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Risk Factors for Contamination of Hotel Water Distribution Systems by *Legionella* Species▽

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The *Legionella* colonization frequency at 385 Greek hotel hot and cold water distribution systems was 20.8%. *Legionella* contamination was associated with the presence of an oil heater (odds ratio [OR] = 2.04, 95% confidence interval [CI] = 1.12 to 3.70), with the sample temperature (OR = 0.26, 95% CI = 0.1 to 0.5), with seasonal operation (OR = 3.23, 95% CI = 1.52 to 6.87), and with the presence of an independent disinfection system (OR = 0.30, 95% CI = 0.15 to 0.62). The same water temperatures, free-chlorine levels, and pHs differently affect the survival of various *Legionella* spp.

Legionnaires' disease is often associated with travel and with staying in hotels. Few reports, covering a small number of hotels, have studied *Legionella* colonization of water distribution systems of hotels.

In Greece, *Legionella pneumophila* was isolated and identified in hotel water distribution systems associated with cases of legionellosis for the first time in 1989 (2). Moreover, 344 cases of *Legionella* infection were associated with traveling in Greece by the European Surveillance Scheme for Travel Associated Legionnaires' Disease network from 1987 to 2005 (http://www.ewgli.org/).

To investigate legionella contamination of the water distribution systems of hotels accommodating athletes and tourists during the Athens 2004 Olympic Games, a descriptive multicentric study was conducted at seven locations in Greece. The aims of this study were to evaluate the frequency of *Legionella* colonization of water distribution systems of hotels, to identify risk factors for *Legionella* contamination associated with water distribution systems and water characteristics, and to identify remedial action needed to improve hotel water distribution systems.

MATERIALS AND METHODS

From January 2003 through September 2004, 1,086 samples were collected from 385 hotels in Athens, Volos, Chalkida, Korinthos (central Greece), Thessaloniki (northern Greece), Patras (western Greece), and Iraklio (southern Greece). The sample collection and microbiological analysis were part of the Environmental Health Surveillance Program developed by the Olympic Planning Unit and implemented for the Athens 2004 Olympic Games, which is described elsewhere (8, 9). Methods used for sample collection, storage conditions, and microbiological analysis have been described elsewhere (7).

Microbiologic analyses for *Legionella* spp. were performed by the National Legionella Reference Laboratory of Southern Greece in Athens and the National Legionella Reference Laboratory of Northern Greece in Thessaloniki. Microbiological testing for total plate count, coliform bacteria, *Escherichia coli*, intestinal enterococci, and *Clostridium perfringens* (including spores) was conducted in the Central Public Health Laboratory in accordance with the methods specified in the standing European legislation (5a).

A detailed standardized form was developed to register the hotel water supply systems at the seven locations (8, 9). Data included in the registry were used to evaluate risk factors possibly associated with *Legionella* colonization.

Data were analyzed with Epi-Info 2000 (Centers for Disease Control and Prevention, Atlanta, GA) and SPSS for Windows release 11.0.1 software (SPSS Inc., Chicago, IL) by *t* test or Mann-Whitney test for quantitative data and by chi-square test or Fisher exact test for qualitative data. Relative risk (RR) and 95% confidence interval (CI) were calculated to assess categorical risk variables associated with legionella-positive test results. Variables that were significant in the univariate analysis were entered into a multiple logistic regression model. By using conditional logistic regression models, independent predictors of colonization were established. Variables were retained in the model if the likelihood ratio test result was significant ($P < 0.05$).

RESULTS

In 80 hotel hot water distribution systems (20.8%, 95% CI = 16.9 to 25.2) the *Legionella* count was >500 CFU liter^{-1} in at least one sample, while in 25 hotels (6.5%) the *Legionella* count was $\geq 10^4$ CFU liter^{-1} in at least one sample. About 35 hotel hot water distribution systems (9%) needed remedial action because the *Legionella* count was $\geq 10^4$ CFU liter^{-1} in at least one sample or $>10^3$ but $<10^4$ CFU liter^{-1} in more than two samples (6).

A total of 268 water samples (24.7%) were contaminated by *Legionella* spp. with concentrations of ≥500 CFU liter^{-1} (Table 1). Of the total of 277 isolates, *L. pneumophila* was the most frequently isolated species (87%), while 14.9% of the positive samples contained $\geq 10^4$ CFU liter^{-1} *Legionella* spp. and 92.5% of these samples were contaminated by *L. pneumophila*.

