Gram-negative bacilli in burns

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SYNOPSIS In a period of two years, 865 strains of Gram-negative bacilli other than *Pseudomonas aeruginosa* isolated from burns were identified by a range of tests. The commonest species were *Proteus mirabilis*, *Escherichia coli*, and *Enterobacter cloacae*. Many strains of *Klebsiella aerogenes*, *Enterobacter aerogenes*, and *Bacterium anitratum* were also found.

A large proportion of the strains were tested for sensitivity to nalidixic acid, ampicillin, kanamycin, chloramphenicol, tetracycline, and carbenicillin, and smaller numbers of strains were tested for sensitivity to cephaloridine, polymyxin, streptomycin, sulphadiazine, sulfamylon, and trimethoprim. The proportion of strains sensitive and resistant to different antibacterial agents varied widely with species of bacteria. A large proportion of the strains of *E. coli* and *P. mirabilis* were resistant to ampicillin, which was much used in treatment; resistance appeared least often towards nalidixic acid, kanamycin, trimethoprim, and gentamicin. Multiple resistance occurred less often among strains of *E. coli* than among *Klebsiella* spp, *Enterobacter* spp, and *P. mirabilis*.

Phage and serological typing of *Ps. aeruginosa* showed that most infections of burns with this organism were due to strains previously found in other patients in the same ward. Taken with other evidence, this supported the view that most infections with *Ps. aeruginosa* were not acquired from the patient's own flora but from sources in the hospital environment.

Gram-negative rods are known to be among the commonest types of bacteria isolated from burns (Colebrook, Clark, Gibson, and Todd, 1944; Langohr, Owen, and Cope, 1947; Jackson, Lowbury, and Topley, 1951; Liedberg, Reiss, Kuhn, Amspacher, and Artz, 1955). The special importance of *Pseudomonas aeruginosa* has been widely reported (eg, Jackson *et al.*, 1951; Markley, Gurmendi, Chavez, and Bazan, 1957; Tumbusch, Vogel, Butkiewicz, Graber, Larson, and Mitchell, 1961; Jones, Jackson, and Lowbury, 1966), but other Gram-negative bacilli are also commonly found in burns. Some of these are potentially important human pathogens, especially in the urinary and respiratory tracts, and occasionally they are the cause of invasive infection and bacteraemic shock. Infection with these organisms is reported to have increased during the era of antibiotics (Finland, 1960; Watt and Okubadejo, 1967). Following the successful control of infection with *Ps. aeruginosa*, other Gram-negative bacilli have been a commoner cause than *Ps. aeruginosa* of septicaemia and death in severely burned patients (Cason and Lowbury, 1968).

Ps. aeruginosa forms a homogeneous and easily identified 'cluster' of bacteria (Liston, Wiebe, and Colwell, 1963) and *Proteus* can be subdivided by a

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few simple tests into species, but the identification of other Gram-negative bacilli requires more elaborate study and the conclusions are sometimes uncertain. For this reason strains isolated on routine examination of burn or wound flora have commonly been grouped together as 'coliform bacilli'.

We report here the identification and antibiotic sensitivity patterns of a series of Gram-negative bacilli isolated from burns of patients in the hospital over a period of two years; this series includes strains of *Proteus* spp but not of *Ps. aeruginosa*, the incidence of which in the same period, under various conditions of treatment, has been reported elsewhere (Cason, Jackson, Lowbury, and Ricketts, 1966; Lowbury and Jackson, 1968). We include, however, a note on the types of *Ps. aeruginosa* present in the burns wards during the period of study, since a large number of these but not of other Gram-negative bacilli were typed, allowing some discussion on the probable sources and routes of transfer of *Ps. aeruginosa*.

MATERIALS AND METHODS

SOURCE OF BACTERIAL STRAINS Burns were examined for bacterial flora by methods described elsewhere (Lowbury, 1960; Brown and Lowbury, 1965; Cason *et al.*, 1966).

Swabs moistened with peptone water were used for sampling, and samples were taken on admission, at all changes of dressings and operations, and daily from burns treated by the exposure method.

METHODS OF ISOLATION Swabs were inoculated on horse blood agar made with infusion broth, containing concentrated (4%) New Zealand agar to prevent swarming of *Proteus*, also on improved cetrimide agar (Brown and Lowbury, 1965), and into Robertson's cooked meat medium. After overnight incubation at 37°C, the blood agar plates were examined for aerobic growth and one colony of every colonial form was picked for confirmatory tests and for morphological examination of a Gram-stained film; a similar examination was made of subcultures from cooked meat medium to blood agar.

A rough sorting of Gram-negative bacilli was made by examining blood agar and cetrimide agar plates under ultraviolet irradiation for growth and fluorescence (Lowbury, Lilly, and Wilkins, 1962) and by tests for oxidase production (Kovacs, 1956); fluorescent, oxidase-producing growth was further examined for characters of *Ps. aeruginosa* (Brown and Lowbury, 1965). Urease tests were made for presumptive recognition of *Proteus* spp. One isolate of every colony form of Gram-negative bacilli from each patient per week was selected for further examination to determine the species of *Proteus* and, when possible, the genus or the genus and species of other types of Gram-negative bacilli.

