5	58	24	7	5
Cephaloridine		2	56	27	12	3
Cephradine	1	1	66	27	1	4
Cephalothin		2	56	33	6	3

[a]: tests performed in Atlanta, Georgia (inoculum about 1×10^5 CFU/ml) compared with those performed in Sacramento, California (inoculum about 5×10^5 CFU/ml). Differences expressed as number of 2-fold dilutions. Positive values = Sacramento values greater; negative values = Atlanta values greater

[b]: figures represent % of strains in each category. Total of 133 strains, including 30 *S. aureus* (10 methicillin resistant) 7 *S. epidermidis*, 19 *E. coli*, 19 *Klebsiella* sp., 20 *P. mirabilis*, 16 *Proteus* spp. (indole positive), 18 *Enterobacter* spp. and 4 *Serratia* sp.

standard. This was further diluted to give about 10^4 CFU/ml and 0.1 ml added to each well. The trays were incubated under increased CO_2 for testing both streptococci and *Hæmophilus influenzæ*.

Neisseria gonorrhœæ and *N.meningitidis* were tested by an agar dilution technique. Proteose peptone agar with 1% hæmoglobin and 1% Kellogg's supplement (White & Kellogg 1965) was prepared with the appropriate antibiotic concentrations. The inoculum was prepared by suspending colonies in Mueller-Hinton broth, diluted to contain about 5×10^6 CFU/ml. The plates were then inoculated with Steer's inoculum replicator (Steers *et al.* 1959). The MICs were determined after 24 h of incubation in 5% CO_2.

The effect of varying the inoculum density was studied with 139 of the less fastidious isolates. Trays were inoculated in order to achieve a final cell concentration of 10^3, 10^5 and 10^7 CFU/ml. The MICs were recorded as the lowest concentration showing complete inhibition of growth after 18–24 h at 35°C.

The bactericidal end-points were determined for a limited series of isolates by subculturing approximately 5 μl from each well. The 5 μl subcultures were transferred to Mueller-Hinton agar in one laboratory (SMC) and to trypticase soy agar with 4% rabbit blood in the other laboratory (CDC). The subcultures were accomplished with a multiple inoculum replicator and were spot inoculated on to 15 cm petri plates, without spreading. After 24 or 48 h of incubation, the end-point was read as the lowest concentration which yielded no more than 0.1% survivors (99.9% kill). Upon occasions a larger number of survivors could be recovered from concentrations greater than that which was defined as the MBC. A limited series of tests was undertaken to retest growth from colonies recovered from concentrations greater than the MIC. The end-points selected for defining the MBC in this study were reasonably reproducible both from an inter-laboratory and intra-laboratory point of view.

RESULTS

Inter-laboratory Variation

Since there were minor differences in the methods by which the microdilution tests were performed in the two laboratories, we first compared the data obtained from isolates tested in both laboratories. Throughout the study both laboratories tested the 'Seattle' strains of *S. aureus* and *E. coli* (originally derived from ATCC 25922 and 25923). The results of such control tests are summarized in Table 1. The MICs were usually within a 2-fold dilution step and always within 1 dilution above or below the mean. The most common (mode) MIC value for each cephalosporin was the same in both laboratories, except for *S. aureus* tested against cephalothin and *E. coli* tested against cefoxitin and cephaloridine. With the latter exceptions, values obtained at Sacramento Medical Center tended to be slightly higher than those obtained at the Center for Disease Control but the differences were never greater than 1 doubling dilution. These data suggest that MICs obtained in the two laboratories are essentially comparable.

Table 3

Range of MICs with Gram positive cocci, *Neisseria* and *Hæmophilus* tested against 7 cephalosporins. Microdilution technique; inoculum about 1–5 × 10^5 CFU/ml.

