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Legionella pneumophila serogroup 1 population in Italy by monoclonal subtyping

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SUMMARY

The Oxford panel of monoclonal antibodies was used to subtype 83 strains of *Legionella pneumophila* serogroup 1 of human and environmental origin. The International panel was also used to subtype 50 of them. All the 18 patients' isolates were of the Pontiac subgroup, and 40/43 of the environmental strains of the Pontiac subgroup were associated with human infection. The remaining environmental strains were subgroups Olda (15 strains), Camperdown (5 strains), and Bellingham (2 strains). The Philadelphia subgroup was the commonest among the environmental strains tested with the international MABs panel.

This study confirms previous findings that *L. pneumophila* serogroup 1 isolates with the Pontiac (Oxford panel) or MAB-2 (international panel) reacting antigen marker seem to be more virulent than the other subgroups.

INTRODUCTION

Legionella pneumophila serogroup 1 is the most frequently recognized cause of legionellosis both in outbreaks and sporadic cases. However, the same microorganism is also frequently isolated from natural and man-made environments apparently unassociated with human infection.

L. pneumophila serogroup 1 is antigenically highly variable [1–4]. Different epitopes have been used to prepare monoclonal antibodies, and to identify monoclonal subtypes of this microorganism [5–11]. Typing schemes by indirect immunofluorescence have been developed using different panels of monoclonal antibodies [6, 7, 11]; recently three laboratories have devised a collaborative standardized subgrouping protocol using seven monoclonal antibodies [12].

Subgrouping schemes have been used in epidemiological studies, and in several instances have been useful in identifying the source and mode of transmission of human infections [6–7, 13–20]. Some subtypes have been more frequently associated with human infection than others [14, 17, 20, 21]. Other subtypes appear to be less commonly associated with disease. These results, however, may vary in different countries. Examination of a large number of strains is therefore

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necessary, to determine the real prevalence of different subtypes, their capability to survive in different environmental niches, and their correlation with human infections. For this reason we examined by monoclonal subtyping *L. pneumophila* serogroup 1 strains isolated throughout Italy.

MATERIALS AND METHODS

A total of 83 *L. pneumophila* serogroup 1 strains were stored between 1981 and May 1989, which had been isolated by us or sent to the Istituto Superiore di Sanità by other laboratories for identification. Eighteen were from patients, and 65 were environmental isolates, some of which were associated with Legionnaires' disease in the above-mentioned patients.

Isolates were identified as *L. pneumophila* serogroup 1 by their ability to grow on buffered charcoal-yeast extract agar supplemented with 1% alpha-keto-glutarate, their inability to grow in the absence of cysteine, and by direct immunofluorescence using specific fluorescent antiserum kindly proved by the Centers for Disease Control, Atlanta, USA. All the strains were positive for catalase, hippurate hydrolysis, β-lactamase production, and production of a brown pigment on tyrosine-supplemented medium; were variable for oxidase; and did not exhibit autofluorescence when exposed to long wave length ultraviolet light. The strains had been repeatedly passaged on buffered charcoal yeast-extract agar before storage at $-80°$.

The monoclonal antibodies (MABs) used were those of the Oxford panel [11]. The strains were typed at the Istituto Superiore di Sanità in Rome and the results confirmed in Oxford. Some were typed by Dr P. J. Dennis, PHLS, Porton Down, Salisbury (personal communication). The majority of the strains was also tested with the standardized international MABs panel [12] at the laboratory in Oxford. The indirect antibody test was performed with heat killed (65 °C for 45–60 min) bacterial aqueous suspensions (turbidity equivalent to a McFarland 2 standard).

RESULTS

Eighteen of the 83 strains were isolated from post mortem lung tissue or from respiratory secretions of patients with Legionnaires' disease. Forty-two of the environmental isolates were from the water supply or respiratory equipment of hospitals; 18 from the water supply of hotels; 3 came from a public facility for elderly and handicapped persons, 1 of which was isolated from the water of a garden fountain; 1 was from the water supply of a cultural centre, and 1 from the water of our Institute.

All the strains isolated from patients belonged to the Pontiac subgroup (Table 1), as did the majority of the environmental strains (66%). With the exception of three strains these were all associated with Legionnaires' disease. Fifteen isolates were Oldas, five were Camperdown, and two were Bellingham. The 17 isolates for which information was available were apparently unassociated with human infection.

