A Taxonomic Study of Clinical Isolates of *Pseudomonas pickettii*, 'P. thomasii' and 'Group IVd' Bacteria

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On the basis of cluster analysis, average similarities within and between groups, and DNA base composition of selected strains, *Pseudomonas pickettii* appeared to be a distinct species comprising several biotypes. Although some or all of these biotypes, represented by sub-clusters in our computer study, may ultimately warrant recognition as separate species, our results were not conclusive enough to warrant such a proposal at present. Most strains tentatively named 'P. thomasii' could be included in *P. pickettii* so that the name *P. pickettii*, which was validly published whilst 'P. thomasii' was not, takes priority over 'P. thomasii'. Strains of Group Va (Tatum *et al.*, 1974) examined were also included in *P. pickettii*. Group IVd (King, 1964) did not appear to be a natural group but some of the strains could also be included in *P. pickettii*.

INTRODUCTION

King (1964) described as Group IVd 16 strains of Gram-negative non-fermentative bacteria that gave similar biochemical reactions. Whether these strains corresponded to an existing species was not established and no proposals were made relating to their taxonomic position. Strains of Group IVd have been isolated from various clinical sources including blood (King, 1964; Graber *et al.*, 1968), spinal fluid (King, 1964) and sputa (Knuth *et al.*, 1969).

Phillips & Eykyn (1972) reported a cross-infection episode, associated with contaminated autoclave cooling-water, caused by an apparently new species of *Pseudomonas* related to *P. cepacia*; although this new organism was tentatively named 'P. thomasii' the name was not validly published (see Discussion). A full account of this outbreak of infection, including additional cases, was given by Phillips *et al.* (1972) who did not adequately describe the organism in the publication but stated their intention to do this elsewhere. Further possible cross-infection episodes associated with organisms similar to 'P. thomasii' have been described by Dowsett (1972), O'Meara *et al.* (1974) and Baird *et al.* (1976).

Pseudomonas pickettii was described and named by Ralston *et al.* (1973) from 20 strains received from M. J. Pickett, 10 of which were from patients who had undergone tracheotomies. Riley & Weaver (1975) described 62 strains of *P. pickettii* isolated from clinical material and drew attention to the similarity between this organism and a group of unclassified bacteria designated Group Va-2 (Tatum *et al.*, 1974), but they did not mention any possible relationship between *P. pickettii* and strains of Group IVd. Strains of Group Va, whose description also resembles that of *P. pickettii*, have been isolated from clinical material (Fass & Barnishan, 1976; Parent & Mitchell, 1978).

We have received strains of Group IVd from R. E. Weaver (Center for Disease Control,

