ORIGINAL ARTICLE

Occurrence of Legionella in beach shower facilities

S. DELIA, P. LAGANÀ, E. MINUTOLI
Department of Hygiene, Preventive Medicine and Public Health "R. De Blasi", University of Messina, Italy

Key words

Legionella pneumophila • Beach shower facilities

Summary

It has been analyzed 36 samples of water proceeding from beach shower facilities: 7 of these were found to be contaminated with Legionella (19.44%). In three showers, L. pneumophila 1 was reported, in two L. pneumophila 2-14; in another two cases L. pneumophila 2-14 was found together with Legionella sp. and L. pneumophila 1 together with L. pneumophila 2-14.
This study, which confirms the circulation of Legionella in our region of southern Italy, extends the list of the possible sources of infection, including also public and private beaches among the contaminated sites.
It is the Authors opinion that when evaluating the risk in these cases we should take into account that the crowding of the beaches in the summer months and the communal nature of shower use in these periods increases the frequency of exposure of children and old people. These vulnerable categories are significantly more likely to develop the disease once infected.

Introduction

Around 30 years after the first epidemic cluster of Legionellosis, there is still widespread uncertainty regarding certain clinical-epidemiological aspects, the biology of its causes and, above all, preventive strategies.
For hospitalised subjects, risk assessment on the basis of levels of exposure to contaminated water pipes should, in our opinion, be calculated following constant environmental monitoring, and critically correlated with strict clinical surveillance.
We in fact maintain that the concentration of 10^3 ufc/l of water, established in Table 5 of the ministerial Guidelines [1], may not necessarily represent a "High risk of hospital infections", if the figure refers to micro-organisms, even those of *L. pneumophila* serotype 1, whose levels in bodies of water should drop significantly and spontaneously. Conversely, it may be imprudent to underestimate findings of even low bacterial concentrations of *L. pneumophila*, whether or not of serotype 1, which could achieve much higher concentrations over a short time.
As is known, Legionella is able to survive for long periods in water and even to replicate in the presence of chlorine, if it manages to create suitable conditions (biofilms, parasitism of amoebas and protozoic cysts, etc.) [2-7].
The basic problem is to ascertain how it may penetrate into water pipes and air-conditioning systems.
Given the documented environmental diffusion of Legionella, the possible existence of animal reservoirs should be investigated, as should the possible role of specific carriers in the dynamics of contamination. With this in mind, we are considering the experimentation of various research hypotheses in our Laboratory.
Further study on the biology of the germ might clarify how its virulence factors [8, 9] and existing immune system deficiencies in the human host may interrelate to influence the evolution of the micro-organism after its penetration into the upper respiratory tract [10-13] in one of its two known clinical manifestations (Pontiac Fever and Legionnaires' Disease).
Moreover, it would be useful if the bacteriological analyst could demonstrate the cause of Legionellosis in episodes of phlogosis of the upper respiratory tract, diagnosing pharyngotonsillitis with coughing in which classic pathogens cannot be isolated as cases of Pontiac Fever. Such studies, which could significantly extend our knowledge of the possible interhuman circulation of the germ and of the incidence of the disease, are unfortunately hindered by the high cost of Legionella research.
The potential role of carrier-diffuser is difficult to demonstrate in the patient affected by Legionellosis, in which the micro-organisms, after invading the lungs, dwell inside the monocytes and the alveolar macrophages [14-17]. Such a role could, meanwhile, be hypothesised in the incubation period of Pontiac Fever, during which the patient could expel Legionella germs by coughing, thus infecting subjects whose clinical condition may become much more serious.
If this hypothesis were to be demonstrated, it could explain those cases (confirmed clinically, in culture and in serological tests) in which the germ was not detected in the water.
Lastly, as far as regards techniques for the purification of contaminated water, it is generally acknowledged that neither chemical or physical methods ensure certain eradication of the germ. Hyperchlorination, meanwhile, perhaps the most widely used method in hospitals, in some cases is not only unsuccessful but actually causes

an increase in Legionella levels in the water [18-21]. We have encountered disappointing instances of this, both in hospitals and in the community at large.
This could be related to the fact that increased concentrations of chlorine are not only ineffective against the Legionella germs protected within organised biofilms, but also act against coliforms and other heterotrophic bacteria, whose death determines an increase in Assimilable Organic Carbon (AOC), which is exploited by autotrophic germs such as Legionella and Pseudomonas.
We are also considering the possibility of performing specific examinations on this aspect.
What is certain is that as long as the entire issue remains so undefined, any epidemiological studies aimed at assessing the environmental circulation of Legionella will be crucial in assessing microbiological danger and risk.
In the past, our research in this field has shown the presence of numerous serotypes of Legionella inside public and private health facilities [22-27].
At present, we are carrying out a surveillance project on cruise and liner ships, in collaboration with the Marine Health Department of Messina and Catania.
This note reports on the results obtained in the course of research performed in beach shower facilities.

