Sensitivity to carbenicillin and ticarcillin, and the β -lactamases of *Pseudomonas aeruginosa* in the UK in 1978-79

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SUMMARY A total of 438 strains of *Pseudomonas aeruginosa* supplied by 10 hospitals in the UK reporting an increase in resistance to carbenicillin was tested for sensitivity to carbenicillin and ticarcillin. It was found that 85% of the strains were inhibited by 125 μ g carbenicillin/ml and 87% by 50 μ g ticarcillin/ml, and that ticarcillin was from two- to four-fold more active than carbenicillin against the majority of these strains. Strains with a high level of resistance to carbenicillin (MIC > 1000 μ g/ml) possessed constitutive β -lactamases, and five different types of enzyme were identified. There was good correlation between minimum inhibitory concentrations and the results of disc sensitivity tests in this study, 82% with the 100 μ g carbenicillin disc and 90% with the 75 μ g ticarcillin disc, but results reported in the hospital laboratory tests with the carbenicillin disc were less satisfactory (64% correlation). From a comparison with data reported in 1967 there does not appear to have been a significant increase in the incidence of carbenicillin-resistant strains of *Ps aeruginosa* in the UK.

The development of resistant strains of *Pseudomonas aeruginosa* during treatment with carbenicillin¹ and the emergence of strains with a high level of resistance² were reported relatively soon after the introduction of the broad-spectrum penicillin into clinical practice in 1967. Not long after, strains of *Ps aeruginosa* possessing chromosomal or plasmid-mediated β -lactamases, which readily inactivated carbenicillin, were isolated,^{3 4} and concern was expressed regarding the possible rapid development of bacterial resistance to carbenicillin.

This report describes the results of tests to determine the sensitivity of strains of *Ps aeruginosa* isolated in 1978 and 1979 from 10 hospitals in the United Kingdom reporting an increase in the incidence of resistance to carbenicillin. The opportunity was also taken to measure the sensitivity of the organisms to ticarcillin (α -carboxy-3-thienyl-methyl penicillin), an analogue of carbenicillin with improved antipseudomonas activity⁵ which became available in the UK in 1979.

Material and methods

CULTURES

Eight hospitals in England, one in Scotland, and one in Wales each agreed to supply 50 consecutive strains of *Ps aeruginosa* isolated in the routine laboratory for inclusion in this study. A total of 438 cultures from a wide variety of clinical sources was received between October 1978 and February 1979.

ANTIBIOTICS

Carbenicillin and ticarcillin (disodium salts) were laboratory reference preparations (Beecham Pharmaceuticals). Antibiotic sensitivity discs containing carbenicillin (100 μ g) and ticarcillin (75 μ g) were commercial samples supplied by Mast Laboratories.

MINIMUM INHIBITORY CONCENTRATIONS

Serial dilutions of carbenicillin and ticarcillin were added to 18 ml volumes of Blood Agar Base (Oxoid, CM55) in petri dishes. The plates were dried for 30 minutes at 55° and inoculated with 0.001 ml of a

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1/50 dilution of an overnight broth culture of the test organism by means of an inoculating device delivering 20 cultures to each plate. The final inoculum was approximately 10^4 cells per drop, as recommended for the ICS method.⁶ The plates were incubated for 18 hours at 37°C, and the minimum inhibitory concentrations were determined as the lowest concentrations preventing visible growth.

ANTIBIOTIC DISC SENSITIVITY TESTS

Antibiotic disc sensitivity tests were performed by the method described by the Association of Clinical Pathologists.⁷ Petri dishes (90 mm) containing Blood Agar Base to a depth of 4 mm were flooded with a 1/5000 dilution of an overnight broth culture of the test organism. The excess fluid was removed with a pasteur pipette, and the plates were dried at 37°C for 30 minutes. The antibiotic sensitivity discs were pressed firmly on the surface of the agar, and zones of inhibition were measured after 18 hours at 37°C. Each test included the control strain, *Ps aeruginosa* NCTC 10662, and the sensitivity of the test cultures was interpreted by comparison with the control strain, as shown below:

Sensitive—zone diameter similar or larger than that of the control.

Moderately sensitive—zone diameter of at least 12 mm but reduced by more than 6 mm when compared with the control.

Resistant—zone diameter of 10 mm or less.

