CONCISE COMMUNICATION

Distribution of *Legionella* Species and Serogroups Isolated by Culture in Patients with Sporadic Community-Acquired Legionellosis: An International Collaborative Survey

Victor L. Yu, ¹ Joseph F. Plouffe, ² Maddalena Castellani Pastoris, ⁵ Janet E. Stout, ¹ Mona Schousboe, ⁶ Andreas Widmer, ⁸ James Summersgill, ⁴ Thomas File, ³ Christopher M. Heath, ⁹ David L. Paterson, ^{1,10} and Annette Chereshsky^{7,a} ¹Veterans Affairs Medical Center, Pittsburgh, Pennsylvania; ²Ohio State University, Columbus, and ³Northeastern College of Medicine, Akron, Ohio; ⁴University of Louisville, Louisville, Kentucky; ⁵Instituto Superiore di Sanita, Rome, Italy; 6Canterbury Health Laboratories, Christchurch, and ¹Communicable Disease Centre, Porirua, New Zealand; ⁸Kantonsspital, Basel, Switzerland; ⁹Royal Perth Hospital, Perth, and ¹OPrincess Alexandra Hospital, Brisbane, Australia

This international collaborative survey identified culture-confirmed legionellosis in 508 patients with sporadic community-acquired legionellosis. *Legionella pneumophila* constituted 91.5% of the isolates. Serogroup 1 was the predominant serogroup (84.2%), and serogroups 2–13 (7.4%) accounted for the remaining serogroups. The *Legionella* species most commonly isolated were *L. longbeachae* (3.9%) and *L. bozemanii* (2.4%), followed by *L. micdadei*, *L. dumoffii*, *L. feeleii*, *L. wadsworthii*, and *L. anisa* (2.2% combined). *L. longbeachae* constituted 30.4% of the community-acquired *Legionella* isolates in Australia and New Zealand.

Forty-two *Legionella* species with 64 serogroups have been classified to date [1]. The incidence of community-acquired pneumonia caused by these species and serogroups remains largely unknown because many bacterial isolates cannot be diagnosed by current microbiologic tests. Direct fluorescent antibody (DFA) stains and serologic tests are available only for a limited number of species, whereas the urinary antigen test has high sensitivity only for *Legionella pneumophila* serogroup 1.

In 1984, the Centers for Disease Control and Prevention (CDC) published a retrospective survey of *Legionella* serogroups and species in the United States, on the basis of 335 specimens submitted to the CDC or 11 state health departments [2]. The conclusion was that *L. pneumophila* was responsible for 80%–85% of *Legionella* infections and that serogroups 1 and 6 were responsible for two-thirds of all *Legionella* infections. However, there were limitations to this survey. Culture, the most sensitive test, was not widely available, so DFA staining of respiratory tract secretions and tissue was the diagnostic test performed. Background information for most isolates was not available, and clinical information concerning community versus hospital acquisition and sporadic versus outbreak-related cases was not

available [2]. Furthermore, new serogroups and species of *Legionella* have been discovered since 1984. A contemporary assessment of *Legionella* species and serogroups causing pneumonia would be important, so we initiated a prospective and retrospective tabulation of sporadic cases of culture-confirmed *Legionella* infection in patients with community-acquired legionellosis in an international multicenter survey.

Methods

Twenty hospital laboratories in North America, Europe, Australia, and New Zealand with expertise in laboratory diagnosis of *Legionella* infections were invited to participate in a survey of sporadic cases of community-acquired pneumonia due to *Legionella* species. The 20 hospitals were identified by review of literature reports in which cases of community-acquired legionellosis were identified by culture methodology. Twelve hospitals accepted the invitation.

The most common reason given for nonparticipation was that culture results could not be classified as sporadic versus outbreak and community-acquired versus hospital-acquired because of a lack of clinical data. One hospital laboratory was removed at the outset of the study because respiratory tract specimens were accepted from other hospital laboratories, and the investigators were unable to confirm that all cases were sporadic rather than outbreak-related. One hospital laboratory withdrew because of failure to enroll any cases in the first year of the study. Government or public health agency laboratories were not invited to participate, to rule out the possibility that outbreak-related cases might inadvertently be included.

Thus, 10 hospital laboratories with expertise in culture of *Legionella* species performed both prospective and retrospective surveys of *Legionella* species isolated from the respiratory tracts of consecutive patients with sporadic community-acquired pneumonia. Data were

The Journal of Infectious Diseases 2002;186:127-8

© 2002 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2002/18601-0020\$15.00

Received 11 October 2001; revised 29 January 2002; electronically published 21 May 2002.

Presented in part: Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, 17–20 September 2000 (abstract 593).

^a A.C. is currently a consultant in Melbourne, Australia.

Reprints or correspondence: Dr. Victor L. Yu, Veterans Affairs Medical Center, Infectious Disease Section, University Dr. C, Pittsburgh, PA 15240 (vly+@pitt.edu).

collected prospectively from 1997 through 2001 and retrospectively from as early as 1980–1993, depending on the hospital.

