Distribution in clinical material and identification of *Pseudomonas maltophilia*

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SUMMARY During the 10-year period ending March 1976, 128 (8.5%) of 1506 strains of Gram-negative non-fermentative bacteria submitted to the National Collection of Type Cultures for computer-assisted identification have been strains of *Pseudomonas maltophilia*. These figures suggest that *Ps. maltophilia* is both a relatively common species in clinical material in the United Kingdom and also that many laboratories experience difficulty in identifying this species. We report the sources from which our strains were isolated and also characteristics of the species by which it may be recognised. The clinical significance of *Ps. maltophilia* is discussed and also the susceptibility of this species to certain antimicrobial agents.

Hugh and Ryschenkow (1960) proposed the name *Pseudomonas maltophilia* for 34 strains they had examined from river water, raw milk, rabbit and human faeces, blood, pericardial fluid, ascitic fluid, chest fluid, spinal fluid, and oropharyngeal swabs. Subsequently, Hugh and Ryschenkow (1961) published an amended and more complete description of the species and designated the type strain (NCTC 10257). A detailed morphological and physiological description of the type strain of *Ps. maltophilia* was given by Hugh and Leifson (1963).

After publication of the above descriptions of *Ps. maltophilia* the species was reported with increasing frequency in clinical material by workers in the United States of America, and with less frequency elsewhere (Table 1). Although Steel and Midgley (1962) and Snell *et al.* (1972) in the United Kingdom described some characteristics of *Ps. maltophilia*, while Snell (1973) and Cowan (1974) each produced schemes for the identification of non-fermenting bacteria, which included *Ps. maltophilia*, there has been, as far as we can tell, only a single report of one isolate of *Ps. maltophilia* from clinical material in the UK (Patrick *et al.*, 1975). The strain described by Patrick *et al.* (1975) was isolated from a case of meningitis, and these authors stated both that *Ps. maltophilia* does not appear to be a common cause of infection in the UK and that it might be, as Hugh and Ryschenkow (1961) had suggested, that *Ps. maltophilia* is sometimes misidentified as *Alcaligenes faecalis* or as *Bordetella bronchiseptica*. Over a

10-year period up to March 1976, 1506 strains of Gram-negative non-fermentative bacteria were submitted to the National Collection of Type Cultures (NCTC) for identification. Of these, 128 strains (8.5% of the total) were identified as *Ps. maltophilia*, which suggests both that the organism is not uncommon in clinical material in the UK and that many laboratories encounter difficulty in identifying this species. We therefore report here the results of biochemical tests carried out on 133 strains of *Ps. maltophilia*.

Material and methods

STRAINS

One hundred and thirty-three strains of *Ps. maltophilia* were examined. They comprised 128 field strains (duplicate cultures excluded), isolated from clinical material in the UK, and sent to the NCTC for computer-assisted identification; the other five strains were reference cultures maintained in the NCTC. The sources of isolation for the field strains are given in Table 2. The five reference cultures comprised the holotype strain NCTC 10257 and four paratype strains, NCTC 10258, 10259, 10498, and 10499, all of which were included in the description of *Ps. maltophilia* given by Hugh and Ryschenkow (1961).

BACTERIOLOGICAL STUDIES

The 128 field strains were identified in some cases solely on the test results obtained in the sending laboratory, in other cases on the test results obtained

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Table 1 Summary of previous reports on identification and incidence of *Ps. maltophilia*

Country of origin of report	Reference	Common sources of isolates reported (where appropriate)
United States of America	King (1964, 1967)	Blood, pus, respiratory tract, spinal fluid, urine
	Gilardi (1968a, 1968b, 1971a, 1971b, 1971c, 1972a, 1973, 1976)	
	Pickett and Pedersen (1968, 1970a, 1970b)	
	Sutter (1968)	Respiratory tract, urine
	von Graevenitz and Redys (1968)	
	Zabransky and Day (1969)	
	Pickett and Manclark (1970)	
	von Graevenitz (1971)	
	von Graevenitz and Pantelick (1971)	
	Weaver <i>et al.</i> (1972)	
	von Graevenitz (1973)	
	Hugh and Gilardi (1974)	
	Sandlin (1974)	
	Kantor <i>et al.</i> (1975)	
	Blazevic (1976)	Blood, respiratory tract
	von Graevenitz (1965)	
	Yeh <i>et al.</i> (1967)	
	Gilardi (1969)	Pus
	Gardner <i>et al.</i> (1970)	Pus, respiratory tract, urine
	Pedersen <i>et al.</i> (1970)	Respiratory tract, urine
Canada	Sonnenwirth (1970)	
	Gilardi (1972b)	Pus, respiratory tract, urine
Australia	Harlowe (1972)	
	Fischer (1973)	
France	Southern and Schneider (1974)	Pus, urine
	Narasimhan <i>et al.</i> (1977)	
Switzerland	Wishart and Riley (1976)	Urine
	Dyte and Gillians (1977)	
West Germany	Chabbert and Courtieu (1961)	
	Thibault (1961)	
	Debette and Blondeau (1977)*	Soil
	Kékessy (1972)	Blood, pus, respiratory tract, urine
	Fritsche <i>et al.</i> (1974)	Respiratory tract