β-LACTAMASES

Ehrlenmeyer flasks (500 ml) containing 100 ml of a sterile tryptone medium (3.2% tryptone (Oxoid), 2% yeast extract (Oxoid), and 0.5% NaCl) were inoculated with 1% v/v additions of overnight broth cultures (Oxoid No. 2) of the test organisms. For each of the cultures two flasks were inoculated and shaken at 30°C and 240 rpm. After 3.5 hours' incubation, 6-aminopenicillanic acid (500 µg/ml) was added to one of the flasks in order to induce β-lactamase production. After 7 hours' incubation the cells were collected by centrifugation at 22 500 g for 15 minutes at 5°C and resuspended in 2.5 ml of distilled water. These suspensions were ultrasonicated

for 3 minutes (MSE ultrasonic disintegrator, MSE Scientific Instruments, Crawley, Sussex) using an ice bath to cool the samples. The cell debris was removed by centrifugation at 38 000 g for 1 hour at 5°C. The supernatants were examined for β-lactamase activity.

Isoelectric focusing of β-lactamases⁸ was carried out using LKB Ampholine PAG plates (pH 3.5-9.5) and the LKB Multiphor system (LKB Instruments Ltd, Croydon). Focusing was carried out at 30 W and 1.4 kV settings with an LKB 2103 power supply for 1.5 hours at 5°C. Enzyme zones were visualised by overlaying with filter paper soaked in the chromogenic cephalosporin, nitrocefin (500 µg/ml). The paper was left in contact with the gel, and the zones formed were observed by inverting the plate.

Substrate profiles were determined by measuring initial rates of substrate hydrolysis at 37°C in 0.05 M sodium phosphate buffer, pH 7.3. Using a spectrophotometric assay,⁹ rates of hydrolysis of the test penicillins were determined at a concentration of 500 µg substrate/ml and at 240 nm for ampicillin, benzylpenicillin, and carbenicillin, and 263 nm for oxacillin. Hydrolysis of cephaloridine (250 µg/ml) was followed by measuring the loss of absorbance at 290 nm.

Results

MINIMUM INHIBITORY CONCENTRATIONS

The distribution of the minimum inhibitory concentrations of carbenicillin and ticarcillin against 438 isolates of *Ps aeruginosa* is illustrated in Table 1, which shows the cumulative percentage strains inhibited by the compounds. For comparison, the table also includes corresponding data reported for carbenicillin in 1967¹⁰ and for ticarcillin in 1970.⁵ Most strains were inhibited by concentrations readily achievable with recommended doses of the compounds. For instance, 85% of the strains were inhibited by 125 µg carbenicillin/ml and 87% by 50 µg ticarcillin/ml. Ticarcillin was twice as active as carbenicillin against half of the strains tested and four-fold more active against one-third of the strains (Table 2). In general, strains with a high level of

Table 1 Sensitivity of *Pseudomonas aeruginosa* to carbenicillin and ticarcillin

Antibiotic	Date of study	No. of strains	Cumulative % strains inhibited by (μg/ml)										
			≤1.0	2.5	5.0	10	25	50	125	250	500	1000	> 1000
Carbenicillin	1978/79	438	2	3	5	20	51	74	85	94	96	97	100
	1967*	206	2	3	4	7	38	82	93	100			
Ticarcillin	1978/79	438	5	10	30	64	77	87	93	94	96	97	100
	1970†	74	1	4	7	26	80	95	100				

*Reference 10.

†Reference 5.

Table 2 Relative activities of carbenicillin and ticarcillin against 438 strains of *Pseudomonas aeruginosa*

	-fold increase in activity of ticarcillin compared with carbenicillin*				
	0.5	1	2	4	8
No. of strains	8	48	214	155	13
%	2	11	49	35	3

*Calculated from $\frac{\text{Carbenicillin MIC}}{\text{Ticarcillin MIC}}$ for each strain.

resistance to carbenicillin (MIC > 1000 µg/ml) were equally resistant also to ticarcillin.

A comparison of MIC values of the penicillins against the current isolates with those reported originally for the compounds shows that there had been only a relatively small increase in the incidence of resistant strains (Table 1). Thus, in 1967, 7% of the strains tested were not inhibited by 125 µg carbenicillin/ml, while in 1978/79 15% of the strains were resistant to this concentration of carbenicillin. However, although the incidence of resistance was relatively unaltered, there was a change in that in 1967 all strains were inhibited by 250 µg carbenicillin/ml, whereas 6% of the current isolates were not sensitive to this concentration and 3% of the total were resistant to 1000 µg carbenicillin/ml. Biochemical studies revealed that the strains with a high level of resistance (MIC > 1000 µg/ml) possessed β-lactamases capable of destroying carbenicillin and ticarcillin (see below).

