

Legionella spp. Colonization on Non-Passenger Ships Calling at Belgian Ports [†]

Leonidas Kourentis ¹, Lemonia Anagnostopoulos ¹, Zafeiris Tsinaris ¹, Achilleas P. Galanopoulos ^{1,2}, Diederik Van Reusel ³, Raf Van den Bogaert ³, Björn Helewaut ³, Inge Steenhout ³, Hasse Helewaut ³, Dion Damman ⁴, Christos Hadjichristodoulou ¹ and Varvara A. Mouchtouri ^{1,*} 

¹ Department of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, 41222 Larissa, Greece

² Department of Immunology & Histocompatibility, Faculty of Medicine, University of Thessaly, 41500 Larissa, Greece

³ Seaport Cell, 2018 Antwerp, Belgium

⁴ Federal Environmental Inspection (Biocides and Pesticides, Dangerous Products), Federal Public Services Health, Safety of the Food Chain and Environment, Victor Hortaplein 40/10, 1060 Brussel, Belgium

* Correspondence: mouchtourib@med.uth.gr; Tel.: +30-2410565009

[†] Presented at the Public Health Congress on Maritime Transport and Ports 2022: sailing to the post-COVID-19 era, Athens, Greece, 21–22 October 2022.

Abstract: The association of cases of Legionnaires' disease and ships has been well documented. Results from potable water samples collected for microbiological analysis during SSC inspections conducted from 2010 to 2018 at Belgian ports were analyzed in order to evaluate the level of colonization on non-passenger ships. Results indicate a high degree of colonization (77.2% of the ships were found to be colonized with *Legionella* spp. at least once) and further analysis is recommended to examine possible factors associated with colonization. Inspections for issuance of SSC should focus on water safety and prevention of Legionnaires' disease.

Keywords: *Legionella*; potable water; ships; SSC inspections



Citation: Kourentis, L.; Anagnostopoulos, L.; Tsinaris, Z.; Galanopoulos, A.P.; Van Reusel, D.; Van den Bogaert, R.; Helewaut, B.; Steenhout, I.; Helewaut, H.; Damman, D.; et al. *Legionella* spp. Colonization on Non-Passenger Ships Calling at Belgian Ports. *Med. Sci. Forum* **2022**, *13*, 15. <https://doi.org/10.3390/msf2022013015>

Academic Editor: Volker Harth

Published: 1 December 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

From 1977 to 2001 about 200 cases of Legionnaires' disease (LD) were associated with ships [1,2]. Since then, many other cases have been reported [3–14]. The majority of these events occurred on passenger ships (cruise ships, ferries and river cruise ships) and only a small number occurred on non-passenger ships. These included oil drilling platforms, training ships, naval ships, fishing vessels and cargo ships [1,9,12,14,15].

A systematic review of the literature conducted in 2015 showed that eight ships (one river ship and seven cruise ships) were linked to nine events of Legionnaires' disease and involved a total of 83 cases [11]. In almost all of the outbreaks, *Legionella* positive samples were associated with high colony forming units per liter (>1000 CFU/L).

There are a number of studies investigating the prevalence of *Legionella* spp. on passenger ships and ferries [16–18]. To our knowledge only two studies investigated *Legionella* spp. colonization on non-passenger ships. The first one investigated the occurrence of *Legionella* in Norwegian naval vessels [9] and the second study examined the microbiological quality of non-passenger merchant vessels [19].

In this study, the results from samples collected during the inspection of non-passenger ships from 2010 to 2018 by the port health authorities in Belgium, are used in order to assess the level of *Legionella* spp. colonization on this type of ships.

2. Materials and Methods

The results of the microbiological analysis along with other information including name of the ship, sample location, temperature and pH, were provided by the Belgian port

health authority. Information related to the ships, including ship type, year build, flag, were acquired from an online database [20]. Classification of the ship types followed the categories used in the EU Common Ship Sanitation Database [21].

3. Results

From 2010 to 2018 the port health authorities collected 777 potable water samples for *Legionella* analysis during 536 SSC inspections performed on 401 different ships calling at Belgian ports. Most of the samples for *Legionella* were collected from 2015 to 2018 (81.5%).