*Strains described only as showing a close affinity to *Ps. maltophilia*.

Table 2 Sources of *Ps. maltophilia* field strains studied

Source	No. of strains	Source	No. of strains
Blood	37	Cerebrospinal fluid	3
Heart valve	2		
Respiratory tract (Sputum 10)	18	Ear swab	2
(Pleural aspirate 2)		Eye swab	2
(Throat swab 1)			
Respirator	4	Faeces	2
Wound swab	18	Hospital environment	7
Urine	7	Miscellaneous	11
Dialysis fluid	7		
Urethral pus	2	Unknown	6

in the sending laboratory combined with further test results obtained in the NCTC, and in the remaining cases solely on the test results obtained in the NCTC. We report for the field strains only the biochemical test results obtained in the NCTC so that for strains identified solely on the test results obtained in the sending laboratory no results are given. In addition, as different numbers of tests were carried out on each strain, we give in Table 3 the number of strains examined in each test. The

method of computer-assisted identification used in the NCTC has been described elsewhere (Lapage, 1974). The five reference cultures were examined in all the 68 tests of Table 3; the methods used for these tests have been described previously (Holmes *et al.*, 1975). The tests were incubated at 37°C except where otherwise required by the specification for the test.

The susceptibility of 12 of the field isolates to antimicrobial agents was determined by an agar dilution technique. Six of the 12 strains were selected because they were reported as being resistant to gentamicin by the sending laboratories; the susceptibility of the remaining six strains (selected to cover the same time period as the first six strains) was not reported to us by the sending laboratories. Twofold dilutions of antimicrobial agents were added to Diagnostic Sensitivity Test (DST) agar (Oxoid Ltd., London), to give a range of concentrations of 128–0.006 µg/ml. Lysed horse blood (2 ml/100 ml) was added to media containing sulphonamide or co-trimoxazole. The medium was dispensed into petri dishes which were inoculated, using a replicating device, with 0.003 ml volumes of overnight broth cultures of bacteria diluted to con-

