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Prevalence of anti-legionella antibodies among Italian hospital workers

P. Borella [a,*], A. Bargellini [a], I. Marchesi [a], S. Rovesti [a], G. Stancanelli [b], S. Scaltriti [a,b], M. Moro [b], M.T. Montagna [c], D. Tatò [c], C. Napoli [c], M. Triassi [d], S. Montegrosso [d], F. Pennino [d], C.M. Zotti [e], S. Ditommaso [e], M. Giacomuzzi [e]

[a] *Department of Public Health Sciences, University of Modena and Reggio Emilia, Italy*
[b] *S. Raffaele Hospital, Milan, Italy*
[c] *Department of Internal Medicine and Public Health, University of Bari, Italy*
[d] *Department of Preventive Medical Sciences, University Federico II of Naples, Italy*
[e] *Department of Public Health and Microbiology, University of Turin, Italy*

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Summary This study evaluated the prevalence of anti-legionella antibodies in workers at hospitals with a long-term history of legionella contamination. The hospitals are located in Milan and Turin, northern Italy, and in Naples and Bari, southern Italy. Antibody prevalence and titres of healthcare workers, medical and dental students and blood donors were assessed. In total 28.5% of subjects were antibody positive, most frequently to *L. pneumophila* serogroups 7–14. Major differences were observed in seroprevalence and type of legionella antibody in persons from different geographic areas. Healthcare workers had a significantly higher frequency of antibodies compared with blood donors in Milan (35.4 vs 15.9%, $P < 0.001$), whereas in Naples both groups exhibited high antibody frequency (48.8 vs 44.0%) and had a higher proportion of antibodies to legionella serogroups 1–6. Dental workers had a higher seroprevalence than office staff in Bari, but not in Turin, where daily disinfecting procedures had been adopted to avoid contamination of dental unit water. No association was found between the presence of antibodies and the presence of risk factors for legionellosis, nor with the occurrence of pneumonia and/or flu-like symptoms. In conclusion, the presence of legionella antibodies

* Corresponding author. Address: Dipartimento di Scienze di Sanità Pubblica, Via Campi 287, I-41100 Modena, Italy. Tel.: +39 059 2055474; fax: +39 059 2055483.
E-mail address: borella.paola@unimore.it

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may be associated with occupational exposure in the hospital environment, but there was no evidence of any association with disease.

Introduction

Legionella spp. should be considered among the occupational hazards of healthcare workers since these bacteria are widespread in healthcare facilities.[1,2] They have been isolated in the majority of hospital water distribution systems in Italy, perhaps reflecting the large number of old buildings in this country.[3,4] Other potential sources of infection in healthcare facilities include aerosol-generating devices, particularly those used during dental care, since legionella frequently colonises dental unit waterlines.[5–7] Patients are known to be at risk of acquiring legionellosis in contaminated hospitals, but little is known about the risks to hospital staff.[8]

The production of anti-*Legionella* spp. antibodies reflects exposure to legionella, and increased antibody titres can be used retrospectively to gauge the infectious risk. Seroprevalence studies have been undertaken during clusters of Legionnaires' disease,[1,9–12] and on people living and working in contaminated environments.[13–15] Recent studies have documented a higher prevalence of *Legionella* spp. antibodies among people who work in hospitals, thermal spas and dental practices but another study found no increase in antibody prevalence among dental practitioners.[16–19]

The aim of this investigation was to explore the prevalence of anti-*Legionella* spp. antibodies in employees of large hospitals with a history of legionella contamination located in different Italian regions. Professional and personal characteristics likely to affect both exposure to *Legionella* spp. and host susceptibility to legionellosis were investigated, and variability in seroprevalence according to local contamination and geographic area was evaluated. The results of the study may clarify whether exposed workers in the health environment are at risk of acquiring disease, which factors are associated with the presence of antibodies, and which measures can be advocated to avoid infection.