Factors associated with contamination by *Legionella* spp. upon application of univariate analysis and logistic regression are shown in Tables 2 and 3, respectively.

Legionellae were not isolated when the sample temperature was above 60.3°C or below 23.7°C. *Legionella* contamination was not associated with positive microbiological results for coliform bacteria, *E. coli*, intestinal enterococci, and *Clostridium perfringens*, including spores (RR = 1.4, 95% CI = 0.3 to 7.0) but was associated with the total plate count (Table 4).

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TABLE 1. *Legionella* contamination in hot and cold water samples examined

Organism	No. of samples/total (%)							
	Hot water samples				Cold water samples			
	Positive	With 500–999 CFU liter^{-1}	With 10^3–9,999 CFU liter^{-1}	With $\geq 10^4$ CFU liter^{-1}	Positive	With 500–999 CFU liter^{-1}	With 10^3–9,999 CFU liter^{-1}	With $\geq 10^4$ CFU liter^{-1}
Legionella spp.	260/962 (27.0)	89/260 (34.2)	131/260 (50.4)	40/260 (15.4)	8/124 (6.4)	3/8 (37.5)	5/8 (62.5)	0/8 (0.0)
L. pneumophila serogroup 1	120/962 (12.5)	42/120 (35.0)	59/120 (49.7)	19/120 (15.8)	1/124 (0.8)	0/1 (0.0)	1/1 (100.0)	0/1 (0.0)
L. pneumophila serogroups 2–14	117/962 (12.2)	38/117 (32.5)	61/117 (52.1)	18/117 (15.4)	3/124 (2.1)	1/3 (33.3)	2/3 (66.7)	0/3 (0.0)
Legionella spp. other than *L. pneumophila*	32/962 (3.3)	19/32 (59.4)	10/32 (31.2)	3/32 (9.4)	4/124 (3.2)	2/4 (50.0)	2/4 (50.0)	0/4 (0.0)

Concentrations of *Legionella* spp. in positive immediate samples and postflush samples are shown in Table 5.

Legionella spp. and the number of positive samples differed with relation to the geographic location. In Athens, Thessaloniki, and Patras, the isolation rates were 19.6% (CI = 16.3 to 23.3), 17.4% (CI = 12.7 to 23.0), and 40.0% (CI = 24.9 to 56.7), respectively. In Volos, Chalkida, Korinthos, and Iraklio, the isolation rates were 28.1% (CI = 17.0 to 41.5), 70.0% (CI = 45.7 to 88.1), 65.0% (CI = 40.8 to 84.6), and 6.1% (CI = 0.7 to 20.2), respectively. Hotels in Athens and Thessaloniki, the two largest cities in Greece, had lower contamination rates than the ones in small towns (RR = 0.42, CI = 0.30 to 0.57, $P > 0.00001$).

The isolation rates per positive hotel distribution system were higher during summer (24.1%) than in winter, autumn, or spring (the isolation rates were 17.5%, 16.2%, and 17.4%, respectively).

No cases of Legionnaires' disease were linked to the hotels inspected during the study period.

TABLE 2. Association of hot water distribution systems and hotel characteristics with *Legionella* contamination by univariate analysis

Characteristic	No. (%) of samples:		RR (95% CI)
	With *Legionella* spp.	Without *Legionella* spp.	
No. of rooms, ≥80	31 (27.0)	49 (18.2)	1.5 (1.0–2.2)[a]
Hotel age, ≥30 yr	34 (17.2)	42 (28.0)	0.6 (0.4–0.9)[a]
Boiler age, ≥20 yr	5 (20.8)	58 (22)	0.9 (0.4–2.1)
Apartments	73 (21.2)	6 (23.1)	0.9 (0.4–1.9)
Bungalows	11 (44)	68 (19.8)	2.2 (1.3–3.6)[b]
Seasonal operation	16 (42.1)	58 (18.4)	2.3 (1.5–3.5)[b]
Water source, municipal supply	70 (20.5)	10 (23.3)	0.8 (0.5–1.6)
Water source, well	3 (25.0)	77 (20.6)	1.2 (0.4–3.3)
Water tank	30 (27.5)	44 (17.1)	1.6 (1.0–2.4)[a]
Operation temp, ≥55°C	15 (27.3)	58 (19.0)	1.4 (0.9–2.3)
Thermal insulation of system	50 (20.4)	19 (19.4)	1.0 (0.6–1.7)
Steel plumbing	5 (26.3)	75 (20.5)	1.2 (0.6–2.8)
Cu–PVC plumbing	3 (23.1)	77 (20.7)	1.1 (0.4–3.0)
Cu plumbing	32 (21.1)	48 (20.6)	1.0 (0.7–1.5)
PVC plumbing	9 (24.3)	71 (20.4)	1.2 (0.6–2.2)
Independent disinfection system	9 (10.8)	245 (29.3)	0.4 (0.2–0.7)[c]
Independent water filtration	10 (23.8)	49 (21.6)	1.1 (0.6–2.0)
Water storage tank protected	22 (30.1)	3 (18.8)	1.6 (0.4–4.7)
Oil heat	55 (25.1)	25 (15.1)	1.7 (1.0–2.5)[a]
Electric heat	8 (14.8)	72 (21.8)	0.6 (0.3–1.3)
Solar heat	8 (17.4)	72 (21.2)	0.8 (0.4–1.6)

