Cefoxitin sensitivity as a marker for inducible beta-lactamases

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Summary. Inducibility of β -lactamase activity by cefoxitin was examined in 626 gram-negative clinical isolates selected for amoxycillin and cephalothin resistance. The results indicated that precise identification and cefoxitin sensitivity or resistance could be used to predict the inducibility of β -lactamase. Of 326 organisms from species capable of β -lactamase induction, induction was shown in 68% and was predictable from the cefoxitin-sensitivity and identification data. No induction of β -lactamase occurred in the remaining species. A comparison of β -lactamase activities against cefotaxime, cefoperazone and latamoxef showed that induction of enzyme activity against cefotaxime and cefoperazone occurred at similar rates. Induction of activity against latamoxef did not occur or was minimal with three bacterial species. The data show that of 119 strains of Enterobacteriaceae displaying inducible β -lactamase, 113 would have been reported as unequivocally sensitive to cefotaxime, 109 as sensitive to cefoperazone and 116 as sensitive to latamoxef if the disk-diffusion technique alone had been used. The majority of *Pseudomonas* strains examined produced inducible enzyme and they were more resistant to the three cephalosporins tested than were the Enterobacteriaceae.

Introduction

Because of their broad spectrum of activity, cephalosporins are widely prescribed in this hospital, both for prophylaxis and therapy. Chemical modifications of the β -lactam nucleus have led to the expanded spectrum of the second, third- and now fourth-generation cephalosporins (Jones *et al.*, 1984) and the related β -lactams such as the oxacephem latamoxef. (For purposes of simplicity latamoxef will be classed as a cephalosporin in this paper.) Recent attention has focused on the role of inducible β -lactamase enzymes in the inactivation of these antibiotics. Clinical and animal studies suggest that induction of β -lactamases may result in treatment failures (Goering *et al.*, 1982; Sanders and Sanders, 1983). Of particular interest is the presence of these enzymes in those groups of organisms which are now encompassed by the expanded spectrum of the third-generation cephalosporins but which were resistant to earlier β -lactam antibiotics, for instance, *Serratia* spp., the indole-positive *Proteus* and *Providencia* spp., *Enterobacter* spp. and *Pseudomonas* spp. (Sanders, 1982).

This study was undertaken to examine the preva-

lence of inducible β -lactamases in routine clinical isolates, to determine whether there were any differences in induction of β -lactamase activity against cefotaxime, cefoperazone and latamoxef, and to examine whether the organisms with inducible β -lactamases would have been reported as sensitive to third generation cephalosporins in routine disk-diffusion sensitivity tests.

Materials and methods

Bacterial strains

Routine laboratory isolates of gram-negative bacilli were collected over a 4-month period and selected because they were resistant to amoxycillin and cephalothin. Identification of these organisms was performed primarily by the "Repliscan" method (Cathra International Inc.). The identity of some unusual strains was confirmed by API 20E and some non-fermentative strains were identified by use of the 9-digit profile of API 20E. Care was taken when selecting organisms to prevent retesting of isolates from the same patients.

Sensitivity tests

Antibiotic-sensitivity tests were performed by the disk-diffusion method on Isosensitest Agar (Oxoid). The

methods were those of Barry and Thornsberry (1980). The zone diameters that represented sensitivity to each agent were determined by reference to break points derived from regression curves produced at the Repatriation General Hospital, Heidelberg (RGH(H)).

Cefoxitin sensitivity was determined with a 30- μ g disk (Oxoid). Furthermore, 25 cefoxitin-sensitive isolates were tested for induction of β -lactamase activity with 10- μ g and 1- μ g disks prepared at RGH(H) from cefoxitin reference powder (Eli Lilly and Co.).

Induction of β -lactamase

The selected strains were tested for inducible β -lactamases by the method of Sanders and Sanders (1979). This involved placing 30- μ g disks of cefotaxime, cefoperazone and latamoxef (all from Oxoid) on a seeded plate at an optimal distance from a 30- μ g cefotoxin disk (Oxoid). Induction of β -lactamase was defined as antagonism between cefoxitin and the cephalosporin being tested so that there was a 4-mm reduction in zone size around the cephalosporin disk compared with the zone produced on the side opposite to the cefoxitin disk.

