SENSITIVITY OF RESPIRATORY STRAINS OF *PSEUDOMONAS AERUGINOSA* TO CARBENICILLIN

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THE minimal inhibitory concentration (MIC) of carbenicillin for *Pseudomonas aeruginosa* is generally found to be of the order of 25–50 µg per ml. Knudsen, Rolinson and Sutherland (1967) determined the MIC for 111 strains isolated from unspecified clinical material and found them to be 25 µg per ml in 31 instances and 50 µg per ml in 61 instances. Only six strains were inhibited by 12.5 µg per ml and none by concentrations lower than this. The MIC for 13 strains was 125 µg per ml or higher. Jones and Lowbury (1967) reported the MIC for 80 miscellaneous strains, mostly isolated from burns; it was 128 µg per ml for 11 strains, 64 µg per ml for 47 strains, 32 µg per ml for 21 strains and 1 µg per ml for one strain. The last strain (Elsworth) had been isolated from the sputum of one of our patients with bronchiectasis.

In the course of routine antibiotic sensitivity testing of strains of *P. aeruginosa* isolated from sputum, we noticed that the MIC of carbenicillin was often less than 6 µg per ml and sometimes as low as 0.7 µg per ml. The occurrence of such strains and their relationship to other strains with an "ordinary" MIC forms the subject of this report.

METHODS

Minimal inhibitory concentrations of carbenicillin were determined by making serial doubling dilutions of the antibiotic in nutrient broth and seeding these with *P. aeruginosa*. Two strengths of inoculum were tested for each strain—approximately 10^{-4} and 10^{-6} of an overnight broth culture. In most instances an identical MIC was obtained with both inocula but occasionally the light one gave a lower figure. In the results shown below the lowest MIC for each strain is quoted.

RESULTS

The distribution of the MIC of carbenicillin for 111 strains of *P. aeruginosa* is shown in the figure. The strains fall precisely into two groups: 72 (65 per cent. with "ordinary" MIC (25 µg per ml or higher), and 39 (35 per cent.) with "low" MIC (6 µg per ml or lower). Six strains were picked at random from each group and sent to Mr R. Sutherland of Beecham Research Laboratories, who confirmed our results.

The strains had been isolated from the sputum of 78 patients; and more than one isolate from the sputum of 25 of the patients was tested. Table I shows the clinical diagnosis of the patients and the MIC of carbenicillin for the

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first strain isolated from each patient. The proportion of strains with a low MIC does not differ significantly from that in the whole collection of strains.

FIGURE.—Distribution of the minimum inhibitory concentration (MIC) of carbenicillin for 111 strains of *Pseudomonas aeruginosa* isolated from sputum.

TABLE I

Minimum inhibitory concentration (MIC) of carbenicillin for strains of Pseudomonas aeruginosa isolated from the sputum of patients with various diseases of the chest

Clinical diagnosis	Number of patients*	Number (and percentage) of strains for which the MIC was	
		" low "†	" ordinary "†
Cystic fibrosis	48	13	35
Bronchiectasis	13	8	5
Chronic bronchitis	17	5	12
Total	78	26 (33)	52 (67)

* The results shown were obtained with the earliest isolate from each patient.

† See text.

TABLE II

Minimum inhibitory concentration (MIC) of carbenicillin for mucoid and non-mucoid strains of P. aeruginosa

Colonial morphology	Number of strains examined	Number (and percentage) of strains for which the MIC was	
		" low "	" ordinary "
Mucoid	49	15 (31)	34 (69)
Non-mucoid	62	24 (39)	38 (61)

Table II shows the MIC of carbenicillin for " mucoid " and " non-mucoid " strains of *P. aeruginosa*; its distribution is similar in the two groups. The occurrence of serum precipitins to *P. aeruginosa* was no different in patients

TABLE III
Carbenicillin sensitivity, source, and results of typing of 25 strains of *P. aeruginosa*

Strain number	Date of isolation	Location of patient*	MIC of carbenicillin†	Pyocine type	Serotype	Phage-typing pattern
1	11.3.69	1/Vic.	L	..	PA	16/44/F8/109/352/1214/Col. 11
2	26.1.69	Home	O	10	7	NT
3	24.1.69	1/Fou.	O	1/D	2A	7/68
4	3.7.69	...	O	1/F	9	31/44/68/F8/109/352/1214/Col. 11
5	10.3.69	1/Fou.	L	1/C	7	7/21/68
6	24.1.69	1/Lind	L	8	1	7
7	26.11.69	1/OPD	O	3	2A	21/44/F8/109/352/1214/M6
8	27.11.69	1/York	O	NT	13	31/44/F8/109/352/Col. 21
9	7.10.69	1/OPD	L	3	2A	NT
10	15.10.69	Home	L	5	PA	NT
11	16.10.69	1/OPD	O	3	2A	68/119X
12	11.12.69	1/Vic.	O	UC	1	16/44/F8/109/352/1214
13	11.12.69	Home	O	UC	8	44/68/F8/109/352/1214
14	24.12.69	1/OPD	O	UC	6	7/21/24/44/68/F7/F8/109/119X/352/1214/M4/Col. 11
15	2.1.70	1/OPD	O	UC	3	24/31/Col. 11
16	22.1.70	1/OPD	O	1/A	9	7/21/24/44/68/F7/F8/109/119X/352/1214/M4/Col. 11
17	22.1.70	1/Vic.	O	NT	3	44/F8/109
18	22.1.70	2	O	36	13	31/44/68/F8/119X/1214/M4
19	26.2.70	1/OPD	L	1/F	13	16/44/68/F8/109/352/1214/Col. 11
20	26.2.70	1/OPD	L	1/F	10	31/44
21	28.1.70	1/Vic.	O	1/C	2A/5C	44/68/109/352/1214
22	3.2.70	1/Vic.	L	UC	13	21/31/68/119X/352/Col. 18
23	5.3.70	3	L	3	PA	1214/M4/Col. 11
24	12.3.70	1/OPD	O	22	PA	16/21/31/44/68/73/F8/109/119X/352/1214
25	12.3.70	Home	L	1/B	7	