METHODS OF IDENTIFICATION Colonies of Gram-negative bacilli picked from blood agar plates were subcultured to a second blood agar plate and to a spot-plate of cetrimide agar. Strains which failed to grow at 37°C or to give yellow-green fluorescence on cetrimide agar and which gave a negative oxidase reaction, that is, those which were not classed as *Ps. aeruginosa* or presumptive *Ps. aeruginosa*, were examined, in subcultures from colonies on blood agar, by the methods and identified according to the criteria described by Cowan and Steel (1961, 1965); similar tests were used for oxidase-producing strains which failed to grow on cetrimide agar or to produce pigments typical of *Ps. aeruginosa*.

Tests were made for the following characters: (1) urease production in six hours on Christensen's medium; (2) phenyl alanine deaminase (PPA) and malonate utilization, using the combined malonate-phenyl alanine medium of Shaw and Clarke (1955); (3) gelatinase production, using gelatin-charcoal discs (Oxoid) incubated for seven to 10 days (Kohn, 1953); (4) H₂S production by lead acetate papers over peptone water cultures examined after 48 hours; (5) indole production by the use of ether and Ehrlich's reagent; (6) fermentation of glucose, mannite, maltose, dulcitol, and sucrose in two to three days; (7) oxidation-fermentation test (Hugh and Leifson, 1953); (8) gluconate oxidation (Shaw and Clarke, 1955); (9) citrate utilization (Koser's and Simmons' methods); (10) motility, by hanging drop and by stab inoculation in semi-solid agar; (11) methyl red and Voges-Proskauer reactions (Barritt's method) after two to three days' incubation at 37°C; (12) growth in KCN broth (Rogers

and Taylor, 1961); (13) nitrate reduction in four to seven days; and (14) arginine, lysine, and ornithine decarboxylase tests (Møller, 1955).

A composite table, based largely on the tables of Cowan and Steel (1961, 1965), was used for identification of strains. Those classified as *Proteus* spp (including *Providencia* or *Proteus inconstans*) because they gave a strong urease reaction on Christensen's medium in six hours were allocated to species on the basis of findings in tests 1 to 6.

ANTIBIOTIC SENSITIVITY TESTS Strains were tested by a ditch plate diffusion method that has been used in this Unit for many years (Topley, Lowbury, and Hurst, 1951), with a sensitive control organism (*Staph. aureus*, Oxford strain) on each plate; in tests with nalidixic acid a sensitive strain of *E. coli* was used as the control. Sensitivity to ampicillin, carbenicillin, cephaloridine, streptomycin, kanamycin, gentamicin, tetracycline, chloramphenicol, nalidixic acid, sulphadiazine, and trimethoprim was tested by this method. Ampicillin, carbenicillin, and sulphadiazine were included in the agar of the ditch at 100 µg per ml, trimethoprim lactate at 10 µg per ml (prepared with a solution heated to 60° to 70°C), and the other agents at 50 µg per ml. A loopful of overnight broth culture was streaked across the plate at right angles to the ditch; the culture was undiluted except in tests with sulphadiazine and trimethoprim, for which a 1 in 250 dilution was used. Ditch plates were examined after 18 hours' incubation at 37°C and the result was assessed as 'sensitive' (zone of inhibition similar to that of control), 'moderately sensitive' (reduced zone of inhibition), and 'resistant' (growth up to or across the ditch).

To assess the significance of these tests and adjust antibiotic concentration in the ditch where necessary, a selection of strains of each identified type and of some unidentified species were tested for sensitivity to these agents also by a tube dilution method (or, in the case of sulphadiazine and of trimethoprim, by a plate dilution method), with an inoculum in the tube dilution test of one drop of a 1 in 1,000 dilution and in the plate dilution test of a small loopful of 1 in 250 dilution of an overnight broth culture.

Because of the unsuitability of diffusion tests for sensitivity to polymyxin (Bushby, 1955), strains were tested for sensitivity to this antibiotic by a plate dilution test. The same method was used for tests of a proportion of the strains with sulfamylon (p. amino methyl benzene sulphonamide hydrochloride). The 'sensitivity' was assessed in relation to possible value in therapy for infections of soft tissues by antibiotics given orally or parenterally.

With the exception of trimethoprim and sulphadiazine, antibacterial agents were added to nutrient agar for plate dilution tests and to peptone water agar for ditch plate tests. Trimethoprim and sulphadiazine hydrochloride were added, for these tests, to lysed blood agar with infusion broth.