	Microdilution MICs (μg/ml) minimum–maximum and (mode)				
Antibiotic	Staphylococcus aureus *Meth. sens. (25)*	Staphylococcus aureus *Meth. resist. (10)*	Streptococcus *epidermidis (18)*	Neisseria gonorrhœæ[a] *(27)*	Neisseria meningitidis[a] *(27)*
Cefuroxime	1.0–2.0 (1.0)	2.0–> 32 (32)	0.12–4.0 (0.5)	≤0.06–0.5 (≤0106)	≤0.06–< 0.06 (≤0.06
Cefamandole	0.25–1.0 (0.5)	2.0–16 (8)	0.12–2.0 (0.25)	≤0.06–2.0 (≤0.06)	≤0.06–0.26 (≤0.06)
Cefazolin	0.25–8.0 (0.5)	2.0–> 32 (8)	0.12–4.0 (0.25)	0.12–4.0 (0.25)	0.25–1.0 (0.5)
Cefoxitin	0.25–4.0 (4.0)	8.0–32 (16)	0.5–8.0 (2.0)	≤0.06–2.0 (0.12)	0.12–0.25 (0.25)
Cephaloridine	≤0.06–0.25 (0.25)	1.0–4.0 (2.0)	< 0.06–1.0 (≤0.06)	0.25–8.0(0.25)	0.15–1.0 (1.0)
Cephradine	1.0–16 (2.0)	4.0–> 32 (32)	0.25–16.0 (2.0)	Not done	Not done
Cephalothin	0.12–0.5 (0.25)	1.0–32 (1.0)	≤0.06–4.0 (0.12)	≤0.06–4.0 (≤0.06)	0.25–0.5 (0.5)
	Streptococcus fæcalis *(11)*	Streptococcus bovis *(5)*	Streptococcus pyogenes *(22)*	Streptococcus pneumoniæ *(23)*	Hæmophilus influenzæ *(29) (10 amp. resist.)*
Cefuroxime	> 32–> 32 (> 32)	≤0.03–0.12 (0.12)	≤0.03–0.12 (≤0.03)	≤0.03–0.25 (< 0.03)	0.25–0.5 (0.5)
Cefamandole	32–> 32 (> 32)	0.06–0.25 (0.09)[b]	≤0.03–0.06 (0.06)	0.06–0.5 (0.06)	0.12–1.; (0.25)
Cefazolin	32–> 32 (32)	0.12–0.25 (0.25)	0.06–0.12 (0.12)	≤0.03–0.5 (0.06)	1.0–8.0 (2.0)
Cefoxitin	32–> 32 (> 32)	1.0–2.0 (2.0)	0.5–2.0 (0.5)	0.5–4.0 (1.0)	0.5–8.0 (2.0)
Cephaloridine	16–> 32 (16)	≤0.03–0.06 (≤0.03)	≤0.03–≤0.03 (≤0.03)	≤0.03–0.12 (≤0.03)	2.0–16.0 (4.0)
Cephradine	32–> 32 (> 32)	0.25–1.0 (1.0)	0.12–1.0 (0.12)	0.5–2.0 (1.0)	4.0–16.0 (8.0)
Cephalothin	32–> 32 (32)	0.25–1.0 (0.5)	0.06–0.12 (0.12)	0.06–0.5 (0.12)	0.5–4.0 (1.0)

[a] = MICs determined by an agar dilution technique.
[b] = when tests are equally distributed among two MIC values, the mode is listed as the value half way between the two MICS.

A total of 133 reference strains was tested by both laboratories and the two MIC values were directly compared (Table 2). Although the majority of the MIC values were identical, there were some differences. Where there were differences, a disproportionate number of strains tended to give higher values at SMC than at the CDC. The majority of strains with discrepant results differed by 1 doubling dilution step. A small but significant number of strains gave major discrepancies of more than 2 doubling dilution steps. Many of these major discrepancies involved tests with *Enterobacter* spp., *Proteus* spp. and methicillin resistant strains of *S. aureus*. The latter represent strains for which inoculum density is especially critical. For that reason, it should be safe to assume that the discrepancies between MICs obtained in the 2 laboratories reflect relatively minor differences in the inoculum density.

Minimal Bactericidal Concentrations (MBCs) were also determined in both laboratories and directly compared. Although there were some 1 or 2 dilution discrepancies between the 2 laboratories, there was no general trend for one centre to report MBC values which consistently exceeded those obtained in the other centre.

Minimal Inhibitory Concentrations

The microdilution data are summarized in Tables 3 and 4. Most of the Gram positive cocci were relatively susceptible to all 7 cephalosporins. Against the staphylococci, cefuroxime was less active than cephalothin, cefamandole, cefazolin and cephaloridine but more active than cefoxitin and cephradine. The methicillin resistant strains were relatively resistant to all cephalosporins. The streptococci were all susceptible, except for the enterococci (*S. fæcalis*) which were highly resistant. Three strains of *S. durans* were also tested but not included in the Tables. Two of the strains were resistant to all 7 drugs and the other strain was susceptible to all 7 cephalosporins.

