

# Controlling *Legionella* and *Pseudomonas aeruginosa* re-growth in therapeutic spas: implementation of physical disinfection treatments, including UV/ultrafiltration, in a respiratory hydrotherapy system

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## ABSTRACT

The study aimed to assess the efficacy of an integrated water safety plan (WSP) in controlling *Legionella* re-growth in a respiratory hydrotherapy system located in a spa centre, supplied with sulphurous water, which was initially colonized by *Legionella pneumophila*. Heterotrophic plate counts, *Pseudomonas aeruginosa*, *Legionella* spp. were detected in water samples taken 6-monthly from the hydrotherapy equipment (main circuit, entry to benches, final outlets). On the basis of the results obtained by the continuous monitoring and the changes in conditions, the original WSP, including physical treatments of water and waterlines, environmental surveillance and microbiological monitoring, was integrated introducing a UV/ultrafiltration system. The integrated treatment applied to the sulphurous water (microfiltration/UV irradiation/ultrafiltration), waterlines (superheated stream) and distal outlets (descaling/disinfection of nebulizers and nasal irrigators), ensured the removal of *Legionella* spp. and *P. aeruginosa* and a satisfactory microbiological quality over time. The environmental surveillance was successful in evaluating the hazard and identifying the most suitable preventive strategies to avoid *Legionella* re-growth. Ultrafiltration is a technology to take into account in the control of microbial contamination of therapeutic spas, since it does not modify the chemical composition of the water, thus allowing it to retain its therapeutic properties.

**Key words** | *Legionella* spp., *P. aeruginosa*, respiratory hydrotherapy, sulphurous spa water, UV/ultrafiltration, water safety plan

## INTRODUCTION

Therapeutic spas are widespread throughout Italy, where there are around 380 certified spa centres; of these, about a third includes respiratory hydrotherapy. The thermal waters are used for both recreational and therapeutic purposes in the form of baths, muds, hydro-massage and respiratory hydrotherapy. In some cases they may be contaminated by micro-organisms of environmental origin which can represent a potential health risk. Among the bacteria most commonly responsible for this contamination are the *Legionella* species (Verissimo *et al.* 1991; Martinelli *et al.* 2001; Alim *et al.* 2002; Kao *et al.* 2013; Zbikowska *et al.* 2013) and *Pseudomonas aeruginosa* (Moore *et al.* 2002; Leoni

*et al.* 2005; Brousseau *et al.* 2013). *L. pneumophila* has been the cause of sporadic cases or outbreaks of Legionnaires' disease associated with spa water (Molmoret *et al.* 2001; Ito *et al.* 2002; CDC 2004; Okada *et al.* 2005; Armstrong & Haas 2008; Campese *et al.* 2010), while *P. aeruginosa* has proved responsible for cases of infection related to the contact with spa water, including both folliculitis and pneumonia (Huhulescu *et al.* 2011; Uldall Pallesen *et al.* 2012). The risk of infection increases when the water is nebulized and administered in the form of aerosol for respiratory hydrotherapy, which has been associated with cases of Legionnaires' disease (Arnou *et al.* 1982; Mastro

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et al. 1991; Woo et al. 1992; Leoni et al. 2006). A recent case of pneumonia due to *L. pneumophila* serogroup 1 was reported in a 4-year old girl exposed to a home humidifier used for aerosol therapy and supplied with contaminated water (Bonilla Escobar et al. 2014).

