Original article

Risk assessment of legionellosis in cardiology units

P. LAGANÀ [1], S. DELIA [1], E. AVVENTUROSO [1], M. CASALE [2], G. DATTILO [2]
1 Department of Biomedical and Dental Sciences and Morphofunctional Imaging. University Hospital of Messina, Italy;
[2] Department of Clinical and Experimental Medicine, Cardiology Unit, University Hospital of Messina, Italy

Keywords

Infective endocarditis • Legionella • Cardiac valve prosthesis • Environment

Summary

Infective Endocarditis (IE) is a disease with high morbidity and mortality. Nowadays, in addition to classic pathogens were isolated exigent Gram negative bacteria as A. baumannii, A. lwoffii, C. burnetii, Bartonella, Chlamydia and Legionella. We present our experience of Legionella isolations in environmental sample (water and air) collected from the Cardiology units belonging to two hospitals in Messina (Italy). A total of 80 samples were carried out, 30 and 50, respectively in the first and in the second structure: 55 of water and 25 of aerosol.
The positivity of 30% of the water samples analyzed and 15% of those aerosol strengthens the conviction of the need for greater environmental monitoring, especially in the wards at high risk.

Introduction

The continuous alert of legionellosis disease occurred in hospitals around the world and the clusters observed in major public places mean that the *"Legionella"* phenomenon became of primary importance for Public Health [1, 2]. The risk of illness increases dramatically if the germ is found in certain wards such as intensive care, cardiology, pulmonology for critical conditions of their hospitalized patients [3-8]. As reported in many articles in the scientific literature, we would like to emphasize as *Legionella* may be potentially related to endocarditis when it is found in the hospital environment.
The Infective Endocarditis (IE) is an infection of the endocardial surface of the heart, which may involve one or more heart valves, the mural endocardium, or a septal defect. If left untreated, IE is generally fatal. IE is in continuous evolution: in the face of an increasing role of degenerative valvular disease and iatrogenic factors such as the presence of prosthetic and intracardiac devices, factors like rheumatic disease have become secondary. The average age of patients has increased dramatically and with it the comorbidity. The frequency at which a particular organism causes endocarditis depends on how frequently can gain access to the circulatory system and its ability to survive in the bloodstream and stick to the components of NBTE (non-bacterial thrombotic endocarditis), to subendothelial structures exposed or to the endothelial surface. Over the past few years, despite the improvement recorded in both clinical-diagnostic and therapeutic circle, the incidence and mortality of infective endocarditis have not been substantially reduced [9, 10].
From an etiological point of view there are significant changes: in addition to classic pathogens (*S. aureus*, *Streptococcus* sp, *Enterococcus* sp) Gram negative bacteria are isolated, for example those belonging to the group HACEK (*Haemophilus* sp, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella kingae*). Moreover, there are studies reporting cases of endocarditis caused *by A. baumannii, A. lwoffii, C. burnetii, Bartonella, Chlamydia* and *Legionella* spp as the responsible of associated forms with negative blood culture [11-16]. Concerning *Legionella*, in the literature are describes cases which *L. micdadei*, *L. bozemanii*, *L. anisa*, *L. dumoffi* and *L. cardiac*, in addition to *L. pneumophila*, are implicated in the genesis of cardiovascular disease, with involvement of valvular structures, both prosthetic that native [17-28].
In hospital and other health care facilities, waterborne diseases may originate from the bacterial colonization of water pipes, taps, cooling towers, showers and water supplies [29-35]. For hospitalized subjects, risk assessment on the basis of levels of exposure to contaminated water pipes should be calculated following constant environmental monitoring, and critically with strict clinical surveillance. 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 (biofilm, parasitism of amoebas and protozoic cysts, etc.) [36, 37].
The Italian National Institute of Health (in italian, Istituto Superiore di Sanità) reported that during 2015 in Italy nosocomial cases of legionellosis were 82 (5.3%) of total cases reported, of which 33 (40%) were of certain nosocomial origin, and 49 (60%) possibly of nosocomial origin [38].
It follows, therefore, that environmental surveillance of *Legionella* spp. is needed for risk assessment and prevention of hospital-acquired legionellosis.

The objectives of the present investigation were to carry out the risk assessment of Legionellosis in Cardiology Units, verifying the presence and distribution of *Legionella* in water and air samples, in order to optimize the prevention program in higher risk areas.