From the 401 ships, 132 (32.9%) were general cargo ships, 91 (22.7%) were chemical tankers and 65 (16.2%) were container ships. Half of the ships (50%) were registered in a port that belonged to a country that was declared as Flag of Convenience (FoC) by the International Transport Workers' Federation [22]. A large proportion of the ships (38%) were registered in EU countries and the rest in non-EU countries that are not considered as FoC. Half of the ships (50.4%) were one to 10 years old when the samples were collected, 32.9% of the ships were 11 to 20 years old, 13.5% of the ships were 21–30 years old and 3.2% of the ships were more than 30 years old.

The potable water samples were collected from the hospital (29.1%), the galley (23.8%), the cabins (16.3%), the bridge (14.3%), and other places on the ship. A total of 162 (20.8%) samples were collected from the cold water line, 187 (24.1%) were collected from the hot water line and for 428 (55.1%) of the samples this information could not be determined.

The pH ranged from 4.93 to 10.56 (median = 7.9, SD = 0.97). The cold water line temperatures ranged from eight to 39 °C with an average of 25.1 °C (SD = 5.3 °C, $n = 187$), while the hot water line temperatures ranged from 26 to 75 °C with an average of 48.4 °C (SD = 9.5 °C, $n = 162$). The presence of heterotrophic plate count (HPC) bacteria at 22 °C ranged from 0 to 3001 CFU/100 mL with a median of 34 CFU/100 mL (SD = 1031.3 CFU/100 mL), while the HPC at 37 °C ranged from 0 to 3001 CFU/100 mL with a median of 125 CFU/100 mL (SD = 1061.7 CFU/100 mL).

From the total number of samples, 396 (51.0%) were positive (≥ 1 CFU/L) for *Legionella* spp. and 381 (49.0%) were negative. Figure 1 shows the number of samples per *Legionella* spp. count levels and per serogroup.

From the 536 different samplings conducted during inspections, 302 (56.3%) had at least one positive sample for *Legionella* spp. From the 302 ships, 233 (77.2%) were found to be colonised at least once, with at least one sample positive. Samples from 133 ships had low levels of *Legionella* spp. (<1000 CFU/L), 107 had ≥ 1000 CFU/L and $<10,000$ CFU/L, and 55 were found with $\geq 10,000$ CFU/L.

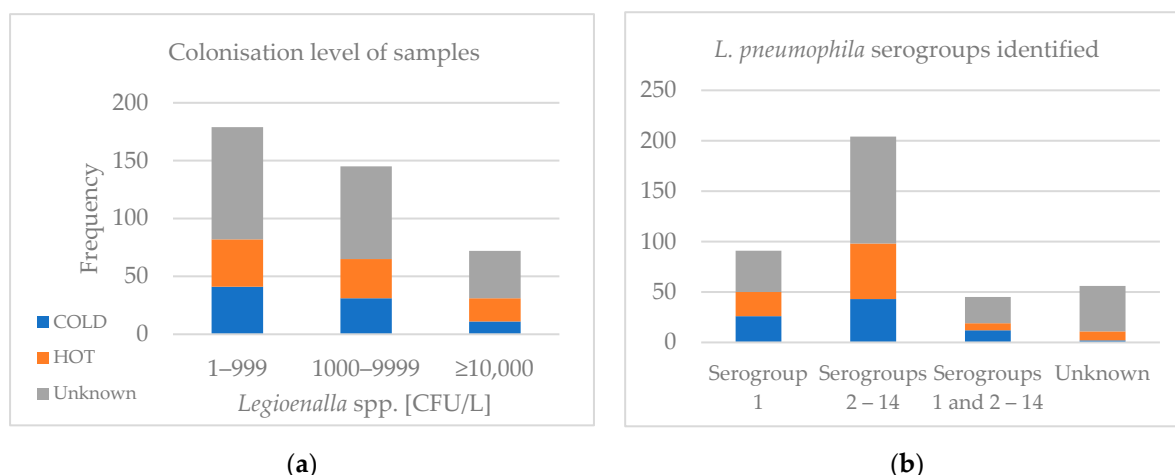


Figure 1. (a) Frequency of samples found positive as per the level of *Legionella* spp. colonization; (b) Frequency of samples found positive as per the *L. pneumophila* serogroup.

4. Discussion and Conclusions

Information on whether the samples were collected directly or after flushing was not available and thus it is not possible to determine whether the colonization for these ships was local or systemic. Additionally, for most of the samples it was unclear whether the sample was collected from the hot water line or the cold water line. The median pH was close to the upper limit for effective chlorination ($\text{pH} < 8$) [1]. Information regarding the disinfection method used for the potable system or measurements of the chlorine levels was not available.