Table 1. *Bacterial strains studied*

Taxon/strain nos*	
<i>Pseudomonas pickettii</i> : K-214, K-230, K-279, K-286, K-288, K-298, K-472, K-507, K-615, K-619, K-633	049063, 069131, 069221, 079332, 080212, 080222, 080262 089022, 089452, 089502, 089512, 089522, 089562, 089572, 090240, 100190, 109191, 110140, 120080, 120419
' <i>P. thomasi</i> ': 010010, 020391, 039042, 039070, 039122, 039182, 049192, 080821†, 090231, 090250, 098991, 110110, 110400, 118680, 118700, 120139, 129181, CL77/72, CL523/72, CL549/72, CL605/72, CL24/74, CL78/74, CL120/74, B7795, B7796, C3807	<i>P. diminuta</i> : NCTC 8545, B7211 <i>P. fluorescens</i> : NCTC 3756, NCTC 10038 <i>P. fragi</i> : NCTC 10689 <i>P. mallei</i> : NCTC 120, NCTC 10230 <i>P. maltophilia</i> : NCTC 10257, 010012, 010121, 010132, 010242, 010252, 010262, 010272, 010371, 010411, 010441, 010531, 010551, 020061, 020161, 020261, 020352, 030221, 050322, 050412, 050422, 050552, 050642, 060200, 070491, 080171, 080291, 080311, 080331, 090280, 090402, 100162, 100182, 100652, 100832, 100842, 100862, 100882, 110192, 120012, 120069, 120219
Group IVd, type 1: A9800, A9838	<i>P. mendocina</i> : NCTC 10897, NCTC 10898
Group IVd, type 1a: A8164, B1483	<i>P. ovalis</i> : NCTC 912
Group IVd, type 1b: A1875	<i>P. pseudoalcaligenes</i> : NCTC 10860, 020113
Group IVd, type 2: A4801, B2304	<i>P. pseudomallei</i> : NCTC 1688, NCTC 4845
Group IVd, type 3: A8932, A9010	<i>P. putida</i> : NCTC 10936
Group IVd, type 4: B1049, B1707	<i>P. putrefaciens</i> : B3181, B5944
Group IVd, type 5: B9001, B1255	<i>P. stutzeri</i> : NCTC 10450, NCTC 10475
Group Va, type 1: B19, B665	<i>P. taetrolens</i> : NCTC 10697
Group Va, type 2: A5310, A8661	<i>P. testosteroni</i> : KC 1071, T6
<i>Acinetobacter calcoaceticus</i> : NCTC 7250, NCTC 7844	<i>P. vesicularis</i> : NCTC 10900, KC 1099
<i>Pseudomonas acidovorans</i> : NCTC 10683, KC 1049	
<i>P. aeruginosa</i> : NCTC 6750, NCTC 10332	
<i>P. alcaligenes</i> : NCTC 10367	
<i>P. cepacia</i> : NCTC 10661, NCTC 10734, NCTC 10743, NCTC 10744, B7617, KC 984, 010180, 010381, 010451, 020151, 020241, 020251, 020271, 020341, 020351, 020421, 020431, 020441, 020451, 030021, 030031, 030081, 030201, 040041, 040081, 040191,	

* Strain numbers of *Pseudomonas pickettii* strains prefixed by the letter K were received from M. J. Pickett, Department of Bacteriology, University of California, Los Angeles, California 90024, U.S.A.; T6 and six-digit strain numbers without any prefix are strains isolated at St Thomas' Hospital; strain numbers of '*Pseudomonas thomasi*' strains prefixed by the letters CL are field isolates from various laboratories in the U.K., originally submitted to the National Collection of Type Cultures (Computer Identification Laboratory) for computer-assisted identification; various reference strains prefixed by the letters A, B, C or KC were received from R. E. Weaver, Center for Disease Control, Atlanta, Georgia 30333, U.S.A.; strain numbers prefixed by the letters NCTC are reference cultures maintained in the National Collection of Type Cultures.

† This strain represents 25 biochemically identical isolates.

Atlanta, U.S.A.) and found that most of them resembled the organism tentatively named '*P. thomasi*'. A taxonomic study was begun with the intention of formally proposing '*P. thomasi*' as a new species. While this study was in progress, *P. pickettii* was described and named by Ralston *et al.* (1973) and subsequently Riley & Weaver (1975) drew attention to the similarity between *P. pickettii* and strains of Group Va-2; we therefore included strains of *P. pickettii* and Groups Va-1 and Va-2 in our study. In the present paper, a numerical analysis of phenotypic characteristics and determinations of DNA base compositions have been used to establish the taxonomic relationship between *P. pickettii*, '*P. thomasi*' and Groups IVd and Va.

METHODS

Strains used. The 174 bacterial strains studied (Table 1) included 11 of *P. pickettii*, 27 of '*P. thomasi*', 13 of Group IVd and two of each of types 1 and 2 of Group Va. The remaining 119 strains included 117 strains of various species of *Pseudomonas* and two of *Acinetobacter calcoaceticus*.