Materials and methods

Ethical Approval was not required as this research did not involve human beings and/or animals.

From October 2015 to September 2016, 55 samples drawn from the water distribution system of Cardiology wards belonging a two hospitals sited in Messina (Italy) were examined for *Legionella* spp.

To recover *Legionella* spp. from water samples the standard procedures reported in the Italian Guidelines for the prevention and control of legionellosis, approved State-Regions Conference, in its meeting of May 7, 2015, were used [39].

In addition to water samples, *Legionella* has also been searched in 25 samples collected from the aerosol formed around the faucet when the water flows. In this case two Petri dish with a diameter of 9 cm were placed on the sites of the tap. Simulating handwashing, the health-care worker responsible for sampling has caused the formation of aerosol. The plates were inoculated only for the passive fall of the aerosol and this imply an even greater risk for patients who use the washbasins [40-43]. The result was an average of values measured on 2 plates/1 h and expressed as CFU/dm^2/h.

The isolates were further identified as *L. pneumophila* serogroups using the microagglutination kit '*Legionella pneumophila antisera set 1 and 2' and Legionella* antisera for several *Legionella* species as *L. dumoffii, bozemani, micdadei*, etc (Biogenetics, Denka Seiken co., Ltd, Tokio, Japan).

Results

A total of 80 samples were carried out, 30 and 50, respectively in the first and in the second structure: 55 of water and 25 of aerosol. Of 55 water samples analyzed (20 in the Hospital 1 and 35 in the Hospital 2), 18 (33%) were positive for the presence of *L. pneumophila*, 7 (35%) in the first and 11 (31%) in the second.

With regard to samples of air, of 25 samples taken, in 8 (32%) *L. pneumophila* was detected. Besides *L. pneumophila* 1, the identified serogroups were 3 and 6, the latter only in the second hospital. The mean concentration of *Legionella* did not show significant differences between the two structures (p>0.05). As expected, the same serogroups isolated from water were found, in much smaller quantities, in respective aerosol samples.

The results are summarized in Table 1.

Discussion and conclusions

The positivity of more than 30% of the water and air samples analyzed, strengthens the conviction of the need for greater and continuous environmental monitoring, especially in the wards at high risk.

The relevance of this alert is focused on the fact that IE sustained by *Legionella* genus is not a frequent but always a possible cause of sudden death in patients undergoing cardiac surgery, especially for prosthesis implantation [44]. Despite the analytical results discovered, strongly indicative of a risk situation, in cardiology wards considered, no cases of Legionnaires' disease had been reported among patients.

Despite the amount of Legionella found in the aerosol is considerably low, an efficient air sampling combined with water surveillance is beneficial for preventing legionellosis. Monitoring the air around aerosol producing devices may assist in tracking the greatest potential for *Legionella* spp. aerosolization, identifying plausible infection sources, and assessing the distance that Legionella has spread.

Attention should be given in increasing environmental surveillance in higher risk areas, otherwise you could apply to new methods of estimation of the spread of germs, such as Geostatistics [45]. Environmental monitoring, carried out for nearly a year, has certainly been useful to underline the problem, optimizing corrective measures, some of which are already active at the time of sampling, but clearly not sufficient to contain the risk

Tab. I. Distribution of water and air samples taken on Cardiology wards, positivity (%) and serogroups identified.

	total number of samples	samples of water	samples of water (% POS)	Serogroups (CFU/l min-max)	samples of air	samples of air (% POS)	Serogroups (CFU/dm^2/h min-max)
Hospital 1	30	20	7 (35)	L. pneumophila 1 (100-4000e+1) L. pneumophila 3 (100-2000e+1)	10	3 (30)	L. pneumophila 1 (0,78-5,5) L. pneumophila 3 (0,78-3,14)
Hospital 2	50	35	11 (31)	L. pneumophila 1 (200-1000e+2) L. pneumophila 3 (100-1000e+1) L. pneumophila 6 (100-2000e+1)	15	5 (33)	L. pneumophila 1 (0,78-6,28) L. pneumophila 3 (0,78-3,93) L. pneumophila 6 (0,78-2,36)
Total	80	55	18 (33)		25	8 (32)	

(as de-calcification, sanitization with chlorine agents, etc.), implementing a more accurate assessment of the state of the water system, considering the replace of some of its components (valves, end portion of the water pipe system, sanitation in recirculation water pipes in the interested departments concerned, etc.), as well as an increase of the level of active epidemiological surveillance on patients and staff of the departments concerned.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
The authors declare that they have no conflict of interest.