[a] $P = 0.01$ to 0.03.
[b] $P = 0.001$ to 0.007.
[c] $P = 0.00001$.

DISCUSSION

Our data indicate that at the time of this study, 20.8% of the water distribution systems in Greek hotels were positive for *Legionella* spp. (≥500 CFU liter^{-1}). Other investigators have observed percentages of *Legionella* contamination ranging from 63.6% (11) to 75% (4) in 11 and 40 Italian hotels, respectively, but the detection limit of the microbiological examination procedure was 25 CFU liter^{-1} while in our study it was 500 CFU liter^{-1}. Moreover, in our study the numbers of hotels and samples examined were higher (385 hotels, 1,086 samples), resulting in a relatively narrow range of CI limits of the percentage of positive distribution systems (20.8%) of 16.9 to 25.2.

In our study 4.2% of the hot water samples had concentrations of $\geq 10^4$ CFU liter^{-1}, while in the Italian studies 11.8% (4) and 17.4% (11) of the samples examined had concentrations of $\geq 10^4$ CFU liter^{-1}. Furthermore, *L. pneumophila* was isolated from 24.6% of the samples examined, while in the studies of the Italian hotels *L. pneumophila* was found in 62.2% (4) and 60.9% (11) of the samples examined. To assess if a difference in the colonization rates of hotels in Italy and Greece does exist, we compared the cases of legionellosis among tourists who visited Greece and Italy reported to the European Surveillance Scheme for Travel Associated Legion-

TABLE 3. Multiple logistic regression of hot water distribution system and water characteristics associated with *Legionella* contamination

Characteristic	OR[c]	95% CI
Hotel age, ≥30 yr	0.65	0.37–1.15
No. of rooms, ≥80	1.14	0.60–2.15
Bungalows	1.74	0.61–4.90
Seasonal operation	3.23	1.52–6.87[a]
Water tank present	1.55	0.82–2.93
Oil heat	2.04	1.12–3.70[a]
Sample temp, ≥55°C	0.26	0.1–0.5[b]
Independent disinfection system	0.30	0.15–0.62[a]

[a] $P = 0.01$ to 0.02.
[b] $P = 0.00001$.
[c] OR, odds ratio.

TABLE 4. Association of physical, chemical, and microbiologic water characteristics with *Legionella* contamination

Characteristic and organism(s)	No. of samples/total (%)		RR	*P* value
	Positive for *Legionella* spp.	Negative for *Legionella* spp.		
pH of ≥7.8				
Legionella spp.	41/176 (24.1)	210/828 (25.4)	0.9	0.4
L. pneumophila serogroup 1	28/170 (16.5)	84/128 (10.1)	1.6	0.01
L. pneumophila serogroups 2–14	12/170 (7.1)	102/828 (12.3)	0.57	0.02
Legionella spp. other than *L. pneumophila*	1/170 (0.6)	33/828 (4.0)	0.14	0.012
Sample temp of ≥55				
Legionella spp.	15/145 (10.3)	239/773 (30.9)	0.33	<0.00001
L. pneumophila serogroup 1	2/145 (1.4)	114/773 (14.7)	0.09	<0.00001
L. pneumophila serogroups 2–14	10/145 (6.9)	105/773 (13.6)	0.50	0.01
Legionella spp. other than *L. pneumophila*	3/145 (2.1)	29/773 (3.8)	0.55	0.2
Free-chlorine level of ≥0.2 mg/liter				
Legionella spp.	14/91 (15.4)	143/460 (31.1)	0.49	0.001
L. pneumophila serogroup 1	3/91 (3.3)	68/460 (14.8)	0.22	0.0008
L. pneumophila serogroups 2–14	8/91 (8.8)	73/460 (15.9)	0.55	0.05
Legionella spp. other than *L. pneumophila*	3/91 (3.3)	10/460 (2.2)	1.51	0.3
Total plate count of ≥400 CFU				
Legionella spp.	83/213 (39.0)	141/569 (24.8)	1.57	0.00008
L. pneumophila serogroup 1	34/213 (16.0)	78/569 (13.7)	1.16	0.2
L. pneumophila serogroups 2–14	39/213 (18.3)	56/569 (9.8)	1.86	0.0013
Legionella spp. other than *L. pneumophila*	13/213 (6.1)	13/569 (2.3)	2.67	0.01