The colonization by *Legionella* spp. of water systems is difficult to eradicate, due to the resistance of these microorganisms to the most commonly applied physical and chemical disinfection treatments (Saby et al. 2005; Cooper & Hanlon 2010; Tzu-Hsin et al. 2014). Furthermore, the peculiar chemical composition of the spa water that determines its therapeutic properties prevents the use of traditional chemical disinfectants which would modify its characteristics by generating unwanted insoluble compounds and/or disinfection by-products that are potentially toxic (Zwiener et al. 2007; LaKind et al. 2010; Florentin et al. 2011). Several different physical methods of disinfection such as superheat and ultraviolet radiations have been used, with varying levels of success (Stout et al. 1998; Hall et al. 2003; Costa et al. 2010). Another technique that has been applied for the decontamination of various types of water is ultrafiltration, a process that makes use of semipermeable membranes with pores measuring 0.001–0.1 micrometers, driven by the force of the trans membrane pressure (TMP). Ultrafiltration has been proposed as an advanced treatment for drinking water (Flores et al. 2013; Guo et al. 2013) and wastewater (Margot et al. 2013; Raffin et al. 2013), while Barbot & Moulin (2008) proposed a method combining ultrafiltration and activated carbon absorption in the treatment of swimming pool water, obtaining a satisfactory water disinfection and clarification and also a decrease in the concentration of combined residual chlorine.

This study describes the results of the monitoring of the microbiological quality of the sulphurous spa water coming from a system for respiratory hydrotherapy, which in 2003 was found to be colonized by *L. pneumophila*. The contamination was abated by making some structural adjustments and implementing a water safety plan (WSP) that included a protocol of periodic heat disinfection and the systematic microbiological monitoring of the water quality. A description of these interventions and the results obtained in the first year of follow-up (2004–2005) have been previously reported (Leoni et al. 2006). This paper now reports the results of the subsequent follow-up, during which the

bacteriological monitoring of the respiratory hydrotherapy system, in the context of a more general WSP, proved fundamental in showing the re-growth of *Legionella* spp. and in guiding decisions for the prevention of *Legionella* re-colonization of the waterlines.

The aim of this study is to assess the efficacy of an integrated WSP and the importance of its continuous review and adaptation on the basis of the changing conditions shown by systematic monitoring. A further aim is to evaluate the feasibility of the ultrafiltration process which, in combination with other physical methods, can be particularly efficacious in the control of the microbial contamination of spa water used for therapeutic purposes. In fact, by non-affecting the mineral content in salts and metal ions, this technique is able to guarantee the maintenance of therapeutic properties of the water, while it provides an effective barrier for bacteria, viruses, suspended particles and colloids (CDC 2008; Pizzichini et al. 2009; Molelekwa et al. 2014).

## MATERIALS AND METHODS

### Respiratory hydrotherapy system

The respiratory hydrotherapy system is supplied by natural sulphurous water. In the period 2003–2004 the system was found to be colonized by *L. pneumophila* serogroup 1 and 5 and consequently underwent corrective measures that were able to control the *Legionella* contamination, as previously described in the article reporting the results at 1 year of follow-up (Leoni et al. 2006). Review and adaptations to the initial WSP are detailed in Table 1. Initially, the sulphurous water, at a source temperature of 16–17 °C, was pumped from the well and carried to a boiler, where it was heated to 30 ± 2 °C. The heated water was then fed into a stainless steel ring circuit (main circuit), with branches leading to five benches where the final distribution points were situated in the form of devices for nasal irrigation and nebulizers for aerosol-therapy. The water lines of the benches are made of Teflon (main pipe) and silicone (flexible connections with the devices). The control plan was then adjusted, in consequence of *L. pneumophila* re-growth, by introducing a system of treatment with UV radiations and ultrafiltration and eliminating the shock heat

