Antibiotic sensitivity of *Proteus* species

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SYNOPSIS A study has been made of the antibiotic sensitivity pattern of 96 strains of *Proteus* isolated from clinical material and a further 29 strains kindly supplied by Dr. Patricia Carpenter. The results have been analysed in relation to the different species. The effect of electrolytes on the penicillin sensitivity of *Proteus* species has also been examined.

The chemotherapy of infection due to organisms of the *Proteus* group is often discussed as if the problem were the same for all species and indeed differentiation of the individual species is frequently not recorded in investigations of this nature. In fact the different species show marked differences in antibiotic sensitivity, and the present study was undertaken to investigate the problem more carefully.

Another point which seemed worth investigating was the effect of electrolytes on the antibiotic sensitivity of *Proteus* species. Both Naylor (1960a) and Sandys (1960) have recommended the use of an electrolyte-deficient medium to prevent the swarming of *Proteus*. Subsequently Naylor (1960b) reported that strains of *Proteus* species showed an increased sensitivity to penicillin when tested on such a medium.

MATERIALS AND METHODS

Ninety-six strains were isolated from specimens sent to a routine clinical bacteriological laboratory and as might be expected the majority of these (82) were strains of *Pr. mirabilis*; seven were *Pr. vulgaris* and seven *Pr. rettgeri*. In addition 12 strains of *Pr. morgani*, eight of *Pr. vulgaris*, and nine of *Pr. rettgeri* were kindly sent to us by Dr. Patricia Carpenter from the Central Public Health Laboratory.

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Identification of the different species was based on the scheme adopted by Dr. Carpenter at the Dysentery Reference Laboratory (unpublished). All strains were urease positive. Their other reactions are given in Table I.

ANTIBIOTIC SENSITIVITY TESTS The minimum inhibitory concentrations were estimated by the technique of serial dilution in agar and the inoculum, unless otherwise stated, was a standard 1 mm. loopful of approximately 1 in 500 dilution of an overnight broth culture.

Bactericidal tests were carried out by the cellophane transfer technique.

The cephalosporins used were kindly supplied by Glaxo Laboratories. They are all derivatives of cephalosporin C and have the nucleus 7-amino-cephalosporanic acid. Phenylacetyl cephalosporin has the same side chain as benzyl-penicillin. Cephalothin has a thienyl acetyl side chain. G/87/4 is the pyridine salt of cephalothin.

CULTURE MEDIA For most purposes the culture medium consisted of Lab-lemco 1%, peptone 1%, and NaCl 0.5% with or without 1.2% Davis New Zealand agar.

For testing the effect of electrolytes two basic electrolyte-deficient media were used: 1 Lab-lemco 0.3%, peptone 0.5% as recommended by Naylor (1960a) referred to as Lab-lemco 0.3% medium; 2 Lab-lemco-peptone, tryptone, and mannitol 0.4% each as recommended by Sandys (1960) referred to as Lab-lemco-tryptone-mannitol medium. When solid medium was required 1.2% agar was added to either.

TABLE I
REACTIONS OF STRAINS TESTED

	<i>Pr. vulgaris</i>	<i>Pr. mirabilis</i>	<i>Pr. rettgeri</i>	<i>Pr. morgani</i>
Glucose	AG ₁	AG ₁	A ₁	AG ₁
Maltose	AG ₁	—	—	—
Sucrose	AG ₁	(AG) ¹	v	—
Mannitol	—	—	A ₁	—
Indole	+	— ¹	+	+
Gelatin	+	+	—	—
Swarming at 37°C.	+	+	—	—

A = acid G = gas 1 = in one day () = delayed v = variable ¹ = occasional strains atypical

RESULTS

INITIAL SCREENING OF STRAINS FROM THE ROUTINE LABORATORY The 96 strains isolated from the routine laboratory, together with six strains of *Pr. morgani* from Dr. Carpenter, were tested for sensitivity to various antibacterial agents by the agar cup method. The concentration of each drug used was high, but except in the case of novobiocin represents the concentration likely to be obtained in the urine after systemic administration. The results are given in Table II. The majority of the strains (82) were *Pr. mirabilis* and 60 of these were sensitive to the high concentration of benzylpenicillin, whereas all strains of other species were resistant. All 102 strains were sensitive to neomycin, and probably would have been to kanamycin had this been included. A high proportion of strains were also sensitive to sulphonamides. Furadantin was relatively inactive, except against strains of *Pr. morgani*.

