Sensitivity tests with cephalosporins

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SUMMARY The sensitivity of 127 strains of miscellaneous Gram-negative bacilli to various cephalosporins was determined by three methods. Disc tests with cephaloridine (CLOR), cephalothin (CTIN) and cephalexin (CLEX) made most strains of beta-lactamase-producing *Escherichia coli* appear sensitive. Eight cephalosporins were tested by the agar break-point method using concentrations of 16 and 4 mg/l for all except cefotaxime (CTAX), when 8 and 1 mg/l were used. In repeated tests there was considerable variation in the response of many strains to the lower concentration of CLOR, CTIN, CLEX and to a lesser extent, cefoxitin (CFOX) and cefuroxime (CROX), and β -lactamase-producing *E coli* appeared as sensitive or moderately sensitive to all. Cephalosporins CLOR, CLEX, and CROX were then tested by inoculating 2 ml volumes of broth containing a 30 μ g disc; all the β -lactamase-producing *E coli* grew in CLOR and CLEX but were inhibited by CROX.

For many years some workers have questioned the reliability of sensitivity tests with the earlier cephalosporins, particularly with ampicillin-resistant coliform bacilli; organisms which give inhibition zones little smaller than that of the standard sensitive strain may fail to respond to treatment. Greenwood *et al*¹ investigated this problem, using 103 strains of ampicillin-resistant enterobacteria, by comparing the zones of inhibition produced by cephaloridine (CLOR) and cephalothin (CTIN) with the rate of regrowth of a dense bacterial population in broth containing 250 mg/l of these drugs. Taking regrowth in eight hours as indicating resistance, many strains found to be resistant by the turbidimetric system had been considered to be sensitive by the disc test, and they questioned whether such strains should be classed as sensitive to these drugs.

Williams recognised this as a clinical problem² and suggested that organisms having a minimum inhibitory concentration (MIC) of 1 or 2 mg/l could be expected to respond to treatment, but those requiring 4 or 8 mg/l may fail. The introduction of Adatabs (Mast Laboratories Ltd) has greatly simplified the preparation of antibiotic-containing media, and the present work was undertaken to see whether more satisfactory results could be obtained by using a simple dilution method employing two break-point concentrations of each drug.

Material and methods

ORGANISMS

Of the 127 organisms tested, 96 were urinary isolates and the remainder stock cultures. They comprised *Escherichia coli* (67), *Klebsiella aerogenes* (13), *Enterobacter* spp (11), *Proteus mirabilis* (13), *Proteus vulgaris* (3), *Proteus morgani* (3), *Proteus rettgeri* (3), *Serratia* spp (4), *Providencia* (4), *Acinetobacter* (2), *Pseudomonas aeruginosa* (3) and *Citrobacter freundii* (1). All organisms were grown overnight in broth, and unless otherwise stated were diluted by adding a 2 mm loopful of this to 4-5 ml sterile water for use in a Steers, Foltz and Graves multiple replicator.

MINIMUM INHIBITORY CONCENTRATIONS

These were determined by the plate dilution method using Isosensitest agar (Oxoid) with an added 0.8% Oxoid agar No 1.

Cefotaxime (CTAX) was kindly supplied by Roussel and cefamandole (CMAN) and cephalexin (CLEX) by Eli Lilly. Pharmaceutical preparations of CLOR, CTIN, CROX, CFOX and cefazolin (CZOL) were used.

DISC TESTS

Tests were done on Isosensitest agar (Oxoid). Plates were inoculated by applying a 2 mm loopful of broth culture to the surface, streaking this across the plate with a dry sterile swab, then spreading uniformly over the whole plate with the other side of the swab using a

rotary plater. Discs (Oxoid) containing 30 µg of each drug were applied to the plates which were incubated without delay. After overnight incubation zone diameters were measured and these were plotted against the MIC of the organism. Any organism giving a zone as large as that given by the control *E. coli* (NCTC 10418) or not > 6 mm smaller was classed as sensitive (S). Organisms giving a zone of at least 12 mm (disc 6 mm) but > 6 mm smaller than the control were classed as moderately sensitive (M), and those giving zones of < 12 mm as resistant (R).

BREAK-POINT TESTS

These were done by multiply inoculating plates containing 16 and 4 mg/l of each cephalosporin, except CTAX, when the concentrations used were 8 and 1 mg/l. Organisms inhibited by both concentrations were classed as sensitive (S) those inhibited only by the higher as moderate (M) and those growing on both plates were considered resistant (R).