Table 2. Characteristics of the 44 strains of the *P. pickettii*/*P. thomasii*' cluster

Test*	No. of strains positive	Results for type strain of <i>P. pickettii</i> (K-288)
Hugh and Leifson O-F test, oxidative reaction	43	+
Acid from ASS arabinose	43	+
Nitrate reduction (1)	41	+
Acid from ASS glycerol	41	+
Poly β -hydroxybutyrate inclusion granules (Snell <i>et al.</i> , 1972)	37	+
Nitrite reduction	35	+
Urease production (1)	33	—
Acid from ASS lactose	33	—
Acid from ASS cellobiose	30	—
Acid from ASS maltose	29	—
Motility at 30 °C (King & Phillips, 1978)	19	—
Motility at 37 °C (King & Phillips, 1978)	18	—
Acid from ASS mannitol	15	—
Gelatinase production (3)	14	—
Acid from ASS trehalose	11	—
Growth at 42 °C (King & Phillips, 1978)	6	—
Casein digestion	2	—
Antibiotic sensitivity (King & Phillips, 1978):		
Tetracycline	43	S
Carbenicillin	28	S
Cephaloridine	21	S
Gentamicin	12	R
Colistin	0	R

All strains were positive in the following tests:

Acid from ASS fructose	Growth at 37 °C (King & Phillips, 1978)	Growth on β -hydroxybutyrate (Snell <i>et al.</i> , 1972)
Acid from ASS galactose		
Acid from ASS glucose	Growth at room temperature	Opalescence on lecithovitellin agar
Acid from ASS mannose	(King & Phillips, 1978)	Tween 20 hydrolysis (1)
Acid from ASS xylose	Growth on MacConkey's agar	Tween 80 hydrolysis (1)
Catalase production (1)	Growth on Simmons' citrate (2)	Tyrosine hydrolysis
Cytochrome oxidase production (1)		Sensitivity to sulphafurazole
		Sensitivity to cephalixin

All strains were negative in the following tests:

Acid from ASS adonitol	Acid from PWS glucose	KCN tolerance
Acid from ASS dulcitol	Aesculin hydrolysis	Lysine decarboxylase (1)
Acid from ASS ethanol	Arginine desiminase (1)	Malonate utilization (1)
Acid from ASS inositol	Arginine dihydrolase (2)	Ornithine decarboxylase (1)
Acid from ASS inulin	Gluconate oxidation (2)	Phenylalanine deamination (1)
Acid from ASS raffinose	Growth at 4 °C (King & Phillips, 1978)	Pigment production on nutrient agar
Acid from ASS rhamnose		
Acid from ASS salicin	Growth on cetrimide	Pigment production on tyrosine agar
Acid from ASS sorbitol	Hydrogen sulphide production (1)	β -Galactosidase production (ONPG)
Acid from ASS sucrose		
Acid from PWS arabinose	Indole production (2)	

* Unless otherwise stated, the methods were those of Cowan (1974); numbers in parentheses indicate which of Cowan's methods was used. ASS denotes ammonium salt sugar medium (4); PWS denotes peptone water sugar medium (1).

Of the 27 strains of '*P. thomasii*', one strain (080821) represented 25 biochemically identical isolates from blood, urine or the respiratory tracts of patients involved in the outbreak of infection described by Phillips *et al.* (1972), or from associated environmental sites. The latter were almost all contaminated directly from the hospital's supply of purified water, and, on the assumption that all were derived from the same source, only 25 of the several hundred isolates were examined in detail. The remaining 26 strains comprised 16 isolated at St Thomas' Hospital following the outbreak described by Phillips *et al.* (1972), seven originally isolated

elsewhere and submitted to the NCTC for computer-assisted identification, and three thought to conform to '*P. thomasi*' by R. E. Weaver (personal communication).

Bacteriological investigations. The bacteria were maintained on nutrient agar (25 g Oxoid nutrient broth powder CM67 and 12 g New Zealand agar per litre distilled water) under aerobic conditions and were tested at their optimum growth temperature – usually 37 °C, but in some cases room temperature (18 to 22 °C). Pigment production was recorded after 4 d growth on nutrient agar and tyrosine agar (Cowan, 1974). The Gram reaction of 24 h cultures was determined by Lillie's modification as described by Cowan (1974). Motility was tested by phase contrast microscopy of wet films on 6 to 7 h cultures grown at room temperature (18 to 22 °C) and at 37 °C in peptone water.

