

Legionella risk assessment in cruise ships and ferries

Pasqualina Laganà¹, Maria Elsa Gambuzza², Santi Delia¹

¹ Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy

² Ministry of Health, USMAF, Territorial Office of Messina, Sicily, Italy

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Abstract

Introduction. The increasing development of marine traffic has led to a rise in the incidence of legionellosis among travellers. It occurs in similar environments, especially closed and crowded, and aboard ships *Legionella* survives and multiplies easily in water pipes, spreading into the environment through air conditioning systems and water distribution points. Although in recent years in the construction of cruise ships preventive measures aimed at curbing the proliferation of *Legionella* (design, materials, focus on the operation and maintenance of the water system), have been taken account, little or no attention has been paid to small ships which, in many cases, are old and not well maintained.

Objective. The aim of the study was to evaluate the frequency and severity of *Legionella* contamination in ferries and cruise ships in order to adopt more specific control measures.

Materials and method. A prevalence study was carried out on 10 ferries and 6 cruise ships docking or in transit across the port of Messina (Sicily, Italy). Water and air samples collected from many critical points were tested for qualitative and quantitative identification of *Legionella*.

Results and conclusions. *Legionella pneumophila* sg 1 was isolated from the samples of shower and tap water in 7 (70%) of the 10 ferries examined, and in 3 (33%) of the 6 cruise ships examined, and *L. pneumophila* sg 2–14 in 8 (80%) and 1 (16.7%) of these ships, respectively. No *Legionella* contamination was found in whirlpool baths, air and ice samples. In conclusion, the data obtained confirm higher levels of *Legionella* contamination in local ferries and cruise ships, underlining the need to adopt corrective actions more specific for these smaller vessels.

Key words

Legionella pneumophila, Cruise ships, Ferries, Preventive measures

INTRODUCTION

National and international maritime transport is very important in Italy and worldwide [1]. Tourism has played an important role in the economic growth of developed and developing countries, which also contributes to the increased traffic of cruise ships. In addition, ferries represent an important part of the public transport systems of many waterside cities and islands, since they carry primarily passengers as well as vehicles and cargo across bodies of water, linking many waterside cities and islands, allowing direct transit between different points. In Italy, a country with a total of almost 8,000 km of coastline, including the peninsula itself and numerous islands, there are many ferry services linking the islands by sea to the mainland. In addition, ships from countries around the Mediterranean are moored in the well-equipped Italian ports. Among the main Italian ports, Messina occupies an important place, since it represents the main departure port from Sicily to the Italian mainland and the Eolian islands. In particular, along the Strait of Messina, the body of water of about 3 km that separates Sicily from the rest of Italy, both international cruise and national ferry traffic are particularly intense every day, especially during the warmer months. In addition, Messina is included among the itineraries of major international cruise lines. Consequently, there is an increased risk for the acquisition of infectious

diseases frequently associated with both the international/national marine traffic, and modern high-speed transport.

Outbreaks of measles, rubella, varicella, meningococcal meningitis, hepatitis A, legionellosis, and other respiratory and gastrointestinal illnesses, in addition to the most recently reported outbreaks of influenza and norovirus, occurred among the passengers and crews of ships [2]. Such outbreaks represent a serious hazard, not only for their potentially dangerous health consequences, but also for the high costs incurred by the industry. Among the different travel-related infectious diseases, legionellosis plays an important role. Legionellosis is a potentially fatal lung infection caused by the inhalation or possibly aspiration of warm, aerosolized water containing *Legionella* organisms. Until recently, it was thought to be a non-contagious disease, but recently there has been a report with evidence of a possible inter-human contagion [3].

The legionellosis alert and negative influence on tourism are witnessed by the increasing number of cases contracted on board cruise ships, so much so that, according to the CDC in Atlanta, USA, they represent 20% of the total [4]. The greatest danger for crew and cruise passengers is represented by the pools and whirlpool baths, as well as by the cabin showers and, potentially, all water points on board ships [5, 6, 7, 8, 9, 10, 11, 12, 13, 14].