Some strains were tested for penicillinase production, using benzyl penicillin, ampicillin, and carbenicillin as substrates; 2 ml amounts of 24-hour broth cultures were incubated at 37°C with 1 ml of an aqueous solution of

TABLE I

IDENTIFICATION OF 865 STRAINS OF GRAM-NEGATIVE BACILLI (EXCLUDING *Ps. aeruginosa*) ISOLATED FROM BURNS (SEPTEMBER 1965 TO SEPTEMBER 1967)

Organism	No. of Strains	Percentage of Total Strains	Organism	No. of Strains	Percentage of Total Strains
<i>Proteus</i> spp			<i>Enterobacter cloacae</i>	119	13.8
<i>P. mirabilis</i>	280	32.4	<i>Enterobacter aerogenes</i>	54	6.2
<i>P.morganii</i>	17	2.0	<i>Enterobacter</i> spp	6	0.7
<i>P. vulgaris</i>	10	1.2	<i>Klebsiella aerogenes</i>	58	6.7
<i>P. inconstans</i> (Providencia)	38	4.4	<i>Bacterium anitratum</i>	37	4.3
Unclassified <i>Proteus</i> strains	15	1.7	<i>Serratia</i>	14	1.6
Other Gram-negative bacilli ('coliform bacilli')			<i>Alkalescens dispar</i>	14	1.6
<i>Escherichia coli</i>	153	17.7	<i>Citrobacter</i>	9	1.0
			<i>Alcaligenes</i>	5	0.6
			Unclassified coliform bacilli	36	4.2

TABLE IIa

SENSITIVITY TO SEVEN ANTIBIOTICS BY DITCH PLATE TEST ON 730 STRAINS OF GRAM-NEGATIVE BACILLI FROM BURNS (SEPTEMBER 1965 TO SEPTEMBER 1967)

Strain	Percentage Strains										
	Nalidixic Acid			Ampicillin			Kanamycin			Chloramphenicol	
	S ¹	'S' ²	R ³	S	'S'	R	S	'S'	R	S	'S'
<i>Proteus mirabilis</i>	96.7	3.3	0	3.2	1.8	95	99.1	0	0.9	79.6	6.1
<i>Proteus vulgaris</i>	100	0	0	55	0	45	100	0	0	100	0
<i>Proteus morganii</i>	100	0	0	5.9	0	94.1	100	0	0	5.9	5.9
<i>Proteus</i> unclassified	100	0	0	0	0	100	100	0	0	100	0
<i>Providencia</i>	96.9	0	3.1	0	0	100	87.5	0	12.5	43.7	9.4
<i>Escherichia coli</i>	98.1	1.3	0.6	6.3	0	93.7	95.6	0.6	3.8	88.7	3.8
<i>Enterobacter cloacae</i>	94.6	2.7	2.7	2.7	0	97.3	100	0	0	90	2.7
<i>Enterobacter aerogenes</i>	93.9	2	4.1	14.3	0	85.7	100	0	0	96	2
<i>Klebsiella aerogenes</i>	84.8	4.3	10.9	2.2	0	97.8	100	0	0	66.6	0
<i>Bact. anitratum</i>	66.7	23.3	10	0	0	100	86.7	0	13.3	10	0
<i>Serratia</i>	100	0	0	14.3	0	85.7	100	0	0	100	0
<i>Alkalescens dispar</i>	100	0	0	0	0	100	83.3	0	16.7	100	0
<i>Alcaligenes</i>	100	0	0	0	0	100	20	0	80	60	40
<i>Citrobacter</i>	87.5	12.5	0	0	0	100	100	0	0	87.5	0
Unclassified	100	0	0	15.8	0	84.2	100	0	0	94.7	0
Mean percentage of total	94.8	3.15	2.05	5.5	0.5	94	96.8	0.1	3.1	78.9	4.0

¹S=sensitive, ²'S'=moderately sensitive, ³R=resistant

benzyl penicillin (100 units), ampicillin (100 µg), and carbenicillin (100 µg). After one and a half hours the mixtures were transferred to Heatley cylinders on plates seeded with a 1 in 1,000 dilution of a broth culture of the Oxford strain of *Staph. aureus*. Absence or reduction of zones of inhibition around cylinders after overnight incubation of the plates was taken to indicate inactivation of the antibiotic by a penicillinase.

TYPING OF *Ps. aeruginosa* Many strains of *Ps. aeruginosa* were typed by phage and serological methods at the Central Public Health Laboratory, Colindale, using techniques described elsewhere by Thom (see Lowbury, Thom, Lilly, Babb, and Whittall, 1969).

RESULTS

Table I shows the results of identification of a series of 865 strains of Gram-negative bacilli, excluding *Ps. aeruginosa*, isolated from burns in this hospital between September 1965 and September 1967.

Proteus was the genus most frequently isolated, and the commonest species, *P. mirabilis*, was more common than any of the other Gram-negative bacilli. Next in frequency was *Escherichia coli*. Only 51 (5.9%) of the strains (including 15 described as *Proteus* spp) could not be classified.

Table IIa shows the proportion of 730 of the strains listed in Table I which were sensitive, moderately sensitive, and resistant to seven antibiotics or chemotherapeutic agents by ditch-plate tests. The same data in respect of smaller numbers of four other antibiotics are shown in Table IIb. The interpretation of these tests is shown in Table III, which summarizes the results of sensitivity tests done in parallel by ditch plate and tube dilution methods on a large number of strains, including a representative sampling of each genus or species; the range of minimum inhibitory concentrations (MIC) corresponding to ditch plate readings S, 'S', and R was narrower in relation to individual species tested

than in the collective results shown in Table III. A larger proportion of strains was sensitive to kanamycin, nalidixic acid, trimethoprim, and gentamicin than to the other agents; the lowest proportion of sensitive results was obtained with ampicillin. Different species varied considerably in their sensitivity patterns; eg, the majority of *P. mirabilis* were sensitive to cephaloridine, but the majority of *P.morganii* were resistant to this agent. Sensitivity to tetracycline was found in 50% of *E. coli* strains, but in only 4% of the strains of *Enterobacter aerogenes*.

