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Taxonomic Investigation of *Legionella pneumophila* Using Monoclonal Antibodies

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A panel of 19 monoclonal antibodies was used to produce patterns of immunofluorescent staining of 468 isolates of *Legionella pneumophila*. Twelve monoclonal antibodies were selected that divided *L. pneumophila* into 17 phenons which, in the majority of cases, conform to serogroup divisions. These phenons are more easily defined than the present serogroups, and isolates can be placed in them with little ambiguity. The standardized set of monoclonal antibodies was also used to define the subgroups of serogroup 1.

There has continued to be a proliferation in the number of serogroups of *Legionella pneumophila* which is rivaled only by the discovery of new *Legionella* species. New serogroups have been defined on the basis of three or four isolates and the presence of a common antigen, but the relationships of these isolates to other *L. pneumophila* serogroups have not been extensively examined. The first serogroups described were easily distinguishable by unabsorbed polyclonal antisera, but this state of affairs ended with the description of serogroup 8 (3) and has since become more complex.

Monoclonal antibodies (MAbs) have a clear role in separating serogroup 1 strains into subgroups with different biological properties. They also provide a reproducible method of identifying and comparing strains (13). Only recently has this approach been directed towards the non-serogroup 1 serogroups (4). It is now apparent that a large number of isolates produce patterns of immunofluorescence that do not exactly match those of serogroup reference strains, and this highlights the inadequacy of the present serogrouping system. Consequently, it was felt that a numerical taxonomic study might provide a more appropriate and reliable classification scheme and would illustrate the relationships between the serogroups of *L. pneumophila*.

MATERIALS AND METHODS

A total of 468 isolates of *L. pneumophila* were examined. Thirty of these were reference strains (Table 1), and the remainder were isolates from environmental and clinical sources collected as part of a nationwide survey over a period of 2 years. The majority of these isolates came from the United Kingdom, but a small number were isolates from France, the Middle East, and Finland. All of the clinical specimens originated in the United Kingdom, but there was strong epidemiological evidence that half the infections were contracted outside the United Kingdom. The environmental samples were from potable-water supplies and cooling and heating systems.

Formalin (1%) suspensions were made of each isolate and were then used for studies with the MAbs by indirect fluorescence. Fluorescence was recorded on a scale from 0 to 3. Variation in the degree of fluorescence between isolates was either because a smaller proportion of organisms fluoresced or because the majority of the organisms fluoresced less brightly. These two states were not differentiated in this study. The reference strains and some of the environmental strains were examined by fluorescence on more than one occasion by several workers. Between workers, the only variation in observed fluorescence was a change of 1 unit, nearly always from 0 to 1, or 2 to 3, or vice versa.

The data were analyzed by using the CLUSTAN packages, version 2, release IC (14), which were run on the University of Southampton IBM 3090/150 computer using the VM/CMS operating system. Resemblance between isolates was calculated in several ways. (i) All 19 MAbs were coded both in multistate format (0 to 3) and in binary format (0 and 1). The binary format was derived from the multistate data by recoding 0 and 1 as 0 and recoding 2 and 3 as 1 (i.e., either negative or positive fluorescence). (ii) A subset of 12 MAbs was selected from the original 19 and again analyzed in both binary and multistate formats.

Euclidean distance was used to calculate the resemblance between isolates by using the multistate coding scheme and the simple matching coefficient (S_{sm}) for the binary coding scheme. The resemblance matrices were sorted by using the UPMGA (group average) method.

Phenons defined from the dendrograms produced were characterized by using the program GPROPS (5), and the mean fluorescence or the proportion fluorescing was calculated for each MAb depending on whether the data were multistate or binary.

RESULTS

The dendrogram derived using all 19 MAbs with the data in multistate format is shown in Fig. 1. The first major division is between serogroup 1 and non-serogroup 1 isolates. The first division within serogroup 1 is between Philadelphia-like and Olda-like strains. The first division between the non-serogroup 1 strains results in a cluster containing serogroups 2, 6, 7, 11, 13, 3, and 9, in that order, and 10, 4, 8, 12, 14, and 5, also in that order.