The serogroups and species were confirmed by established methods, as described elsewhere [3]. In brief, colonies morphologically consistent with *Legionella* species were subcultured to buffered charcoal yeast extract agar and selective media with and without cefamandole. Culture plates were incubated for 10–14 days. DFA staining was performed to identify individual species and serogroups.

Results

In total, 508 cases of culture-confirmed community-acquired legionellosis were identified. Isolates were from the United States (72.2%), Italy (12.6%), Switzerland (6.1%), Australia (4.7%), and New Zealand (4.3%) (table 1). *L. pneumophila* was the most frequently isolated species (91.5% of isolates). *L. long-beachae* comprised 3.9% of the organisms; however, 14 of those 20 isolates originated in Australia and New Zealand.

Discussion

Hospital-acquired outbreaks of legionnaires disease have involved serogroups other than *L. pneumophila* serogroup 1 (especially serogroups 4 and 6) and *Legionella* species other than *L. pneumophila*, especially *L. micdadei*, *L. dumoffii*, and *L. bozemanii* [4]. However, this survey documents that community-acquired legionnaires disease is dominated by *L. pneumophila* serogroup 1. Table 1 shows that 84.2% of all isolates were *L. pneumophila* serogroups accounted for only 7.4% of the isolates. Species other than *L. pneumophila* were rare: *L. longbeachae* (3.9%) and *L. bozemanii* (2.4%) accounted for most of the nonpneumophila cases. *L. micdadei*, *L. feeleii*, *L. dumoffii*, *L. wadsworthii*, and *L. anisa* combined accounted for 2.2% of the remaining cases.

This distribution of species and serogroups has implications for the development of molecular tests that will have the widest utility. For example, the Binax urinary antigen appears to be clinically useful for community-acquired pneumonia outside Australia and New Zealand, since *L. pneumophila* serogroup 1 accounted for 88.2% (407/461) of American and European *Legionella* isolates. In contrast, for Australia and New Zealand, *L. pneumophila* serogroup 1 accounted for only 45.7% of cases of community-acquired legionellosis, and *L. longbeachae* accounted for 30.4% of cases.

A major limitation of this study is the relatively small number of laboratories in only a few countries, so the results cannot be considered representative. We were surprised by how few reference laboratories for *Legionella* compiled clinical data on culture-confirmed cases of legionellosis. At the present time, it is not possible to perform a truly representative international survey of culture-confirmed cases of community-acquired le-

Table 1. Classification of respiratory tract *Legionella* species and serogroups isolated from consecutive patients with community-acquired pneumonia.

Legionella species,		
serogroup	Species	Serogroup
L. pneumophila	91.5 (465)	
1		84.2 (428)
6		1.7 (9)
5		1.3 (7)
4		1.0 (5)
3		1.0 (5)
7		0.6(3)
2		0.4(2)
8		0.4(2)
13		0.4(2)
Unknown ^a		0.6(3)
L. longbeachae	3.9 (20)	
Unknown		2.6 (13)
1		1.2 (6)
2		0.1(1)
L. bozemanii	2.4 (12)	
Unknown		2.0 (10)
1		0.2(1)
2		0.2(1)
L. micdadei	0.6(3)	
L. dumoffii	0.6(3)	
L. feeleii	0.4(2)	
L. wadsworthii	0.2(1)	
L. anisa	0.2(1)	
Unknown species	0.2(1)	
Total	100 (508)	

NOTE. Data are percentage (no.) of isolates.

gionellosis, since most public health and government laboratories fail to collect clinical data on consecutive cases or fail to differentiate between outbreak and sporadic cases of legionnaires disease. A second limitation is that the culture methodology may favor growth of *L. pneumophila* over that of other species [5]; however, 8 of the 10 hospital laboratories isolated 7 species other than *L. pneumophila*. Nevertheless, this study is now the largest survey of pathogenic *Legionella* serogroups and species ever reported and is the only study that used the undisputed reference standard of culture confirmation.

References

- Benson RF, Fields BS. Classification of the genus Legionella. Semin Respir Infect 1998: 13:90-9
- Reingold A, Thompson B, Brake B, et al. Legionella pneumonia in the US: the distribution of serogroups and species causing human illness. J Infect Dis 1984; 149:819–24.
- Vickers RM, Stout JE, Yu VL, Rihs JD. Culture methodology for the isolation of *Legionella pneumophila* and other *Legionellaceae* from clinical and environmental specimens. Semin Respir Infect 1987;2:274–9.
- Fang GD, Yu VL, Vickers RM. Disease due to the Legionellaceae (other than Legionella pneumophila): historical, microbiological, clinical and epidemiological review. Medicine (Baltimore) 1989;68:116–32.
- Lee TC, Vickers RM, Yu VL, Wagener MM. Growth of 28 Legionella species on selective culture media: a comparative study. J Clin Microbiol 1993; 31: 2764–8.

^a Serogroup unknown but not serogroup 1.