Legionella grows readily in man-made biofilm formed inside plumbing fixtures and pipes where warm temperatures and the build-up of nutrients and microorganisms on surfaces provide an ideal environment, which are also populated by other microorganisms involved into a multitude of trophic interactions [15, 16, 17, 18]. Several factors can contribute to the potential risk of infection: water contamination levels,

Address for correspondence: Pasqualina Laganà, Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy
E-mail: plagan@unime.it

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host factors (older age, tobacco smoke, chronic degenerative diseases, state of immunodeficiency, etc.), and specific virulence factors that can often be enhanced by a protozoa – or *amoeba* – bacterium relationship [19]. Most cases of legionellosis are sporadic, but outbreaks can always occur, due to the contamination of hot and cold water systems, spa pools, natural pools, thermal springs, evaporative condensers and respiratory therapy equipment [20, 21, 22]. Biofilm formation can provide a further means for *Legionella* survival and dissemination [23, 24].

In the light of these considerations, the Regional Reference Laboratory for Clinical and Environmental Surveillance of Legionellosis, Messina branch, in collaboration with the Maritime Health Office in Messina, undertook an epidemiological survey on ferries and cruise ships, in order to assess the impact of *Legionella* spp. contamination in vessels. More specifically, this study assessed the frequency and severity of *L. pneumophila* contamination in cruise ships and ferries in order to evaluate both the risk exposure for crews and passengers belonging to these different categories of vessels, and the control measures adopted.

MATERIALS AND METHOD

The survey was carried out on 16 ships docked at the port of Messina, from March – October: 10 ferries, including 7 belonging to a national company and 3 to a local shipping company, linking the ports of Messina and Villa San Giovanni (Strait of Messina), in addition to 6 cruise ships belonging to national companies and temporarily docked at the port of Messina.

During the investigation, a very high number and types of sites were examined. In addition, due to the diversity of plumbing and mechanical systems used on the ships, in some cases more than one sample from the suspected source was collected. Any water source that might be aerosolized was considered a potential means for the transmission of *Legionella*, and was consequently analyzed.

Both water and air sampling were collected from many critical sites, including crew cabins, kitchens, coffee bars, rooms connected to the central air conditioning system, tanks, and hot baths.

On board all ships, the epidemiological investigation was divided in three phases.

Phase I – Visual inspection. A preliminary inspection was performed at all recreational and potable water points, according to the inspection outlines of the European Ships Manual for Hygiene Standard and Communicable Disease Surveillance on Passenger Ships [25]. Visual inspection criteria included cleanliness, water turbidity, wall slime, sludge, general repair, access, and location of the sampling points. Moreover, air outlets where air samples should be collected were identified.

Phase II – Delivery of a questionnaire. A questionnaire was given to the master of each ship to collect the following information:

- characteristics of the ship, mainly concerning the slips, the latest water supplies, etc.;
- recent crew and passenger respiratory diseases, or other infectious diseases;

- routine water pipes maintenance and repair;
- chemical water treatment and analytical tests carried out;
- any suspected case of legionellosis.

Phase III – Sampling and analysis (water and air).

Water sampling. A high number of sites were tested in both ferries and in cruise ships. In addition, due to the diversity of plumbing and mechanical systems in use, in some cases more than one sample from the suspected source was collected. All water sources that might be aerosolized were considered a potential means for the transmission of *Legionella* and consequently analyzed.

Hot water samples were collected using 1 L-sterile glass bottles, after flushing for 5–10 min and sterilization of the terminal portion of the tap. Sampling was carried out taking care not to neglect any part of the ship: stern, bow, starboard and port side, and each deck, to obtain a sampling representative of the hygienic-sanitary conditions.

Air sampling. As previously mentioned, at the main points considered at risk, active air sampling was performed by using Surface Air System (SAS 100, PBI International, Milan, Italy), located about 20 cm from the point of entry of conditioned air in the various premises. The SAS was set to aspirate 100 L/min, and aspirated for 10 minutes, for a total of 1,000 L. A total of 64 samplings were carried out. The number of colonies present on each plate was calculated according to the conversion table provided by the manufacturer, and expressed in cfu/m³.