Results

Induction of β -lactamase activity against cefotaxime

A total of 634 clinical isolates was tested. In 222 (35%) of these selected strains inducible β -lactamase

activity was demonstrated by antagonism between cefoxitin and cefotaxime. Cefotaxime was chosen as the representative cephalosporin because it has been reported to be highly susceptible to inactivation by inducible β -lactamases (Sanders *et al.*, 1982). The strains in which inducible β -lactamase was not detected were: *Escherichia coli* (180), *Klebsiella pneumoniae* (43), *K. oxytoca* (32), *P. mirabilis* (17), *Providencia* spp. (6), *Aeromonas hydrophila* (4) and *Acinetobacter calcoaceticus* (18).

The strains in which inducible β -lactamase activity against cefotaxime was demonstrated with cefoxitin as the inducer are listed in table I. To ensure that all inducible strains were detected with a 30- μ g cefoxitin disk, 25 isolates from species likely to show induction but which were sensitive to cefoxitin (30- μ g disk) and in which β -lactamase was not induced, were retested with 1- μ g, and 10- μ g cefoxitin disks. In none of the 25 strains tested (*Enterobacter* spp., *Citrobacter* spp., indole-positive *Proteus* spp. and *Hafnia alvei*) was induction demonstrated with the lower concentration of cefoxitin.

Several of the species of Enterobacteriaceae listed in table I (*Enterobacter* spp., *C. freundii*, *H. alvei* and *S. liquefaciens*) shared a common characteristic. Cefoxitin-resistant or intermediate strains were invariably producers of inducible β -lactamase but

Table I. Organisms in which induction of β -lactamase activity against cefotaxime was demonstrated

Species	Number tested	Number of strains whose cefoxitin sensitivity category was					
		sensitive		intermediate		resistant	
		Total	Number with inducible enzyme	Total	Number with inducible enzyme	Total	Number with inducible enzyme
<i>Ent. cloacae</i>	46	11	0	0	0	35	35
<i>Ent. aerogenes</i>	10	4	0	0	0	6	6
<i>Ent. agglomerans</i>	5	3	0	0	0	2	2
<i>C. freundii</i>	19	10	0	0	0	9	9
<i>C. diversus</i>	17	17	0	0	0	0	0
<i>S. marcescens</i>	37	13	0	19	17	5	4
<i>S. liquefaciens</i>	5	4	0	1	1	0	0
<i>H. alvei</i>	10	8	0	0	0	2	2
<i>P. vulgaris</i>	26	23	12	2	2	1	1
<i>M. morgani</i>	43	32	17	7	7	4	4
<i>Ps. aeruginosa</i>	113	6	0	1	1	106	98*
<i>Ps. cepacia</i>	3	0	0	2	2	1	1
<i>Ps. putrefaciens</i>	1	1	1	0	0	0	0
<i>Ps. maltophilia</i>	5	0	0	0	0	5	2†
<i>Ps. fluorescens</i>	3	0	0	0	0	3	0

* Five strains were constitutively resistant; in three strains, induction was not demonstrated but there was a small degree of synergy.

† These two strains were resistant to cefotaxime but β -lactamase activity was induced against cefoperazone.

Table II. Induction of β -lactamase active against three β -lactam antibiotics

Species	Number tested	Number in which β -lactamase activity was induced against		
		cefotaxime	cefoperazone	latamoxef
<i>Ent. cloacae</i>	46	35	35	35
<i>Ent. aerogenes</i>	10	6	6	6
<i>Ent. agglomerans</i>	5	2	2	2
<i>C. freundii</i>	19	9	9	9
<i>S. marcescens</i>	37	21	17	0
<i>S. liquefaciens</i>	5	1	1	1
<i>H. alvei</i>	10	2	2	2
<i>P. vulgaris</i>	26	15	15	0
<i>M. morgani</i>	43	28	24	2*
<i>Ps. aeruginosa</i>	113	99	95	94
Other <i>Pseudomonas</i> spp.	12	3	6†	3
Total	326	222‡	212	153

* Both strains were resistant to cefoxitin.

† Three strains were resistant to cefotaxime.