Authors' contributions

PL and SD carried out: study design, laboratory analysis, data interpretation. EA and MC provided the bibliography and wrote the manuscript. GD performed manuscript revision and handled cardiological aspects.

References

[1] Phin N, Parry-Ford F, Harrison T, Stagg HR, Zhang N, Kumar K, Lortholary O, Zumla A, Abubakar I. *Epidemiology and clinical management of Legionnaires' disease*. Lancet Infect Dis 2014;14(10):1011-21. doi: 10.1016/S1473-3099(14)70713-3.

[2] McCormick D, Thorn S, Milne D, Evans C, Stevenson J, Llano M, Donaghy M. *Public health response to an outbreak of Legionnaires' disease in Edinburgh, United Kingdom, June 2012*. Euro Surveill 2012;17(28).

[3] Lanternier F, Ader F, Pilmis B, Catherinot E, Jarraud S, Lortholary O. *Legionnaire's Disease in Compromised Hosts*. Infect Dis Clin North Am 2017;31(1):123-35. doi: 10.1016/j.idc.2016.10.014.

[4] Cunha BA, Burillo A, Bouza E. *Legionnaires' disease*. Lancet 2016;(23);387:376-85. doi: 10.1016/S0140-6736(15)60078-2.

[5] Francois Watkins LK, Toews KE, Harris AM, Davidson S, Ayers-Millsap S, Lucas CE, Hubbard BC, Kozak-Muiznieks NA, Khan E, Kutty PK. *Lessons From an Outbreak of Legionnaires' Disease on a Hematology-Oncology Unit*. Infect Control Hosp Epidemiol 2017;38(3):306-13. doi: 10.1017/ice.2016.281.

[6] Laganà P, Delia S. *Environmental sources of Legionella pneumophila: incubators in a Premature Unit*. J Prev Med Hyg. 2000; 41:34-6.

[7] Nazemi S, Mirzaii M, Yaslianifard S, Darban-Sarokhalil D, Khoramrooz SS, Norozi P, Davardoost F. *Microbiological qualification of air, water and dialysate in a haemodialysis centre: a new focus on Legionella spp*. Iran J Microbiol 2016;8(4):219-25.

[8] Guyot S, Goy JJ, Gersbach P, Jaton K, Blanc DS, Zanetti G. *Legionella pneumophila aortitis in a heart transplant recipient*. Transpl Infect Dis 2007;9(1):58-9.

[9] Prendergast BD. *The changing face of infective endocarditis*. Heart 2006;92:879-85.

[10] Slipczuk L, Codolosa JN, Davila CD, Romero-Corral A, Yun J, Pressman GS, Figueredo VM. *Infective Endocarditis Epidemiology over five decades: a systematic review*. PLoS One 2013;8(12):e82665. doi: 10.1371/journal.pone.0082665.

[11] Valero C, Fariñas MC, Palomo DG, Mazarrasa JC, Macías JG. *Endocarditis due to Acinetobacter lwoffi on native mitral valve*. Int J Cardiol 1999;69:197-9.

[12] Gonzalez-Juanatey C, Testa-Fernandez A, Lopez-Alvarez M. *Isolated pulmonary native valve infectious endocarditis due to Enterococcus faecalis*. Int J Cardiol 2006;26:113(1):E19-20.

[13] Matthews PC, Missouris CG, Jordaan J, Lessing MP. *S. lugdunensis endocarditis following cardiac catheterization International.* Int J Cardiol 2008;30;130(1):87-8.

[14] D.R. Murdoch, G.R. Corey, B. Hoen, J.M. Miró, V.G. Fowler Jr., A.S. Bayer, et al. *Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Prospective Cohort Study*. Arch. Intern Med 2009;169:463-73.

[15] Durante-Mangoni E, Andini R, Agrusta F, Iossa D, Mattucci I, Bernardo M, Utili R. *Infective endocarditis due to multidrug resistant gram-negative bacilli: single centre experience over 5 years*. Eur J Intern Med 2014;25(7):657-61. doi: 10.1016/j.ejim.2014.05.015.

[16] Laganà P, Melcarne L, Delia S. *Acinetobacter baumannii and endocarditis, rare complication but important clinical relevance*. Int J Cardiol 2015;187:678-9. doi: 10.1016/j.ijcard.2015.04.019.