Hæmophilus influenzæ was effectively inhibited by low concentrations of cefuroxime and cefamandole, whereas cephradine and cephaloridine were the least effective. The 10 ampicillin-resistant strains did not differ in their susceptibility to the cephalosporins, as compared to the strains not producing β-lactamase. Apparently these cephalosporins are all relatively resistant to the β-lactamases produced by ampicillin resistant strains of *H. influenzæ*

Cefuroxime was extremely effective against all strains of *N. gonorrhœæ* and *N. meningitidis*. Cephaloridine and cefazolin were the least effective against these isolates. However, they were all inhibitory at concentrations readily achieved in the blood during therapy.

Among the Enterobacteriaceæ, cefuroxime was more active than cephalothin but less active than cefamandole. *Proteus mirabilis* and most *P. rettgeri* isolates were very susceptible to cefamandole and to cefuroxime. Most isolates of *P. morganii*, *P. vulgaris* and *Providencia* sp. were resistant. Cefamandole and cefuroxime appeared to be effective against a large proportion of *Enterobacter* spp. *E. agglomerans* was much

Table 4

Range of MICs with Gram negative bacilli tested against 7 celphalosporins. Microdilution technique; inoculum about 1–5 × 10^5 CFU/ml

	Microdilution MICs (μg/ml) minimum–maximum and (mode)				
Antibiotic	Escherichia coli (*25*)	Klebsiella pneumoniæ (*25*)	Proteus mirabilis (*25*)	Proteus *spp. indole positive* (*26*)	Aeromonas hydrophilia (*6*)
Cefuroxime	1.0–16 (4.0)	1.0–16 (2.0	0.5–2.0 (0.75)[a]	0.12–> 32 (> 32)	0.25–2.0 (1.0)
Cefamandole	0.12–16 (0.5)	0.25–8.0 (1.0)	0.25–1.0 (0.5)	⩽0.06–> 32 (> 32)	0.25–2.0 (0.5)
Cefazolin	0.5–32 (1.0)	0.5–32 (1.0)	1.0–8.0 (4.0)	0.5–> 32 (> 32)	4.0–> 32 (> 32)
Cefoxitin	1.0–32 (2.0)	0.5–> 32 (4.0)	1.0–4.0 (4.0)	1.0–> 32 (8.0)	0.5–8 (8.0)
Cephaloridine	1.0–32 (2.0)	0.5–> 32 (4.0)	4.0–8.0 (4.0)	8.0–> 32 (> 32)	4.0–> 32 (> 32)
Cephradine	4.0–> 32 (8.0)	4.0–> 32 (8.0)	4.0–16 (16)	8.0–> 32 (> 32)	8.0–> 32 (8.0)
Cephalothin	2.0–> 32 (6.0)[a]	0.5–> 32 (8.0)	1.0–4.0 (4.0)	2.0–> 32 (> 32)	1.0–> 32 (32)
	Citrobacter (*12*) C. diversus (*6*) C. freundii (*6*)	Enterobacter (*24*) E. cloacæ (*10*) E. ærogenes (*10*) E. agglomerans (*4*)	Serratia (*17*) S. marcescens (*16*) S. rubicea (*1*)	Pseudomonas (20) P. æruginosa (*10*) P. cepacia (*5*) P. maltophilia (*5*)	Miscellaneous (*17*) Edwardsiella (*3*) Shigella *sp.* (*8*) Salmonella *sp.* (*6*)
Cefuroxime	2.0–> 32 (4.0)	2.0–> 32 (4.0)	16–> 32 (> 32)	16–> 32 (> 32)	0.12–4.0 (0.5)
Cefamandole	0.5–> 32 (0.5)	0.25–> 32 (1.0)	8.0–> 32 (32)	32–> 32 (> 32)	0.06–0.5 (0.12)
Cefazolin	1.0–> 32 (> 32)	1.0–> 32 (> 32)	32–> 32 (> 32)	32–> 32 (> 32)	0.5–1.0 (1.0)
Cefoxitin	2.0–> 32 (2.0)	2.0–> 32 (> 32)	8.0–> 32 (16)	32–> 32 (> 32)	0.5–2.0 (1.0)
Cephaloridine	2.0–> 32 (> 32)	2.0–> 32 (> 32)	32–> 32 (> 32)	32–> 32 (> 32)	1.0–2.0 (2.0)
Cephradine	8.0–> 32 (8.0)	4.0–> 32 (> 32)	32–> 32 (> 32)	32–> 32 (> 32)	4.0–8.0 (4.0)
Cephalothin	2.0–> 32 (> 32)	1.0–> 32 (> 32)	32–> 32 (> 32)	32–> 32 (> 32)	1.0–4.0 (4.0)

[a] = when tests are equally distributed between 2 MIC values, the mode is listed as the value halfway between the two MICs

more susceptible to the cephalosporins than *E. cloacæ* or *E. ærogenes*. All 7 cephalosporins were relatively inactive against *Serratia* spp. and all 3 *Pseudomonas* species.