Methods

Participating units

This study was conducted as part of a wider survey of legionella infection that was aimed at better defining environmental contamination in Italian hospitals, providing data on the prevalence of community and nosocomial infections and evaluating the risk to personnel working in contaminated hospitals.[20] Four research groups recruited subjects in their respective university hospitals. Two were located in northern Italy (Turin and Milan) and two in southern Italy (Naples and Bari). The participating units were selected on the basis of a history of legionella contamination.

Hospital dental workers ($N = 61$) were recruited in Turin, where *Legionella pneumophila* serogroup 1 and *L.* non-*pneumophila* species have been found sporadically in the water of drill units subjected to daily disinfection procedures, and in Bari ($N = 44$), where drill units were not subjected to regular disinfection and were frequently contaminated by *L.* non-*pneumophila* species and *L. pneumophila* serogroup 1.[21,22] In both cases, office staff from the same hospital ($N = 70$ and 44 respectively) were selected as controls to evaluate specific and/or additive exposure. In addition, dental students ($N = 58$) were included in Turin. Water distribution systems in both hospitals have been found highly contaminated by *Legionella* spp. and hospital-acquired infection has occurred.

In Milan and Naples, ward personnel ($N = 65$ and 41 respectively) were selected as subjects while blood donors ($N = 132$ and 75 respectively) served as controls. In addition, a group of office staff ($N = 15$) was studied in Milan, and a group of medical students was studied in Naples ($N = 59$), according to local co-operation. The water supplies in the hospitals in Milan and Naples hospitals have both been shown to be heavily colonised with legionella, and in both cases this has been associated with hospital-acquired infection.[23,24] In Milan *L. pneumophila* serogroups 2–14 were most prevalent (serogroup 1 was rarely detected), whereas in Naples *L. pneumophila* serogroups 3 and 1, and *L.* non-*pneumophila* species were mainly detected.

Guidelines for healthcare facilities have been available in Italy since 2000, making recommendations for the appropriate management of legionellosis, the assessment of environmental contamination in at-risk wards, and the implementation of preventive measures on the basis of legionella concentration.

Recruited subjects

Different groups were selected in each city as described in the previous section. A total of 340 hospital workers (131 males, 209 females) with various occupations (physician, dentist, nurse, healthcare assistant, cleaner, and clerk) were examined. They were classified into three groups: persons working in dentistry wards (dental staff, $N = 105$), those working in medical/surgical wards (ward staff, $N = 106$) and those employed in administrative and/or technical services (office staff, $N = 129$). Students and doctors attending specialisation courses in medicine and/or dentistry were also included ($N = 117$). As controls in Milan and Naples, 207 subjects were randomly selected among blood donors and paired for age. Participation in the study was voluntary with written informed consent, and the study received the formal approval of the local Ethical Committees.

Hospital staff (dental workers, ward staff and office staff) and blood donors did not differ in mean age (39.4 ± 10.1 vs 39.1 ± 10.0 years), but there were significantly fewer men among hospital staff (38.5% vs 64.7%, $P < 0.001$). Smoking habits were similar (27.8% vs 25.1%), whereas significantly fewer hospital workers than blood donors declared a daily alcohol intake (48.1 vs 65.2%, $P < 0.005$). The students were similar to hospital staff in sex composition and alcohol intake, but fewer of them smoked (23.1%). This group was younger (27.4 ± 6.0 years, $P < 0.001$) and had a shorter length of service (3.6 ± 1.9 vs 12.6 ± 10.2 years, $P < 0.001$).

A detailed questionnaire was used to collect information on demographic characteristics (sex, age, civil state, education), personal habits (smoking, alcohol consumption, type of drinking water), present and past diseases, personal hygiene (bathtub and/or shower, jacuzzi, sauna use), sporting activities and related water exposure, type of job, length of service, exposure to water at work (use of shower and/or sink, use of aerosol-producing devices, pressured water jets, etc.), housing (flat or house, year of construction/refurbishment, water supply), water-heating systems and related characteristics (central or independent, electric or gas heater, existence of a tank, service frequency, existence of a softening system, etc.). Particular attention was paid to recording episodes of pneumonia during the previous five years (time of occurrence, antibiotics and hospital admissions) and flu-like symptoms in the previous year.