**Table 1** | Main interventions established by the WSP and its adaptations

	Interventions initially established by the WSP (Leoni et al. 2006)	Review and adaptation of WSP
Description of water treatment	Microfiltration: multi-cartridge filters (MIDI, Itaca Company, Italy) porosity 20 µm	Unvaried
	Water heat shock: the sulphurous water in the boiler is heated to 70–75 °C twice a week for at least 3 hours	Heat shock eliminated; UV/ Ultrafiltration introduced
	Waterlines heat shock: 3 times a week, on alternate days, the water lines of the benches are treated with superheated steam (1 atm.) which is introduced in the circuit for 1 hour and released from the nebulizers at a temperature of at least 80 °C	Unvaried
	Chemical shock treatment in the event of re-growth: descaling with nitric acid and shock disinfection with chlorine dioxide. Chlorine dioxide is produced from the reaction between 9% hydrochloric acid and 7.5% sodium chlorite and introduced into the network in order to obtain a concentration of 20 ppm for 12 hours at the points of use. After treatment, waterlines are rinsed with sulphurous water until complete removal of chlorine dioxide	Unvaried
Definition of modalities and frequency of plant maintenance	Every day the nozzles of the nebulizers and nasal irrigators are taken off, descaled by immersion in nitric acid and disinfected by means of immersion in stabilized chlorine dioxide solution	Unvaried
	Every 10 cycles of disinfection with pressurized steam, the tubes, connections and electrovalves of the devices are descaled and disinfected following the same method. Periodically the water pipes of the benches are replaced. Review of the list of maintenance companies and suppliers of disinfection systems, for prompt contact if necessary	Unvaried
Planning of environmental monitoring	Microbiological monitoring every 6 months and each time the activity recommences after a period of closure (according to the Italian guidelines for tourist-reception centres and spas) (Conferenza permanente 2005)	Unvaried

previously used (Table 1). Chemical treatment with chlorine dioxide was introduced as a corrective emergency measure only in the event of *Legionella* re-growth. In the modified protocol, the waterlines of each bench continued to be treated with superheat steam for 1 hour on alternate days. However, the periodic heat shock was withdrawn due to the undesirable salt precipitation, and the water was no longer heated to  $30 \pm 2$  °C since this tended to create favourable conditions for microbial development.

### UV treatment/ultrafiltration system

The water coming from the well is first treated with microfiltration in order to suppress the largest impurities. The microfilter consists of a cylindrical stainless steel shell (AISI 316L, size Midi) containing three filtering cartridges of polypropylene with a porosity of 20 µm. After microfiltration, the water undergoes treatment with UV radiation produced

by a Wallenius AOT 42, 219-type quartz lamp (Wallenius Water AB, Sweden; [www.walleniuswater.com](http://www.walleniuswater.com)) with a titanium catalyzer that favours the production of free radicals and thus enhances the disaggregation of the organic substance and of the micro-organisms (Kim et al. 2013). The running conditions foresee a maximum capacity of  $5 \text{ m}^3 \text{ h}^{-1}$  and a pressure of 0.2–7 bar, for an exercise period of about  $8 \times 10^5$  hours, after which the lamp is replaced. The lamp is certified to meet the strict new BRL K14010-1/01 guidelines issued by Dutch quality assurance agency KIWA. At the entry of each bench containing the irrigators and nebulizers, the water is subjected to a process of ultrafiltration. Each filter unit has a total capacity of  $0.48 \text{ m}^3 \text{ h}^{-1}$  and consists of a cylindrical PVC shell containing several dozen hydrophilic hollow fiber membranes (4.2 mm in diameter) made of polyethersulfone with multi-bore configuration (UFK 50-510-220; [www.acquaspar.it/acquaspar.html](http://www.acquaspar.it/acquaspar.html)). Each tubular membrane is traversed longitudinally by seven capillaries

0.8 mm in diameter. Under the force of a TMP of 2–8 bar, the water from the capillaries passes through the filtering material, which has a porosity of 0.03  $\mu\text{m}$ . When the capacity decreases due to the deposition of impurities onto the membrane, an automatic backwash is triggered whereby a valve opens that creates a preferential exit route for the water and the impurities that have accumulated. Every 6 months the ultrafiltration units are cleaned with alkaline and acid products.

### Sampling

Water samples were taken from different points of the hydrotherapy plant, from the well to the final outlets (nasal irrigators and aerosol devices). Samples were collected from the well, after the microfiltration/UV treatment, from the main ring circuit of the plant, on entry to benches (directly or after the ultrafiltration unit of each bench), and from the final outlets. The water from the well was sampled once a year, while the water from the other sampling points was collected every 6 months, in accordance with the protocol of the internal WSP. If monitoring showed an increase of heterotrophic plate counts (HPCs) and the detection of *P. aeruginosa* or *Legionella* spp., the checks were intensified on the basis of the corrective measures adopted, in order to assess their efficacy. After the first year of follow-up previously described (Leoni et al. 2006), monitoring was carried out for a further period of about 9 years, before and after the implementation of the new UV/ultrafiltration system.