The biochemical characteristics investigated are listed in Table 2 and the methods used were those of Cowan (1974), Snell *et al.* (1972) or King & Phillips (1978).

Sensitivity of the organisms to carbenicillin, cephalixin, cephaloridine, colistin, gentamicin, sulphafurazole and tetracycline was assessed by the disc method, standardized by the determination of minimum inhibitory concentrations for representative strains, for which suitable concentrations of the antibiotics were incorporated in Diagnostic Sensitivity Test agar (DST; Oxoid CM261). Inocula consisted of about 10⁴ colony-forming units and the results were read after overnight incubation.

Computation of data. Of the 68 characteristics listed in Table 2, 64 were used: in the remaining four, strains were either all positive (catalase production) or all negative (malonate utilization, indole production and acid production from inulin in ammonium salt medium).

Most tests were treated as two-state characters and the results were coded as + or – (weakly positive results were coded as +; doubtful positive results were coded as –). For two-state related tests, implied negative results were coded as 'no comparison' (Sneath & Sokal, 1973). The Hugh and Leifson O–F test and colony pigmentation were treated as qualitative multi-state characters and the test results for antibiotic sensitivity were treated as a single quantitative multi-state character (resistant = 1, intermediate = 2, sensitive = 3). The between-strain similarities were calculated with Gower's (1971) general similarity coefficient (S_g in the notation of Sneath & Sokal, 1973) and negative matches were included for the two-state characters. The strains were clustered by median sorting cluster analysis (Gower, 1967; Sneath & Sokal, 1973) and by single linkage cluster analysis (Sneath & Sokal, 1973); in addition, a minimum spanning tree (Gower & Ross, 1969; Sneath & Sokal, 1973) was constructed. Programs developed in the NCTC were used. The centrotpe of the *P. pickettii*/*P. thomasi*' strains was determined by the method of Lapage & Willcox (1974) and the within- and between-taxon average similarities were calculated as arithmetic means of the relevant similarities (identical strains excluded).

DNA base composition. The determinations were done by J. J. S. Snell. DNA was extracted by the method of Marmur (1961) and further purified by treatment with protease and phenol as described by Brenner *et al.* (1969). The T_m determinations were made as described by Holmes *et al.* (1978).

RESULTS

Numerical taxonomic analysis

The dendrogram (Fig. 1) derived by median sorting cluster analysis shows the similarities between strains (detail of the *P. pickettii*/*P. thomasi*' cluster is shown in Fig. 2); the order of the strains was obtained from the minimum spanning tree. All the *P. pickettii* strains, most of the '*P. thomasi*' strains (24/27), some of the Group IVd strains (5/13) and all the Group Va strains formed a cluster at the 93 % similarity level. Table 3 lists the average within- and between-taxon similarities of this cluster and for three of the single strains which failed to join a cluster at this level. Where two reference strains of a species had not joined at or above this level they were treated as a single cluster for the calculation of the between- and within-taxon average similarity. The average within-taxon similarity of 89.8 % for the *P. pickettii*/*P. thomasi*' cluster was similar to that of two other taxa also represented by a large number of isolates (*P. cepacia*, cluster 2, 96.2 %; *P. maltophilia*, 93.7 %) and was within the range of values determined for the other taxa. The centrotpe of the *P. pickettii*/*P. thomasi*' cluster was strain CL77/72.

Most strains in the *P. pickettii*/*P. thomasi*' cluster oxidized glucose in Hugh and Leifson O–F medium and produced acid from arabinose in ammonium salt sugar medium. Strain K-619 effected no change to the medium in the O–F test and strain 049192 failed to produce acid from arabinose and in this respect they were the least typical members of the

Fig. 1. A simplified dendrogram showing the relationship between clusters based on median sorting cluster analysis. Percentage similarity was calculated with the general similarity coefficient of Gower (S_G), negative matches included.