* Hospitals 1, 2 and 3; wards in hospital 1 indicated separately; OPD = out-patient department.

† L = "Low"; O = "ordinary".

NT = Untypable; UC = unclassifiable; PA = polyagglutinable.

from whose sputum strains with a low MIC were isolated than in those carrying strains with an ordinary MIC. In other words, there was no evidence that the virulence of the highly carbenicillin-sensitive strains differed from that of "normal" strains (Burns and May, 1968).

Fifty-eight strains were typed serologically by Habs' method and with bacteriophages at the Central Public Health Laboratory, Colindale, and 52 strains were pyocine-typed at the Department of Microbiology, Edinburgh University. No correlation was apparent between the degree of resistance to carbenicillin and the typing results. Indeed, the strains formed a remarkably heterogeneous collection, and observations of the dates of isolation, and the situation of the patients at the time, provided no evidence of cross-infection

TABLE IV
Relationship between the sensitivity of P. aeruginosa strains to carbenicillin and the presence or absence of enterobacteria in the sputum

Associated enterobacteria	Number of patients*	Number (and percentage) of strains for which the MIC was	
		"low"	"ordinary"
present	19	4 (21)†	15 (79)‡
absent	59	22 (37)	37 (63)

* The results shown were obtained with the earliest isolate from each patient.

† All patients had *Proteus* spp. in sputum.

‡ 7 patients had *Klebsiella*, 4 *Proteus*, 2 *Escherichia coli*, 1 *Enterobacter*, 1 *Citrobacter* in sputum.

between them. In table III are shown, for example, the MIC, the date of isolation and the results of typing for the first 25 strains in the series, together with the whereabouts of the patients when the sputum culture was made.

Table IV shows the frequency of strains with a low and with an ordinary MIC in patients with and without associated enterobacteria in their sputum. Although strains with an ordinary MIC were found more often in patients with enterobacteria in their respiratory tract than in those without, the difference was not statistically significant.

DISCUSSION

Mention in the literature of strains of *P. aeruginosa* that are as sensitive to carbenicillin as those described here is uncommon, and the explanation for our findings that immediately comes to mind is that our patients were the victims of a high rate of cross-infection by one or two strains with an exceptionally low MIC. However, the results of typing exclude this possibility (table III). Further inspection of the literature did not reveal any reports in which the MIC for strains isolated from chronic respiratory disorders was recorded separately. Strains isolated from urinary-tract infections (Brumfitt, Percival and Leigh, 1967) and infected burns (Jones and Lowbury, 1967),

conform in the distribution of the MIC to the pattern of the unspecified "clinical isolates" studied by Knudsen *et al.* (1967).

Our observations suggest that one-third of the strains inhabiting the respiratory tract of patients with such disorders as bronchiectasis and cystic fibrosis are, by currently accepted criteria, exceptionally sensitive to carbenicillin. Two possible explanations for this come to mind. First, the sensitive strains may be selectively favoured by the environment in the respiratory tract, but the lack of evidence that these strains are more virulent there than are strains with "ordinary" carbenicillin sensitivity does not support this possibility. Secondly, the comparative resistance of most strains of *P. aeruginosa* to carbenicillin might be determined by the presence of a plasmid, and this might sometimes be lost through exposure to a factor in the respiratory environment, in a manner analogous to the loss of the penicillinase plasmid by skin staphylococci (Noble, 1972). Examination of this hypothesis in detail would necessitate a study, not only of the influence of respiratory factors on the carbenicillin sensitivity of *P. aeruginosa*, but also of the relationship between carbenicillin sensitivity and transferable R-factors—or perhaps of "sensitivity" factors—in associated bacterial species elsewhere in the patients. As a preliminary observation we found no significant relationship between the carbenicillin sensitivity of the *P. aeruginosa* strain and the presence or absence of enterobacteria in the respiratory tract of the patients from whom they had been isolated.

SUMMARY

The MIC of carbenicillin for 111 strains of *Pseudomonas aeruginosa* isolated from the sputum of patients with chronic respiratory infections was estimated. For 72 strains it corresponded to that reported for strains isolated from non-respiratory sites (25–>100 µg per ml), but for 39 strains it ranged from 6 µg per ml to <0.7 µg per ml.

About half of the strains were typed serologically, by bacteriophages and by pyocine production. No evidence for cross-infection between the patients was found, nor was there any correlation between sensitivity to carbenicillin and any particular type of organism.

There was no evidence that strains with an abnormally low MIC were more virulent in the respiratory tract than were strains with an "ordinary" MIC; nor did it appear that coexistence with enterobacteria in the respiratory tract affected the carbenicillin resistance of *P. aeruginosa*.

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