Isolation and identification of *Legionella* spp. To isolate *Legionella* spp. from water samples, the standard procedures reported in the Italian Guidelines for the Prevention and Control of Legionellosis were used [26]. More specifically, 1 L water samples were concentrated to 10 mL through 0.2 µm porosity membrane filters, and incubated at 50 °C for 30 min in a thermostatic bath. Concentrated and unconcentrated samples were spread on duplicate plates of Buffered Charcoal-Yeast Extract (BCYE) Agar Base Medium (Oxoid, Ltd., Milan, Italy), incubated for 10 days at 36–37 °C in a moist chamber with 2.5% CO₂. The suspected colonies were isolated and confirmed as *Legionella* spp. after screening their inability to grow on a culture medium without cysteine. *Legionella* spp. counts were reported in colony forming units/litre (CFU/L) according to the number of colonies *per* plate and to the dilutions performed on the original sample. The isolates were further identified as *L. pneumophila* serogroup 1, *L. pneumophila* serogroups 2–14, or *Legionella* spp using the microagglutination *Legionella* Latex Test Kit (Oxoid).

STATISTICAL ANALYSIS

The data obtained were processed using Microsoft Office Excel in order to produce graphics and tables.

RESULTS

From a total of 66 water samples collected from the showers in crew cabins, 32 (48%) were positive for *Legionella*. In water coming from the taps of sinks located at various sites on the

ships (including the WC inside the cabins of passengers and crew, bars, gyms, bridge, etc.), *Legionella* was isolated in 20 cases out of 46 (43%). *Legionella* presence in 3 (23%) of the 13 reservoirs controlled, was indexed as high risk, since from this point water branches in the pipes to the whole ship. Only 1 of 13 (8%) samples of drinkable water (gushing fountain) was positive, but it has not yet been shown that *Legionella* is transmitted by ingestion of contaminated water. It is also true that to drink from the gushing fountains, they must be approached by the face which means inhaling droplets that could potentially be contaminated [27, 28].

No *Legionella* contamination was found in whirlpool baths, air and ice samples.

Among 92 water samples obtained from ferries, 59 (64%) resulted positive for *L. pneumophila*, in contrast to cruise ships where 6 (4%) of 136 water samples resulted positive (Tab. 1).

Detailed sampling plans were made on board the ferries and cruise ships, and the CFU/L range of the *L. pneumophila* 1 and 2–14 isolated also indicated (Tab. 1).

Figure 1(a) shows the percentage distribution of positive samples according to the source. Figure 1(b) shows the percentage of *Legionella* positive samples isolated from ferries (91%), compared to cruise ships (9%). Figure 1(c) shows the distribution percentages of serogroups of *Legionella* isolated.

In detail, of 92 water samples taken on board of ferries, 59 (64%) were positive for the presence of *Legionella*; of these, 21 (36%) were *L. pneumophila* sg 1 and 30 (51%) *L. pneumophila*

sgs 2–14. In addition must be counted 8 (13%) samples in which both *L. pneumophila* sg 1 and sgs 2–14 were isolated. Of the 29 samples positive for the presence of *Legionella pneumophila* 1, 11 (39%) contained a bacterial load of between 10^3 and 10^4 CFU/L; 3 (10%) between 10^4 – 10^5 and 14 (48%), less than 10^3 CFU/L. A value greater than 10^5 CFU/L was detected only in 1 (3%) sample. According to Italian guidelines in force at the time of surveillance, this level is considered critical, even in the absence of clinical cases.

Of the 38 samples positive for the presence of *Legionella pneumophila* 2–14, 19 (50%) contained a bacterial load of between 10^3 and 10^4 CFU/L; 14 (37%) between 10^4 – 10^5 and 5 (13%) less than 10^3 CFU/L. No sample exceeded the value of 10^5 .

Of 136 water samples taken on board cruise ships, only 6 (4%) were positive for the presence of *Legionella*; of these, 4 (67%) were *L. pneumophila* sg 1 and 2 (33%) *L. pneumophila* sgs 2–14.