[17] McCabe RE Baldwin JC, McGregor CA, Miller DC, Vosti KL. *Prosthetic valve endocarditis caused by Legionella pneumophila*. Ann Intern Med 1984;100(4):525-7.

[18] Tompkins LS, Roessler BJ, Redd SC, Markowitz LE, Cohen ML. *Legionella prosthetic-valve endocarditis*. N Engl J Med 1988;318(9):530-5. doi: 10.1056/NEJM198803033180902.

[19] Domingo C, Roig J, Seres J. *Pericardial effusion as a clinical sign of legionnaires' disease*. Int J Cardiol 1989;23(3):407-9.

[20] Armengol S, Domingo C, Mesalles E. *Myocarditis: a rare complication during Legionella infection*. Int J Cardiol 1992:37(3):418-20

[21] Park D, Pugliese A, Cunha BA. *Legionella micdadei prosthetic valve endocarditis*. Infection 1994;22(3):213-5.

[22] Chen TT, Schapiro JM, Loutit J. *Prosthetic valve endocarditis due to Legionella pneumophila*. J Cardiovasc Surg 1996;37(6):631-3.

[23] Muder RR, Yu VL. *Infection due to Legionella species other than L. pneumophila*. Clin Infect Dis 2002;35(8):990-8. doi:10.1086/342884.

[24] Massey R, Kumar P, Pepper JR. *Innocent victim of a localized outbreak: Legionella endocarditis*. Heart. 2003;89(5):e16.

[25] Pearce MM, Theodoropoulos N, Noskin GA, Flaherty JP, Stemper ME, Aspeslet T, Cianciotto NP, Reed KD. *Native valve endocarditis due to a novel strain of Legionella*. J Clin Microbiol 2011;49:3340-2. doi:10.1128/JCM.01066-11.

[26] Samuel V, Bajwa AA, Cury JD. *First case of Legionella pneumophila native valve endocarditis*. Int J Infect Dis 2011;15(8):e576-7. doi:10.1016/j.ijid.2011.04.007.

[27] Fukuta Y, Yildiz-Aktas IZ, Pasculle AW, Veldkamp PJ. *Legionella micdadei prosthetic valve endocarditis complicated by brain abscess: case report and review of the literature*. Scand J Infect Dis 2012;44(6):414-8. doi: 10.3109/00365548.2011.645506.

[28] Compain F, Bruneval P, Jarraud S, Perrot S, Aubert S, Napoly V, Ramahefasolo A, Mainardi JL, Podglajen I. *Chronic endocarditis due to Legionella anisa: a first case difficult to diagnose*. New Microbes New Infect 2015;22(8):113-5. doi: 10.1016/j.nmni.2015.10.003

[29] Leoni E, De Luca G, Legnani PP, Sacchetti R, Stampi S, Zanetti F. *Legionella waterline colonization: detection of Legionella species in domestic, hotel and hospital hot water systems*. J Appl Microbiol 2005;98:373-379. DOI 10.1111/j.1365-2672.2004.02458.x

[30] Perola O, Kauppinen J, Kusnetsov J, Heikkinen J, Jokinen C, Katila ML. *Nosocomial Legionella pneumophila serogroup*

5 outbreak associated with persistent colonization of a hospital water system. Acta Pathol Microbiol Immunol Scand 2002;110:863-8.

[31] Pignato S, Coniglio MA, Faro G, Cantaro P, Carini SA, Mangano G, Cunsolo R, Coco G, Giammanco G. *Legionella contamination in the hospital environment: monitoring of the hot water distribution systems of three hospitals in Catania (Italy)*. Ig Sanità Pubbl 2006;62(6):635-52.

[32] Spagnolo AM, Orlando P, Perdelli F, Cristina ML. *Hospital water and prevention of waterborne infections*. Reviews in Medical Microbiology. 2016;1:25-32. doi: 10.1097/MRM.0000000000000060.

[33] Stojek NM, Szymanska J, Dutkiewicz J. *Gram-negative bacteria in water distribution systems of hospitals*. Ann Agric Environ Med 2008;15:135-42.

[34] Sikora A, Wójtowicz-Bobin M, Kozioł-Montewka M, Magryś A, Gładysz I. *Prevalence of Legionella pneumophila in water distribution systems in hospitals and public buildings of the Lublin region of eastern Poland*. Ann Agric Environ Med 2015;22(2):195-201.