### Physical–chemical parameters

Water temperature, pH (potentiometric method UNI 1050/1996) and conductivity (UNI EN 27888/1995) were determined at the time of sampling. In the first year after the implementation of the UV irradiation/ultrafiltration system, chemical parameters were measured in the water sampled four times a year, once per season, from the well (source untreated water) and at the entry to benches (treated water). Acid-preserved glass bottles were used for sampling.

The UNI ISO methods were used for the determination of the residual level at 180 °C (UNI 10506/1996) and the oxydability (UNI EN ISO 8467/1995), while the

standardized methods stated by the Italian Agency for Environment Protection and Technical Services (APAT) were used for the determination of the metal ions and salts. Total concentrations of Ca, Mg, Na, K, Fe, and SiO<sub>2</sub> were measured by Inductively Coupled Plasma emission spectrometry (APAT IRSA/CNR 2003, 3020). Chlorides and sulphates were measured using the technique of ion chromatography (APAT IRSA/CNR 2003, 4020). Finally, the concentrations of bicarbonates and sulphides were measured by the potentiometric method (APAT IRSA/CNR 2003, 2010/A) and the technique of oxidation by iodine (APAT IRSA/CNR 2003, 4160), respectively.

### Microbiological parameters

Samples were taken in 1 L sterile bottles and analysed within 6 hours of collection. The following process indicators were chosen: the total heterotrophic plate count at 22 °C (HPC 22 °C), the total heterotrophic plate count at 36 °C (HPC 36 °C), *P. aeruginosa* and *Legionella* spp. The HPCs were performed by pour plate method on Plate Count Agar (Bio-life) at 36 °C and 22 °C, for 48 hours and 72 hours, respectively, according to the ISO standard techniques (EN ISO 6222, 1999). Testing for bacteria belonging to *Pseudomonas* species was carried out by means of the standard membrane filtration technique using Pseudomonas Agar Base with CFC supplement (Oxoid) as culture medium. For each test, a volume of 100 mL was filtered using cellulose acetate filters with a porosity of 0.45  $\mu\text{m}$  (Millipore). After incubation at 30 °C for 48 hours, colonies with morphology typical of *P. aeruginosa* were tested for growth at 42 °C and submitted to the oxidase test and the biochemical tests in the API 20NE system (BioMerieux). *Legionella* spp. was detected according to the ISO11731 standard technique (1998) by pouring 1 L of water through a nylon filter with 0.22  $\mu\text{m}$  diameter pores (Millipore). The concentrate was suspended in 10 mL of sample water and vortexed for 15 minutes. An aliquot of the concentrate was examined as such, another aliquot was subjected to decontamination treatment with heat at 50 °C for 30 minutes. Both the concentrated and decontaminated samples were plated on *Legionella* GVPC selective Agar (Oxoid) and incubated at 35 °C in microaerophilic conditions for 14 days. The isolates were identified on the basis of cultural, biochemical and

serological features as previously described (Leoni & Legnani 2001; Leoni et al. 2006).

### Statistical analysis

For the statistical analysis the bacteriological data (cell forming units – CFU) were converted into  $\text{Log}_{10}\times$  to normalize the non-normal distributions. For all negative samples, the detection limits were used. The analysis of variance (ANOVA) test was applied to compare the HPCs detected in the various sampling points, from the well to the final devices (nasal irrigators and nebulizers) and before/after the introduction of the ultrafiltration treatment. All descriptive and statistical analyses were carried out using the StatView program (Albacus Concepts Inc., Berkely, CA, USA) for Apple Macintosh computer.