Serological study

Serum anti-*Legionella* spp. antibodies were detected by indirect immunofluorescence (IFA, RIDA® FLUORlegionella IgG, R-Biopharm AG, Darmstadt, Germany). This method distinguishes IgG antibodies against *L. pneumophila* serogroups 1–6, *L. pneumophila* serogroups 7–14, and *L.* non-*pneumophila* (*L. bozemanii*, *dumoffii*, *gormanii*, *jordanis*, *longbeachae* and *micdadei*). Laboratory workers in the different cities were trained to ensure standardisation of test performance. A cut-off for positivity of 1:128 was selected to reduce the risk of false-positive results although cross-reaction with other micro-organisms cannot be completely ruled out.[25]

Statistical analysis

Statistical analyses were conducted by SPSS/pc (SPSS Inc., Chicago, IL, USA). Pearson's Chi-squared test was used to evaluate the relationship between anti-*Legionella* spp. antibodies (positive/negative, titres and species) in the examined groups and the investigated variables, and odds ratios (ORs) and [95% confidence intervals (CI)] were calculated on bivariate variables to assess the risks associated with the presence of antibody. Conditional logistic regression was used to evaluate independent predictors of seropositivity.

Results

Table I summarises the anti-*Legionella* spp. antibody frequency, type and titres in the groups examined. In total, 189 out of 664 subjects (28.5%) had detectable antibodies. Seven of these had detectable titres to both *L. pneumophila* serogroups 7–14 and serogroups 1–6, while 19 had detectable titres to both *L. pneumophila* serogroups 7–14 and to *L.* non-*pneumophila* species. Since multiple antibodies were found in both hospital staff and blood donors (5.0 vs 3.4%, respectively), the total number of antibodies was used for statistical analysis. A slightly higher positivity was observed among hospital staff, but direct comparison between groups was not performed due to differences in selection by the participating units. In all groups the most frequently detected antibodies were directed against *L. pneumophila* serogroups 7–14 and the most frequent titre was 1:256, but titre distribution differed between groups.

Table II presents the results separately. In Turin no difference was observed between dental and

Table I *Legionella* spp. antibodies in the examined groups

	Hospital staff	Students	Blood donors	Total
Positive subjects No. (%)	106/340 (31.2)	29/117 (24.8)	54/207 (26.1)	189/664 (28.5)
Antibody type				
LP 1–6				
No. (% of total)	9/340 (2.6)	4/117 (3.4)	7/207 (3.4)	20/664 (3.0)
(% of positive)	(8.5)	(13.8)	(13.0)	(10.6)
LP 7–14				
No. (% of total)	74/340 (21.8)	17/117 (14.5)	34/207 (16.4)	125/664 (18.8)
(% of positive)	(69.8)	(58.6)	(63.0)	(66.1)
LS				
No. (% of total)	40/340 (11.8)	8/117 (6.8)	20/207 (9.7)	68/664 (10.2)
(% of positive)	(37.7)	(27.6)	(37.0)	(36.0)
Total antibodies				
No. (% of total)	123/340 (36.2)	29/117 (24.8)	61/207 (29.5)	213/664 (32.1)
Antibody titre[a]				
No. (% of positive)				
1:128	24/106 (22.6)	17/29 (58.6)	20/54 (37.0)	61/189 (32.3)
1:256	54/106 (50.9)	7/29 (24.1)	12/54 (22.2)	73/189 (38.6)
1:512	28/106 (26.4)	5/29 (17.2)	22/54 (40.7)	55/189 (29.1)

LP 1–6, *L. pneumophila* serogroups 1–6; LP 7–14, *L. pneumophila* serogroups 7–14; LS, other *Legionella* spp.
[a] When multiple antibody types were detected, only the highest titre was selected.