The antibiotics were supplied by Mast Laboratories as Adatabs containing 1.6 and 0.4 mg (CTAX 0.8 and 0.1 mg). One Adatab was added to bottles containing 100 ml melted, and cooled agar, according to the manufacturers' instructions, and five plates were poured from each bottle. All organisms were tested five times, using Oxoid Isosensitest agar + 0.8% No 1 agar twice and Mast DST agar + 50 mg/l paranitrophenyl glycerol (PNPG, also supplied as an Adatab) three times. Plates were stored up to three days at 4°C and dried well at 37°C before use.

BROTH TESTS

Broth tests were done by adding a 30 µg disc to 2 ml broth (Oxoid No 2) and inoculating the tube with a 2 mm loop of broth culture.

BETA-LACTAMASE PRODUCTION

Production was tested for using Oxoid Beta-lactamase detection papers according to the manufacturers' instructions.

Table 1 Minimum inhibitory concentration of eight cephalosporins showing the proximity of the MIC of large numbers of strains to the break-points signified by the vertical lines

Cephalosporin		No of strains inhibited by eight cephalosporins (mg/ml)										
		Resistant					Moderate		Sensitive			
		>256	256	128	64	32	16	8	4	2	1	0.5
Cephaloridine	(CLOR)	16	9	5	6	3	2	12	50	24		
Cephalothin	(CTIN)	15	8	6	5	1	7	37	31	15	2	
Cephalexin	(CLEX)	14	1	9	6	2	13	45	35	2		
Cefoxitin	(CFOX)	2	1	6	1	6	4	21	51	26	8	1
Cefuroxime	(CROX)	6	3	3	3	5	4	18	44	27	12	2
Cefamandole	(CMAN)	4	2	6	1		5	4	8	8	18	71
Cefazolin	(CZOL)	12	7	5	3	1	3	8	11	20	51	6
Cefotaxime	(CTAX)						2		2	1	2	120*

*102 strains were inhibited by ≤ 0.06 mg/l.

Results

Minimum inhibitory concentrations of each drug for all the organisms were determined and the results are given in Table 1. All organisms were tested for β-lactamase production; 15/67 strains of *E. coli*, 12/13 *Kl. aerogenes*, 3/13 *P. mirabilis*, and 9/11 *Enterobacter* spp were found to be positive by this method, together with five strains of the remaining species.

BREAK-POINT TESTS

All the organisms were tested against all the drugs on five occasions and the number appearing resistant (R), moderate (M) or sensitive (S) in all five tests in 4/5 tests and in 3/5 tests are given in Table 2. It will be seen that a very large proportion of strains varied between S and M to CLOR and CTIN, and results with CFOX and CROX were little better. This variation is almost certainly the result of the proximity of the lower break-point used (4 mg/l) to the MIC of many of the strains (see Table 1). In contrast, the results with CMAN, CZOL and CTAX were much more constant because the MICs were considerably lower. The MIC of the β-lactamase-producing strains was 4–8 mg/l; two strains were consistently S to CLOR, two varied between S and M and 10 were always M.

There is no evidence that the results were affected by either of the media used; both appeared satisfactory and the swarming of *Proteus* spp was adequately controlled.

EFFECT OF INOCULUM

Plates containing 16 and 4 mg/l of CLOR, CTIN, CLEX, CFOX and CROX were inoculated with overnight broth cultures diluted 1/10 and 1/1000; 102 strains were tested. With CLOR 13 strains changed from S to M and 10 from M to R with the heavier inoculum, but these included only three of the β-lactamase-producing *E. coli*, the remainder being unaffected. With CTIN six strains changed from S to M and 10 from M to R. Six strains changed from S to

Table 2 No of strains appearing resistant, moderate, or sensitive in five tests by the break-point method

	CLOR	CTIN	CLEX	CFOX	CROX	CMAN	CZOL	CTAX
Resistant								
5 tests	36	29	23	12	15	12	22	0
4/5 tests	0	4	6	5	3	2	2	2
3/5 tests	2	4	3	0	1	0	3	1
Moderate								
5 tests	17	25	48	0	19	0	5	1
4/5 tests	3	13	9	9	9	4	2	6
3/5 tests	14	8	12	7	7	0	2	9
Sensitive								
5 tests	27	19	6	62	44	98	66	120
4/5 tests	16	16	12	22	18	4	14	0
3/5 tests	12	9	8	10	11	2	4	0

CLEX to M and two strains of *P mirabilis* went from M to R. With both CFOX and CROX 10 strains changed from S to M. In almost all these changes the MIC of the organism was close to the break-point.