Cultures on nutrient agar slopes of 125 strains of *E. coli* and 83 strains of *P. mirabilis* from this series

were stored for 15 months at room temperature and then tested again for sensitivity to ampicillin. Of the strains of *E. coli*, 117 (94%) were resistant in the first test, but only 51 (41%) were resistant in the second test; of the *P. mirabilis* strains, 79 (95%) were resistant in the first test, and 62 (75%) were resistant in the second test. A series of more recent isolates of *E. coli* (24) and *P. mirabilis* (21) from the Burns Unit were tested and retested after two months' storage at room temperature; 11 (46%) of the *E. coli* strains and 16 (76%) of the *P. mirabilis* strains were resistant at the time of sampling; after two months' storage 10 (42%) of *E. coli* and 15 (71%) of *P. mirabilis* were resistant to ampicillin. The anti-

TABLE IIa—continued

SENSITIVITY TO SEVEN ANTIBIOTICS BY DITCH PLATE TEST ON 730 STRAINS OF GRAM-NEGATIVE BACILLI FROM BURNS (SEPTEMBER 1965 TO SEPTEMBER 1967)

R	Percentage Strains						Number of Strains					
	Tetracycline			Carbenicillin			Gentamicin					
	S	'S'	R	S	'S'	R	S	'S'	R			
14.3	83.3	0.5	16.2	94.9	0	5.1	99.5	0.5	0	216		
0	36.3	0	63.7	55	0	45	100	0	0	11		
88.2	52.9	11.8	35.3	94.1	0	5.9	94.1	5.9	0	17		
0	50	0	50	100	0	0	100	0	0	2		
46.9	6.3	3.1	90.6	93.7	6.3	0	62.5	25	12.5	32		
7.5	50	5.1	44.9	76.1	1.3	22.6	99.4	0.6	0	159		
7.3	35.5	9	55.5	86.4	2.7	10.9	98.2	0.9	0.9	110		
2	4.1	40.8	55.1	77.5	2	20.5	97.9	0	2.1	49		
33.4	52.2	6.5	41.3	15.2	0	84.8	97.8	2.2	0	46		
90	6.7	3.3	90	63.4	23.3	13.3	83.4	6.6	10	30		
0	28.6	0	71.4	85.7	0	14.3	100	0	0	14		
0	75	0	25	50	0	50	100	0	0	12		
0	0	0	100	100	0	0	100	0	0	5		
12.5	12.5	0	87.5	50	12.5	37.5	100	0	0	8		
5.3	42.1	10.5	47.4	68.4	10.5	21.1	100	0	0	19		
17.1	52.6	4.1	43.3	79.4	2.7	17.9	96.7	2.1	1.2	Total 730		

TABLE IIb

SENSITIVITY TO FOUR ANTIBIOTICS BY DITCH PLATE TEST ON STRAINS OF GRAM-NEGATIVE BACILLI (SEPTEMBER 1965 TO SEPTEMBER 1967)

Strain	Percentage Strains												Total Strains Tested with		
	Cephaloridine			Streptomycin			Trimethoprim			Sulphadiazine			Cephaloridine	Trimethoprim and Streptomycin	Sulphadiazine
	S	'S'	R	S	'S'	R	S	'S'	R	S	'S'	R			
<i>Proteus mirabilis</i>	67.8	0	32.2	83.3	8.3	8.4	85.4	14.6	0	29.8	6.4	63.8	59	48	47
<i>Proteus vulgaris</i>	0	0	100	100	0	0	33.3	33.3	33.3	0	33.4	66.6	4	3	3
<i>Proteus morganii</i>	9	0	91	50	50	0	90	10	0	0	7.1	92.9	11	10	14
<i>Providencia</i>	13.6	0	86.4	5.9	0	94.1	70.6	23.5	5.9	84.6	7.7	7.7	22	17	13
<i>Escherichia coli</i>	71.0	1.3	27.7	62.7	26.5	10.8	99	1	0	76	5.2	18.8	126	102	96
<i>Enterobacter cloacae</i>	12.2	0	87.8	58.4	37.7	3.9	94.8	5.2	0	59.4	10.9	29.7	98	77	64
<i>Enterobacter aerogenes</i>	17.1	0	82.9	82.8	8.6	8.6	97.1	2.9	0	82.2	7.1	10.7	35	35	28
<i>Klebsiella aerogenes</i>	40.9	9.1	50	90	0	10	90	10	0	22.2	22.2	55.6	22	10	9
<i>Bact. anitratum</i>	8.7	0	91.3	41.7	33.3	25	33.3	25	41.7	92.3	0	7.7	23	12	13
<i>Serratia</i>	50	0	50	75	25	0	100	0	0	28.5	0	71.5	4	8	7
<i>Alkalescens dispar</i>	71.4	0	28.6	33.3	0	66.7	100	0	0	60	0	40	7	3	5
<i>Alcaligenes</i>	25	0	75	75	0	25	0	0	100	100	0	0	4	4	3
<i>Citrobacter</i>	25	0	75	75	12.5	12.5	87.5	12.5	0	37.5	12.5	50	8	8	8
Unclassified	66.7	0	23.3	58.8	41.2	0	88.2	5.9	5.9	64.3	7.1	28.6	18	17	14
Mean percentage of total	41.9	0.7	57.4	64.1	23.2	12.7	89.5	7.1	3.4	59.6	7.4	33	441	354	324