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TABLE 1. Reference strains of *L. pneumophila* used in analyses

Strain	Serogroup	Type culture no.[a]
Philadelphia	1	33152
Allentown	1	43106
Benidorm	1	43108
Knoxville	1	33153
France	1	43112
Olda	1	43109
Oxford	1	43110
Heysham	1	43107
Camperdown	1	43113
Bellingham	1	43111
Togus 1	2	33154
Bloomington 2	3	33155
Los Angeles 1	4	33156
Kingston 4	4	NA
Dallas 1E	5	33216
Cambridge 2	5	11417 (N)
Chicago 2	6	33215
Oxford 3	6	11287 (N)
Chicago 8	7	33823
Concorde 3	8	35096
P157	8	NA
P183	9	NA
F1356 (PE)	9	NA
Leiden 1	10	NA
Portland 1	?4	NA
CDC 11	11	43130
CDC 12	12	43290
CDC 13	13	43736
1169 MN-H	14	43703
1586-SCT-H	14	NA

[a] The type culture numbers are ATCC or NCTC (N). NA, Not available.

The dendrogram shown in Fig. 2 was produced by using only 12 MAbs in the binary format. The MAbs that were excluded from this analysis were LA3, which fluoresced with a wide range of serogroup reference strains; JR4, which distinguished between the two serogroup 9 type strains used; and the last five MAbs in the standardized set, which divided serogroup 1 into a number of minor subgroups. This dendrogram is divided into 19 phenons, whereas the corresponding dendrogram produced by using multistate format results in 20 phenons. There is considerable concordance between the phenons produced in the two ways; 12 of the binary phenons are identical to multistate groups (phenon 1 binary equals phenons 1 and 2 multistate), a one-isolate overlap occurs within each of the two phenons of each serogroup 5 subgroup, and an overlap of two isolates occurs in the serogroup 4 and 8 cluster. Three of the phenons are single-member clusters; one of them includes the SG7 reference strain, and the other two (phenons 7 and 19) have been excluded from consideration since only Formalin suspensions were available and the confirmation of their purity was not possible. The concordance between binary and multistate groupings is 90%, and if the phenons within each serogroup 5 subgroup are merged the concordance rises to 96%. The fluorescence patterns for the phenons in binary format are shown in Table 2.

The section of the dendrogram in Fig. 3, which was derived from binary data using all 19 MAbs, shows the serogroup 1 cluster and demonstrates groupings equivalent to the named subgroups (8). There is a subgroup added to those previously described, of which three isolates were available that reacted to both W32 and MAb2. These isolates