GENERAL PATTERN OF SENSITIVITY Representative strains of each species were tested for sensitivity to

the various antibiotics most likely to be effective, by serial dilution in routine Lab-lemco agar medium. The results are given in Table III. All strains of all species were sensitive to from 1 to 8 µg./ml. kanamycin and from 0.5 to 16 µg./ml. neomycin. With streptomycin some strains of all species were sensitive to 4 µg./ml. or less, but other strains of each species were highly resistant. With the other antibiotics tested different results were obtained with the different species. Thus all strains of *Pr. vulgaris* were inhibited by from 4 to 68 µg./ml. chloramphenicol, 4 to 32 µg./ml. tetracycline, and 2 to 128 µg./ml. novobiocin. The strains of *Pr. mirabilis* were similar in their sensitivity to chloramphenicol but tended to be slightly more resistant to tetracycline and novobiocin. The strains of *Pr. morgani* and *Pr. rettgeri* gave very variable results, and the majority were highly resistant to these three antibiotics.

SENSITIVITY TO THE PENICILLINS The minimum inhibitory concentration of seven penicillins and two cephalosporins for the different species when tested with the standard moderately small inoculum are shown in Table IV. All strains of *Pr. vulgaris* and

TABLE II
SENSITIVITY OF *PROTEUS* SPP. TO CONCENTRATIONS OF DRUGS OBTAINABLE IN THE URINE

Concentration in Cup	No. of Strains	Penicillin (100 units/ml.)	Streptomycin (250 µg./ml.)	Tetracycline (250 µg./ml.)	Neomycin (250 µg./ml.)	Chloramphenicol (250 µg./ml.)	Novobiocin ¹ (50 µg./ml.)	Sulphonamides (250 µg./ml.)	Furadantin (200 µg./disc.)
<i>Pr. mirabilis</i>	82								
Sensitive		60	73		82	80	45	52	19
Slightly sensitive		12		9		2	36	13	58
Resistant		10	9	73			1	17	5
<i>Pr. vulgaris</i>	7								
Sensitive			6	3	7	7	1	4	
Slightly sensitive				2			4	1	3
Resistant		7	1	2			2	2	4
<i>Pr. rettgeri</i>	6								
Sensitive					6				
Slightly sensitive						2			
Resistant		6	6	6		4	6	6	6
<i>Pr. morgani</i>	7								
Sensitive			3	2	7	4	2	3	5
Slightly sensitive		1					2		2
Resistant		6	3	5		3	3	4	

¹This concentration of novobiocin is not likely to be obtained in the urine.

TABLE III
ANTIBIOTIC SENSITIVITY PATTERN OF *PROTEUS* SPP.¹

	No. of Strains	Streptomycin	Neomycin	Kanamycin	Chloramphenicol	Tetracycline	Novobiocin	Benzylpenicillin	Ampicillin	Cephalothin
<i>Pr. mirabilis</i> a	8	4-8	2-8	4-8	4-16	32-64	8-32	8-32	2-8	4
<i>Pr. mirabilis</i> b	6	4->256	4-16	4-8	4-16	32-64	8-32	>256	>256	4
<i>Pr. vulgaris</i>	15	2->512	0.5-8	1-8	4-256	2-64	8-256	16-512	16-512	64->256
<i>Pr. rettgeri</i>	15	2->256	2-16	1-4	8->256	32->256	64->256	4->256	2->256	2->256
<i>Pr. morgani</i>	12	4->256	1-8	2-4	4-256	4-64	8->256	128->256	128->256	256->256

¹Minimum inhibitory concentration with a moderate inoculum

TABLE IV

SENSITIVITY OF *PROTEUS* SPECIES TO THE PENICILLINS¹

Species	No. of Strains	Benzylpenicillin	Phenoxy-methyl Penicillin	Phen-ethicillin	Phen-benicillin	Ampicillin	Methicillin	Cloxacillin	Cephalosporins	
									Phenyl-acetyl	Cephalothin
<i>Pr. mirabilis</i> a	8	8-32	32-128	128->500	128-256	2-8	128-500	256->500	16	4
<i>Pr. mirabilis</i> b	6	>500	>500	>500	>500	>256	>500	>500	16	4
<i>Pr. vulgaris</i>	15	64->500	>500	>500	128->500	16->512	256->500	500->500	128->500	64->256
<i>Pr. rettgeri</i>	15	4->500	16->500	16->500	16-256	2-500	32->500	32->500	2->256	2->256
<i>Pr. morgani</i>	12	128->500	>500	256->500	64-256	64->500	256->500	500->500	>256	>256

¹Minimum inhibitory concentration with a moderate inoculum.

