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Legionella pneumophila: monoclonal antibody typing of clinical and environmental isolates

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SUMMARY

Forty-one clinical isolates of *Legionella pneumophila* from sporadic cases of legionella pneumonia were collected from laboratories throughout the United Kingdom and were compared with 300 routine environmental isolates using two panels of monoclonal antibodies, covering serogroups 1–10. Eighty-five per cent of the clinical isolates belonged to the subgroup Pontiac of serogroup 1, whilst only 13% of the environmental isolates did. Approximately half of the clinical isolates tested came from patients with a recent history of foreign travel, mainly to southern Europe.

INTRODUCTION

The use of monoclonal antibodies (MAbs) for the subgrouping of *Legionella pneumophila* serogroup 1 is recognized as a valuable epidemiological tool (McKinney *et al.* 1983; Joly & Ramsay, 1985; Edelstein *et al.* 1986). We have extended the use of MAbs to the identification of the non-serogroup 1 serogroups and compared the relative prevalences of the various serogroups and subgroups in isolates from throughout the United Kingdom.

MATERIALS AND METHODS

Isolates of *L. pneumophila* from sporadic cases of legionella pneumonia which had been reported to the Communicable Diseases Surveillance Centre (CDSC) of the Public Health Laboratory Service (PHLS) from 1979 to the end of 1985 were collected following a written request to all reporting laboratories. Replies were received from 20 laboratories out of 26 who were contacted and out of 46 reported cases 20 viable isolates were obtained, 5 of the cases had not been culture positive, 8 were no longer viable and 3 could not be traced. Clinical isolates of *L. pneumophila* were also obtained from Dr R. J. Fallon of Ruchill Hospital, Glasgow, Scotland and from a number of other laboratories. Most of these other isolates were from cases of pneumonia that occurred in late 1985 and 1986 and a few were from cases that had not been reported to CDSC. Three hundred consecutive isolates were recovered from a set of environmental water samples from over 150 sites throughout the United Kingdom received in Oxford from April to December 1986. The environmental samples were either filtered or centrifuged

Table 1. *Non-serogroup 1 (NSG 1) monoclonal antibody panel*

NSG1 type strains	Sero-group	Togus 1/1	3-2	K13	LA3	Cam 2	Dal 1	OX3/ SG6	SG7/ P10F	188-2	JR4	183/24	Leiden 5
Togus	2	+++*	—	—	—	—	—	—	—	—	—	—	—
Bloomington 2	3	—	+++	—	—	—	—	—	—	—	—	—	—
Los Angeles	4	—	—	+++	+++	—	—	—	—	—	—	—	—
Kingston 4	4	—	—	+++	+++	—	—	—	—	—	—	—	—
Dallas 1E	5	—	—	—	—	+/++	+++	—	—	—	—	—	+
Cambridge 2	5	—	—	—	++	+++	—	—	—	—	—	—	—
Chicago 2	6	—	—	—	—	—	—	+++	—	—	—	—	—
Oxford 3	6	—	—	—	—	—	—	+++	—	—	—	—	—
Chicago 8	7	—	—	—	—	—	—	—	++	—	—	—	—
Concorde 3	8	—	—	+++	+++	—	—	—	—	++	—	—	—
P157	8	—	—	+++	+++	—	—	—	—	+++	—	—	—
P183	9	—	—	—	++	—	—	—	—	—	+++	+++	—
F1356 (PE)	9	—	—	—	+	—	—	—	—	—	—	+++	—
Leiden	10	—	—	++	—	—	—	—	—	—	—	—	+++

* +/+++/+++, strength of fluorescence.