Although the overall incidence of resistance to carbenicillin (15%) and ticarcillin (13%) remained low there was considerable variation in the proportion of resistant strains in the individual hospitals (Table 3). In most cases the incidence of resistance

Table 3 Distribution of sensitive strains of *Pseudomonas aeruginosa*

Hospital	No. of strains tested	% Strains inhibited by	
		Carbenicillin 125 µg/ml	Ticarcillin 50 µg/ml
1	32	100	100
2	43	81	84
3	49	61	61
4	47	89	87
5	18	100	100
6	53	89	91
7	50	78	76
8	50	98	98
9	56	84	89
10	40	88	95
Total	438	85	87

lay between 10% and 20% of isolates, but in hospital 3 two-fifths of the strains were relatively insensitive to the penicillins. On the other hand, in two of the hospitals (1 and 5) all isolates tested were sensitive to carbenicillin and ticarcillin.

DISC SENSITIVITY TESTS

Data in Table 4 show the correlation between MIC values and the results of the antibiotic disc sensitivity tests. Overall, there was relatively good correlation between the results of the disc tests carried out in this study and the MIC values. With carbenicillin, 82% of the 365 strains sensitive to 125 µg carbenicillin/ml were classified as being sensitive to the 100 µg carbenicillin disc, and the remainder (save one strain) were classified as being moderately sensitive to the carbenicillin disc. The performance of the ticarcillin disc was slightly better in that 90% of the 372 strains inhibited by 50 µg ticarcillin/ml were considered to be sensitive to

Table 4 Correlation between results of serial-dilution (MIC) tests and antibiotic disc sensitivity tests

Hospital	Number of strains							
	Carbenicillin				Ticarcillin			
	MIC ≤ 125 µg/ml	100 µg disc			MIC ≤ 50 µg/ml	75 µg disc		
		S	MS	R		S	MS	R
1	31	20	11	0	32	29	3	0
2	34	25 (22)*	9	0	35	31	4	0
3	29	27 (21)	2	0	29	26	3	0
4	39	28 (29)	11	0	38	35	3	0
5	16	13 (13)	3	0	16	13	3	0
6	46	42 (3)	4	0	47	46	1	0
7	39	36 (32)	3	0	38	36	2	0
8	49	35 (40)	13	1	49	42	7	0
9	47	44 (30)	3	0	50	44	6	0
10	35	31 —	4	0	38	31	7	0
Total	365	301	63	1	372	333	39	0
		(82%) (64%)*				(90%)		

*Number of strains reported sensitive by hospital laboratory.

the 75 µg ticarcillin disc, the remaining 10% being classified as moderately sensitive. The results of the disc sensitivity tests reported by eight hospital laboratories showed that 190 of 253 strains inhibited by 125 µg carbenicillin/ml were reported to be sensitive to the 100 µg carbenicillin disc, an overall correlation of 64% correct results. There was considerable variation among the laboratories, ranging from 7% to 82% correlation between the disc tests and MIC values (Table 4).

β-LACTAMASES

The β-lactamases produced by 17 carbenicillin-resistant isolates (MIC > 125 µg/ml) are shown in Table 5. Identification was based upon Iso-Electric Focusing (IEF) characteristics and by substrate-profile data.⁸ All organisms (except strain 13) produced an inducible enzyme with a high isoelectric point (pI 7.9-8.8) and the six strains possessing this enzyme solely gave substrate profiles corresponding to that obtained with the Sabath type β-lactamase. The rest of the strains produced a variety of constitutive β-lactamases which were identified tentatively as the Dalglish enzyme, TEM 1, the isoxazolyl-hydrolysing enzymes OXA 1 and OXA 2, and enzymes in strains 9, 10, and 11 which might have been either RMS 139¹¹ or RPL 11,¹² as these latter have identical pIs (5.7) and similar substrate profiles. The six strains producing the Sabath β-lactamase exclusively were usually less resistant to carbenicillin (MIC 250-1000 µg/ml) than the strains that also possessed constitutive β-lactamases (MIC > 1000 µg/ml).