Table 3 Biochemical characters of *Ps. maltophilia*

Test	Results of type strains* (No. positive/ No. tested)	Results of field strains (No. positive/ No. tested)	Percentage positive of all strains	Sign†
Catalase production	5/5	111/111	100	+
Deoxyribonuclease production	5/5	33/33	100	+
Gelatinase production (plate method)	5/5	70/70	100	+
Growth at 37°C	5/5	95/95	100	+
Growth at room temperature (18°C-22°C)	5/5	102/102	100	+
Growth on MacConkey's agar	5/5	52/52	100	+
Motility at room temperature (18°C-22°C)	5/5	101/101	100	+
Tween 80 hydrolysis	5/5	54/54	100	+
Tween 20 hydrolysis	5/5	88/89	99	+
Acid from ASS maltose	5/5	69/71	97	+
Casein digestion	5/5	26/27	97	+
Alkali production on Christensen's citrate	5/5	51/56	92	+
Tyrosine hydrolysis	5/5	78/87	90	+
Lysine decarboxylase	3/5	71/79	88	+
Pigment production on tyrosine	5/5	72/87	84	d
Gelatin liquefaction (stab method)	5/5	42/53	81	d
Growth on β -hydroxybutyrate	5/5	64/88	74	d
Aesculin hydrolysis	3/5	39/53	72	d
Opalescence on LV agar	4/5	16/25	67	d
Motility at 37°C	5/5	59/94	65	d
Nitrate reduction	3/5	34/58	59	d
Acid from ASS glucose	4/5	40/71	58	d
Acid from ASS fructose	4/5	11/23	54	d
Hydrogen-sulphide production (lead acetate paper)	3/5	39/94	42	d
KCN tolerance	3/5	20/55	38	d
Growth on cetrimide	4/5	12/40	36	d
Hugh and Leifson O-F test no reaction	0/5	38/101	36	d
Malonate utilisation	4/5	17/55	35	d
Hugh and Leifson O-F test alkaline reaction	2/5	33/101	33	d
Growth at 42°C	1/5	16/49	31	d
Hugh and Leifson O-F test oxidative reaction	3/5	30/101	31	d
Oxidase production	1/5	24/112	21	d
β -Galactosidase production (ONPG)	1/5	8/54	15	-
Urease production	0/5	9/63	13	-
Acid from ASS cellobiose	0/5	7/66	10	-
Growth at 5°C	0/5	5/46	10	-
Yellow pigment production	0/5	7/113	6	-
Nitrite reduction	0/5	1/50	2	-
Acid from ASS arabinose	0/5	1/63	1	-
Acid from ASS adonitol	0/5	0/25	0	-
Acid from ASS dulcitol	0/5	0/23	0	-
Acid from ASS ethanol	0/5	0/61	0	-
Acid from ASS glycerol	0/5	0/29	0	-
Acid from ASS inositol	0/5	0/23	0	-
Acid from ASS lactose	0/5	0/23	0	-
Acid from ASS mannitol	0/5	0/25	0	-
Acid from ASS raffinose	0/5	0/22	0	-
Acid from ASS rhamnose	0/5	0/22	0	-
Acid from ASS salicin	0/5	0/22	0	-
Acid from ASS sorbitol	0/5	0/22	0	-
Acid from ASS sucrose	0/5	0/23	0	-
Acid from ASS trehalose	0/5	0/63	0	-
Acid from ASS xylose	0/5	0/61	0	-
Acid from PWS glucose	0/5	0/53	0	-
Acid from glucose (10 g/100 ml)	0/5	0/28	0	-
Acid from lactose (10 g/100 ml)	0/5	0/46	0	-
Arginine desiminase	0/5	0/45	0	-
Arginine dihydrolase	0/5	0/54	0	-
Fluorescence on King's B medium	0/5	0/42	0	-
Gas from PWS glucose	0/5	0/53	0	-
Gluconate oxidation	0/5	0/51	0	-
Growth on Simmons' citrate	0/5	0/91	0	-
Hydrogen-sulphide production (TSI)	0/5	0/51	0	-
Indole production	0/5	0/88	0	-
Ornithine decarboxylase	0/5	0/55	0	-
Phenylalanine deamination	0/5	0/54	0	-
Poly- β -hydroxybutyrate inclusion granules	0/5	0/87	0	-
Selenite reduction (0.4 g/100 ml)	0/5	0/48	0	-
Starch hydrolysis	0/5	0/22	0	-
3-ketolactose production	0/5	0/22	0	-

*Holotype strain: NCTC 10257; paratype strains: NCTC 10258, 10259, 10498, 10499.

†+85% or more of strains positive; d 16-84% of strains positive,

- 15% or less of strains positive.

ASS = ammonium salt sugar; PWS = peptone water sugar; TSI = triple sugar iron agar.

tain about 10^5 cells/ml. Each plate was also inoculated with three control strains of known susceptibility: *Escherichia coli*, NCTC 10418; *Pseudomonas aeruginosa*, NCTC 10662; and *Staphylococcus aureus*, NCTC 6571. The minimum inhibitory concentration (MIC) of an antimicrobial agent for each strain was defined as the lowest concentration completely inhibiting growth after overnight incubation at 30°C.

Results

The strains were Gram-negative rods generally producing non-pigmented colonies on nutrient agar (seven strains were yellow-pigmented). The biochemical test results are shown in Table 3 while Table 4 shows that the 12 strains, whose susceptibility to antimicrobial agents was determined, were resistant to clinically attainable levels of streptomycin, kanamycin, gentamicin, tobramycin, amikacin, cephaloridine, erythromycin, tetracycline, and chloramphenicol; resistant or moderately sensitive to ampicillin, carbenicillin, polymyxin B, and nalidixic acid; and sensitive to sulphamethoxazole and co-trimoxazole.