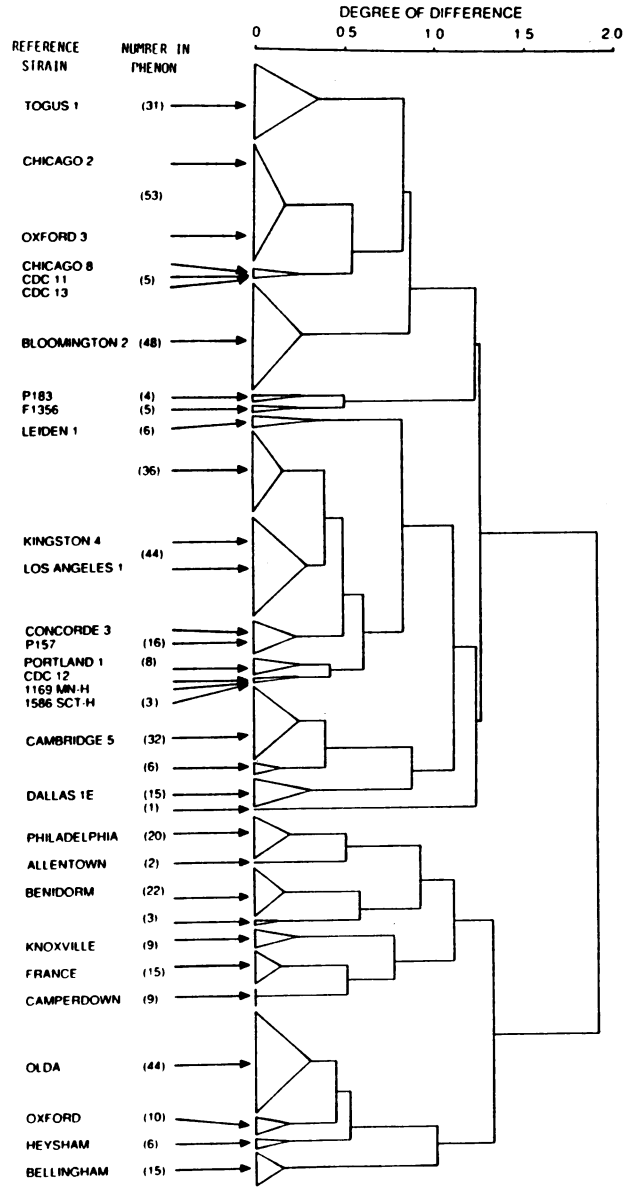

FIG. 1. Dendrogram derived by using all 19 MAbs with the data in multistate format.

were rechecked, and the results were confirmed, but they represent only 2% of the serogroup 1 isolates tested. The fluorescence patterns of the subgroups in multistate format are shown in Table 3. Concordance between binary and multistate phenons is 100%.

DISCUSSION

The most common environmental and clinical isolates are members of serogroup 1. MAb typing has shown that one MAb can identify a subset of strains that cause the majority of cases of legionella pneumonia (13). The specificity, reproducibility, and ease of manufacture have made MAbs valuable tools in the identification and typing of bacteria in general, and it appears reasonable to extend their role to the other serogroups of *L. pneumophila*.

The dendrograms constructed from the fluorescence data produced clusters that, in most cases, agree well with the