TABLE III

ANTIBIOTIC SENSITIVITY OF GRAM-NEGATIVE BACILLI BY DITCH PLATE AND TUBE DILUTION TESTS¹

Antibiotic	Minimal Inhibitory Concentrations (μ g/ml) of Antibiotics for Strains Shown in Ditch Plate as			Number of Strains Tested
	S	'S'	R	
Ampicillin	1-16	16-32	(8) ² 16-512	180
Carbenicillin ³	6.25-16	8-32	16-512	180
Cephaloridine	1-16	8-32	16->512	180
Kanamycin	0.5-16	32	16-64	180
Gentamicin	0.25-2	1-32	4-64	180
Tetracycline	2-32	16-64	32-256	180
Chloram- phenicol	1-16	8-32	32-512	180
Nalidixic acid	0.25-16	8-32	32-64	180
Streptomycin	4-16	8-32	64-512	97
Trimethoprim	0.5-10	10-20	20-500	49
Sulphadiazine	2.5-50	25-100	150->500	47

S=Sensitive 'S'=Moderately sensitive R=Resistant.

¹The results are based on tests made on strains representing 14 genera or species and a group of unclassified organisms.²Only in *Alcaligenes* and *Alkalescens dispar*.³Carbenicillin has been used in the treatment of pseudomonas infections, which were found S and 'S' by the ditch test when MIC was 32 μ g/ml or less, and 64 μ g/ml respectively.

biotic was commonly used in the treatment of patients in the Burns Unit. A large proportion of the strains tested were sensitive to trimethoprim; the proportions of strains sensitive to sulphadiazine and to streptomycin were considerably smaller. Trimethoprim had not yet been used, and streptomycin and sulphadiazine were rarely used in the Unit.

SULFAMYLYON SENSITIVITY Because of its reported value as a topical prophylactic agent (Lindberg, Moncrief, Switzer, Order, and Mills, 1965; Lowbury and Jackson, 1969), sulfamylon was included in these tests. Table IV shows the MIC of sulfamylon hydrochloride for 389 strains of Gram-negative

TABLE IV

SENSITIVITY OF 389 GRAM-NEGATIVE BACILLI TO SULFAMYLYON HYDROCHLORIDE

Organism	Number of Strains Showing MIC (mg per ml) of							
	Total Strains	0.2	0.5	1	2	5	10	20
<i>Proteus mirabilis</i>	54					3	19	32
<i>Proteus vulgaris</i>	3							3
<i>Proteus morganii</i>	8		1		2	2	3	
<i>Providencia</i>	19					2	10	7
<i>Ent. liquefaciens</i>	1							1
<i>E. coli</i>	116	1		2		3	62	48
<i>Ent. cloacae</i>	89					36	46	7
<i>Ent. aerogenes</i>	39					11	22	6
<i>Kleb. aerogenes</i>	6					1	4	1
<i>Bact. anitratum</i>	11		2	1	3	1	3	1
<i>Serratia</i>	8					3		5
<i>Alk. dispar</i>	3						3	
<i>Alcaligenes</i>	5	2		1		2		
<i>Citrobacter</i>	9						7	2
Unclassified	18		1	3	1	8	3	2

bacilli. All the strains were sensitive to 20 mg per millilitre or less of the compound, which was lower than the concentration used for local application (100 mg per gram), though in many strains the margin was not great.

POLYMYXIN SENSITIVITY Table V shows the results of plate dilution sensitivity tests with polymyxin. Strains of *Proteus* spp (including *Providencia*) and *Serratia* were usually resistant (strains not inhibited by 12.8 μ g colistin sulphomethate per ml; see Bushby, 1955). The majority of the strains of other species were sensitive.

PENICILLINASE PRODUCTION AND RESISTANCE TO ANTIBIOTICS Many strains of *Proteus* spp, *Escherichia*, and other coliform bacilli were sensitive to carbenicillin but resistant to ampicillin. Since both ampicillin and carbenicillin are known to be inactivated by penicillinases, a selection of strains was tested for diffusible penicillinase production, using benzyl penicillin, carbenicillin, and ampicillin as the test substrates, and for sensitivity to these antibiotics. From these tests it was hoped to obtain evidence about the association of penicillinase production with resistance, about the similarity of patterns of inactivation of the three penicillins by bacterial enzymes, and about the frequency of intrinsic resistance.