Discussion

In an early publication on carbenicillin from this

laboratory, it was reported that 94% of 206 strains of *Ps aeruginosa* isolated in the UK in 1967 were inhibited by 125 µg carbenicillin/ml.¹⁰ The results reported here show an apparent slight reduction in the incidence of sensitivity, 85% of the 438 strains of *Ps aeruginosa* tested being inhibited by 125 µg carbenicillin/ml, but there appears to have been no great increase overall in the incidence of carbenicillin-resistant strains of *Ps aeruginosa* in the UK since the introduction of the compound in 1967. This finding is in agreement with the results of a recent survey in four hospitals in the United States where 86% of 718 isolates of *Ps aeruginosa* were reported to be sensitive to 128 µg carbenicillin/ml.¹³ However, there was considerable variation from hospital to hospital, ranging from 39% of strains resistant in one hospital to 100% sensitivity in two hospitals, which may reflect the volume of usage and type of patient or infection treated.

The isolation of strains with high-level resistance to carbenicillin and possessing constitutive β-lactamases capable of inactivating the compound, in addition to the Sabath enzyme typical of this organism,⁸ was reported in 1970^{3, 4} but only one-fifth of the carbenicillin-resistant strains studied here were found to have enzymes of this type. The majority of strains classified as being resistant showed MIC values of 250-1000 µg carbenicillin/ml and possessed only the inducible Sabath enzyme, against which carbenicillin is relatively stable. In view of the small numbers of resistant strains isolated, demonstration of the presence of five different constitutive β-lactamases in the 17 strains studied is unexpected. The pattern of distribution appears to suggest that a single enzyme type predominated in the hospital of isolation but the numbers involved are very small.

Table 5 β-Lactamases produced by carbenicillin-resistant isolates of *Pseudomonas aeruginosa*

Hospital	Strain no.	Carbenicillin MIC (µg/ml)	Main band isoelectric point (pI)		Probable β-lactamase	
			1	2	1	2
2	1	> 1000	8.0	5.1	Sabath	Dalglish
	2	> 1000	8.0	5.1	Sabath	Dalglish
	3	> 1000	8.0	5.1	Sabath	Dalglish
3	4	1000	8.0	—	Sabath	—
	5	1000	8.5	—	Sabath	—
	6	1000	8.5	—	Sabath	—
4	7	> 1000	8.8	5.4	Sabath	TEM 1
6	8	250	8.0	—	Sabath	—
7	9	> 1000	8.2	5.7	Sabath	RMS 139
	10	> 1000	8.1	5.8	Sabath	or
	11	> 1000	8.1	5.8	Sabath	RPL 11
	12	500	8.0	7.4	Sabath	OXA 1
9	13	> 1000	—	5.4	—	TEM 1
	14	> 1000	8.6	—	Sabath	—
10	15	> 1000	8.4	8.1	Sabath	OXA 2
	16	> 1000	8.4	8.1	Sabath	OXA 2
	17	250	8.5	—	Sabath	—

It is of interest that the cultures tested here were supplied by 10 hospitals thought to have experienced an increase in the incidence of carbenicillin-resistant isolates of *Ps aeruginosa*, although in fact the overall level of resistance was found not to be high, being no more than 10-20% of the strains supplied by the majority of hospitals. It may be that the batches of isolates tested were unrepresentative, but it could also be that there are problems in sensitivity testing, as is evidenced by the fact that only 64% of the strains inhibited by 125 µg carbenicillin/ml were classified as being sensitive to carbenicillin on the basis of hospital disc sensitivity tests. There are difficulties in testing the sensitivity of *Ps aeruginosa* to carbenicillin as carbenicillin sensitivity discs are relatively unstable and readily inactivated unless the correct storage conditions are used,¹⁴ and a large inoculum yields small inhibition zones, possibly with apparently resistant colonies.¹⁵ Another factor that may contribute is that *Ps aeruginosa* NCTC 10662, the control strain recommended in the UK, may be more sensitive to carbenicillin than are many isolates that may be considered clinically sensitive (MIC ≤ 125 µg/ml). For instance, the culture of *Ps aeruginosa* NCTC 10662 used in this study was inhibited in eight out of 10 tests by 25 µg carbenicillin/ml, and consequently a number of strains with MIC values of 50-125 µg carbenicillin/ml were classified as being only moderately sensitive.

The opportunity was also taken in this study to test these recent clinical isolates for sensitivity to ticarcillin, and the results of the tests confirmed that ticarcillin was two to four times more active than carbenicillin against the majority of strains tested.⁵ Strains with an increased level of resistance to carbenicillin usually showed a similar increase in resistance to ticarcillin, and those strains with a high level of resistance to carbenicillin were equally resistant to ticarcillin. In the disc sensitivity tests, there was rather better correlation between minimum inhibitory concentrations and the results of the disc tests with the 75 µg ticarcillin disc than with the 100 µg carbenicillin disc, in keeping with reports from the USA.^{16 17}

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