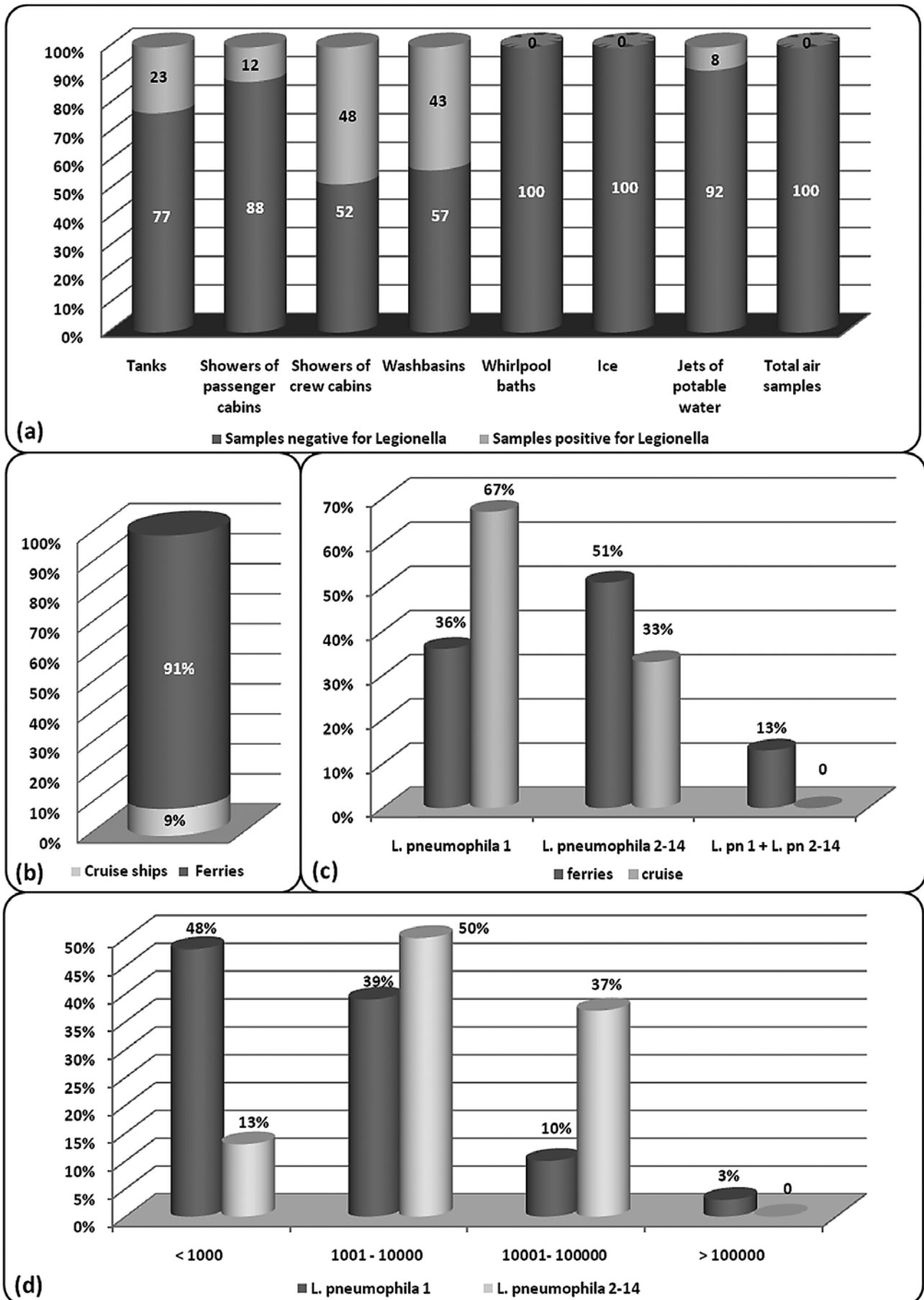
Of the 4 samples positive for the presence of *Legionella pneumophila* 1, 3 (75%) contained a bacterial load of between 10^3 and 10^4 CFU/L, and 1 (25%) less than 10^3 CFU/L. Regarding *L. pneumophila* 2–14, both samples contained less than 10^3 CFU/L. Distribution of *Legionella* positive samples according to serogroups and CFU/L isolated are presented in Figure 1(d).

Legionella pneumophila 1 was isolated in 7 (70%) ferries of the 10 examined, and in 2 (33%) cruise ships of 6 examined.