Pr. morgani were resistant to all the penicillins and the cephalosporins. With *Pr. rettgeri*, using this inoculum, the minimum inhibitory concentration of benzylpenicillin and ampicillin ranged from 2 or 4 to 512 µg./ml. However, with a large inoculum all strains of *Pr. rettgeri* were resistant to 100 µg./ml. or more and after only one passage in penicillin all strains were highly resistant even when tested with the standard inoculum. Different strains of *Pr. rettgeri* showed similar variations in sensitivity to the cephalosporins, but sensitive strains developed resistance to these antibiotics rather less readily.

Strains of *Pr. mirabilis* fell into two distinct groups in relation to penicillin sensitivity. Eight of the strains (group a) were sensitive to from 8 to 32 µg./ml. benzylpenicillin and 2 to 8 µg./ml. ampicillin. The remaining six strains (group b) tested were highly resistant to both these penicillins. All 14 strains, regardless of their sensitivity to penicillin, were inhibited by 16 µg./ml. phenyl acetyl cephalosporin and 4 µg./ml. cephalothin.

EFFECT OF INOCULUM SIZE ON PENICILLIN SENSITIVITY
The sensitivity to benzylpenicillin and cephalothin of a number of strains of each species was compared using the standard inoculum and an inoculum 500 times larger. The fold increase in resistance obtained with the large inoculum was as follows:—

	Benzylpenicillin	Cephalothin
<i>Pr. mirabilis</i> a	0.2	0.2
<i>Pr. mirabilis</i> b	32	4
<i>Pr. vulgaris</i>	32	8-32
<i>Pr. rettgeri</i>	2	16
<i>Pr. morgani</i>	0.8	2.4

These results are probably related to penicillinase activity (Ayliffe, 1963). Thus with *Pr. mirabilis* strains of group 'a' do not produce penicillinase and the effect of inoculum size is negligible. Strains of group 'b' produce a penicillinase, which is moderately active against benzylpenicillin, but has only slight activity against cephalothin, and this is reflected in a 32-fold increase in resistance of a

large inoculum with penicillin, but only a four-fold difference with cephalothin.

Strains of *Pr. vulgaris* and *Pr. morgani* produce an enzyme which is moderately active against penicillins and cephalosporins, and the effect of inoculum was similar with both antibiotics although less marked with *Pr. vulgaris* than with *Pr. morgani*. With *Pr. rettgeri* penicillinase activity is rather less against penicillins than it is against cephalosporins (Ayliffe, personal communication) and the effect of inoculum was greater with cephalothin than with benzylpenicillin.

BACTERICIDAL TESTS These are grouped as follows:

Streptomycin and kanamycin In tests with streptomycin-sensitive strains a concentration of 32 µg./ml. of either antibiotic almost completely sterilized all species in 18 hours and killed the majority of cells of all species in seven hours. A concentration of 8 µg./ml. caused a substantial reduction in the inoculum in 18 hours.

Penicillin and ampicillin With group 'a' strains of *Pr. mirabilis* a concentration of 32 µg./ml. benzylpenicillin or 16 µg./ml. ampicillin killed the majority of cells in 18 hours but even with concentrations as high as 512 and 256 µg./ml. respectively there were large numbers of survivors after seven hours.

Cephalothin With both group 'a' and 'b' strains of *Pr. mirabilis* cephalothin had a similar bactericidal activity to that of ampicillin against group 'a' strains.

EFFECT OF ELECTROLYTES

EFFECT OF SALT ON SENSITIVITY OF VARIOUS BACTERIAL SPECIES TO VARIOUS ANTIBIOTICS Strains of all species were tested for sensitivity to streptomycin, neomycin, kanamycin, chloramphenicol, tetracycline, and novobiocin in 0.3% Lab-lemco with and without 0.5% sodium chloride. The results are summarized in Table V, together with those obtained with a number of bacteria of unrelated species tested under similar conditions. It will be seen that the

absence of salt caused an increase in sensitivity to streptomycin, neomycin, and kanamycin with all bacteria tested. This depressant effect of salt on the action of streptomycin is a well known, if not wholly explained, phenomenon (see Klein and Kimmelman, 1946; Berkman, Henny, Housewright, and Henry, 1948). Only *Proteus* species showed an increase in sensitivity to penicillin in the absence of salt. No such increase was noted with chloramphenicol, tetracycline, and novobiocin with any of the bacteria tested.