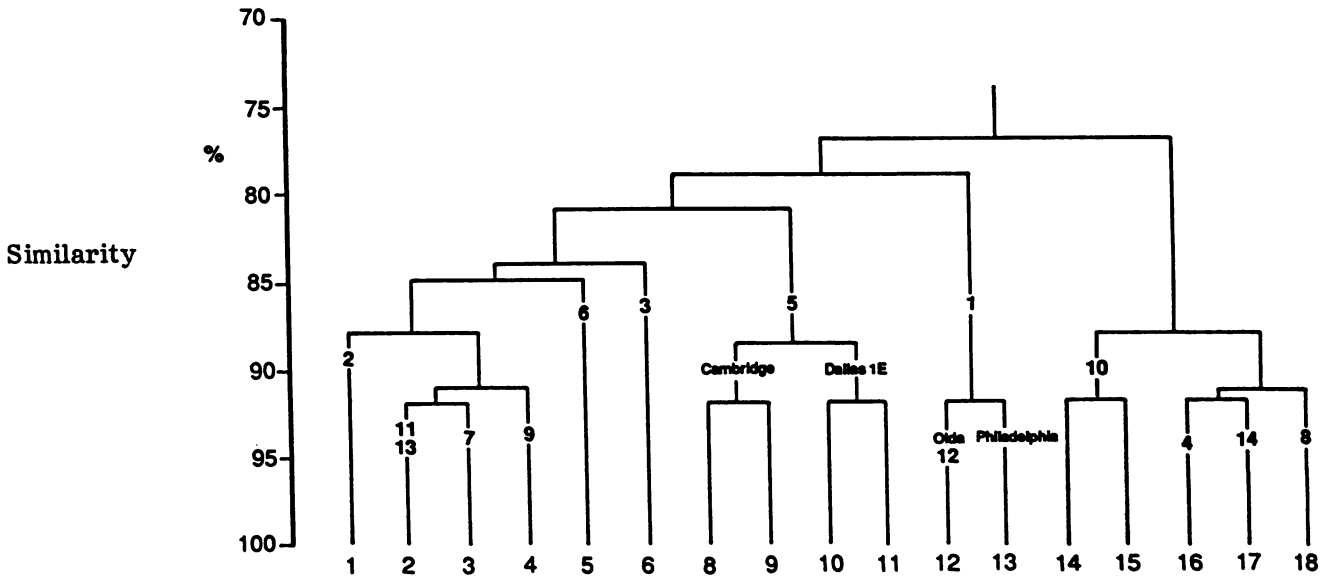

FIG. 2. Dendrogram showing the phenons derived by using binary data obtained from 12 MAbs. The numbers within the borders refer to the serogroup reference strains. The numbers on the *x* axis refer to the phenons (note that phenons 7 and 19 are excluded as there was only one isolate in each).

present serogrouping system. Serogroup 1 was divided into subgroups in the way described by Joly et al. (8), and this confirms the reproducibility of this system. Serogroups 2, 3, and 6 (9, 10) are valid entities confirmed by the high degree of similarity within the cluster. Serogroup 7 (2) was poorly represented in this study and appears to be an infrequent environmental isolate.

There has been evidence for some time that serogroup 5 is composed of two very different groups of organisms. Watkins and Tobin (12) showed that MAbs differentiate the Cambridge 2-like strains from those resembling Dallas 1E. Garrity et al. (7) have shown by serological and DNA homology studies differences sufficient to accord Dallas 1E-like strains species status. This was confirmed by Selander et al. (11) using the electrophoretic mobility of isoenzymes as a typing scheme.

Serogroup 9 (6) can be separated into two subgroups by MAb JR4, though whether this is of practical value is doubtful. The subgroups are closely related, and the group is uncommon in the United Kingdom, with only nine isolates included in this study.

TABLE 2. 12 MAbs with the serogroups of *L. pneumophila*

Serogroup no. and subgroup[a]	Phenon no.		MAb(s) giving positive fluorescence
	Binary	Multistate	
1 Olda	12	17	MAb1
1 Philadelphia	13	19	MAb1 and MAb2
2	1	1 and 2	TOG1
3	6	8	S3-2
4	16	15	K13
5 Cambridge	9	11	K13 and CAM2
5 Cambridge	8	10	CAM2
5 Dallas 1E	10	12	CAM2 and DAL1
5 Dallas 1E	11	13	DAL1
6	5	6	OXF 3
7	3	5	P10F
8	18	16	K13 and 188
9	4	7	183
10	14	3	LDN5
10	15	14	K13 and LDN5
11	2	4	NONE
12	12	17	MAb1
13	2	4	None
14	17	18	MAb1 and K13

[a] Serogroups 7, 11, 12, and 13 are represented by only one isolate. Isolates from serogroups 11 and 13 did not react with any MAb in the panel. Isolates from serogroup 12 reacted with LA3, but isolates from serogroup 1 did not.