Materials and methods

In the summer of 2006 (June-August) random samples of water were taken from 36 beach shower facilities, both public and private, in the province of Messina and from towns in nearby Calabria.
Most of the showers were located directly on the beach, while only few were installed on the pavements of the immediately adjacent streets. Each sample was taken without letting the water run, and without flaming at the outlet point, since all the showers examined were in continuous use by the bathers ("searching for Legionella in conditions of communal use": Annex 2, section 2.1 of the Ministerial Guidelines).
The samples were placed in 2-litre sterile containers, containing a 10% solution of sodium thiosulphate to neutralise the chlorine, and taken to the laboratory, where we proceeded with the filtration of 1 litre of water through a membrane with 0.45 μ diameter pores (Millipore, MA, USA).
The bacterial patinas were then collected using sterile swabs which, together with the filters, were inserted into test tubes containing 10 ml of the same water and held at a temperature of 50°C for 30 minutes. After heat treatment, 50 microlitres were taken from each of them for culture (BCYE, Oxoid).
We adopted standard culture methods, although made a change which we applied right from the first studies. This consisted in the prior culture of the colonies developed with Legionella-like characteristics on Agar Triple Sugar Iron (TSI, Oxoid) and test-tubes of Brain Heart Infusion (BHI, Oxoid).
At times it is possible to observe on the plate the development of numerous varieties of micro-organisms belonging to certain species of Pseudomonas, or to other autotrophic species not rare in water, which grow similarly to Legionella. Their rapid growth on the aforementioned supports (6-12 hours in BHI; 12-24 hours on TSI agar) allows us to exclude them from the course of the investigation, thus reducing research costs.
The failure to develop in these mediums, which presupposed the presence of Legionella, was subsequently confirmed by the inability to grow on unsupplemented CYE agar base and by the final agglutination with Legionella latex test kit (*L. pneumophila 1*, *L. pneumophila 2-14*, *Legionella species*) (Oxoid).

Results and considerations

As can be observed in Table I, which for each date specifies sampling sites, together with serotypes and quantities recorded, 7 of 36 the shower facilities were found to be contaminated with Legionella (19.44%). In three showers, *L. pneumophila 1* was reported, in two *L. pneumophila 2-14*; in another two cases *L. pneumophila 2-14* was found together with *Legionella sp.* and *L. pneumophila 1* together with *L. pneumophila 2-14*.
This further study, which confirms the circulation of Legionella in our region of Southern Italy, extends the list of the possible sources of infection, including also public and private beaches among the contaminated sites.
We found no similar experience reported by other authors in literature, either regarding similar environmental isolations, or cases of Legionellosis in which the subjects were reported to have stayed in beach resorts.
It is our opinion that when evaluating the risk in these cases we should take into account that the crowding of the beaches in the summer months and the communal nature of shower use in these periods increases the frequency of exposure of children and old people. These vulnerable categories are significantly more likely to develop the disease once infected.
For these reasons, next summer we intend to repeat this exercise, while widening the study catchment area and increasing the number of sampling campaigns, which will be accompanied by any other useful information acquired.
We therefore consider it helpful to publish the results of this study, in order to encourage similar studies, and also to bring attention to these sites in the event of epidemiological investigations being carried out consequent to clinical episodes of Legionellosis.
The isolated strains are conserved, and in the near future will be examined using PFGE and compared with strains isolated from other sources in order to identify possible patterns in common.

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Tab. I. Results of Legionella research in beach showers facilities.

Date	Seaside resort	Showers		Serotype and quantity (u.f.c./l)
		Negative	Positive	
01/06/06	Place 1 (Sicily)		1	L. pneumophila 1 (1.000)
12/06/06	Place 2 (Sicily)	1		
30/06/06	Place 3 (Sicily)	1		
"	Place 4 (Sicily)	2		
"	Place 5 (Sicily)		1	L. pneumophila 1 (200)
"	Place 6 (Sicily)		1	L. pneumophila 1 (200)
05/07/06	Place 7 (Sicily)	2		
15/07/046	Place 8 (Calabria)		1	L. pneumophila 2-14 (200)
20/07/06	Place 9 (Sicily)	2		
22/07/06	Place 10 (Calabria)	2		
02/08/06	Place 11 (Sicily)	1		
04/08/06	Place 12 (Sicily)		1	L. pneumophila 2-14 (1.200) Legionella sp. (6.000)
06/08/06	Place 13 (Sicily)	1		
"	Place 14 (Calabria)	3		
09/08/06	Place 15 (Calabria)	1		
"	Place 16 (Calabria)	1		
15/08/06	Place 17 (Calabria)	1		
16/08/06	Place 18 (Calabria)		1	L. pneumophila 2-14 (2.800)
16/08/06	Place 19 (Calabria)	1		
17/08/06	Place 20 (Calabria)	1		
18/08/06	Place 21 (Sicily)	1		
24/08/06	Place 22 (Sicily)		1	L. pneumophila 1 (1.000) L. pneumophila 2-14 (1.000)
28/08/06	Place 23 (Calabria)	1		
29/08/06	Place 24 (Calabria)	4		
30/08/06	Place 25 (Sicily)	1		
	Total	29	7	

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■ Correspondence: Dr P. Laganà, Department of Hygiene, Preventive Medicine and Public Health "R. De Blasi", University of Messina, via C. Valeria, 98125 Messina, Italy - Tel. +39 090 2213358 - 2212444 - Fax +39 090 2213351 - E-mail: plagana@unime.it