## RESULTS

Table 2 shows the mean composition in oligo-elements of sulphurous water at the source (samples collected from the

**Table 2** | Physical and chemical parameters of the sulphurous water taken from the well and after the UV irradiation/ultrafiltration treatment (UV/UF)

Physical-chemical parameters	Units of measure	Mean $\pm$ SD	
		Well (n: 4)	After UV/UF (n: 4)
pH	pH unit	7.2 $\pm$ 0.2	7.1 $\pm$ 0.2
Conductivity 20 °C	$\mu\text{S}/\text{cm}^{-1}$	713.2 $\pm$ 29.8	696.5 $\pm$ 47.4
Residual level at 180 °C	$\text{mg L}^{-1}$	484.6 $\pm$ 10.4	478.0 $\pm$ 17.0
Oxydability ( $\text{O}_2$ )	$\text{mg L}^{-1}$	0.5 $\pm$ 0.1	0.6 $\pm$ 0.0
Chloride ( $\text{Cl}^-$ )	$\text{mg L}^{-1}$	15.1 $\pm$ 1.6	15.9 $\pm$ 2.8
Sulphate ( $\text{SO}_4^{2-}$ )	$\text{mg L}^{-1}$	41.9 $\pm$ 5.1	37.6 $\pm$ 3.5
Dissolved iron (Fe)	$\text{mg L}^{-1}$	0.02 $\pm$ 0.00	<0.02
Bicarbonate ( $\text{HCO}_3^-$ )	$\text{mg L}^{-1}$	470.8 $\pm$ 11.3	465.5 $\pm$ 16.3
Calcium ( $\text{Ca}^{2+}$ )	$\text{mg L}^{-1}$	118.1 $\pm$ 3.2	116.8 $\pm$ 5.9
Magnesium ( $\text{Mg}^{2+}$ )	$\text{mg L}^{-1}$	24.0 $\pm$ 1.3	22.9 $\pm$ 1.6
Sodium ( $\text{Na}^+$ )	$\text{mg L}^{-1}$	25.4 $\pm$ 0.6	25.4 $\pm$ 0.8
Potassium ( $\text{K}^+$ )	$\text{mg L}^{-1}$	2.0 $\pm$ 0.1	2.1 $\pm$ 0.1
Silicon dioxide ( $\text{SiO}_2$ )	$\text{mg L}^{-1}$	19.6 $\pm$ 2.6	20.4 $\pm$ 4.8
Hydrogen sulphide ( $\text{H}_2\text{S}$ )	$\text{mg L}^{-1}$	3.0 $\pm$ 0.4	2.8 $\pm$ 0.4

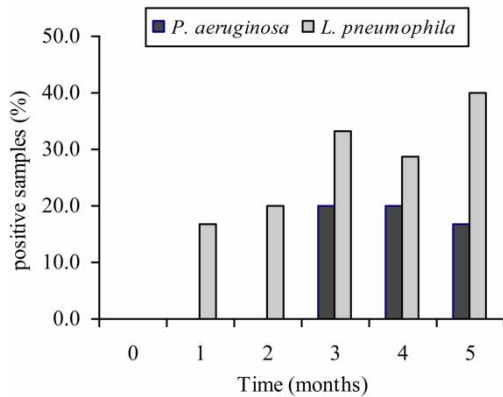
well) and after the treatment with the combined UV irradiation and ultrafiltration processes (samples collected at entry to benches). The main physico-chemical characteristics of the thermal water were not substantially altered by the combined treatments. In particular, the sulphur content which gives the water its peculiar therapeutic properties ranged between 2.60 and 3.40  $\text{mg L}^{-1}$  in the well and between 2.55 and 3.40  $\text{mg L}^{-1}$  after the combined UV/ultrafiltration process.

Table 3 compares the results of the microbiological follow up, in the 4 years before the *Legionella* re-growth and the 4 years after the introduction of the UV/ultrafiltration system. Results are given at the various stages of the water treatment. In the water of the well, the HPCs were very low and *P. aeruginosa* and *Legionella* spp. were not detectable throughout the entire period. Also in the main circuit water, these bacteria were never detectable. However, before the introduction of the UV/ultrafiltration system, the HPCs significantly increased in recirculation water of the main circuit (22°:  $P < 0.01$ ; 37°:  $P < 0.01$ ), at entry to the benches (22°:  $P < 0.001$ ; 37°:  $P < 0.001$ ) and in the final points of use (22°:  $P < 0.001$ ; 37°:  $P < 0.001$ ), suggesting that a microbial biofilm had formed inside the tubes. In the benches, both at entry and in the distal devices, *P. aeruginosa* was occasionally isolated at low concentrations. At the end of the 4-year period, *L. pneumophila* serogroup 1 and 3 was recovered at the entry and from the distal distribution points of two benches with aerosol devices, while it was never detected in the main circuit water.