Table 1. *L. pneumophila* serogroup 1 isolates by monoclonal antibody typing

(Oxford and international panels)

Strains *n*	City	Origin	Actual source	Associated with disease (outbreak or sporadic)	Monoclonal subgroup	
					Oxford	International
4	Lido di Savio	Hotel 1	Ps	Yes (o)	Pontiac	Philadelphia
1	Lido di Savio	Hotel 2	Ps	Yes (o)	Pontiac	Philadelphia
1	Diano Castello	Hotel	Ps	Yes (s)	Pontiac	Knoxville
1	Rimini	Hospital	Pa		Pontiac	Benidorm
3			Ps	Yes (o)	Pontiac	Benidorm
1	Bari	CAI	Pa		Pontiac	Benidorm
12	Torino	Hospital 1	Pa		Pontiac	Not Tested
2			Oh	Yes (o)	Pontiac	Not Tested
14			Ps	Yes (o)	Pontiac	Philadelphia (2 strains tested)
1			Ps		Bellingham	Bellingham
2	Torino	Hospital 2	Po	NK	Pontiac	Philadelphia
5	Paestum	Hotel	Ps	Yes (o)	Pontiac	Knoxville (1 strain tested)
2	Meldola	Public Institution	Ps	Yes (o)	Pontiac	Knoxville
1			Fo		Pontiac	Not Tested
1	Roma	CAI	Pa		Pontiac	Philadelphia
1	Roma	Cultural Centre	Ps	Yes (o)	Pontiac	Philadelphia
1	Roma	ISS	Ps	No	Bellingham	Bellingham
1	Monza	CAI	Pa		Pontiac	Benidorm
1	Monza	CAI	Pa		Pontiac	Allentown
1	Como	CAI	Pa		Pontiac	Allentown
1	Molveno	Hotel 1	Ps	Yes (s)	Pontiac	Philadelphia
1	Molveno	Hotel 2	Ps	Yes (s)	Pontiac	Philadelphia
1	Cattolica	Hotel	Ps	Yes (s)	Pontiac	Benidorm
4	Folgaria	Hotel	Ps	Yes (o)	Pontiac	Philadelphia
7	Firenze	Hospital 1	Ps	no	5 Olda, 2 Camperdown	5 Olda, 2 Camperdown
1			Ac	No	Olda	Olda
7	Firenze	Hospital 2	Ps	*	5 Olda, 2 Camperdown	5 Olda, 2 Camperdown
5	Messina	Hospital	Ps	NK	4 Olda, 1 Camperdown	4 Olda, 1 Camperdown

CAI, Community acquired infection.
ISS, Istituto Superiore di Sanità, Rome.
Ps, Plumbing system; Pa, patient; Oh, oxygen humidifiers; Po, pools, Fo, fountain; Ac, air conditioner.
NK, Not known.
*, One case serologically diagnosed, no isolate available.

Using the international typing scheme 6 patient isolates subtyped as Benidorm (3 strains), Allentown (2 strains), and Philadelphia (1 strain). The subgroups of the environmental isolates associated with disease were Philadelphia (14 strains), Benidorm (5 strains), and Knoxville (2 strains). There were no differences between strains isolated during outbreaks or associated with sporadic cases.

DISCUSSION

Sixty-one (73·5 %) of the 83 *L. pneumophila* serogroup 1 strains isolated in Italy were of the Pontiac subgroup by the Oxford MABs panel. All of the 18 patient isolates were of the Pontiac subgroup; 40/43 of the environmental strains of the Pontiac subgroup were associated with human infection. Twenty-two of the 65 environmental strains tested belonged to the subgroups Olda (15 strains), Camperdown (5 strains), and Bellingham (2 strains) and were not associated with cases of Legionnaires' disease. *L. pneumophila* serogroup 1 isolates from water supply of an hospital in Firenze (hospital 2) were of the subgroups Olda and Camperdown. In that hospital one nosocomial case was diagnosed (seroconversion), but unfortunately no clinical isolate was available for examination. In another hospital in Firenze (hospital 1) the plumbing system was found to be colonized by the subgroups Olda and Camperdown and the air conditioner by the subgroup Olda, but no cases occurred among the patients. However it should be noted that clinicians used erythromycin immediately the first respiratory symptoms appeared. Among 17 environmental isolates of an hospital in Torino (hospital 1) one was of the Bellingham subgroup. This strain was isolated from a shower after control measures were adopted raising the hot water temperature. At that time no further nosocomial cases of Legionnaires' disease were detected at the hospital.

There was a relatively high number of isolates of the Camperdown subgroup which is usually rare.

The Philadelphia subgroup is commonest among the environmental strains tested with the international MABs panel.

L. pneumophila serogroup 1 is most commonly associated with Legionnaires' disease, although it is ubiquitous in the environment. A number of monoclonal antibody subtyping schemes have been developed for epidemiological studies [6, 7, 11, 12]. These have also been useful in identifying possible correlations between antigenic subgroups and virulence [17, 20–22].

This study confirms previous findings that *L. pneumophila* serogroup 1 isolates with the Pontiac (Oxford panel) or MAb-2 (international panel) reacting antigen marker are more virulent than the other subgroups [20–22] in that they are more commonly isolated from cases of Legionnaires' disease. In this study all of the strains isolated from patients were of the Pontiac subgroup, as were a high proportion of environmental isolates associated with outbreaks.

More information on strains isolated from epidemic and sporadic cases, and from different ecosystems associated with human disease will increase our understanding of virulence factors in subgroups and the capability of subgroups to survive in different environmental niches.

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