EFFECT ON PENICILLIN SENSITIVITY OF *PROTEUS* SPECIES A penicillin-sensitive strain of *Pr. mirabilis* was tested for sensitivity to benzylpenicillin, ampicillin, streptomycin, and chloramphenicol by the agar-cup diffusion method, on 0.3% Lab-lemco agar with and without sodium chloride, on routine 1.0% Lab-lemco plus horse blood alone and plus additional agar, and on McConkey agar. It will be

seen from Table VI that on the salt-free medium the diameters of inhibition with all the antibiotics, except chloramphenicol, were significantly larger than those on any of the other media. No significant differences were caused by the presence or absence of horse blood, or by the addition of 6% agar or by use of McConkey agar.

The same strain of *Pr. mirabilis* was tested for sensitivity to benzylpenicillin and ampicillin by the serial dilution method in 0.3% Lab-lemco broth with or without salt, in horse serum, and in normal human urine containing 1% glucose. It will be seen from Table VII that the absence of salt caused a four-fold increase in sensitivity to both penicillins and that in urine (which has a high salt content) the organisms were four times more resistant than in Lab-lemco agar with 0.5% salt. Results in serum were similar to those in Lab-lemco plus salt in the case of ampicillin, but there was a two-fold decrease in sensitivity with benzylpenicillin.

TABLE V
FOLD INCREASE IN SENSITIVITY TO VARIOUS ANTIBIOTICS IN 0.3% LAB-LEMCO WITHOUT ADDED NaCl

	Penicillin	Ampicillin	Streptomycin	Neomycin	Kanamycin	Chloramphenicol	Tetracycline	Novobiocin
<i>Pr. mirabilis</i>	4	8	8	4	8	2	0	0
<i>Pr. vulgaris</i>	8	8	8	4	8	0	0	0
<i>Pr. rettgeri</i>	4	2	Not tested	4	8	2	0	0
<i>Pr. morgani</i>	8	8	4	4	4	2	0	0
<i>Esch. coli</i>	0	2	4	4	4	2	0	Not tested
<i>Klebsiella</i>	0	0	4	4	4	0	0	Not tested
<i>Salm. typhimurium</i>	2	2	4	4	4	0	2	Not tested
<i>Salm. paratyphi B</i>	0	2	4	8	2	0	0	Not tested
<i>Salm. typhi</i>	0	0	4	8	2	0	0	Not tested
<i>Staph. aureus</i>	0	0	4	4	4	0	2	0
<i>Str. faecalis</i>	0	0	4	4	2	0	0	0

TABLE VI
SENSITIVITY OF *PR. MIRABILIS* TO FOUR ANTIBIOTICS ON VARIOUS MEDIA BY AGAR-CUP METHOD

Basic Medium	Salt (%)	Agar (%)	Blood (%)	Diameter of Zones of Inhibition (mm.)			
				Penicillin (50 µg./ml.)	Ampicillin (50 µg./ml.)	Streptomycin (200 µg./ml.)	Chloramphenicol (250 µg./ml.)
Lab-lemco 0.3%	0	1.2	0	29	26	28	26
Lab-lemco 0.3%	0.5	1.2	0	22	19	23	24
Lab-lemco 1.0%	0.5	1.2	5	20	19	24	24
Lab-lemco 1.0%	0.5	6.0	5	19	17	23	24
McConkey	0.5	1.2	0	18	17	23	25

TABLE VII
SENSITIVITY OF *PR. MIRABILIS* TO BENZYLPENICILLIN AND AMPICILLIN IN VARIOUS MEDIA BY SERIAL DILUTION METHOD (MODERATE INOCULUM)

Basic Medium	Salt (%)	Minimum Inhibitory Concentration (μg./ml.)												
		Benzylpenicillin						Ampicillin						
		16	8	4	2	1	0.5	8	4	2	1	0.5	0.25	0.12
Lab-lemco 0.3%	0	—	—	—	—	—	++	—	—	—	—	—	—	++
Lab-lemco 0.3%	0.5	—	—	—	+	++	++	—	—	—	—	++	++	++
Horse serum	—	—	—	++	++	++	++	—	—	—	—	++	++	++
Urine + 1% glucose	—	—	++	++	++	++	++	—	++	++	++	++	++	++

TABLE VIII

SENSITIVITY OF *PROTEUS* SPECIES TO BENZYLPENICILLIN BY SERIAL DILUTION IN LAB-LEMCO BROTH WITH AND WITHOUT VARIOUS ELECTROLYTES

Minimum Inhibitory Concentration ($\mu\text{g./ml.}$)

