

Legionnaires' Disease, its Bacteriological Characteristics, Distribution and Clinical Features in Japan

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DISTRIBUTION OF LEGIONELLA SPECIES FROM ENVIRONMENTAL SOURCE IN JAPAN

Each 500 ml of 408 water samples including 343 colling tower water from sites widely distributed over Japan, 25 samples of paddy field, 29 samples of river and 11 samples of lake, fountain, puddle and shower water in Nagasaki area, was obtained and centrifuged at 10,000 rpm for 20 min. The sediments were cultured in a selective medium¹⁾ for isolation of *Legionella* spp. after low pH treatment. In some negative samples, 3 ml of the sediments were inoculated to guinea pigs intraperitoneally and they were sacrificed four days later. The peritoneal swab and homogenate of spleen were inoculated to B-CYE agar, Each 200 g of soil was suspended in 400 ml of distilled water with 0.5% of Tween 60 for 20 min. The supernatant was centrifuged at 1,000 rpm for 10 min., and again the supernatant was centrifuged at 3,000 rpm for 10 min. The sediment were obtained and used for isolation of *Legionella* spp.

One hundred and sixty-six strains of *Legionella* spp. were isolated from 126 (36.7%) out of 343 samples. The origin of positive samples ranged from Hokkaido (Northern

Table 1 Distribution of *Legionella* species from Cooling Tower in Japan

Area	No. of samples	positive samples(%)	L. pneumophila						L. bozemanii	L.L.O.
			I	II	III	IV	V	VI		
Hokkaido	12	3(25.0)	2			1				
Tohoku	30	4(13.3)	4							
Kanto	43	22(51.2)	19			3		3	8	
Chubu	29	11(37.9)	10			2		1		
Kinki	34	14(41.2)	13						3	
Chugoku	38	9(23.7)	8						1	
Shikoku	26	11(42.3)	9			1		1	6	
Kyushu	131	52(39.7)	45		2	5		3	7	
	343	126(36.7)	110		2	12		3	12	
									27	

Japan) to Okinawa (Southern Japan) (Table 1). *L. Pneumophila*, serogroup 1 was the most frequent isolate (110 strains) and *Legionella-like* organisms (L.L.O.) were second in frequency (27 strains). We used the DFA reagents for *L. pneumophila* (serogroup 1 to 6), *L. micdadei*, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, and *L. longbeachae* (serogroup 1) which were obtained from CDC. *L. pneumophila* (serogroup 7 and 8), *L. longbeachae* (serogroup 2), *L. jordanis*, *L. wadsworthii* and *L. oakridgensis* were not examined.

No *Legionella* spp. was isolated from water samples of paddy field (25 samples), river (29 samples), lake (3 samples), fountain (one sample), well (4 samples) or shower water (one sample). In soil, three out of 13 samples from sites under construction and two of 3 samples from river side were positive. All 8 strains isolated were *L. pneumophila*, serogroup 1 (5 strains), serogroup 4 (one strain) and serogroup 5 (2 strains). *L. pneumophila* and *L. bozemanii* were widely distributed over Japan, but we could not obtain *L. micdadei*, *L. dumoffii*, *L. gormanii*, and *L. longbeachae*, serogroup 1. Yield was highest in urban city locations (Tokyo, Osaka, etc.) and lowest in rural areas. There was no relationship between ambient temperature and culture yield. The main reason of negative isolation of *Legionella* species by the method of low pH treatment and use of selective medium, was considered to be over growth of the other bacteria or fungi.

Legionnaires' disease in Japan

Five cases of pneumonia were confirmed by isolation of *L. pneumophila* from pleural effusion of the patients. The four cases experienced by us in Nagasaki area were all caused by *L. pneumophila*, serogroup 1 and the remaining case in Tokyo Metropolitan Hospital was caused by serogroup 3.