Fig. 2. A portion of the dendrogram illustrated in Fig. 1, showing the *P. pickettii*/*P. thomasi*' cluster in detail.

cluster (see Table 2). Of those species included in the present study for references purposes, *P. stutzeri* was most similar to the *P. pickettii*/*P. thomasi*' cluster (80.0 %; Table 3).

Except for *P. cepacia* and *P. maltophilia*, insufficient strains were included to draw any conclusions about inter-relationships amongst the various reference strains. Whilst the *P. maltophilia* strains formed a distinct cluster, the *P. cepacia* strains formed two clusters (between-taxon similarity, 84.7 %), the first (*P. cepacia* cluster 1) consisting of five of the six reference strains of the species included (within-taxon similarity, 94.1 %), and the second (*P. cepacia* cluster 2) containing all the field strains (all isolated at St Thomas' Hospital) of that species (within-taxon similarity, 96.2 %).

Characteristics of organisms in the P. pickettii/'P. thomasi' cluster

The 44 strains, comprising 11 strains of *P. pickettii*, 24 strains of '*P. thomasi*', five strains of Group IVd and four strains of Group Va, were strictly aerobic, Gram-negative rods with

Table 3. Within- and between-taxon average similarities of some taxa included in this study (identical strains excluded)

Taxon	No. of strains	Percentage similarity				
		Group IVd, type 2	A9010	<i>P. pickettii</i> / ' <i>P. thomasi</i> '	B1049	C3807
<i>Acinetobacter calcoaceticus</i>	2	69.9	71.1	72.6	70.3	72.6
<i>Pseudomonas putrefaciens</i>	2	62.9	70.6	69.5	68.1	65.7
<i>Pseudomonas pseudomallei</i>	2	77.3	76.9	75.7	76.9	79.3
<i>Pseudomonas cepacia</i> , cluster 1	5	72.1	61.6	63.5	73.6	74.4
<i>Pseudomonas cepacia</i> , cluster 2	22	82.5	71.8	72.5	81.8	82.1
Group IVd, type 2	2	97.7	76.9	75.7	80.1	78.5
Unclassified group IVd, type 3, strain A9010	1		100.0	88.8	86.7	79.7
<i>Pseudomonas pickettii</i> /' <i>P. thomasi</i> '	40			89.8	86.2	86.9
Unclassified group IVd, type 4, strain B1049	1				100.0	91.4
Unclassified ' <i>P. thomasi</i> ' strain C3807	1					100.0
<i>Pseudomonas stutzeri</i>	2	66.4	76.2	80.0	72.3	77.7
<i>Pseudomonas acidovorans</i>	2	66.0	72.6	75.1	69.5	75.0
<i>Pseudomonas pseudoalcaligenes</i>	2	53.5	62.9	65.3	62.1	62.1
<i>Pseudomonas diminuta</i>	2	57.9	64.7	61.5	64.7	60.7
<i>Pseudomonas testosteroni</i>	2	58.3	66.0	69.3	67.5	69.2
<i>Pseudomonas maltophilia</i>	37	55.2	60.0	61.0	66.6	61.5
<i>Pseudomonas fluorescens</i>	2	70.7	65.6	71.0	71.1	76.6
<i>Pseudomonas aeruginosa</i>	2	69.9	66.8	68.4	66.4	64.0
<i>Pseudomonas vesicularis</i>	2	54.8	67.2	65.5	64.8	65.6
<i>Pseudomonas mallei</i>	2	64.7	68.2	67.0	57.2	64.3

Table 4. DNA base compositions of selected *P. pickettii*/'*P. thomasi*' strains

Strain no.	Sub-cluster		T_m (°C)	% G + C
	no.			
K-472	1		81.3	63.8
CL77/72	2		81.3	63.8
010010	3		81.4	64.0
CL605/72	7		81.1	63.4

parallel sides and rounded ends. Their colonies failed to produce pigment on nutrient agar or tyrosine agar.