Minimal Bactericidal Concentrations

The bactericidal activity of the cephalosporins was determined with 161 isolates. Table 5 summarizes the results of these studies. An antimicrobial agent may be considered bactericidal if it is able to kill 99.9% of the cells in the original inoculum at concentrations 1 or 2 doubling dilutions above that required for inhibition. With all 7 cephalosporins, a bactericidal effect was usually observed at the MIC or at 1 doubling dilution above the MIC. Cefuroxime was clearly bactericidal against all isolates except some strains of *Proteus* spp. With cefamandole, on the other hand, the bactericidal activity was less clear cut. Cefazolin and cephaloridine were the least effective as bactericidal agents.

Inoculum Size

The importance of inoculum density is summarized in Table 6. With a 100-fold increase or decrease in inoculum density, the proportion of strains inhibited at any given concentration was markedly altered. With a very heavy inoculum (10^7 CFU/ml) all 7 cephalosporins are relatively ineffective. A decrease in inoculum density from 10^5 to 10^3 CFU/ml did not change the proportion of susceptible strains as markedly as did an increase from 10^5 to 10^7 CFU/ml. The effect of increasing the inoculum density was about the

Table 5

Bactericidal activity of 7 cephalosporins. Minimal bactericidal concentration expressed as number of 2-fold dilutions above the MIC

		Cefuroxime				*Cefamandole*				*Cefazolin*				*Cefoxitin*				*Cephaloridine*				*Cephradine*				*Cephalothin*			
Organism	*No. tests*[a]	*0*	*1*	*2*	*⩾3*	*0*	*1*	*2*	*⩾3*	*0*	*1*	*2*	*⩾3*	*0*	*1*	*2*	*⩾3*	*0*	*1*	*2*	*⩾3*	*0*	*1*	*2*	*⩾3*	*0*	*1*	*2*	*⩾3*
Staphylococcus aureus	25	16	6			15	9	1		15	4	2	1	21	4			9	3	2		12	4	2		20	2	2	
Escherichia coli	27	23	2			21	4	1		23	2			23	4			18	5	2		11	7			16	5	1	
Klebsiella pneumoniæ	24	21	2			10	11	3		7	9	5	3	17	6			8	8	8		17	6			12	7	5	
Enterobacter spp.	25	9	4			7	0	3	2	4	0	1		2	1			2			1	2	4			2			1
Proteus mirabilis	26	13	10	1	2	6	15	1		14	5	4	2	16	5	4		17	6			10	9			18	6		
Proteus spp.	27	8	0	2	4	2	4	0	2	3	1			16	3	3	1	0				2				1	1		
Streptococcus bovis & durans	7	3				5				5				6				2				5				5			
Total	161	93	24	3	6	66	43	9	4	71	21	12	6	101	23	7	1	56	22	12	1	59	30	2		74	21	8	1

[a] = tests with MBCs or MICs outside the range tested (⩾0.06 or > 32 μg/ml) were excluded

same for the different Gram negative bacilli. With the staphylococci, MICs did not change dramatically with changes in inoculum size. However, with the methicillin resistant strains of *S. aureus*, MICs were very dependent on inoculum density. With a heavy inoculum, all methicillin-resistant trains were resistant to all 7 cephalosporins.

Effect of Prolonged Incubation

The extent to which the MIC would be increased by an additional 24 h of incubation was determined. An increase in MICs may be the result of delayed growth that occurs if the drug is inactivated, or may represent selection of more resistant variants that may be present in small numbers. Table 7 summarizes the result of such an experiment with 35 representative isolates. With the majority of isolates, the MICs either remained the same or increased by 1 doubling dilution step. In no case did the MIC increase more than 2 doubling dilutions. An increase in MICs after 48 h was seen most frequently with cefamandole and least frequently with cephaloridine. With cefuroxime, about one-third of the strains showed an increase in MICs after 48 h, whereas nearly half of the strains showed an increase with cephalothin and two-thirds increased MICs with cefamandole.