	No Salt	Sodium Chloride	Ammonium Phosphate	Magnesium Sulphate	Potassium Chloride	Potassium Sulphate	Sodium Citrate
<i>Pr. mirabilis</i>	1	4	2	4	4	4	2
<i>Pr. vulgaris</i>	8	32	8	32	32	32	16
<i>Pr. morgani</i>	32	256	64	32	512	256	256
<i>Pr. rettgeri</i>	128	512	64	128	256	512	256
Swarming	(+)	++	+	+	++	++	+

The effect of other electrolytes on the sensitivity of *Proteus* to benzylpenicillin was next studied. One strain of each of the four species was tested by the serial dilution method in Lab-lemco-tryptone-mannitol broth without salt, and with 0.5% of various electrolytes. The results are given in Table VIII from which it will be seen that with all four species the minimum inhibitory concentration in the absence of any added salt was only one quarter that in the presence of sodium chloride. Potassium chloride and potassium sulphate had a similar effect to that of sodium chloride. Intermediate results were obtained with magnesium sulphate, sodium citrate, and ammonium phosphate.

DISCUSSION

SENSITIVITY PATTERN OF *PROTEUS* SPECIES The sensitivity pattern is discussed in relation to the streptomycin group, the penicillins, and the cephalosporins.

Streptomycin group The streptomycin group of antibiotics are the most active agents against all species of *Proteus*. Many strains of all species were found to be resistant to streptomycin itself, but all strains tested were sensitive to from 0.5 to 16 $\mu\text{g./ml.}$ neomycin or from 1 to 8 $\mu\text{g./ml.}$ kanamycin. Strains of *Pr. mirabilis* tended to be slightly less sensitive to neomycin and kanamycin than those of other species. This was noted by Potee, Wright, and Finland (1954) in relation to neomycin. These antibiotics were also bactericidal in concentrations not much higher than the minimum bacteriostatic concentrations.

Penicillins With the penicillins the position is complicated by the fact that resistance is dependent both on an increased tolerance to the unchanged penicillin and to penicillin inactivation by penicillinases. Partly for this reason and partly because individual strains may contain a few highly resistant cells, the results obtained are dependent on the size

of inoculum. Our results confirm the finding of Potee *et al.* (1954) that only strains of *Pr. mirabilis* are sufficiently sensitive to the penicillins to be likely to respond to penicillin therapy. The strains of *Pr. mirabilis* tested by us fell clearly into two groups. One group was sensitive to from 2 to 8 $\mu\text{g./ml.}$ ampicillin, regardless of the size of inoculum used, whereas strains of the other produced a penicillinase and were highly resistant to all the penicillins. As will be seen from the initial screening results shown in Table II, the majority of strains from clinical sources belong to the former group.

Cephalosporins It is of interest that the results obtained with the two cephalosporins tested differ from those with the penicillins and suggest that the penicillinases of different species of *Proteus* differ in their action on the two groups of antibiotics. Thus all 16 strains of *Pr. mirabilis* were sensitive to the cephalosporins, whether or not they produced penicillinase, whereas strains of *Pr. rettgeri* appeared to be even more resistant to the cephalosporins than to the penicillins.

Although both the penicillins and the cephalosporins are inactivated by bacteria by hydrolysis of the β -lactam ring, it has been shown that 'penicillinase' and 'cephalosporinase' activity are not necessarily associated and may possibly be due to different enzymes (Newton and Abraham, 1956; Crompton, Jago, Crawford, Newton, and Abraham, 1962). In a recent report on the distribution of cephalosporinase activity among different bacterial species Fleming, Goldner, and Glass (1963) record that strains of *Pr. mirabilis* were consistently negative, whereas *Pr. morgani* were consistently positive, and *Pr. vulgaris* and *Pr. rettgeri* variable.

EFFECT OF ELECTROLYTES Naylor (1960b) suggested that the increase in penicillin sensitivity of *Proteus* species on an electrolyte-deficient medium and the prevention of swarming are related phenomena. The results recorded here do not support this suggestion, since there was no corresponding increase in sen-

sitivity on other media which inhibit swarming (*e.g.*, 6% agar, McConkey medium). Our results suggest that penicillin is more actively bacteriostatic against *Proteus* species in the absence of electrolytes. Whatever the cause of the phenomenon, salt-free media are unsuitable for penicillin-sensitivity tests of these organisms. No similar phenomenon was noted with bacteria of other genera and the penicillins, or with other antibiotics, except for the streptomycin group, the activity of which against all bacteria is depressed by sodium chloride.

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