office staff, whereas in Bari dental staff were significantly more likely than office staff to be antibody positive (OR: 4.67; 95% CI: 1.39–15.61; $P < 0.01$), mainly due to a higher frequency of *L.* non-*pneumophila* antibodies (3.75; 1.10–12.74; $P < 0.005$). In Milan ward staff were significantly more likely than blood donors to be antibody positive (2.89; 1.45–5.77; $P < 0.002$), particularly for *L.* non-*pneumophila* antibodies (4.62; 1.51–14.14; $P < 0.01$). Naples had the highest seroprevalence in both hospital staff and blood donors and a higher frequency of antibodies against *L. pneumophila* serogroups 1–6. Blood donors in Naples were significantly more likely to be antibody positive than those in Milan (44.0 vs 15.9%; $\chi^2 = 19.57$; $P < 0.001$), although age, job and gender were similar. Among blood donors in Naples, antibody prevalence was higher in those who consumed alcohol compared with non-consumers (53.3 vs 30.0%; $P < 0.05$), those living in flats compared with those living in houses (51.8 vs 21.2%; $P < 0.02$), those drinking tap water compared with those drinking mineral water (73.3 vs 36.7%; $P < 0.05$) and those taking baths instead of showers (83.3 vs 35.7%; $P < 0.05$). Students had significantly fewer antibodies, probably related to their lower age and shorter length of service.

Table II also presents the distribution of antibody types together with data on local water contamination. *L. pneumophila* serogroups 7–14 prevailed in all groups of subjects living in northern Italy, even though in Turin hospital contamination has mainly involved serogroup 1 and *L.* non-*pneumophila* species. In Milan serogroups 2–14 were frequently isolated in the environment both within and without the hospital, perhaps explaining the high percentage of antibodies to *L. pneumophila* serogroups 7–14, but not the higher prevalence of antibodies to *L.* non-*pneumophila* species in ward staff. Similarly, in Bari antibodies to *L.* non-*pneumophila* species prevailed in both dental and office staff, even though legionellae were frequently detected in the water of dental chair equipment but rarely in the hospital water system. A higher proportion of antibodies to *L. pneumophila* serogroups 1–6 was confirmed in Naples, in accordance with the environmental isolates.

Among hospital staff, subjects older than 50 years were more likely to be seropositive (43.4 vs 28.9%, $P < 0.05$). In addition, seroprevalence increased with length of service: from 19.4% in those working less than 5 years to 41.1% in those working more than 20 years ($\chi^2 = 13.6$, $P < 0.01$). When age, length of service and cities were introduced as independent variables into a multivariate regression model, length of service remained independently associated with an increased risk of

Table II Distribution of anti-*Legionella* spp. antibodies according to group and location

	Subjects positive No. (%)	No. of antibodies detected	Antibody distribution [no. (% total) (% positive)]			Local water contamination
			LP 1–6	LP 7–14	LS	
Turin (North)						
Dental staff ($N = 61$)	18 (29.5)	18	0	17 (27.9) (94.4)	1 (1.6) (5.6)	Dental unit: sporadically LP 1 (25%), LS (75%)
Dental students ($N = 58$)	14 (24.1)	14	3 (5.2) (21.4)	9 (15.5) (64.3)	2 (3.4) (14.3)	
Office staff ($N = 70$)	23 (32.9)	26	1 (1.4) (3.8)	20 (28.6) (77.0)	5 (7.1) (19.2)	Hospital: LP 1 (50%), LP 3 (3.3%), LS (46.6%)
Bari (South)	**				*	
Dental staff ($N = 44$)	14 (31.8)	17	0	5 (11.1) (29.4)	12 (23.3) (70.6)	Dental unit: LS (67%), LP 1 (21%), LP 2–14 (12%)
Office staff ($N = 44$)	4 (9.1)	6	0	2 (4.5) (33.3)	4 (9.1) (66.7)	Hospital: LP 2–14 (65%), LP 1 (30%), LS (5%)
Milan (North)	**				*	
Ward staff ($N = 65$)	23 (35.4)	30	2 (3.1) (6.7)	18 (27.7) (60.0)	10 (15.4) (33.3)	Hospital: LP 2–14 (98.8%), sporadically LP 1
Office staff ($N = 15$)	4 (26.7)	5	0	4 (26.7) (80.0)	1 (6.7) (20.0)	
Blood donors ($N = 132$)	21 (15.9)	24	0	19 (14.4) (79.2)	5 (3.8) (20.8)	Home/hotel: LP 2–14 (73%), LP 1 (17%)[a]
Naples (South)	*		*			
Ward staff ($N = 41$)	20 (48.8)	21	6 (14.6) (28.6)	8 (19.5) (38.1)	7 (17.1) (33.3)	Hospital: LP 3 (70%), LS (17%), LP 1 (13%)
Ward students ($N = 59$)	15 (25.4)	15	1 (1.7) (6.7)	8 (13.6) (53.3)	6 (10.2) (40.0)	
Blood donors ($N = 75$)	33 (44.0)	37	7 (9.3) (18.9)	15 (20.0) (40.5)	15 (20.0) (40.5)	Home/hotel: LP 1 (94%), LP 2–14 (6%)[a]