The strains grew optimally at 37 °C and all biochemical tests, unless specified otherwise were carried out at this temperature. The strains all grew at room temperature (18 to 22 °C) and at 37 °C, but none grew at 4 °C and only six grew at 42 °C. Motility at both 37 and 30 °C could be demonstrated in 14 out of 44 strains, four were motile only at 37 °C and five only at 30 °C.

The biochemical characteristics and the sensitivity to antimicrobial agents of the strains are listed in Table 2. A total of 46 characteristics were common to all strains (either all positive or all negative) and there were 22 characteristics in which one or more of the strains differed. The tests with the largest number of strain differences were motility at 37 °C and at 30 °C, acid from cellobiose, maltose and mannitol, and gelatinase production.

Within the *P. pickettii*/'*P. thomasi*' cluster, seven sub-clusters could be discerned at the 95.5 % similarity level (Fig. 2). The DNA base composition of representative strains from four of these sub-clusters ranged from 63.4 to 64.0 % G + C (Table 4) with a mean of 63.75 %.

Strains similar to P. pickettii/'P. thomasi' but of uncertain taxonomic position

Whereas 44 strains formed the *P. pickettii*/'*P. thomasi*' cluster at the 93 % similarity level on the dendrogram derived from median sorting (Fig. 2) three further strains (A9010,

Table 5. *Characteristics for the differentiation of the seven sub-clusters included in the P. pickettii/'P. thomasi'* cluster

The results indicate the number of strains showing the character, except where the strains in the sub-cluster were either all positive (+) or all negative (—). In tests with antibiotics, strains were classified as sensitive (S), resistant (R) or of intermediate sensitivity (I); where no number is given, all strains in the sub-cluster gave the same result.

Test (see Table 2)	Sub-cluster						
	1 (15)*	2 (6)	3 (6)	4 (2)	5 (2)	6 (3)	7 (7)
Acid from ASS cellobiose	1	+	+	+	+	+	+
Acid from ASS lactose	4	+	+	+	+	+	+
Acid from ASS maltose	—	+	+	+	+	+	+
Acid from ASS mannitol	—	—	—	+	+	+	6
Acid from ASS trehalose	—	—	—	+	—	—	+
Gelatinase production	1	1	—	+	—	1	+
Growth at 42 °C	—	—	—	—	+	+	—
Nitrate reduction	+	+	+	+	+	—	+
Nitrite reduction	+	+	2	+	+	—	5
Urease production	9	+	1	+	+	+	+
Carbenicillin	S	S	5S	S	11/1R	R	R
Cephaloridine	13S	5I	5S	S	I	11/2R	6I
Gentamicin	14R	4S	4S	S	11/1R	R	6R

* Figures in parentheses indicate the total number of strains in the sub-cluster.

B1049 and C3807) were included in the *P. pickettii*/*P. thomasi*' cluster obtained by the single linkage analysis. Strains B1049 and C3807 each had a high similarity to the *P. pickettii*/*P. thomasi*' strains (86.2 % and 86.9 %, respectively; Table 3) and there was no one character in which either strain differed from all the strains in the *P. pickettii*/*P. thomasi*' cluster. Strain A9010 also had a high similarity to the *P. pickettii*/*P. thomasi*' strains (88.8 %; Table 3) but differed from them in failing to produce lecithinase. The minimum spanning tree showed that strains A9010, B1049 and C3807 joined the *P. pickettii*/*P. thomasi*' cluster through typical members of that cluster.