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Table 2. *Proportions of isolates belonging to serogroups and major subgroups*

		Environmental		Clinical	
Group	Subgroup	No.	%	No.	%
SG 1		125	41·7	37	90·3
	Pontiac	40	13·3	35	85·4
	Olda	67	22·3	2	4·9
	Bellingham	18	6·0	0	0
SG 2		17	5·7	0	0
SG 3		24	8·0	0	0
SG 4		42	14·0	1	2·4
SG 5		32	10·7	3	7·3
	Cambridge	25	8·3	3	7·3
	Dallas 1E	7	2·3	0	0
SG 6		34	11·3	0	0
SG 7		0	0	0	0
SG 8		8	2·7	0	0
SG 9		4	1·3	0	0
SG 10		1	0·3	0	0
Not typable by MAbs		13	4·3	0	0
Total		300	99·9	41	100

and the deposit spread onto selective BCYE agar (Edelstein, 1981). Formalin suspensions were made of presumed *Legionella* spp. and using indirect fluorescence, tested with polyclonal rabbit antisera (Division of Microbiological Reagents and Quality Controls, PHLS).

Suspensions of the two sets of isolates were tested with two different panels of MAbs using indirect fluorescence. The first panel divided serogroup 1 into three major subgroups and further minor subgroups (Watkins *et al.* 1985). The second panel (Table 1), which contained MAbs raised against strains of the non-serogroup 1 *L. pneumophila*, was used for any isolate that either failed to group with the first panel or had not been allocated to serogroup 1 using the rabbit antisera.

RESULTS

Of the 300 environmental isolates 58% belonged to serogroups other than 1 and of the 125 that were serogroup 1, 67 (54%) belonged to the Olda subgroup. Thirty-seven (90%) of the 41 clinical isolates belonged to serogroup 1, with 95% of these belonging to the Pontiac subgroup (Table 2). All the clinical serogroup 5 isolates were isolated from patients from different locations and were all of the same MAb pattern (Cambridge 2). No Dallas 1E-like isolates were obtained from patients but seven were found in the environmental samples. It is believed that Cambridge 2 is more representative of serogroup 5 than Dallas 1E as a recent analysis of isoenzyme patterns places the latter in a separate species (Selander *et al.* 1985) despite sharing the serogroup epitope.

Thirteen environmental isolates sent to us as *Legionella* spp. could not be grouped using these two panels of MAbs and on further testing with other polyclonal antisera 3 were identified as *L. pneumophila*, 2 were *L. wadsworthii* and 8 remained unidentified.

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There was a high degree of correlation between the serogroup allocated to isolates using the polyclonal rabbit antisera and the monoclonal antibodies. No polyclonal rabbit antisera against serogroups 9 and 10 was available at the time of testing.

There were 19 serogroup 1 isolates from patients giving a history of recent foreign travel, of which 18 were Pontiac strains and 1 was an Olda strain.

DISCUSSION

Watkins *et al.* (1985) using British and European isolates of *L. pneumophila* serogroup 1 (SG 1) from outbreaks and individual cases of legionella pneumonia have shown that environmental water samples from sites associated with cases were significantly more likely to contain Pontiac strains than samples not associated with cases. This study has confirmed the difference in the relative prevalence of the various serogroups between the strains isolated from patients and those isolated from environmental sources. *L. pneumophila* strains of the Pontiac subgroup of serogroup 1 are by far the commonest cause of legionella pneumonia. They are less frequently isolated from routine environmental samples of water, only 12% in a recent study (Tobin *et al.* 1986). The relatively high number of Pontiac strains (32%) among the SG 1 strains that were isolated from our environmental samples may reflect the examination of water samples following a case of legionella pneumonia rather than routine surveillance. McKinney *et al.* (1983) using their monoclonals had suggested that strains from outbreaks fell into different subgroups from those isolated from mainly sporadic cases. The subgroup from outbreak strains corresponds to our Pontiac subgroup and to the MAb2 positive subgroup of the 'standard' panel proposed by Joly *et al.* (1986). The occurrence of sporadic cases of legionella pneumonia with non-Pontiac SG 1 strains is not common and appears to reflect the relative prevalence of these non-outbreak strains in environmental sources. It is of interest to note that about half the cases from which the clinical isolates came had a history of travel abroad within the preceding few weeks. Most of the travel had been to southern Europe and the Mediterranean. The widespread use of monoclonal antibodies for routine sero- and subgrouping of isolates of *L. pneumophila* should provide valuable information on the epidemiology of legionella pneumonia.

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