DISC TESTS

Disc tests were done with CLOR, CTIN, CLEX, CFOX, and CROX and the results were compared by plotting the zone diameter for each strain against its MIC. The best results were obtained with CTIN and CLEX, when the majority of zones for a given MIC varied no more than 4 mm and there was little overlap between strains with different MIC. Both CFOX and CROX often gave very diffuse edges to the zone, making precise measurement difficult and there was a wider variation in zone size and considerable overlap between different MIC.

The number of strains classed as S and M by these tests is given in Table 3. All but four of the organisms

TESTS IN BROTH

Broth tests with CLOR, CLEX and CROX were done on 67 strains of *E coli*, seven *Kl aerogenes* and one *P mirabilis* all with MIC $\leq$ 16 mg/l. Fifteen of the strains of *E coli* produced beta-lactamase and all grew in CLOR and CLEX but (with the exception of one strain which gave partial growth) were inhibited by CROX; all the remaining strains were inhibited by CLOR. Sixteen gave full growth and 12 partial growth in CLEX, but the MIC of the majority of these was 8–16 mg/l. All the *Klebsiella* produced beta-lactamase, but only two of these grew in CLOR; four gave partial growth in CLEX and two in CROX. The strain of *P mirabilis* gave partial growth in all drugs.

When the tests with the β -lactamase-producing *E coli* were repeated with the inoculum diluted 1/100, all strains were inhibited by CLOR and CLEX as well as by CROX.

Discussion

In the present work, the MIC of CLOR for β -lactamase-producing *E coli* and the results obtained with the disc test are similar to those reported by Greenwood *et al*¹ and by Williams.² Eight strains had MIC of 8 mg/l and only four of these gave zones which were classed even as M, the remainder, together with those having MICs of 4 mg/l appeared as S. It is clearly impossible to detect these potential treatment failures by the disc test; increasing the size of the inoculum did not improve the results as all zones were reduced regardless of β -lactamase production.

It is equally clear that it is impossible to distinguish these strains by an agar dilution method. Nearly half of the 127 strains tested in this study had MIC of 4 or 8 mg/l for CLOR, CTIN, CLEX, CFOX and CROX and it is not surprising that many of these strains varied in their response to a break-point of 4 mg/l; indeed, with CLOR and CTIN only about half the strains in the S and M categories gave the same result in all five tests.

Table 3 No of strains classed as sensitive (S) or moderate (M) by disc tests

	S	M
CLOR	83	8
CTIN	84	6
CLEX	90	7
CFOX	104	9
CROX	103	5

classed as S to CLOR were inhibited by 4 mg/l or less, but with CTIN, CFOX and CROX this category also included all strains inhibited by 8 mg/l. Strains classed as S to CLEX included all those with MIC of 16 mg/l.

The effect of the size of the inoculum was studied by flooding plates with a series of 1/5 dilutions of an overnight broth culture of ampicillin-sensitive and -resistant *E coli*. There was a difference of 5–6 mm between the diameters of the zones produced with the heaviest and the lighter inocula with all five antibiotics, with the sensitive as well as the resistant strain.

The method used by Greenwood *et al*¹ which led them to question whether β -lactamase-producing *E coli* should be classed as S to CLOR or CTIN, is obviously unsuitable for routine sensitivity testing, but it suggests that a break-point method in broth might serve the same purpose. The results obtained in the present study indicate that this is so. When discs containing 30 μ g CLOR or CROX were added to 2 ml broth, all the β -lactamase-producing *E coli* appeared resistant to the former and sensitive to the latter. This concentration (15 mg/l) was obviously too low for use with CLEX and better results would probably have been obtained had the disc been added to only 1 ml broth, all the β -lactamase-producing *E coli* appeared there is always the risk that small numbers of cells having a slightly higher MIC will grow out,³ therefore the presence of only partial growth in these tests should be disregarded.

It is therefore suggested that if sensitivity tests to either CLOR, CTIN, or CLEX are required, they should be performed by adding the disc to broth instead of to an agar plate, and using a heavy inoculum.

References

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