Selection of Resistant Variants by Cefuroxime

Colonies recovered from inhibitory concentrations of cefuroxime were subcultured to trypticase soy broth and then retested in order to determine whether the single passage through cefuroxime selected a more resistant population of cells. Table 8 summarizes the results of such susceptibility tests done with 18 representative strains. Susceptibility to all 7 cephalosporins was determined before and after a single passage through cefuroxime. Two of the 3 *Enterobacter* spp. showed a significant increase in resistance to all cephalosporins for which an end-point was originally obtained. The other *Enterobacter* showed a 4-fold increase in resistance to cefamandole but not to cefuroxime. The *E. coli* and *K. pneumoniæ* developed 2- to 4-fold increases in resistance to most of the cephalosporins. The other genera that were tested showed no significant change in MICs to the cephalosporins. The minimal bactericidal concentration was also determined before and after passage through cefuroxime. The results were essentially the same as those depicted by the MIC values.

Broth Dilution Compared with Agar Dilution

The micro-broth dilution technique was further evaluated by comparing end-points with those obtained with an agar dilution technique. For this study, logarithmic phase broth cultures were diluted to match the turbidity of a MacFarland 0.5 standard. A 1:10 dilution of this suspension was then spot inoculated on to Mueller-Hinton agar plates using a hand-held inoculum replicator which delivers approximately 0.002 ml/spot. Consequently, each spot received about 2×10^4 viable cells. The microdilution wells were inoculated with approximately 5 μl of a 1:25 dilution of the standardized cell suspension. Each well received about 2×10^4 viable cells in 0.1 ml of antibiotic-containing broth, or 2×10^5 CFU/ml. Three

Table 6

Effect of inoculum size on microdilution MICs. Seven cephalosporins against 139 isolates[a]

		% of strains inhibited by (μg/ml)						
Antibiotic	*Inoculum size*	*<0.5*	*1*	*2*	*4*	*8*	*16*	*>32*
Cefuroxime	10^3	10	37	53	76	80	86	100
	10^5	6	25	43	66	72	76	100
	10^7	0	4	13	21	27	33	100
Cefamandole	10^3	57	71	84	88	92	96	100
	10^5	35	53	59	70	80	84	100
	10^7	2	7	19	31	34	48	100
Cefazolin	10^3	19	45	58	74	76	79	100
	10^5	11	35	42	54	71	65	100
	10^7	3	7	9	21	38	42	100
Cefoxitin	10^3	1	11	35	64	71	79	100
	10^5	1	3	18	52	62	71	100
	10^7	0	0	2	21	38	55	100
Cephaloridine	10^3	14	23	42	59	67	70	100
	10^5	10	11	25	50	57	63	100
	10^7	2	3	6	25	46	51	100
Cephradine	10^3	1	5	16	34	59	72	100
	10^5	0	0	9	22	44	55	100
	10^7	0	0	1	10	13	21	100
Cephalothin	10^3	13	22	41	59	68	74	100
	10^5	11	13	22	42	58	72	100
	10^7	9	11	12	12	18	30	100

[a]: includes tests with 22 *E. coli*, 20 *Klebsiella* spp., 20 *Enterobacter* spp., 19 *P. mirabilis*, 20 *Proteus* spp. (indole positive), 3 *P. cepacia*, 5 *P. maltophilia*, 4 *A. hydrophilia* and 26 *S. aureus* (9 methicillin resistant)

Table 7

Change in MIC end-points after 48 h compared to 24 h MIC values[a]

	Increase in MICs at 48 h[b]		
Antibiotic	*Unchanged*	*+1 dilution*	*+2 dilutions*
Cefuroxime	23	10	1
Cefamandole	10	16	5
Cefazolin	11	10	1
Cefoxitin	18	8	3
Cephaloridine	14	4	0
Cephradine	10	15	2
Cephalothin	11	8	2

[a]: 35 isolates were tested including *E. coli*, *K. pneumoniæ*, *P. rettgeri S. epidermidis*, *S. aureus* (3 each) & *E. cloacæ*, *E. ærogenes*, *Serratia* spp., *Providencia* spp., *C. diversus*, *C. freundii*, *S. dysenteriæ*, *S. typhi*, *E. tarda* and *A. hydrophilia* (2 each)