Shock disinfection with chlorine dioxide was immediately performed in the contaminated bench waterlines, in accordance with the internal WSP that established a chemical shock treatment in the event of re-growth of *Legionella* spp. (Table 1). Shock treatment was followed by flushing with the spa water, which was allowed to flow until all residual chlorine dioxide had disappeared. Figure 1 shows the trend of *P. aeruginosa* and *L. pneumophila* contamination in the 5 months following the shock treatment. With a periodicity of about 3–4 weeks, the sulphurous water was collected from the final outlets of the two contaminated benches (for each sampling session: 5–9 samples per bench). Just 1 month after the shock treatment, *L. pneumophila* serogroup 3 was re-isolated from the

**Table 3** | Bacterial contamination of the sulphurous water in the 4 years before (pre-monitoring) and after (post-monitoring) the implementation of the integrated UV/UF process

Microbiological parameters	Well		After microfiltration/ UV irradiation (only in post-monitoring)	Main circuit		At entry to benches (in post- monitoring after ultrafiltration)		Final distribution points	
	Pre n: 4	Post n: 5	Post n: 10	Pre n: 10	Post n: 20	Pre n: 26	Post n: 20	Pre n: 52	Post n: 40
Temperature									
Mean (°C)	15.4	15.8	16.2	30.9	17.8	30.7	17.7	31.4	18.1
SD (°C)	1	0.6	0.4	2.5	0.6	1.4	0.3	1.4	1.3
HPC 37 °C									
Positive samples (%)	100	100	100	100	100	96.2	100	98.1	100
Geometric mean (CFU mL <sup>-1</sup> )	2.9	5	5.4	49.0	6.7	33.7	3.1	89.9	12.5
Range (CFU mL <sup>-1</sup> )	(1–1.5 × 10)	(3–10)	(2–6.0 × 10)	(7–4.1 × 10 <sup>2</sup> )	(2–3.2 × 10)	(0–4.0 × 10 <sup>2</sup> )	(1–1.1 × 10)	(0–3.8 × 10 <sup>3</sup> )	(2–4.4 × 10)
HPC 22 °C									
Positive samples (%)	100	100	100	100	100	100	100	100	100
Geometric mean (CFU mL <sup>-1</sup> )	2.7	5.8	7.6	50.9	5.5	26.8	3.8	56.1	16.8
Range (CFU mL <sup>-1</sup> )	(1–9)	(1–1.5 × 10)	(3–2.9 × 10)	(2–5.2 × 10 <sup>2</sup> )	(2–1.9 × 10)	(3–3.2 × 10 <sup>2</sup> )	(2–1.3 × 10)	(2–6.9 × 10 <sup>3</sup> )	(1–5.4 × 10)
<i>P. aeruginosa</i>									
Positive samples (%)	0	0	0	0	0	7.7	0	15.4	0
Range of positive samples (CFU mL <sup>-1</sup> )						(2–8)		(2–9.0 × 10)	
<i>L. pneumophila</i>									
Positive samples (%)	0	0	0	0	0	7.7	0	3.8	0
Range of positive samples (CFU mL <sup>-1</sup> )						(1.5 × 10 <sup>2</sup> –2 × 10 <sup>3</sup> )		(8.0 × 10 <sup>2</sup> –2 × 10 <sup>3</sup> )	



**Figure 1** | *P. aeruginosa* and *L. pneumophila* occurrence in sulphurous water in the five months following chemical shock treatment with ClO<sub>2</sub> (each sampling session: 5–9 samples collected from both the contaminated benches).

waterlines of the two benches initially contaminated, at levels between  $5 \times 10$  CFU L<sup>-1</sup> and  $4 \times 10^2$  CFU L<sup>-1</sup> respectively. The percentage of positive samples progressively increased over time.