[35] Cristina ML, Spagnolo AM, Casini B, Baggiani A, Del Giudice P, Brusaferro S, Poscia A, Moscato U, Perdelli F, Orlando P. *The impact of aerators on water contamination by emerging gram-negative opportunists in at-risk hospital departments*. Infect Control Hosp Epidemiol 2014;35:122-9.

[36] Casini B, Buzzigoli A, Cristina ML, Spagnolo AM, Del Giudice P, Brusaferro S, Poscia A, Moscato U, Valentini P, Baggiani A, Privitera G. *Long -term effects of hospital water network disinfection on Legionella and other waterborne bacteria in an Italian university hospital*. Infect Control Hosp Epidemiol 2014;35(3):293-9. doi: 10.1086/675280.

[37] Laganà P, Caruso G, Piccione D, Pino R, Gioffrè ME, Delia S. *Legionella spp, amoebae and not-fermenting Gram negative bacteria in an Italian university hospital water system.* Ann Agric Environ Med 2014;21(3):489-93. doi: 10.5604/12321966.1120623.

[38] Rota MC, Caporali MG, Napoli C, Bella A, Giannitelli S, Scaturro M, Ricci ML. *Rapporto annuale sulla Legionellosi in Italia nel 2015*. Not Ist Super Sanità 2016;29(10):3-10.

[39] *Italian Guidelines for the prevention and control of legionellosis*. May 7, 2015. Available online: http://www.salute.gov.it/imgs/C_17_pubblicazioni_2362_allegato.pdf

[40] Chang, CW, Chou FC. *Methodologies for quantifying culturable, viable, and total Legionella pneumophila in indoor air*. Indoor Air 2011;21:291-9.

[41] Montagna MT, Cristina ML, De Giglio O, Spagnolo AM, Napoli C, Cannova L, Deriu MG, Delia S, Giuliano A, Guida M, Laganà P, Liguori G, Mura I, Pennino F, Rossini A, Tardivo S, Torre I, Torregrossa V, Villafrate MR, Albertini R, Pasquarella C. *Serological and molecular identification of Legionella spp in water and surrounding air samples in Italian healthcare facilities*. Environ Res 2016;146:47-50. doi: 10.1016/j.envres.2015.12.015.

[42] Montagna MT, De Giglio O, Napoli C, Cannova L, Cristina ML, Deriu MG, Delia S, Giuliano A, Guida M, Laganà P, Liguori G, Mura I, Pennino F, Rossini A, Tardivo S, Torre I, Torregrossa MV, Villafrate MR, Albertini R, Pasquarella C. *Legionella spp. contamination in indoor air: preliminary results of an Italian multicenter study*. Epidemiol Prev 2014;38(6)Suppl2:62-5.

[43] Montagna MT, De Giglio O, Cristina ML, Napoli C, Pacifico C, Agodi A, Baldovin T, Casini B, Coniglio MA, D'Errico MM, Delia SA, Deriu MG, Guida M, Laganà P, Liguori G, Moro M, Mura I, Pennino F, Privitera G, Romano Spica V, Sembeni S, Spagnolo AM, Tardivo S; Torre I, Valeriani F, Albertini R, Pasquarella I. *Evaluation of Legionella air contamination in healthcare facilities by different sampling methods: an italian multi center study*. Int J Environ Res Public Health 2017;14(7):670; doi:10.3390/ijerph14070670.

[44] Patel MC, Levi MH, Mahadevi P, Nana M, Merav AD, Robbins N. *L. micdadei PVE successfully treated with levofloxacin/valve replacement: case report and review of the literature*. J Infect 2005Dec;51(5):e265-8. Review.

[45] Laganà P, Moscato U, Poscia A, La Milia DI, Boccia S, Avventuroso E, Delia S. *The Geostatistics, tool applied to the distribution of Legionella pneumophila in a hospital water system*. Ann Agric Environ Med 2015;22(4):655-60. doi: 10.5604/12321966.1185769.

■ Received on February 27, 2017. Accepted on April 19, 2017.

■ Correspondence: Pasqualina Laganà, Department of Biomedical and Dental Sciences and Morphofunctional Imaging. Torre Biol. 3p, via C. Valeria snc, AOU 'G. Martino'. University Hospital of Messina, Italy - E-mail: plagana@unime.it