[b]: figures represent the number of strains in each category. Data from strains with MICs outside of the range tested (≤ 0.06 or > 32 μg/ml) were excluded

Table 8

Increased resistance to cephalosporins by a single exposure to inhibitory concentrations of cefuroxime

	MIC values (μg/ml) before–after single exposure to cefuroxime[a]				
Antibiotic	Enterobacter ærogenes	Enterobacter cloacæ	Klebsiella pneumoniæ	Escherichia coli	Enterobacter ærogenes
Cefuroxime	4.0–32	8.0–32	2.0–4.0	4.0–8.0	4.0–4.0
Cefamandole	2.0–16	1.0–8.0	0.5–2.0	0.5–2.0	1.0–4.0
Cefazolin	4.0–16	> 32– > 32	4.0–8.0	1.0–4.0	32–32
Cefoxitin	> 32– > 32	> 32– > 32	2.0–8.0	2.0–16	> 32– > 32
Cephaloridine	> 32– > 32	> 32– > 32	4.0–4.0	4.0–8.0	> 32– > 32
Cephradine	16– > 32	> 32– > 32	4.0–8.0	16–32	> 32– > 32
Cephalothin	> 32– > 32	> 32– > 32	2.0–4.0	8.0–32	> 32– > 32

[a] = microdilution trays were subcultured and a single colony, recoverd from the highest concentration of cefuroxime yielding growth, was retested after overnight incubation in trypticase soy broth. No significant change in MICs was observed with 13 other isolates, including 3 *P. rettgeri*, 2 *C. freundii*, 2 *E. tarda* and 1 each *C. diversus*, *S. dysenteriæ*, *S. typhi*, *Providencia* spp., *S. aureus* and *S. epidermidis*.

cephalosporins (cefuroxime, cefamandole and cephalothin) were tested at concentrations ranging from 0.06–32 μg/ml.

The 35 test strains included 2 *E. cloacæ* and 2 *E. aerogenes*. One strain of *E. cloacæ* was fully resistant (MIC>32 μg/ml) to all 3 cephalosporins by both methods. The 3 other strains of *Enterobacter* spp. were susceptible to cefuroxime (MICs 4–8 μg/ml) and to cefamandole (MICs 1–4 μg/ml) but were resistant to cephalothin (MIC>32 μg/ml).

Table 9 summarizes a direct comparison of agar dilution and broth dilution MICs with the 35 representative isolates. Although the 2 MIC values were generally within a range of ± 1 $\log_2$ dilution step, there was a disproportionally large number of strains with slightly greater agar dilution MICs for cefuroxime and cefamandole but not for cephalothin. With the agar dilution technique used in the present study, all 3 cephalosporins gave rather sharply defined end-points and the slightly larger MIC values could not be attributed to a few isolated colonies that could be detected on agar plates but yet failed to produce visible turbidity in the microdilution trays after 18–24 h.

Table 9

Micro-broth dilution MICs compared to agar dilution MICs with 35 isolates[a] tested against 3 cephalosporins

Differences (dilutions)		*No. strains in each category*		
		Cefuroxime	*Cefamandole*	*Cephalothin*
Broth > agar	3			
	2	1	1	
	1	3	2	3
No difference		13	17	13
Agar > broth	1	11	10	4
	2	4	2	1
	3	2	1	
	4			

[a]: including the 35 isolates described in Table 7

Discussion

Two microbiology laboratories independently tested 133 isolates against 7 cephalosporins. Although slightly different techniques were used to inoculate the microdilution trays, the results of tests with quality control strains indicated that the two laboratories obtained essentially identical results. However, when a wider variety of isolates were tested at the two centres, minor but significant differences were seen. MICs obtained with an inoculum of about 5×10^4 CFU/mol tended to be slightly greater than those observed with an inoculum of about 1×10^4 CFU/ml. The differences in inoculum density would appear to be the most likely explanation for the discrepancies that were observed between the two laboratories. In spite of the general trend for slightly higher MICs in one laboratory, 89% of the end-points were in agreement (± 1 $\log_2$ dilution step). The important lesson learned from this exercise is the error in assuming that if MICs with control strains are in agreement, tests with other microorganisms should also be in agreement. The control strains give clear-cut end-points and their MICs are not markedly influenced by minor changes in the inoculum density.