Almost half of the samples collected were positive for *Legionella* spp. and more than 2/3 of the ships were found to test positive at least once. The level of colonization on board the ship found here is slightly higher than the level of colonization presented in a study that examined the microbiological quality of non-passenger merchant vessels [19]. In that study, 803 samples collected by port health officers from 2013 to 2016 during routine inspections from 360 ships were cultured for *Legionella* spp. and 48.6% of the samples (corresponding to 53.8% of the vessels) were found positive. The level of colonization was reported to be even less in a study investigating the occurrence of *Legionella* in Norwegian naval vessels, where seven out of 41 vessels were found to be positive [9]. A high level of colonization has also been reported on ferries, while cruise ships were not found to be colonized [16–18]. It can be safely assumed that in most cases the ships to be sampled were selected arbitrarily and not after finding major deficiencies.

In this study, the level of *Legionella* spp. colonization of non-passenger ships was assessed by analyzing results of samples collected by the port health authorities of Belgian ports retrospectively, and a high level of colonization was observed. Additional analysis of the data may reveal association of colonization with specific characteristic of the ship. Finally, analysis of the data recorded in the EU Common Ship Sanitation Database may provide further insight into the relationship between the inspection findings and the level of colonization.

Author Contributions: Conceptualization, V.A.M. and C.H.; methodology, L.K. and V.A.M.; formal analysis, Z.T. and A.P.G.; investigation, D.V.R., R.V.d.B., B.H., I.S., H.H. and D.D.; writing—original draft preparation, L.K.; writing—review and editing, L.K., L.A. and V.A.M.; supervision, C.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: 3rd Party Data.

Acknowledgments: We would like thank the port health officers conducted the samplings and staff of the laboratories who performed the laboratory analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. *Sanitation on Ships: Compendium of Outbreaks of Foodborne and Waterborne Disease and Legionnaires' Disease Associated with Ships 1970–2000*; World Health Organization: Geneva, Switzerland, 2001.
2. Rowbotham, T.J. Legionellosis associated with ships: 1977 to 1997. *Commun. Dis. Public Health* **1998**, *1*, 146–151. [[PubMed](#)]
3. Regan, C.M.; McCann, B.; Syed, Q.; Christie, P.; Joseph, C.; Colligan, J.; McGaffin, A. Outbreak of Legionnaires' disease on a cruise ship: Lessons for international surveillance and control. *Commun. Dis. Public Health* **2003**, *6*, 152–156. [[PubMed](#)]
4. Kobayashi, A.; Yamamoto, Y.; Chou, S.; Hashimoto, S. Severe *Legionella pneumophila* pneumonia associated with the public bath on a cruise ship in Japan. *J. Anesth.* **2004**, *18*, 129–131. [[CrossRef](#)] [[PubMed](#)]
5. Centers for Disease Control and Prevention (CDC). Cruise-ship—Associated Legionnaires disease, November 2003–May 2004. *MMWR Morb. Mortal Wkly. Rep.* **2005**, *54*, 1153–1155.
6. Kura, F.; Amemura-Maekawa, J.; Yagita, K.; Endo, T.; Ikeno, M.; Tsuji, H.; Taguchi, M.; Kobayashi, K.; Ishii, E.; Watanabe, H. Outbreak of Legionnaires' disease on a cruise ship linked to spa-bath filter stones contaminated with *Legionella pneumophila* serogroup 5. *Epidemiol. Infect.* **2006**, *134*, 385–391. [[CrossRef](#)] [[PubMed](#)]

