

Prevalence of *Legionella* spp. and *Escherichia coli* in the drinking water distribution system of Wrocław (Poland)

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ABSTRACT

Drinking water should be free from bacterial pathogens that threaten human health. The most recognised waterborne opportunistic pathogens, dwelling in tap water, are *Legionella pneumophila* and *Escherichia coli*. Drinking water samples were tested for the presence of *Legionella* spp., *L. pneumophila*, and *E. coli* in overall sample microbiomes using a quantitative real-time polymerase chain reaction (qPCR) approach. The results indicate a rather low contribution of *Legionella* spp. in total bacteria in the tested samples, but *L. pneumophila* was not detected in any sample. *E. coli* was detected in only one sample, but at a very low level. The *qacEΔ1* gene, conferring resistance to quaternary ammonium compounds, was also not detected in any sample. The results point to generally sufficient quality of drinking water, although the presence of *Legionella* spp. in tap water samples suggests proliferation of these bacteria in heating units, causing a potential threat to consumer health.

Key words | bacterial pathogens, qPCR, tap water

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INTRODUCTION

In many countries, tap water is one of the main sources of drinking water, and therefore, a high quality in terms of physical-chemical and microbiological conditions is necessary. The latter seems to be of even more importance for human health risk assessment because the presence of microbiological pathogens in drinking water may lead to numerous infections (Ashbolt 2015; Banach *et al.* 2019; Liu *et al.* 2019). Moreover, the environmental conditions in drinking water distribution systems may favour the proliferation of some bacteria (September *et al.* 2007; Falkinham *et al.* 2015). The most recognised waterborne pathogens from the kingdom of bacteria include *Legionella pneumophila* (*L. pneumophila*) and *Escherichia coli* (*E. coli*) (WHO 2017). *Legionella* spp. is mostly found in hot tap water, which provides these bacteria with the most appropriate conditions for growth and proliferation (WHO 2007). Nevertheless, some representatives of the genus may also be found in non-heated tap water, distributed to consumers' taps (Pereira *et al.* 2017).

Legionella may cause legionellosis, Pontiac fever and also Legionnaires' disease. Pontiac fever is a non-lethal, self-limited flu-like illness. It was first recognised in the Country Health Department building in Pontiac (Michigan, USA). Legionnaires' disease, which may be fatal, was named after the American Legion convention at a Philadelphia hotel (Principe *et al.* 2017). The most common pathogenic *Legionella* species is *L. pneumophila*, although 27 other *Legionella* species are also recognised as human pathogens (Principe *et al.* 2017). The presence of representatives of the entire genus should therefore be monitored in drinking water samples. In Poland, 39 and 75 cases of legionellosis were reported in 2017 and 2018, respectively. The majority of them were Legionnaires' disease (38 and 70, respectively) (Czarkowski *et al.* 2019). However, there is a suspicion that Polish data regarding legionellosis are underestimated (Stypulkowska-Misiurewicz & Czerwiński 2016), and more attention should therefore be given to this issue.

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E. coli is a natural commensal to humans and inhabits the human and animal intestine. Nonetheless, some strains may become invasive and cause many illnesses, for example moderate to severe diarrhoea (Vásquez-García *et al.* 2019). *E. coli* is also the most important indicator of potential faecal contamination (Paruch & Mæhlum 2012). The presence of *Legionella* spp. and *E. coli* cells in drinking water samples should therefore not be neglected.

The presence of bacteria in disinfected tap water was confirmed in many studies. The bacterial microbiome characteristic for tap water consists mainly of non-pathogenic species (Rudi *et al.* 2010; El-Chakhtoura *et al.* 2015; Douterelo *et al.* 2016). Nevertheless, insufficient source-water treatment and accidental contaminations within the distribution system may lead to the introduction of virulent genera. The most common ones, namely *Escherichia* and *Legionella*, are monitored continuously by local drinking water suppliers and sanitary-epidemiological stations in terms of cultivation of these bacteria on selective agar media. In recent years, *E. coli* was not reported in drinking water samples in Wrocław. Nevertheless, the use of the quantitative real-time polymerase chain reaction (qPCR) method in the detection and quantification of waterborne bacterial pathogens may contribute to the elucidation of the issue. It is worth mentioning, however, that the implementation of the qPCR method does not differentiate between live and dead organisms, unless a special procedure (for example, with propidium monoazide, PMA) is applied (Álvarez *et al.* 2013; Toplitsch *et al.* 2018).