The spectrum of activity of cefuroxime is documented in a separate report (Jones *et al.* 1978). The present study compares cefuroxime to 6 other cephalosporins. In general, cefamandole and cefuroxime ranked among the most active of the cephalosporins studied. Cephaloridine had the greatest *in vitro* activity against the Gram positive cocci. The enterococcus *S. fæcalis*, was uniformly resistant to the cephalosporins. The methicillin resistant strains of *S. aureus* were more resistant to the cephalosporins than were methicillin-susceptible strains.

Against *N. gonorrhœæ* and *N. meningitidis*, cefuroxime was extremely active. The other cephalosporins were also effective but at somewhat higher concentrations.

The *H. influenzæ* strains were all susceptible to the cephalosporins, with cefamandole and cefuroxime being the most active. The cephalosporins seem to be relatively resistant to the β-lactamases produced by *H. influenzæ* since the β-lactamase producing strains gave MICs which were essentially the same as those obtained with the susceptible strains.

Against the *Enterobacteriaceæ*, cefamandole and cefuroxime were both very active, especially against *Proteus* spp. and *Enterobacter* spp. The *in vitro* activity of cefamandole against *Enterobacter* spp. has been investigated rather extensively by Findell & Sherris (1976). Their studies demonstrated a high frequency of resistant variants within cultures of *Enterobacter* spp. These resistant variants were readily detected when a moderately heavy inoculum was used and skipped tubes were often observed. They also observed much higher MICs in a broth dilution test (inoculum, about 7×10^4 CFU/ml) and lower MICs (1–8 μg/ml) with an agar dilution test (inoculum about 1×10^4 CFU/spot). With the microdilution technique, we did not observe the exceptionally high MICs reported by Findell & Sherris (1976). Our broth dilution MICs were the same or slightly lower than those observed with the agar dilution technique. However, in our experiments, the broth and agar dilution tests received comparable inocula (2×10^4 CFU/well or/spot). With a heavy inoculum, the cephalosporins were all essentially ineffective against most of the *Enterobacter* spp. A marked inoculum effect was seen with most other enteric bacilli but not with the staphylococci.

A very limited series of tests with colonies recovered from inhibitory concentrations of cefuroxime suggest a fairly high frequency of resistant variants within broth cultures of *Enterobacter* spp. but not within cultures of other enteric bacilli. Furthermore, resistant populations selected by a single exposure to cefuroxime were also resistant to the other cephalosporins tested. The apparent *in vitro* activity of cefuroxime against *Enterobacter* spp. should be interpreted with caution. Carefully controlled clinical trials are needed before recommending cefuroxime therapy for infections due to *Enterobacter* spp.

The skipped tube phenomenon was seen occasionally, especially in the laboratory that regularly used a slightly denser inoculum. Skipped tubes were seen most frequently with *Enterobacter* spp. and *Proteus* spp. When this occurred, growth in the higher concentrations was checked for purity and then retested. The repeated tests usually confirmed the original MIC and growth in the skipped wells was not often repeated.

In order to obtain reproducible end-points, the MIC was defined as the lowest concentration showing complete inhibition of growth. Low MICs with skipped tubes should be interpreted with caution since the presence of resistant variants must be suspected (Findell & Sherris 1976, Brenner *et al.* 1965). Chance will play a role in determining whether a tube will receive enough resistant variants to produce turbid growth after overnight incubation and therefore the actual MIC observed with such broth cultures is not likely to be very reproducible. This might explain some of the major disagreements between values obtained in the two laboratories.

Spontaneous inactivation of the cephalosporins or inactivation by the surviving microorganisms may also influence the MIC determination. In the present study, no attempt was made to determine the concentration of drugs in the inoculated wells after overnight incubation. Since we were able to recover viable cells from wells containing concentrations several dilution steps above the MIC, we reasoned that secondary regrowth should occur with prolonged incubation, if significant drug inactivation had taken place. The fact that the MICs did not increase more than 1 dilution step after a second period of incubation would speak against the possibility that the cephalosporins were significantly inactivated during incubation in the tests. More resistant strains, with MICs beyond the range tested, might be capable of producing a potent cephalosporinase which would not be detected by this approach.

Because of such difficulties in determining MIC values with the cephalosporins, considerable inter- and intra-laboratory variation should be expected, especially when testing *Enterobacter* spp., *Proteus* spp. and methicillin resistant *S. aureus*. In view of such technical problems, the inter-laboratory reproducibility of the microdilution technique was actually very good.