LP 1–6, *L. pneumophila* serogroups 1–6; LP 7–14, *L. pneumophila* serogroups 7–14; LS, other *Legionella* spp.
*$P < 0.05$; **$P < 0.01$ (Chi-squared test).
[a] References 4 and 26.

seropositivity (OR: 1.04; 95% CI: 1.01−1.06; $P < 0.002$).

The presence of antibodies was not associated with known personal or professional risk factors for legionellosis, except that antibodies to *L. pneumophila* serogroups 1−6 were slightly more common in hospital workers using aerosol-generating devices: 5/75 (6.7%) vs 7/265 (2.6%) ($P = 0.55$). No association was found between seroprevalence and the occurrence of flu-like symptoms and/or pneumonia, except for a higher risk of pneumonia in subjects positive for antibodies to *L. pneumophila* serogroups 1−6 compared with negative subjects: 2/16 (12.5%) vs 6/459 (1.3%) (OR: 10.79; 95% CI: 2.00−58.24; $P < 0.001$). The two subjects were: (i) a 48-year-old woman reporting pneumonia two years previously, living in northern Italy, a housekeeper, heavy smoker and alcohol consumer with hypertension; (ii) a 60-year-old man who had had pneumonia five years previously, working as a health assistant in Naples, ex-smoker, alcohol consumer, regularly taking analgesic drugs.

Discussion

This study has defined an endemic level of *Legionella* spp. exposure in different Italian regions with special reference to hospital staff, and demonstrates geographic variations in both the frequency and type of antibodies to legionella in line with the variety of species and serogroups distributed nationwide.[3,4]

Although much emphasis has been placed on the role of cell-mediated immunity in legionella infection, antibodies acting in concert with polymorphonuclear leucocytes may play a role in overcoming infection.[26] Hence it is not surprising that antibody prevalence was so high in a country where legionella contamination is widespread.[3,4,27] Indeed, the rate might have been underestimated by our cut-off of 1:128 for positivity.

Irrespective of their roles, personnel working in contaminated Italian hospitals were more likely to be seropositive than controls, although differences emerged depending on the local environmental contamination. *Legionella* spp. antibody titres >1:256 have also been found in a significant proportion of hospital staff in Poland, and their specificity reflected the distribution of environmental isolates.[17] Seroprevalence was associated with length of service, whereas no association could be demonstrated with risk factors for disease or with the occurrence of pneumonia or flu-like symptoms. This suggests that the antibodies measured represent the frequency of exposure to the bacterium rather than to disease. Recent surveys of other legionella-contaminated working environments documented increased antibody levels among personnel, but no cases of legionellosis were recorded.[12−14]

As regards dental care services, our data suggest that the risk of legionella infection due to water contamination of dental equipment can be prevented by adopting rigorous disinfection procedures. Antibodies to *L.* non-*pneumophila* species were significantly more common among dental staff in Bari where *L.* non-*pneumophila* have been frequently isolated from dental units. By contrast, in Turin no difference could be observed between office and dental staff, probably because daily disinfection has been implemented. In a similar study carried out in London and Northern Ireland, the prevalence of *L. pneumophila* antibodies in dentists was low and did not exceed the levels seen in blood donors, and environmental contamination was consistently very low.[19]