DISCUSSION

The results of the cluster analysis and the within-group similarities show that the 44 strains of the *P. pickettii*/*P. thomasi*' cluster constitute a distinct but heterogeneous taxonomic group containing several sub-clusters. The within-group similarity of the *P. pickettii*/*P. thomasi*' cluster was lower than that for the *P. cepacia* (clusters 1 and 2) and *P. maltophilia* clusters. This was perhaps to be expected as the strains of *P. cepacia* cluster 2 and of *P. maltophilia* were isolated from the restricted environment of St Thomas' Hospital. The strains of the *P. pickettii*/*P. thomasi*' cluster, however, came from a much wider geographical area; the *P. pickettii*, Group IVd and Group Va strains were from various locations in the U.S.A. and several of the '*P. thomasi*' strains were from locations in the U.K. other than St Thomas' Hospital. The geographical source of isolation may also account for the division of the field and reference strains of *P. cepacia* into separate clusters. That the *P. pickettii*/*P. thomasi*' cluster constitutes a distinct taxonomic group is supported by similarities in the DNA base composition of representative strains. The average figure of 63.75 % G + C is in excellent agreement with that of 64 % G + C obtained by Ralston *et al.* (1973) for *P. pickettii* strains.

Whether due to variation in the geographical source from which our *P. pickettii*/*P. thomasi*' strains were isolated or as a result of some other factor, the cluster contained seven distinct sub-clusters. Characteristics by which these sub-clusters may be differentiated from each other are given in Table 5.

Table 6. *Characteristics for the differentiation of Pseudomonas pickettii**

The results indicate the number of strains showing the character, except where the strains tested were either all positive (+) or all negative (-).

Test (see Table 2)	<i>P. pickettii</i> (including ' <i>P. thomasii</i> ') (44)†	<i>P. aeruginosa</i> (12)	<i>P. cepacia</i> (46)	<i>P. fluorescens</i> (9)	<i>P. mallei</i> (2)	<i>P. maltophilia</i> (42)	<i>P. pseudomallei</i> (2)	<i>P. putida</i> (25)	<i>P. stutzeri</i> (7)
Acid from ASS adonitol	—	—	+	1	—	—	—	—	—
Acid from ASS arabinose	43	11	+	+	—	—	+	24	5
Acid from ASS dulcitol	—	—	+	—	—	—	+	—	—
Acid from ASS ethanol	—	10	—	1	—	—	—	22	4
Acid from ASS galactose	+	11	+	+	+	—	+	+	6
Acid from ASS glucose	+	+	+	+	+	2	+	+	+
Acid from ASS inositol	—	—	+	7	—	—	+	—	—
Acid from ASS mannose	+	11	25	+	+	—	+	+	+
Acid from ASS sorbitol	—	—	+	6	+	—	+	—	—
Acid from ASS sucrose	—	—	4	7	—	—	+	—	—
Acid from ASS xylose	+	+	+	+	—	—	+	24	6
Aesculin hydrolysis	—	—	5	—	1	+	—	—	—
Arginine desiminase	—	+	—	+	1	—	+	+	2
Arginine dihydrolase	—	+	—	+	+	—	+	+	2
Casein digestion	2	+	+	7	—	+	+	1	—
Cytochrome oxidase production	+	+	+	+	—	40	+	+	+
Green pigment on nutrient agar	—	11	—	—	—	—	—	—	—
Growth at 4 °C	—	—	2	+	—	3	—	+	+
Growth on cetrimide	—	+	19	+	—	—	—	+	1
Growth on MacConkey's agar	+	+	+	+	—	+	+	+	+
Growth on Simmons' citrate	+	+	+	+	—	6	+	+	+
Growth on β -hydroxybutyrate	+	+	+	+	+	—	+	+	+
Hugh and Leifson O-F test, oxidative reaction	43	+	+	+	+	—	+	+	+
Hugh and Leifson O-F test, alkaline reaction	—	—	—	—	—	34	—	—	—
KCN tolerance	—	+	6	—	—	32	1	1	—
Lysine decarboxylase	—	—	45	—	—	+	—	—	—
Opalescence on lecithovitellin agar	+	+	+	+	—	+	+	—	+
Ornithine decarboxylase	—	—	41	—	—	—	—	—	—
Pigment production on tyrosine agar	—	—	—	2	—	+	—	24	+
Tween 80 hydrolysis	+	+	+	+	—	41	+	2	6

* The phenotypic results were derived from this study and from St Thomas' Hospital unpublished data.