Among the other microbial species simultaneously

Table 1. Distribution of samples taken on Ferries (a) and Cruise ships (b), positivity (%) and serogroups identified. In the table the UCF/L range of *L. pneumophila* 1 and 2–14 isolated also is indicated

Code ship given by the Laboratory	total number of samples	samples of water	samples of water (% POS)	samples of air (% POS)	samples of ice (% POS)	<i>Legionella</i> Serogroups (UCF/L range)
(a) Ferries						
A	7	6	0	1 (0)	0	0
B	12	10	9 (90)	2 (0)	0	1 (500) 2–14 (1800–80000)
C	14	10	7 (70)	4 (0)	0	1 (100–4000) 2–14 (300–1500)
D	10	10	2 (20)	0	0	2–14 (1500–2500)
E	16	12	4 (33)	4 (0)	0	1 (100–900)
F	10	10	10 (100)	0	0	1 (200–3000) 2–14 (300–40000)
G	15	11	11 (100)	4 (0)	0	1 (100–30000) 2–14 (2600–70000)
H	10	7	4 (57)	3 (0)	0	1 (15000–50000) 2–14 (7500–17500)
I	8	6	2 (33)	2 (0)	0	2–14 (200)
L	14	10	10 (100)	4 (0)	0	1 (1000–15000) 2–14 (3000–25000)
Total	116	92	59 (64)	24 (0)	0	
(b) Cruise ships						
A1	33	25	0	6 (0)	2 (0)	0
B1	31	21	0	8 (0)	2 (0)	0
C1	28	20	0	8 (0)	0	0
D1	30	24	2 (8)	6 (0)	0	1 (100–600)
E1	27	21	4 (19)	6 (0)	0	1 (200–5000) 2–14 (400–500)
F1	31	25	0	6 (0)	0	0
Total	180	136	6 (4)	40 (0)	4 (0)	



Graphic 1. (a) Percentage distribution samples pos/neg for *Legionella* vs source; (b) Percentage positive samples for *Legionella*: Cruise ships vs Ferries; (c) Percentage distribution of *Legionella* Serogroups isolated from ferries and cruise ships; (d) Percentage distribution of *Legionella* positive samples in according to UCF/L isolated

recovered in the same sample, the most frequent was *Pseudomonas* spp. These data (not shown) are more interesting because the presence of *Pseudomonadaceae* is highly indicative for biofilm formation and the consequent induction of *Legionella* proliferation.

Regarding information collected via the questionnaires completed by the masters of both cruise ships and ferries, the results are summarized in Table 2 (ferries) and Table 3 (cruise ships).

Table 2. Data obtained from the questionnaires filled out by the commander of the ferries. The answers are faithfully reported

Code ship given by the Laboratory	A	B	C	D	E	F	G	H	I	L
Number of Crew	11	37	12	22	20	11	13	/	14	12
Number crew cabins	0	41	0	29	0	0	0	0	0	0
Number of passengers	/	150	/	670	-	/	/	/	/	/
Number passenger cabins	0	77	0	76	0	0	0	0	0	0
Cases of infectious diseases and clinical manifestations registered on board ships	N	N	N	N	N	N	N	N	N	N
Chemical and microbiological analyzes on water samples (conformity)	Y (Conform)	Y (Conform)	Y (Conform)	N	Y (Conform)	Y (Conform)	Y (Conform)	Y (Conform)	Y (Conform)	Y (Conform)
Typology of air conditioning system on board	CENTR	CENTR	CENTR and IND	CENTR	CENTR	IND	IND	v	CENTR	CENTR and IND
Maintenance of the air-conditioning system (intervals)	Y (fortnightly)	Y (monthly)	Y (monthly)	Y (fortnightly)	Y (weekly)	Y (monthly)	N	Y (half-yearly)	Y (monthly)	Y (monthly)
Number whirlpool baths	0	0	0	0	0	0	0	0	0	0
Number of pools	0	0	0	0	0	0	0	0	0	0
Pipe material	GALV	GALV	GALV	GALV	GALV	GALV	GALV	GALV	GALV	GALV
Maintenance and reclamation waterworks (intervals)	N	Y (monthly)	Y (monthly)	Y (annual)	Y (annual)	Y (monthly)	Y (monthly)	N	N	Y (monthly)
Maintenance mode waterworks	/	Technico-Mechanics	/	Technico-Mechanics	Disinfection, cleaning, substitution UV lamps	water flow	Disinfection, Cleaning, Substitution UV lamps	/	/	/
Presence of ultraviolet ray lamps	Y	/	Y	N	Y	N	Y	Y	N	Y
Maintenance potable water tanks (intervals)	N	N	N	Y (half-yearly)	N	N	N	N	N	N
Maintenance mode of potable water tanks	/	/	/	cementing				/	/	/
Presence of evaporation towers, aerators and humidifiers (intervals)	Y (fortnightly)	Y (weekly)	/	-	N	Y (monthly)	Y (quarterly)	N	/	/
Maintenance mode	SAN	SAN	/	-	/	Water flow	Cleaning	/	/	/