Discussion

According to Blazevic (1976), *Ps. maltophilia* is the second most common pseudomonad isolated from clinical material, although it occurs much less frequently than *Ps. aeruginosa*. Support for this statement appears in the publications of Gilardi (1968a, 1968b, 1971a, 1971b, 1971c, 1972a, 1972b, 1973, 1976), Pickett and Pedersen (1968, 1970a), Pedersen *et al.* (1970), von Graevenitz and Redys (1968), Gardner *et al.* (1970), and Weaver *et al.* (1972), who, in collecting strains for their respective studies on the identification of pseudomonads other

than typical *Ps. aeruginosa*, isolated more strains of *Ps. maltophilia* than of any other *Pseudomonas* species. We have shown that *Ps. maltophilia* occurs quite commonly in clinical material in the UK, but nevertheless Patrick *et al.* (1975) may be correct in claiming that this species does not appear to be a common cause of infection in the UK. We received few clinical details with each of our strains, and only six appeared to be of clinical significance. One of our strains was isolated from three of eight blood cultures from a patient who some months previously had had a prosthetic cardiac valve inserted; the patient's serum showed a high agglutination titre to the strain. Three other strains, from blood cultures, that we examined were also possibly of clinical significance as one strain was isolated from four blood culture bottles, one from all three bottles of one blood culture set, and one from all of three blood culture bottles. Other strains in our series, of possible clinical significance, include one isolated in profuse pure growth from a gangrenous foot and another isolated as a heavy growth in almost pure culture from the sputum of a patient with pulmonary tuberculosis. Common sources of human clinical isolates of *Ps. maltophilia* are blood, pus, the respiratory tract, and urine (Table 1), and this distribution accords well with the distribution of our strains as given in Table 2. *Ps. maltophilia* can, however, be isolated, although with less frequency, from many other sites (see p. 66 for source of strains examined by Hugh and Ryschenkow, 1960). Most clinical strains of *Ps. maltophilia* appear to be isolated in mixed culture and to be of little clinical significance (Sutter, 1968; Gilardi, 1969; Pedersen *et al.*, 1970; Gardner *et al.*, 1970), often disappearing without the use of antimicrobial agents (Pedersen *et al.*, 1970). Clinically significant isolates of *Ps. maltophilia* have been reported by Yeh *et al.* (1967), Gilardi (1969), Pedersen *et al.* (1970), Harlowe

Table 4 Susceptibility of *Ps. maltophilia* to antimicrobial agents

Antimicrobial agents	Minimum inhibitory concentration ($\mu\text{g/ml}$)								
	1	2	4	8	16	32	64	128	>128
Sulphamethoxazole	1	4	4	2					1
Co-trimoxazole	1	5	5					1	
Streptomycin								1	11
Kanamycin							2		10
Gentamicin					1		3	3	5
Tobramycin					1		3	4	4
Amikacin							1	3	8
Ampicillin			3	3	3		2		1
Carbenicillin			4	1	3	1		1	2
Cephaloridine							1	1	10
Erythromycin						1	9	2	
Chloramphenicol			7	1	4				
Tetracycline					2	9	1		
Polymyxin B	1	2	3	1	3	1	1		
Nalidixic acid			3	2	6	1			

(1972), Fischer (1973), and Wishart and Riley (1976), and the species has been implicated as the cause of pneumonia in patients with serious underlying disease (Gardner *et al.*, 1970). Transient bacteraemia has been reported after dialysis by Pedersen *et al.* (1970), and in association with pneumonia by Sonnenwirth (1970); in both these cases therapy with appropriate antimicrobial agents proved effective. Gardner *et al.* (1970), in discussing 28 strains of *Ps. maltophilia* they had isolated from sputum, noted a striking association with the presence of a tracheostomy or endotracheal tube (26/28 patients) and prior administration of antimicrobial agents (27/28 patients). They also considered that all but two of the strains were hospital-acquired. Southern and Schneider (1974), however, pointed out that *Ps. maltophilia* was not necessarily a nosocomial problem because over 50% of the strains of this species that they examined were isolated at the time of admission or from outpatients and only 30% of their strains were isolated from patients who had been in hospital for more than 15 days. Southern and Schneider (1974) also pointed out that only eight of their 32 patients had received recent antimicrobial therapy; few of their strains, however, unlike those of Gardner *et al.* (1970), were isolated from sputum.