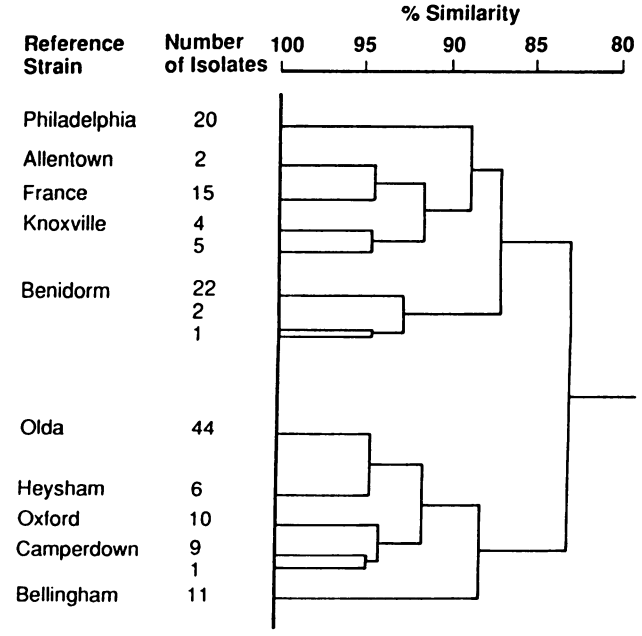

FIG. 3. Dendrogram of the serogroup 1 cluster derived from 19 MAbs with the data in binary format.

TABLE 3. Standardized MAb panel showing the subgroups of *L. pneumophila* serogroup 1

Subgroup	Phenon no.:		No. of isolates	Mean fluorescence[a] with:						
	Binary	Multistate		MAb1	MAb2	MAb3	W32	33G2	32A12	144C
Philadelphia	1	1	20	2.8	3.0	0	0	2.9	2.9	0.1
Allentown	2	2	2	3.0	3.0	0	0	3.0	0	0
France	3	6	15	3.0	3.0	0	0	0	0	0
Knoxville	4 + 5	5	9	2.8	3.0	3.0	0	0	1.1*	0
Benidorm	6	3	22	2.8	3.0	0	0	2.8	0	3.0
Unnamed	7 + 8	4	3	3.0	3.0	0	3.0	1.7**	0	3.0
Olda	9 + 13	8	44	2.9	0	0	0	0	2.8***	2.8
Heysham	10	10	6	3.0	0	2.7	0	0	3.0	2.8
Oxford	11	9	10	3.0	0	0	0	0	2.9	0.2
Camperdown	12	7	9	3.0	0	0	0	0	0	0
Bellingham	14	11	15	2.9	0	0	3.0	0	0	3.0

[a] Fluorescence was scored on a scale from 0 to 3, and the values for mean fluorescence are accurate to the nearest 0.1, rounded down. All of the MAbs with mean scores below 2 produced negative scores except when four isolates were positive and five were negative (*) and when two were positive and one was negative (**). All of the MAbs with mean scores above 2 produced positive scores except when 43 isolates were positive and 1 was negative (***). Consequently, for each of these three multistate phenons two binary phenons exist.

The most interesting group is the 4, 8, and 10 cluster. The description of serogroup 8 by Bisset et al. (3) suggests that this is not an easy group to define with polyclonal antisera. The MAb panel divided this cluster into five groups: 10, 4/10, 4, 14, and 8, with each group almost merging into the next. Serogroup 14 is the latest group to be described (1), and only two strains were examined by using the MAb panel. Portland 1 (a candidate for the serogroup 4 reference strain) does not fit well into this grouping system, mainly because of its poor fluorescence with K13. It is very atypical of serogroup 4, and no other isolate in this study has an exactly similar fluorescence pattern. The other serogroup reference strains (serogroups 11, 12, and 13) were included in the study despite the fact that the panel of MAbs did not include a MAb raised against them. Serogroup 12 was clustered with a small group of LA3 positives when all 19 MAbs were used but otherwise was grouped with serogroup 1. The reference strains of serogroups 11 and 13 were unreactive with the panel.

The value of analyzing the data in the binary and multistate formats is in demonstrating that the phenons are easily defined entities. The same groupings were generated if fluorescence was scored as a binary character or by the strength of fluorescence, and this is a reflection of the ability of MAbs to be highly specific. The present serogrouping system fits well with the phenons produced by this analysis but does not completely recognize the divisions within the serogroups. MAbs have the advantage of being well defined and easily produced in large quantities. In view of these features, we recommend that these or similar MAbs replace the present polyclonal antisera.

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