Legend. CENTR: Centralized; DISINF: Disinfection; IND: independent; GALV Galvanized steel; SAN: sanification; Y: Yes; N: No.

Table 3. Data obtained from the questionnaires filled out by the commander of the cruise ships. The answers are faithfully reported

Code ship given by the Laboratory	A1	B1	C1	D1	E1	F1
Number of Crew	775	509	577	882	394	1002
Number crew cabins	474	308	353	508	207	250
Number of passengers	1577	1220	884	2146	937	2466
Number passenger cabins	964	513	654	1057	410	1080
Cases of infectious diseases and clinical manifestations registered on board ships	N	Y (Fever)	Y (varicella)	Y (Fever)	N	N
Chemical and microbiological analyzes on water samples (conformity)	Y (Conform)	Y (Conform)	Y (Conform)	Y (Conform)	Y (Conform)	Y (Conform)
Typology of air conditioning system on board	CENTR	CENTR	CENTR	IND	CENTR	CENTR and IND

Table 3. Data obtained from the questionnaires filled out by the commander of the cruise ships. The answers are faithfully reported

Code ship given by the Laboratory	A1	B1	C1	D1	E1	F1
Maintenance of the air-conditioning system (intervals)	Y (monthly)	Y (monthly)	Y (monthly)	Y (monthly)	Y (monthly)	Y (monthly)
Number whirlpool baths	5	3	4	4	3	3
Maintenance of whirlpool baths (intervals)	Y (daily/monthly)	Y (daily)	Y (daily)	Y (daily/monthly/half-yearly)	Y (daily/monthly/half-yearly)	Y (daily)
Maintenance mode whirlpool baths	Change filters	Hyperchlorination	Cleaning and Chlorination	Water, sand and filters control	Cleaning and Chlorination	SAN
Number of pools	3	3	2	4	2	3
Maintenance of pools (intervals)	Y (daily/monthly/yearly)	Y (daily)	Y (daily)	Y (daily)	Y (daily)	Y (daily)
Maintenance mode pools	Change filters; Hyperhalogenation tanks	Hyperchlorination	Cleaning and Chlorination	Cleaning and hyperchlorination	Hyperchlorination	Emptying and sanification
Pipe material	Copper, Polyester	Copper, Polyester, Galvanized Iron	Galvanized steel	Copper, Galvanized steel	Galvanized steel	Galvanized steel, Polyester
Maintenance and reclamation waterworks (intervals)	Y (for each load, daily, monthly)	Y (half-yearly)	N	Y (half-yearly)	Y (weekly/quarterly)	Y (fortnightly)
Maintenance mode waterworks	Analyzers control and halogenated residual values	Visual inspection	/	Cleaning, Hyperchlorination of silt	Hyperchlorination	SAN
Presence and number of ultraviolet ray lamps	Y	N	N	Y	N	N
Maintenance potable water tanks (intervals)	Y (half-yearly)	Y (half-yearly)	Y (half-yearly)	Y (annual)	Y (half-yearly)	Y (half-yearly)
Maintenance mode of potable water tanks	/	Visual inspection	DISINF	Cleaning and hyperchlorination	Hyperchlorination	Emptying and sanification
Presence of evaporation towers, aerators and humidifiers (intervals)	N	N	Y (half-yearly)	Y (weekly/quarterly)	Y (weekly)	Y (weekly)
Maintenance mode	/	/	Cleaning and calibration	Visual inspection and replacement	Cleaning and Chlorination	SAN

Legend. CENTR: Centralized; DISINF: Disinfection; IND: independent; SAN: sanification. Y Yes; N No.