‡ Plus two strains in which β -lactamase activity against cefoperazone was induced but which were resistant to cefotaxime.**Table III.** Sensitivities of selected bacterial strains to three β -lactam antibiotics

Species	Number tested	Percentage of strains allocated to the given sensitivity category with								
		cefotaxime			cefoperazone			latamoxef		
		S	I	R	S	I	R	S	I	R
<i>E. coli</i>	180	98.8	1.2	0	93.8	2.8	3.3	99.4	0	0.6
<i>K. pneumoniae</i>	34	100	0	0	85.3	5.8	8.8	100	0	0
<i>K. oxytoca</i>	32	100	0	0	93.8	3.1	3.1	96.9	3.1	0
<i>P. mirabilis</i>	17	100	0	0	94.1	5.9	0	100	0	0
<i>Prov. spp.</i>	6	100	0	0	100	0	0	100	0	0
<i>Ent. cloacae</i> *	46	97.9	0	2.1	97.9	0	2.1	97.9	0	2.1
<i>Ent. aerogenes</i>	10	100	0	0	100	0	0	100	0	0
<i>Ent. agglomerans</i>	5	100	0	0	100	0	0	100	0	0
<i>S. marcescens</i>	37	97.7	2.7	0	100	0	0	100	0	0
<i>S. liquefaciens</i>	5	100	0	0	100	0	0	100	0	0
<i>P. vulgaris</i>	26	100	0	0	96.1	3.9	0	100	0	0
<i>M. morgani</i>	43	97.3	2.3	0	97.7	2.3	0	100	0	0
<i>C. freundii</i>	19	89.5	0	10.5†	89.5	0	10.5†	94.7	0	5.3†
<i>C. diversus</i>	17	100	0	0	100	0	0	100	0	0
<i>H. alvei</i>	10	100	0	0	100	0	0	100	0	0
<i>A. hydrophila</i>	4	100	0	0	100	0	0	100	0	0
<i>Acinetobacter</i> spp.	18	50	44.4	5.6	11.1	27.8	61.1	22.2	72.2	5.6
<i>Ps. aeruginosa</i>	113	45.1	47.8	7.1	92.0	3.6	4.4	71.1	22.1	6.2
<i>Ps. cepacia</i>	3	100	0	0	100	0	0	100	0	0
<i>Ps. putrefaciens</i>	1	100	0	0	100	0	0	100	0	0
<i>Ps. maltophilia</i>	5	20	40	40	60	20	20	100	0	0
<i>Ps. fluorescens</i>	3	33.3	66.7	0	100	0	0	33.3	66.7	0

S = sensitive; I = intermediate; R = resistant.

* One strain was resistant to all three β -lactam antibiotics.

† Induction was also demonstrated in these strains.

cefoxitin-sensitive strains did not produce this enzyme. However, in other species (*S. marcescens*, *P. vulgaris* and *Morganella morganii*) this predictable relationship was not found. In the case of *S. marcescens* not all cefoxitin-resistant strains produced inducible enzyme and 52% and 53% respectively of cefoxitin-sensitive strains of *P. vulgaris* and *M. morganii* produced inducible β -lactamase active against cefotaxime.

Inducible β -lactamase has been reported previously to be common in *Ps. aeruginosa* (Hoffman *et al.*, 1981) and it was detected in 87.6% of the test strains. Five strains that were cefoxitin resistant had zone sizes that precluded detection of the required 4-mm reduction in zone sizes and thus induction could not be demonstrated. In three *Ps. aeruginosa* strains induction was not demonstrated, and there was a small degree of synergy between cefoxitin and the three β -lactam antibiotics tested with these strains. Only small numbers of other *Pseudomonas* spp. were available and those varied in inducibility of β -lactamase.

Inducible β -lactamase activity against three third-generation cephalosporins

A comparison of production of inducible β -lactamases active against three cephalosporins is given in table II. The number of strains in which β -lactamase activity against cefoperazone was demonstrated was slightly less than the number of strains that produced inducible β -lactamase active against cefotaxime. This difference was predominantly due to the smaller number of *S. marcescens* and *M. morganii* strains that produced inducible β -lactamase active against cefoperazone than the number with β -lactamase activity induced against cefotaxime.

Less inducible β -lactamase activity against latamoxef was found than with the other two cephalosporins. None of the *S. marcescens* strains that produced enzyme active against cefotaxime produced β -lactamase active against latamoxef and only two *M. morganii* strains produced enzymes active against this drug.