naires' Disease. In 2003 and 2004, traveling to Greece was associated with 28 and 24 cases of Legionnaires' disease, respectively, while traveling to Italy was associated with 123 and 111 cases, respectively, (http://www.ewgli.org/data/data_tables/year_onset_country_travel.asp). During the 2-year period, the numbers of cases per million tourists were 1.85 (52 cases per 28 million tourists) in Greece and 3.00 (234 cases per 78 million tourists) in Italy. This higher rate of cases in Italy is consistent with the higher rates of *Legionella* colonization of the Italian hotels reported in these studies.

Internationally, about 90% of Legionnaires' disease cases are due to *L. pneumophila*, and predominant serogroup 1 of *L. pneumophila* accounts for 84% of the cases (13). Our findings support the idea that a small proportion of Greek hotels posed a risk for Legionnaires' disease at the time of the study, due to the high concentrations of *L. pneumophila* (24.6% of the total hot water samples examined) and the number of positive distribution systems which exceeded the safety levels of the European Surveillance Scheme for Travel Associated Legionnaires' Disease guidelines (9% of the hotel water distribution systems examined). Regulations regarding the control of *Legionella* spp. in water distribution systems should be established in Greece.

TABLE 5. *Legionella* contamination of immediate samples and postflush samples of the hot water collected

No. of *Legionella* CFU liter^{-1}	No. (%) of positive samples of hot water	
	Immediate sample	Postflush sample
500–999	43 (48)	45 (52)
10^3–9,999	73 (60.5)	54 (39.5)
$\geq 10^4$	27 (73.4)	11 (26.6)
Total	143 (57.5)	110 (42.5)

Seasonal hotel operation was the main predictor of the presence of legionellae in hotel water systems, while in small towns with hotels operating to a major degree seasonally, the isolation rates were higher than in big towns, where hotels generally operate all year round. Control measures should be applied intensively during summer, especially in hotels operating seasonally.

Storage water heaters powered directly by electricity and solar water heaters showed a lower prevalence of colonization by legionellae than those heated by oil. Similar were the findings in previous studies concerning domestic hot water in Wellington, New Zealand (3), and in six towns in Italy (5), while the opposite was observed in a previous study in Quebec City (1). In Greece, oil heaters are part of the hotel central heating system while electric heaters are used as point-of-use heating systems, have minimal storage, and are placed in each hotel apartment or hotel room.

The temperature of the hot water samples was negatively associated with contamination with *L. pneumophila* serogroup 1 and to a lesser degree with contamination with serogroups 2 to 14, suggesting that serogroup 1 is more sensitive to high temperatures. This is the opposite of the findings of the Italian study, which indicates that *L. pneumophila* serogroup 1 is more resistant to higher temperatures (4).

In our study, increases in pH correlated negatively with the counts of *Legionella* spp. other than *L. pneumophila* and of *L. pneumophila* serogroups 2 to 14 and positively with counts of *L. pneumophila* serogroup 1. These findings confirm other reports, which indicated a positive association of *L. pneumophila* with pH (10, 11, 12). The differences in distribution of species according to water characteristics confirm the hypothesis of

other reports, which indicated that *Legionella* strains differ in sensitivity to environmental risk factors and have different ecological niches (4, 5, 11).

Contamination by *Legionella* spp. was associated with total plate counts above the acceptable level in samples collected from the same water distribution system. This association may be explained by the presence in contaminated distribution systems of biofilms consisting of bacteria and other microorganisms embedded in a protective layer with entrained debris attached to a surface (6). The total plate count could be used as an indicator of the presence of *Legionella* spp.

Future studies in Greece should directly evaluate levels of water distribution system contamination and water and system characteristics in relation to Legionnaires' disease cases and outbreaks.

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