Summary

A microdilution technique was used to compare cefuroxime, cefamandole, cefazolin, cefoxitin, cephaloridine, cephradine and cephalothin, tested against 397 bacterial isolates. Two separate laboratories independently tested 133 of the isolates, thus permitting an evaluation of inter-laboratory variability. Additional studies were carried out to determine (1) the bactericidal activity of all 7 drugs; (2) the effect of varying the inoculum density; (3) the effect of prolonged incubation time and (4) the effect of a single passage through inhibitory concentrations of cefuroxime. Exposure to cefuroxime appeared to select resistant variants of *Enterobacter* spp. and those variants were resistant to the other cephalosporins. The skipped tube phenomenon was observed occasionally, especially with *Entero-*

bacter spp. and *Proteus* spp., which also showed a marked inoculum effect. Cefuroxime was especially active against strains of *Neisseria gonorrhœæ* and *Neisseria meningitidis*. The cephalosporins were all active against ampicillin resistant and susceptible strains of *Hæmophilus influenzæ*. Against the *Enterobacteriaceæ*, cefuroxime had a spectrum of activity which resembled that of cefamandole. But with most genera, cefamandole MICs were generally 1 to 2 doubling dilution steps lower than cefuroxime MICs.

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DISCUSSION

Professor W Brumfitt (*London*) noted the difficulty that workers had had with *Proteus*. Professor Goto had shown *P. morganii* resistant and *mirabilis* sensitive but later showed the drug stable to lactamase. Dr Barry had shown that *P. rettgeri* was highly resistant and that *P. morganii* was relatively resistant. He wondered how speakers would approach the practical problem of choosing an antibiotic if they did not agree that (with the exception of *P. mirabilis*) it was necessary to identify the species before treatment could be initiated.

Dr Gaya said that in practice, in the clinical laboratory, the vast majority of infections were with *Proteus mirabilis* and he suspected that it might not be necessary to identify the species, certainly in urinary tract infections. In the case of chronic and wound infections, however, it was necessary to identify the species with the appropriate tests.

Dr Barry said that in his Institution and in many American hospital laboratories, the identification of *Proteus* species was a routine procedure. He agreed that the vast majority of isolates were *Proteus mirabilis*. But there were real differences in susceptibility within species, a problem with which they would have to come to grips.

Professor Goto said that the percentage of sensitive and resistant strains varied with each species. About 50% of the strains that she had studied were sensitive, the remainder resistant. She also felt that there was a certain measure of resistance that could not be fully explained by the β-lactamase.

Dr S H Zinner (*Rhode Island*) asked if Dr Gaya had any data derived from patients with which to calculate his maximal inhibitory dilution (MID).

Dr Gaya said that he hoped to have in the future. As yet it was a theoretical relationship, but he hoped that if the methodology was good it would remain true.

Dr L D Sabath (*Minneapolis*) asked Dr Barry about the increase in MIC values after passage of bacteria in the presence of cephalosporins. He wondered if this might be due to β-lactamase activity, to possible selection of more resistant strains, or to some combination of the two.

Dr Barry assumed that there was a selection of resistant variants but he did not know whether it was due to cephalosporins. The question should be followed up, but he did not currently have the data.

In reply to Dr Gaya, he said that he had not given the strains the chance to revert to sensitive by further passages in the absence of cefuroxime. He had been interested only in the increase induced by a single passage.

Professor C S Goodwin (*Perth*) said that isolates in Western Australia showed resistance patterns closer to those of the second and third speakers than the first. *Serratia* was 50% sensitive to cefuroxime, *Acinetobacter* 40%, and *Enterobacter* 66%. He also commented on the fact that disc tests had proved extremely unreliable in predicting the MIC values of cephalosporins in a detailed study conducted in Sydney.

Professor H C Neu (*New York*) said that in Dr Barry's studies of both *Enterobacter* and *Escherichia coli* it appeared that he had selected out 2 types of strain, both with increased β-lactamase activity. Investigators present had shown that cefamandole was much more susceptible to β-lactamase than was cefuroxime, and organisms appeared to be even less permeable to cefoxitin. He therefore asked the Chairman if it was possible that isolates were indeed being selected and that permeability was an important factor in this process.

Professor Richmond (*Chairman*) said it was possible, but he had seen no evidence, at least from limited studies, that strains from clinics where the drugs had been used had any different β-lactamase activity. If there were differences they were likely to be due to penetrability, but he could not comment on any large body of work.