The biochemical characteristics of the field strains of *Ps. maltophilia* agree very closely with those of the five reference cultures of the species (which include the type strain) maintained in the NCTC and with the original descriptions of the species given by Hugh and Ryschenkow (1960, 1961) and the description of the type strain given by Hugh and Leifson (1963). The biochemical characteristics of all the strains examined also agree well with those presented by other authors except for some minor discrepancies as follows. Very few (7/71) of our strains produced acid from cellobiose and none produced acid from lactose, sucrose, or trehalose but acid production from two or more of these four carbohydrates has been noted in the majority, or a large proportion, of their strains by Gilardi (1968a, 1968b, 1969, 1971a, 1971b, 1972a, 1973, 1976), Pickett and Pedersen (1970a, 1970b), Fritzsche *et al.* (1974), Komagata *et al.* (1974), and Wishart and Riley (1976), probably due to the use of ammonium salt sugar media in our laboratory while most other authors have used Hugh and Leifson O-F media. In addition, Stanier *et al.* (1966), using sole carbon source utilisation tests, found that their strains of *Ps. maltophilia* were able to utilise these four carbohydrates. Lowe and Ingledew (1975) made a detailed study of lactose utilisation by *Ps. maltophilia* and, as *Ps. maltophilia* requires methionine as a growth factor (Iizuka and Komagata, 1964; Stanier *et al.*, 1966), the differing results may be due to vary-

ing amounts of methionine in the media. According to Cowan (1974), the majority of strains of *Ps. maltophilia* are yellow-pigmented and do not hydrolyse Tween 80, but for our strains we observed the converse results in these two tests. The proportion of strains found able to produce cytochrome-oxidase varies considerably between different authors (eg, Hugh and Ryschenkow, 1961: 16/26; Gilardi, 1971b: 0/81) possibly due to differences in sensitivity of the reagents used for the test (we used the very sensitive reagent tetramethyl-*p*-phenylenediamine dihydrochloride). As with other non-fermentative species, insufficient acid is produced in the control tube of the Møller (1955) decarboxylase medium to turn the medium yellow. The unchanged appearance of the control tube can be mistaken for no growth of the strain under test and consequently the decarboxylase tests may be discarded. Decarboxylation of lysine can thus be overlooked, yet it is characteristic of some pseudomonads, chiefly *Ps. maltophilia*, but also of some strains of *Ps. cepacia*.

The susceptibility to antimicrobial agents of the 12 strains we examined is in reasonable accord with those reported by other authors. No authors reported strains of *Ps. maltophilia* resistant to sulphamethoxazole or to co-trimoxazole, the two antimicrobial agents to which all but one of the strains we tested were sensitive. The patient described by Fischer (1973) failed to respond to therapy until placed on a regimen of co-trimoxazole. However, several authors (von Graevenitz and Redys, 1968; Gilardi, 1969, 1971c, 1972b, 1976; Gardner *et al.*, 1970; Pedersen *et al.*, 1970; Moody *et al.*, 1972; Fritzsche *et al.*, 1974; Komagata *et al.*, 1974; Southern and Schneider, 1974; Patrick *et al.*, 1975; Wishart and Riley, 1976; Dyte and Gillians, 1977) reported that all or a large proportion of the strains they examined were sensitive to one or more of streptomycin, kanamycin, gentamicin, erythromycin, tetracycline, and chloramphenicol, antimicrobial agents to which all our strains proved resistant. The resistance to antimicrobial agents of the 12 strains we examined suggests that infections due to *Ps. maltophilia* might prove difficult to treat and that sulphonamides are possibly most useful in treatment.

Strains of *Ps. maltophilia* have been inadvertently assigned to *Alcaligenes bookeri*, a species *incertae sedis* (Hugh and Ryschenkow, 1961) as well as being described under the name *Pseudomonas melanogena*, a junior synonym (Iizuka and Komagata, 1963a; Komagata *et al.*, 1974). Strains of *Ps. maltophilia* have also been misidentified as *Pseudomonas alcaligenes* (Komagata, 1961; Iizuka and Komagata, 1963b, 1963c, 1964; Hugh and Leifson, 1963; Komagata *et al.*, 1974).

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