The silicone waterlines of the contaminated benches were replaced with new pipes of the same material and the new protocol including the integrated system UV irradiation/ultrafiltration was introduced and implemented (Table 1). In the following 4 years *L. pneumophila* and *P. aeruginosa* were no longer isolated in any of the monitored points (Table 3). Also, the HPCs remained significantly lower than those recorded in the 4 years before the installation of the new integrated system, in the main circuit water (22°:  $P < 0.01$ ; 37°:  $P < 0.01$ ), at entry to the benches (22°:  $P < 0.001$ ; 37°:  $P < 0.001$ ) and in the distal points of use (22°:  $P < 0.05$ ; 37°:  $P < 0.001$ ).

## DISCUSSION

The integration of the various physical treatments gave satisfactory results in controlling the contamination of the spa water used for respiratory hydrotherapy. UV irradiation contributed to maintain the low level of microbial contamination in the water circulating in the main circuit of the hydrotherapy equipment: after UV irradiation, HPCs at 22 and 37 °C were significantly lower compared with those observed in the main circuit water before the introduction of UV irradiation. The subsequent passage of

water through the ultrafilters produced a further removal of contamination. The combined processes, associated with the periodic treatment with superheated steam, eliminated on a stable basis the contamination by *Legionella* and *P. aeruginosa* from the final distribution points, thus avoiding a re-colonization of the system. The main physico-chemical characteristics of the thermal water were not altered by the UV irradiation and the passage through the ultrafilters. This represents an important advantage in a water that owes its therapeutic properties to the natural contents of dissolved chemical substances (Salami et al. 2008; Ottaviano et al. 2012; Prandelli et al. 2013). Other types of treatment could, in fact, interfere with these natural substances. Chemical disinfectants, for example, could leave traces of residual disinfectant in the water and generate disinfection by-products (Zwiener et al. 2007; Barbot & Moulin 2008; LaKind et al. 2010; Florentin et al. 2011). Also, the combined process ultrafiltration-activated carbon adsorption used by Barbot & Moulin (2008) for swimming pool water treatment cannot be applied to the sulphurous water used for therapeutic purposes, since the absorption process would lead to a significant removal of the dissolved mineral content. Similarly, the high temperatures, causing the precipitation of the compounds in solution, could alter the composition of the water. Furthermore, it has been shown that heat shock is not able to provide a lasting control of contamination by *Legionella* (Farhat et al. 2010; Cristino et al. 2012). Our findings partly confirm the inefficacy of heat shock in the long-term.

The 10-year experience gained in this respiratory hydrotherapy plant shows that the actions of the WSP must be adapted on the basis of the results obtained by monitoring and the changes in conditions. Continuous monitoring, not only microbiological, but also extended to checking the compliance with the safety plan measures, is fundamental to identify the most suitable and integrated solutions to control and manage the infection risk by *Legionella* spp.

## CONCLUSIONS

In the spa system examined, the internal WSP allowed for the identification at onset of the re-growth of *L. pneumophila* in certain specific points and to avoid re-colonization

through the prompt application of corrective measures. In order for it to be effective, the control system must be dynamic, that is to say open to modifications on the basis of the period results, as well as integrated, that is to say involving different, combined actions for the control of the various critical points.

The treatments of sulphurous water (microfiltration/UV irradiation/ultrafiltration), waterlines (superheated stream) and distal outlets (protocol of descaling/disinfection of nebulizers and nasal irrigators), used in combination, ensured the maintenance over time of a satisfactory microbiological quality in the water and of its natural chemical composition. These findings suggest that ultrafiltration is a technology to take into account in the control of microbial contamination of therapeutic spas, and is also advantageous in the long-term from an economic point of view, since the initial installation costs are compensated over time by the lower maintenance costs compared to the previous periodic heat treatment that generated greater technical setbacks (precipitation of salts, incrustation, blockages, etc.) as well as high energy costs.

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