NOSOCOMIAL LEGIONNAIRES' DISEASE DIAGNOSED BY DFA STAINING

Three hundred and sixty-two out of 1,488 cases autopsied in six general hospitals had bacterial pneumonia in histological examination and 196 cases of them were diagnosed as fatal bacterial pneumonia by both histological and clinical findings. Retrospective analysis was performed by DFA staining of these formalin-fixed lung tissues (7 to 10 different specimens in each case). Forty-two cases (22%) were positive in DFA staining with the reagents of anti-*L. pneumophila*, serogroup 1 to 6. The ratio of male and female cases was 4:1, and average age was 62.3 years. On the other hand, in total 154 cases without Legionnaires' disease, the ratio of male and female was 3:1, and the average age was 62.2 years. There was no seasonal trend in occurrence of both Legionnaires' disease and other bacterial fatal pneumonia cases. Serogroup distribution of *L. pneumophila* in these 42 cases was shown in Table 2. *L. pneumophila*, serogroup 1 was the most frequent pathogen (29 cases, 69.0%), followed by serogroup 4 (9 cases, 21.4%). There were no lung-tissue specimens which had positive DFA staining to multiple serogroups, but 4 of 9 cases with positive DFA for serogroup 4 showed positive DFA for *L. micdadei*. We could not confirm whether these cases had two kinds of organisms or not.

Cough, sputum, chest pain and dyspnea were commonly observed, but high fever

Table 2 Serogroup Distribution of *L. pneumophila* in 42 Cases of Nosocomial Legionnaires' Disease

L. pneumophila, serogroup	Cases
I	29(69.0%)
II	2(4.8)
III	1(2.4)
IV	9(21.4)
V	0
VI	1(2.4)
total	42(100%)

Table 3 Laboratory Findings in Nosocomial Legionnaires' Disease

normal range	positive cases / total cases	0	50%	100%
WBC (4,500—8,500)	9000 ≤ : 19/38		50%	
% Neutrocyte (40—65%)	80% ≤ : 30/34			88.2
LDH (201—344)	: 22/34		64.7	
α ₂ -globulin (5.6—10.0%)	: 21/30		70	
CRP (—)	: 28/28			100
Hyponatremia (135—150mEq/L)	: 12/30		40.0	
Hypophosphatemia (2.3—4.2mEq/L)	: 6/12		50	
T. Bilirubin (<1.0mg%)	: 9/20		45	
LAP (98—195)	: 14/22		63.6	
Total protein (6.4—8.0)	≤ 6.3 : 20/34		58.8	
↑ S-GOT (13—36)	: 13/32		40.6	
↑ S-GOT, S-GPT	: 6/32		18.7	
Proteinuria (—)	: 13/22			59.1
Azotemia: BUN (8—20)	: 19/27			70.4
Creatinine (0.7—1.5)	: 8/21		38.1	

(over 40°C) and relative bradycardia were not characteristic signs in our cases (positive rate was 6.4 and 10.6%, respectively). Hypoxemia with hyperventilation and respiratory alkalosis were seen in early stage of the disease in spite of involving small shadow on chest X-ray (Table 3). Main underlying diseases of the 42 cases were cancers (8 cases of primary lung cancer, 5 cases of stomach cancer, 3 cases of gall bladder cancer and 8 cases of other cancers), followed by lymphoma (4 cases), cerebrovascular disorder (3 cases), heart failure (2 cases) and diabetes mellitus (2 cases). Chest X-ray findings were analyzed in 25 out of 42 cases. Noncircumscribed homogenous shadow (17 cases) was not

most frequent patterns. Pleural effusion was seen in 13 (75%) cases with 8 cases in left, 5 cases in right and 5 cases in both sides (Table 4). There were no trend in involved area.

Table 4 Chest X-ray patterns in 25 out of 42 cases of nosocomial Legionnaires' disease

		cases
Large multiple mass lesion		1
Lobar consolidation		1
Diffuse alveolar consolidation		2
Noncircumscribed lesion		
	homogenous	17
	linear	0
	reticular	0
	reticulonodular	3
	pathy	2
Pleural effusion		
	left	8
	right	5
	both	5
		} 75%
Involved area		
	right	
	upper	3
	middle	2
	lower	3
	left	
	upper	4
	middle	6
	lower	3
	both	
	lower	1
	all	6

REFERENCE

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