Results of conventional disk-sensitivity tests

Sensitivities of all isolates to cefoxitin, cefotaxime, cefoperazone and latamoxef were determined by disk diffusion and the results are presented in table III. As expected, the most resistant strains were amongst the *Pseudomonas* and *Acinetobacter* spp. with occasional resistant strains amongst the Enterobacteriaceae.

Discussion

β -lactamases induced by the disk-diffusion technique of Sanders and Sanders (1979) have been reported previously in some Enterobacteriaceae and in *Ps. aeruginosa* (Hoffman *et al.*, 1981; Sanders *et al.*, 1982). The findings of our study confirm this. No induction of enzyme was demonstrated in *E. coli*, *Klebsiella* spp., *P. mirabilis*, *Acinetobacter* spp. and *Aer. hydrophila*. The six *Providencia* strains examined exhibited no induction although *Prov. rettgeri* has been reported to behave similarly to *M. morganii* with regard to β -lactamase production (Sykes and Bush, 1983). However, all six strains, five of which were *Prov. rettgeri*, were sensitive to cefoxitin and were separate isolates. It is possible that inducible β -lactamase might be demonstrated in cefoxitin-resistant strains.

Within certain species it appeared that resistance to cefoxitin was synonymous with presence of inducible β -lactamase. Cefoxitin-resistant strains of *Enterobacter* spp., *C. freundii*, *M. morganii* and *H. alvei* invariably had inducible enzyme. Similarity between the enzymes produced by *Enterobacter* spp. and *C. freundii* has been reported previously (Gootz *et al.*, 1984). They also noted that *C. diversus* strains are generally sensitive to cephalosporins and do not produce inducible enzyme. All our *C. diversus* strains were sensitive to cefoxitin and did not produce inducible enzyme in contrast to the *C. freundii* strains. If β -lactamase induction is predictable amongst some cefoxitin-resistant species of bacteria, identification and cefoxitin sensitivity alone may be used as a guide to enzyme production. This would preclude the need for disk-diffusion induction testing of large numbers of clinical isolates and also eliminate the extra 24 h required before the presence of a possible inducible enzyme producer may be reported to the clinician.

S. marcescens, *P. vulgaris* and all but two *M. morganii* strains did not produce inducible β -lactamase active against latamoxef, although enzyme active against cefotaxime and cefoperazone was induced in these species. A higher incidence of antagonism between cefoxitin and cefotaxime than between cefoxitin and latamoxef has been noted (Sanders *et al.*, 1982). It is interesting that those species that did not produce inducible enzyme active against latamoxef were the same three species in which cefoxitin sensitivity or resistance did not always correlate with production of inducible enzyme.

Ps. aeruginosa behaved in a manner consistent with reports that a high degree of inducible β -lactamase activity was exhibited against all three

cephalosporins tested. Also, cefoperazone was more active than cefotaxime or latamoxef against *Ps. aeruginosa* (Jones and Barry, 1983).

The Enterobacteriaceae isolates tested were generally sensitive to the three β -lactam antibiotics examined and in the past would have been reported as such, despite the fact that 24% of our selected strains (119 of the 487 Enterobacteriaceae) had inducible β -lactamases. This raises the question of the reporting of strains suspected or known to have inducible β -lactamases. One policy would be to restrict the use of third-generation cephalosporins and withhold the sensitivity reports until approached by the clinical staff. The possibility of the presence of these enzymes may then be raised and each case assessed on its individual merits. If cephalosporins were still the drugs of choice, co-operation with the laboratory would ensure that the

patients' isolates would be followed to detect development of resistance as a result of enzyme induction. An alternative approach would be to report the sensitivities with a footnote that the organism isolated may display inducible β -lactamase activity.

Treatment failures (Sanders and Sanders, 1983) and the presence of inducible enzymes suggest that the newer β -lactam antibiotics cefotaxime, cefoperazone and latamoxef must be used with care against the group of organisms for which they were developed—i.e., the *Enterobacter*, *Serratia* and indole-positive *Proteus* spp., *C. freundii*, *H. alvei* and *Pseudomonas* spp.

This work was supported by the Department of Veterans' Affairs. We also thank J. Infanti for technical assistance and M. Carson and Dr B. Wadham for assistance with the manuscript.

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