Table VI shows the results of these tests. Ampicillinase and carbenicillinase activity were found in the same four strains, which were also highly resistant to both antibiotics as well as to benzyl penicillin. A number of other strains which were sensitive to ampicillin and carbenicillin inactivated benzylpenicillin and were resistant to it. Several strains which did not inactivate ampicillin or carbenicillin had intrinsic resistance to ampicillin but not to carbenicillin. One strain of *E. coli* was more resistant to carbenicillin than to ampicillin, and one strain (of *Serratia*) which did not inactivate any of the three penicillins was more sensitive to benzyl penicillin and carbenicillin than to ampicillin.

MULTIPLE RESISTANCE Strains of the commonest species which were tested for sensitivity to cephaloridine (see Table IIb) were analysed for multiple resistance to two or more of the eight antibacterial agents listed in Table III (see Table VII). *E. coli* was most often resistant to one agent only, but the other species were more often resistant to two or three agents; the resistance patterns of these strains are shown in Table VII. The great majority of strains showing resistance to one antibiotic were resistant to ampicillin.

TABLE V
SENSITIVITY OF 295 GRAM-NEGATIVE BACILLI TO COLISTIN SULPHOMETHATE

Organism	Total Strains	Number of Strains Showing MIC (μ g/ml) of						
		0.05	0.2	0.8	3.2	6.4	12.8	25.6
<i>Proteus mirabilis</i>	45			1	3	1		23
<i>Proteus vulgaris</i>	2						1	1
<i>Proteus morganii</i>	7				1	2		2
<i>Providencia</i>	14							5
<i>E. coli</i>	86		1	25	46	6		1
<i>Ent. cloacae</i>	66			18	30	8		1
<i>Ent. aerogenes</i>	25			8	13	2		1
<i>Kleb. aerogenes</i>	9			1	5	1	1	1
<i>Bact. anitratum</i>	8		1		4	2		1
<i>Serratia</i>	8						1	2
<i>Alk. dispar</i>	3	1		1	1			
<i>Alcaligenes</i>	4				2	1		1
<i>Citrobacter</i>	7	1			3			
Unclassified	11	1		3	3			2

TABLE VI

PENICILLINASE PRODUCTION AND SENSITIVITY OF GRAM-NEGATIVE BACILLI TO BENZYL PENICILLIN, AMPICILLIN AND CARBENICILLIN

Strain	Benzylpenicillin			Ampicillin			Carbenicillin		
	MIC (μ g/ml)	Ditch Plate Result	Inactivation by Strain	MIC (μ g/ml)	Ditch Plate Result	Inactivation by Strain	MIC (μ g/ml)	Ditch Plate Result	Inactivation by Strain
<i>Proteus mirabilis</i>	1	128	R	—	2	S	—	1	S
<i>Proteus mirabilis</i>	2	256	R	+	16	S	—	4	S
<i>Proteus mirabilis</i>	3	256	R	+	1024	R	+	128	R
<i>Proteus mirabilis</i>	4	256	R	+	512	R	—	0.5	S
<i>Proteus morganii</i>	1	512	R	+	512	R	+	256	R
<i>Proteus morganii</i>	2	512	R	+	128	R	—	8	S
<i>Providencia</i>		128	R	+	128	R	—	4	S
<i>E. coli</i>	1	64	R	+	32	R	—	16	S
<i>E. coli</i>	2	512	R	+	2048	R	+	256	R
<i>Klebsiella aerogenes</i>	1	64	R	+	32	R	—	128	R
<i>Klebsiella aerogenes</i>	2	128	R	+	8192	R	+	256	R
<i>Bact. anitratum</i>	1	64	R	+	4	S	—	16	S
<i>Bact. anitratum</i>	2	32	R	+	32	R	—	8	S
<i>Serratia</i>	1	4	S	—	32	R	—	8	S
<i>Serratia</i>	2	32	R	+	64	R	—	16	S

TYPES OF *Ps. aeruginosa* AND PROBABLE SOURCES OF INFECTION For six weeks, a selection of strains of *Ps. aeruginosa* from all patients whose burns were colonized by the organism were examined each week for serotype and phage type.

Each week between three and seven patients were found to have *Ps. aeruginosa* in their burns; sometimes two, three, or even four different types were isolated from one patient, and between three and seven types of *Ps. aeruginosa* were found in the ward each week. Each patient tended to go on carrying strains of the same type for two weeks or longer (Table VIII).

Self infection has been considered by some to be an important source of *Ps. aeruginosa* infection of burns (Sachs and Watson, 1969), but the relative rarity of *Ps. aeruginosa* in the faeces of normal subjects (eg, Lowbury and Fox, 1954; Shooter, Walker, Williams, Horgan, Parker, Asheshov, and Bullimore, 1966) makes it seem more likely that infections are usually

acquired from external sources. This view was supported by typing of *Ps. aeruginosa* in the Burns Unit (see also Lowbury and Fox, 1954), which showed that new infections were almost always due to types that had recently been isolated from other patients in the Unit, more often from patients in the same ward (Table IX). Of the strains which were not found in other patients before they were first detected in a burn, some may have been missed, because not all isolates of *Ps. aeruginosa* were typed. The data strongly support the view that cross infection is the usual mode of infection with *Ps. aeruginosa* in burns.