7. Beyrer, K.; Lai, S.; Dreesman, J.; Lee, J.V.; Joseph, C.; Harrison, T.; Surman-Lee, S.; Lück, C.; Brodhun, B.; Buchholz, U.; et al. Legionnaires' disease outbreak associated with a cruise liner, August 2003: Epidemiological and microbiological findings. *Epidemiol. Infect.* **2007**, *135*, 802–810. [[CrossRef](#)] [[PubMed](#)]
8. Joseph, C.A.; Yadav, R.; Ricketts, K.D. Travel-associated Legionnaires disease in Europe in 2007. *Eurosurveill* **2009**, *14*, 19196. [[CrossRef](#)] [[PubMed](#)]
9. Ahlén, C.; Aas, M.; Nor, A.; Wetteland, P.I.; Johansen, H.; Sørbø, T.; Sommerfelt-Pettersen, J.K.; Iversen, O.J. *Legionella pneumophila* in Norwegian naval vessels. *Tidsskr. Nor Laegeforen.* **2013**, *133*, 1445–1448. [[CrossRef](#)] [[PubMed](#)]
10. de Jong, B.; Payne Hallström, L.; Robesyn, E.; Ursut, D.; Zucs, P. Travel-associated Legionnaires' disease in Europe, 2010. *Eurosurveill* **2013**, *18*, 20498. [[CrossRef](#)] [[PubMed](#)]
11. Mouchtouri, V.A.; Rudge, J.W. Legionnaires' Disease in Hotels and Passenger Ships: A Systematic Review of Evidence, Sources, and Contributing Factors. *J. Travel Med.* **2015**, *22*, 325–337. [[CrossRef](#)] [[PubMed](#)]
12. Inglis, T.J.J.; Spittle, C.; Carmichael, H.; Downes, J.; Chiari, M.; McQueen-Mason, A.; Merritt, A.J.; Hodge, M.; Murray, R.J.; Dowse, G.K. Legionnaires' Disease Outbreak on a Merchant Vessel, Indian Ocean, Australia, 2015. *Emerg. Infect. Dis.* **2018**, *24*, 1345–1348. [[CrossRef](#)] [[PubMed](#)]
13. Arashiro, T.; Nakamura, S.; Asami, T.; Mikuni, H.; Fujiwara, E.; Sakamoto, S.; Miura, R.; Shionoya, Y.; Honda, R.; Furukawa, K.; et al. SARS-CoV-2 and *Legionella* co-infection in a person returning from a Nile cruise. *J. Travel Med.* **2020**, *27*, taaa053. [[CrossRef](#)]
14. Ahlen, C.; Aas, M.; Krusnell, J.; Iversen, O.J. A single *Legionella pneumophila* genotype in the freshwater system in a ship experiencing three separate outbreaks of legionellosis in 6 years. *Microb. Ecol. Health Dis.* **2016**, *27*, 31148. [[CrossRef](#)] [[PubMed](#)]
15. Caylà, J.A.; Maldonado, R.; González, J.; Pellicer, T.; Ferrer, D.; Pelaz, C.; Gracia, J.; Baladrón, B.; Plasencia, A. A small outbreak of Legionnaires' disease in a cargo ship under repair. *Eur. Respir. J.* **2001**, *17*, 1322–1327. [[CrossRef](#)] [[PubMed](#)]
16. Goutziana, G.; Mouchtouri, V.A.; Karanika, M.; Kavagias, A.; Stathakis, N.E.; Gourgoulidis, K.; Kremastinou, J.; Hadjichristodoulou, C. *Legionella* species colonization of water distribution systems, pools and air conditioning systems in cruise ships and ferries. *BMC Public Health* **2008**, *8*, 390. [[CrossRef](#)] [[PubMed](#)]
17. Azara, A.; Piana, A.; Sotgiu, G.; Dettori, M.; Deriu, M.G.; Masia, M.D.; Are, B.M.; Muresu, E. Prevalence study of *Legionella* spp. contamination in ferries and cruise ships. *BMC Public Health* **2006**, *6*, 100. [[CrossRef](#)]
18. Laganà, P.; Gambuzza, M.E.; Delia, S. *Legionella* risk assessment in cruise ships and ferries. *Ann. Agric. Environ. Med.* **2017**, *24*, 276–282. [[CrossRef](#)] [[PubMed](#)]
19. Collins, S.L.; Stevenson, D.; Mentasti, M.; Shaw, A.; Johnson, A.; Crossley, L.; Willis, C. High prevalence of *Legionella* in non-passenger merchant vessels. *Epidemiol. Infect.* **2017**, *145*, 647–655. [[CrossRef](#)] [[PubMed](#)]
20. MarineTraffic. Available online: www.marinetraffic.com (accessed on 26 August 2022).
21. Mouchtouri, V.A.; Van Reusel, D.; Bitsolas, N.; Katsioulis, A.; Van den Bogaert, R.; Helewaut, B.; Steenhout, I.; Damman, D.; Dávila Cornejo, M.; Hadjichristodoulou, C. European Web-Based Platform for Recording International Health Regulations Ship Sanitation Certificates: Results and Perspectives. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1833. [[CrossRef](#)] [[PubMed](#)]
22. International Transport Workers' Federation. Flags of Convenience. Available online: <https://www.itfglobal.org/en/sector/seafarers/flags-of-convenience> (accessed on 26 August 2022).