† Figures in parentheses indicate the total number of strains.

DNA-DNA hybridization studies of the type strain of *P. pickettii* (K-288) and '*P. thomasii*' strain 080821 (carried out by J. L. Johnson of the Virginia Polytechnic Institute and State University, Blacksburg, Virginia, U.S.A.) showed a 50 % base sequence relatedness between these two strains. (This value was determined by a reassociation method in free solution, using S_1 nuclease to degrade unassociated molecules, and so should be equivalent to 60 to 70 % relatedness by membrane competition or hydroxyapatite techniques.) Johnson (personal communication) concluded that the value obtained is comparable to the homology differences in the *P. fluorescens* biotypes. A detailed DNA hybridization study of strains of the *P. pickettii*/*P. thomasii* cluster would assist in evaluating the taxonomic level of its sub-clusters.

The *P. pickettii*/*P. thomasii* cluster contained all the strains of *P. pickettii* (including the type strain of the species K-288 = ATCC 27511) and most (24/27) of the strains tentatively named '*P. thomasii*'. Although the name '*P. thomasii*' appeared in the literature earlier

than the name *P. pickettii*, '*P. thomasi*' was not formally described and no type strain was designated so the name was not validly published in accordance with the Bacteriological Code [Rule 12a(2) and Recommendation 12e, respectively; International Committee on Bacteriological Nomenclature, 1958]. The name *P. pickettii* (Ralston *et al.*, 1973) was validly published, however, and therefore has priority over the name '*P. thomasi*'. We do not formally propose the name '*P. thomasi*' as a separate species here in view of the insufficient evidence to establish it as such.

The *P. pickettii*/*P. thomasi*' cluster also contained the four reference strains representative of Group Va (two each of types 1 and 2). Although we examined only a small number of strains of Group Va, it would appear that this group is also synonymous with *P. pickettii*. Riley & Weaver (1975) considered that strains of Group Va-2 could probably be included in *P. pickettii*. Considering the heterogeneity of the *P. pickettii*/*P. thomasi*' cluster and its division into several sub-clusters, it might be mentioned that Tatum *et al.* (1974) found their strains of Group Va heterogeneous; although most of their strains were divided into types 1 and 2, other strains showed as many as five different additional oxidation patterns which were considered by Tatum *et al.* (1974) to be intermediate or additional biotypes. The 13 strains of Group IVd included in the present study were very heterogeneous, and only five were recovered in the *P. pickettii*/*P. thomasi*' cluster. Indeed, even where a particular biotype of Group IVd was represented by two strains, these two strains often appeared far apart on the dendrogram. Group IVd thus does not appear to be a natural one and though some of the strains of the Group belong to *P. pickettii*, from the dendrogram the remainder of the Group IVd strains examined appear only to be more related to *P. pickettii* than to any other taxon included in this study.

Table 6 lists characteristics for use in the identification of *P. pickettii* (including '*P. thomasi*') and for differentiating strains of this taxon from other saccharolytic pseudomonads that may occur in clinical material: *P. aeruginosa*, *P. cepacia*, *P. fluorescens*, *P. mallei*, *P. maltophilia*, *P. pseudomallei*, *P. putida* and *P. stutzeri*. Oberhofer *et al.* (1977) have published an identification scheme which includes Group Va-1. Garcia *et al.* (1977) have described an organism resembling *P. pickettii* whilst Otto & Pickett (1976) have published an identification scheme which includes *P. pickettii*.

Three of the 25 biochemically identical isolates represented in this study by strain 080821, which were involved in the outbreak of infection described by Phillips *et al.* (1972) and tentatively named '*P. thomasi*', have been deposited in the National Collection of Type Cultures as NCTC 10893 (strain 080821), NCTC 10894 (strain 080771) and NCTC 10895 (strain 080801). The type strain of *P. pickettii* (K-288 = ATCC 27511) has also been deposited, as NCTC 11149.

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