DISCUSSION

The proportions of different genera and species of aerobic Gram-negative bacilli isolated from burns were different from those normally found in the faeces. While the latter include a large preponderance of *E. coli*, the largest proportion of strains

TABLE VII

PATTERNS OF RESISTANCE IN THE COMMONER SPECIES		
Species	No. of Strains	Resistance Patterns Shown by Numbers of Strains
<i>Proteus mirabilis</i>	59	AT (28), ATCe (7), AChT (6), T and ATCaCe (4), AChTce and Tce (3), TCh, ATCa, AKChT and ATCaKCe (1). A (52), AT (22), ATCe (7), ACaCe, ACe and ATCaCe (6) ACa (5), AChTCaCe (4), T and AKTCaCe (3), ACh, AChT and AKCaCe (2), Ce, AKT and AChTca (1). ATCe (12), ACe (9), A and Ce (2), CeCa, T, Tce, ACa, NACaTce, ATCaCe, ATGce and ATCa (1).
<i>Escherichia coli</i>	123	ACe and ATCe (38), A (8), ATCaCe (4), AT (2), AChCe, NACHTce, Ce, AGCe, Tce, NATce and ACaCe (1).
<i>Enterobacter aerogenes</i>	33	ACa (8), ACaCe (6), ATCa and NACHTca (2), NACHCaCe, CaCe, ATCaCe and ATChTcaCe (1).
<i>Enterobacter cloacae</i>	97	
<i>Klebsiella aerogenes</i>	22	

A = ampicillin, Ca = carbenicillin, Ce = cephaloridine, Ch = chloramphenicol, G = gentamicin, K = kanamycin, N = nalidixic acid, T = tetracycline

TABLE VIII

DURATION OF CARRIAGE OF SAME TYPES OF *Ps. aeruginosa* IN BURNS

Ward	Numbers of Patients Carrying Same Types of <i>Ps. aeruginosa</i> for							Total
	One Week	Two Weeks	Three Weeks	Four Weeks	Five Weeks	Six Weeks	Over Six Weeks	
E	1	3	2	2	2	1	1	12
F	1	3	4	1	1	0	0	10

TABLE IX

PREEXISTING INFECTION WITH *Ps. aeruginosa* IN BURNS WARD AS PROBABLE SOURCE OF NEW INFECTION DURING FOUR MONTHS

Patients in whom Infecting Types of <i>Ps. aeruginosa</i> Were ¹			
	Previously Isolated from Other Patients ²		Not Previously Isolated from Other Patients in Wards E or F
	In Ward E	In Ward F	
Ward E	21	5	6
Ward F	3	17	7

¹Patients acquiring more than one type of *Ps. aeruginosa* are represented separately for each type acquired.

²Within previous two months.

from burns were *Proteus* spp, of which *P. mirabilis* was by far the commonest species. In this respect the bacteria of burns resemble those of secondary infection of the urinary tract, which include a large proportion of strains, including *Ps. aeruginosa*, acquired from other patients or from environmental

sources (Gould, 1968). Since *Ps. aeruginosa* is present in the faeces of a relatively small proportion of normal subjects, the high incidence of these bacteria commonly found in burns not specifically protected against them must be attributed to infection from sources outside the patient (Lowbury and Fox, 1954; Wahba, 1965); this view is supported by the presence, in most cases, of the infecting type in other patients before its first appearance in a burn. The relatively high incidence of *Proteus* spp and *Klebsiella* spp may be due, also, to frequent cross infection with these organisms, or it may be the selective result of poorer growth conditions for *E. coli* than for some other Gram-negative bacilli in the burn, while optimal conditions for *E. coli* are found in the colon. The typing of *E. coli* and other Gram-negative bacilli isolated from the faeces of patients on admission to hospital and subsequently from their burns should throw light on this problem.

The antibiotic sensitivity patterns of the strains isolated from burns support the view that a large proportion of these organisms is acquired in hospital. As in secondary urinary tract infection (Gould, 1968), a high proportion of *E. coli* isolated from burns were resistant to ampicillin. More surprising, perhaps, was the high incidence of ampicillin-resistant *Proteus mirabilis* (cf Barber and Waterworth, 1964). This was probably the result of the extensive use of ampicillin in the treatment of patients in the Burns Unit. Tests on ampicillin-resistant strains preserved for 15 months showed some evidence of reversion to sensitivity, but a large proportion retained their resistance. Resistance of *P. mirabilis* to ampicillin is commonly associated with penicillinase production; it was therefore surprising that most of the ampicillin-resistant strains were sensitive to carbenicillin which, like ampicillin, is inactivated by penicillinase. Tests on inactivation of the antibiotics by penicillinase from 15 strains of *Proteus* and other organisms showed that those which inactivated ampicillin also inactivated carbenicillin; some strains were intrinsically resistant to ampicillin without producing a penicillinase active against it, and these were sensitive to carbenicillin. As regards the other antibiotics, nalidixic acid, kanamycin, trimethoprim, and gentamicin were active against the largest number of strains.