It is instructive to examine the different distribution and types of antibodies in the areas studied. Healthcare personnel in Milan were significantly more at risk than blood donors, but this was not the case in Naples where both groups had high seroprevalence (48 and 44%, respectively). One explanation is that persons living in Naples receive more exposure to legionella in the community, perhaps because of the greater use of air conditioners, although this is speculation. However, our data suggest that lifestyle factors such as alcohol consumption, more frequent exposure to tap water and housing characteristics are risk factors for increased exposure.

Antibodies to *L. pneumophila* serogroups 1−6 were found in only 20 samples, i.e. 9.3% of total antibodies and 3% of sera tested, even though these serogroups are those most frequently involved in human disease.[28] Interestingly, 14/20 were detected in Naples, in both hospital workers (28.6% of positive sera) and blood donors (18.8% of positive sera). A previous study conducted in Naples on healthy persons confirmed this trend: using a cut-off of 1:256, antibodies to serogroups 1−6 were found in 23% of samples.[29] This is in line with environmental contamination in Naples, where 83% of hospital isolates were *L. pneumophila* serogroups 1 and 3, and 94% of domestic/hotel isolates were *L. pneumophila* serogroup 1.[4,24,27] By comparison, antibodies to *L. pneumophila* serogroups 7−14 and to *L.* non-*pneumophila* species prevailed in Milan, and the frequency of positive sera was much lower. This partly reflects

the environmental data for Milan, where *L. pneumophila* serogroups 2—14 are the predominant isolates. The reported incidence of Legionnaires' disease is much higher in Milan than in Naples.[30] The reasons for this might be numerous, and include differences in detecting and/or reporting cases, or different age composition of the two populations, but we also suggest that persistent and more frequent contact with *L. pneumophila* serogroups 1—6 from infancy can induce protection against the disease later in life.

Excluding Naples, antibodies to serogroups 1—6 were found in only six subjects out of 489 (1.2%), four of whom were from Turin hospital: three dental students and one office worker. In this hospital contamination by *L. pneumophila* serogroups 1 and 3 and non-*pneumophila* species prevailed, and numerous hospital-acquired cases have been detected in spite of costly environmental control measures.[21] A previous study on blood donors in Turin revealed that 0.3% had antibodies to serogroup 1 (cut-off: 1:16),[31] suggesting sporadic exposure in the community. Under these conditions a marginal risk of developing disease may exist for hospital staff, particularly those with risk factors. In our study, persons positive for antibodies to serogroups 1—6 showed a significantly higher prevalence of pneumonia, not specifically related to their hospital employment but more to typical risk factors for legionellosis (smoking, alcohol and chronic disease).

In conclusion, hospital personnel may be at higher risk of developing anti-*Legionella* spp. antibodies as a result of exposure in the workplace. We did not demonstrate an association between seropositivity and disease, however, probably because workers are generally in good health which helps to prevent the progression of infection to disease.

The public health implications of such widespread exposure to legionella in Italian hospitals also deserve attention. The facilities examined have a long history of hospital-acquired legionellosis, and have adopted preventive strategies to reduce water contamination, but eradication is difficult particularly in old and large buildings.[21—24] For instance in Turin the incidence of healthcare-associated legionellosis has not decreased despite extensive disinfection procedures; we confirmed the importance of daily disinfection of dental unit water to avoid exposure in dental staff.[21] Furthermore, generally only high-risk wards are subjected to control measures according to the national guidelines, such that little information is available for lower-risk environments.[32] Strategies for preventing legionellosis in healthcare workers should include attention to education on the adverse effects of lifestyle factors such as smoking and alcohol consumption, and the use of protective devices in at-risk persons in case of exposure to potentially contaminated aerosols.

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Conflict of interest statement
None declared.

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