CONCLUSIONS AND DISCUSSION

The presented study further confirms data from previous studies showing that both hot and cold water distribution systems on board ferries can be more easily colonized by *Legionella* spp. at high concentrations, compared to cruise ships. Similar results were previously obtained by Azara *et al.* in a study performed on 2 cruise ships and 7 ferries docked at seaports in northern Sardinia in 2004. Data obtained from these studies showed that 6/7 (86%) ferries were positive, 38/90 (42%) of the water samples were contaminated, and 77.8% contained $\geq 10^4$ CFU/L [29]. Another Italian study, performed by Negretto *et al.* at the port of Venice (data unpublished) in 2010, further confirms the data of the current study, and shows that ferries present more high risks of *Legionella* contamination, compared to cruise ships. Similarly, a previous survey carried out by Goutziana *et al.* in 33 water tanks on yachts in Athens, showed that approximately 40% were positive for *Legionella* spp. [30]. The results obtained in the presented study further confirm the frequent and high *L. pneumophila* contamination in water distribution systems of ferries, and underline the need for adopting more specific preventive measures. This was also recently and further confirmed by Mouchtouri and Rudge [31]. Cayla JA *et al.*, reported 2 cases of Legionellosis that

occurred in two mechanics working on a cargo ship under repair [32].

The biggest problems lie with the ferries where the high risk of *Legionella* colonization is mainly caused to their old and always non-optimal conditions of most of these vessels (Tab. 2). In addition, the limited number of crew members and their particular turnover hamper the correct surveillance of the water safety plan. In some cases, the water tanks are not easily accessible due to the small size of the vessels and, consequently, a less accurate sanification may induce the formation of biofilm that represents the main substrate for the growth of both *Legionella* spp. and other pathogens. Finally, the showers and other washing facilities not continuously used, unlike on board the cruise ships, represent a further cause of contamination.

In 2005, the European Commission funded the SHIPSAN project which aims at assessing the usefulness of an EU ship sanitation programme and coordinated action for the control of communicable diseases in cruise ships and ferries [33]. The European Manual for Hygiene Standards and Communicable Diseases, edited by the European ShipSan trainer partnership, contains detailed guidelines to prevent and control legionellosis on passenger ships [34]. However, in the opinion of the authors of this study, it would be advisable to edit a water sampling plan more specific for the ferries in

order to consider their particular requirements. This plan should oversee all water outputs, including showers and sinks. During sanitation practices, the water tanks could be subjected to enzymatic treatment for preventing biofilm formation. In the ferries where the personnel is always subjected to mobility and turnover from a vessel to another, according to seasonal needs and itineraries, it is very difficult to identify a unique and competent person who could be responsible for supervision and health safety, able to control the correct performance of the procedures in compliance with the safety water plan adopted to avoid biofilm formation and *Legionella* contamination.

A specialized external inspection team should be appointed and able to check compliance with specific procedures adopted by each ship. The inspectors should periodically conduct a reassessment of the sites at high risk of contamination in order to evaluate the need to implement further precautions able to control all identified risk from *Legionella*. The team should be composed of members with specific skills (e.g. a microbiologist, environmental health officer or water engineer with specific expertise). These additional control measures could contribute to the maintenance of protection at satisfactory levels, comparable to that of cruise ships. Moreover, to evaluate the potential contamination of *Legionella* in ships (both cruises and ferries), it could be useful to apply georeferential statistical analysis to assess the possible sources of dispersion and, consequently, the risk of exposure for both crew and passengers [35].

Authors' contributions

Prof. Dr. Pasqualina Laganà made substantial contributions to the conception and design analysis and acquisition of data, carried out the analysis in the laboratory for the detection of *Legionella*, and was involved in drafting the manuscript. Dr. Maria Elsa Gambuzza, an expert in the field of maritime health, also involved in drafting the manuscript. Prof. Dr. Santi Delia, an expert in the field of *Legionella*, made substantial contributions to the conception and design, analysis and interpretation of data. He, too, was involved in critically revising the manuscript for important intellectual content, and in the final approval of the version to be submitted.

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