Multiple resistance was commoner among strains of *Klebsiella* spp, *Enterobacter* spp, and *Proteus mirabilis* than among *E. coli*; this may have been due to the greater frequency of *E. coli* in the normal intestinal flora and may indicate a larger incidence of self-infection, as distinct from cross infection, with *E. coli*. A similar observation has been made in respect of urinary tract infection by Talbot,

Cunliffe, and Gower (1957). A large proportion of strains of *E. coli* acquired by infants today have been shown to possess transferable antibiotic resistance (Moorhouse, 1969; Datta, 1969). It seems likely that many of the resistant strains found in our series were brought in by patients and had transferable resistance. Many burns become colonized by several different species of Gram-negative bacilli, which would provide opportunities for resistance to be transferred and to increase even when antibiotics are not used. It seems a likely cause for the high incidence of ampicillin-resistant *Proteus mirabilis*, most strains of which were distinct from each other by the Dienes reaction.

In the absence of data on typing of strains within species it is hard to assess the importance of different sources and routes of infection with Gram-negative bacilli. It was, however, possible to form an opinion on sources of infection with *Ps. aeruginosa* from the result of serological and phage typing. Most patients became infected with types previously found in other patients in the Unit, and especially in the same ward. Since *Ps. aeruginosa* is usually found to be a relatively rare member of the faecal flora of normal subjects, cross infection must be regarded as a much more important route of transfer with these organisms than self infection.

Tests with sulfamylon hydrochloride confirmed the activity of this agent, at the high concentrations (10%) used for local prophylaxis, against a series of strains of Gram-negative bacilli from burns. Some strains, however, were inhibited only by concentrations as high as 2%; the margin between the MIC and the concentration of antibacterial agent applied with success to burns is usually much greater. Some tests for synergy through combined action of sulfamylon with trimethoprim were made, because of the known synergy between sulphonamides and trimethoprim (Bushby and Hitchings, 1968). There appeared to be no synergic action against strains sensitive to trimethoprim, but appreciable synergy was found against some strains showing moderate resistance to trimethoprim, including *Ps. aeruginosa* and *Enterobacter* spp. In view of the known synergy of sulphadiazine with trimethoprim, the use of this combination for prophylaxis and therapy of Gram-negative bacillary infection in burns deserves study. Another combination which has been reported to have a valuable prophylactic effect is that of silver nitrate with sulphadiazine (Fox, 1968). Studies are

in progress to determine, by controlled trial, the local chemoprophylactic effect of silver-sulphadiazine, silver nitrate-trimethoprim, and sulphadiazine-trimethoprim applications on burns.

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a low production of pigment on normal media, according to all the other biochemical tests showed them to be normal *P. aeruginosa* strains; this was confirmed by the results of the growth on the milk agar, both hydrolysis of the casein and pigment production being observed. In the case of the other two strains, 950 and 5940, the position was not so straightforward. Neither produced pigment on any of the solid media, including the milk agar. They did, however, show some signs of hydrolysis of the casein, and this increased on incubation for a further 48 hours, but no pigment was demonstrable. The results of the biochemical tests likewise showed that these two strains did not conform precisely to the *P. aeruginosa* or the *P. fluorescens* patterns, but in fact have some characters common to both, and these can be regarded as intermediate *fluorescens/aeruginosa* types. This is supported by the results of the milk agar media previously discussed. Indeed, in view of the recently demonstrated high transformation frequency within the genus *Pseudomonas* (Khan and Sen, 1967), the existence of such intermediate strains is not surprising. In either case, the milk agar has shown itself to be as reliable as the more conventional biochemical tests.

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Errata

In Table II in the paper entitled, 'Comparison of quick and slow thaw methods of producing cryoprecipitate antihemophilic factor from fresh and 24-hour-old blood' A. L. Bloom (*J. clin. Path.*, **22**, 447-452) the *P* values for the supernatant have been printed under the wrong headings. The correct table 'Factor VIII content of cryoprecipitate and supernatant plasma', is printed below.

	Fresh Blood		Twenty-four Hour Blood	
	Quick-Thaw (A)	Slow-Thaw (B)	Quick-Thaw (C)	Slow-Thaw (D)
Number of samples	101	67	102	76
Factor VIII in cryoprecipitate (units) Mean $\pm$ SD	83 $\pm$ 32	112 $\pm$ 44	53 $\pm$ 25	72 $\pm$ 30
Factor VIII in supernatant (units) Mean $\pm$ SD	42 $\pm$ 21	30 $\pm$ 21	31 $\pm$ 19	25 $\pm$ 12
Statistical significance	<i>A</i> v <i>B</i>	<i>C</i> v <i>D</i>	<i>A</i> v <i>C</i>	<i>B</i> v <i>D</i>
Supernatant	<i>P</i> = <0.001 <i>P</i> = <0.001			
Cryoprecipitate	<i>P</i> = <0.001 <i>P</i> = <0.001 <i>P</i> = <0.001 <i>P</i> = <0.001			

In Table IIa of the paper by Davis *et al.*, *J. Clin. Path.*, 1969, **22**, 634, the figures for *Proteus mirabilis* under the columns for tetracycline should read: S: 9.7%, 'S': 0.9% and R: 89.4%; the mean percentages of total should therefore read: S: 30.8%; 'S': 6.1%; R: 63.1%.