Bacteria dwelling in tap water distribution systems are generally known to be resistant to disinfectants (Khan *et al.* 2016). This feature allows them to overcome the presence of the disinfectant agent remaining in water samples. Despite water disinfection in water treatment plants and the presence of residual disinfectant in a network, some bacteria may enter the distribution system during pipe renovations or replacements. Therefore, even proper water treatment does not provide sufficient microbial quality within the entire network. In addition, genes responsible for the phenomenon of efflux pumps or other resistance mechanisms, such as the *qacEΔ1* gene (conferring resistance to quaternary ammonium compounds) (Szekeeres *et al.* 2018), may indicate the bacterial ability to overcome the disinfectant residual in the tap water distribution system. The *qacEΔ1* gene is known to be the part of class 1 integrons, also known as 'resistance

integrons' (Stalder *et al.* 2014). In this study, this gene was used as an indicator of resistance of bacteria.

If resistant, pathogens belonging to genera *Legionella* and *Escherichia* may cause a serious threat to human health because they might not be removed in the water treatment plant and can enter the distribution system. Bacterial cells could also participate in horizontal gene transfer where they are able to transform some genes (Gyles & Boerlin 2014). The detection of selected genes, which may enhance bacterial resistance to disinfectants in tap water samples, is therefore also important in the estimation of consumer health risk.

This study is aimed at the detection and determination of total quantity of (viable and non-viable) *Legionella* spp., *L. pneumophila* and *E. coli* bacterial cells, and their contribution to overall bacterial load quantified in microbiomes that are cultivated from consumer tap water samples using qPCR. In this study, the assumption was made that if *Legionella* or *E. coli* bacteria are able to proliferate in broth in the presence of other bacteria, they could pose a serious threat to consumers' health.

As mentioned above, some bacteria, including pathogens, are able to overcome the presence of disinfectants because they possess specific genes conferring increased resistance. Therefore, simultaneous monitoring of such genes in tap water microflora may be important. In this study, the presence of the *qacEΔ1* gene in cultivated microflora, as the representative gene conferring resistance to disinfectants, was determined.

Consumer tap water samples were collected from the drinking water distribution system in Wrocław, Poland. This research contributes to estimating the consumer health risk associated with tap water because, in Poland, tap water is generally considered to be drinking water for consumption directly from a tap.

MATERIALS AND METHODS

Consumer drinking water samples were collected from 15 different points of the water supply system in Wrocław (Figure 1). All samples were taken at private homes. The number of measuring points depended on the size of the district and the availability of the collection point. The

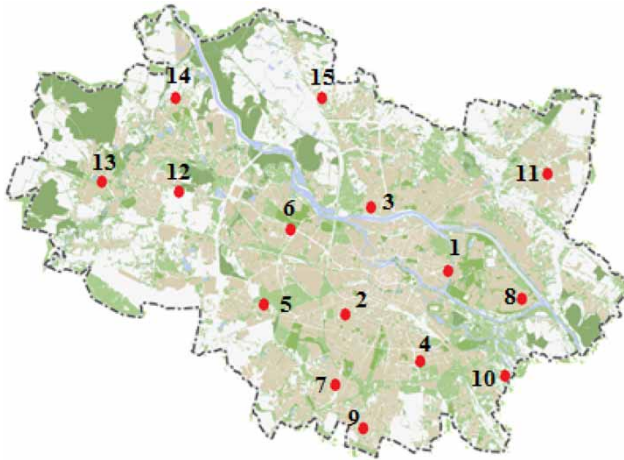


Figure 1 | Map of Wrocław with location of collection points.

sampling points were chosen to cover the entire supply network evenly. Each sample was taken once. The sampling period was the spring and the summer of 2017. Before sampling, the taps were disinfected with 96% ethanol (Sigma), and water was flushed for 5 minutes. Each sample of 3 litres was filtrated on Whatman membranes (mixed-cellulose esters, diameter 0.2 µm). The membranes were submerged in sterile broth and incubated for 48 h at room temperature. This step was performed to cultivate the microflora dwelling in each tap water sample. Afterwards, the suspension was used for DNA extraction.

Genomic DNA was extracted using an Isolate II Genomic Kit (BIOLINE). The concentration of DNA was measured on a NanoPhotometer N60 (Implen).

The qPCRs (MIC, Bio Molecular Systems) were carried out to quantify *Legionella* spp., *L. pneumophila* and *E. coli* genomes, as well as 16S rRNA and *qacEΔ1* genes.

The reactions for the quantification of *Legionella* spp., *L. pneumophila* and *E. coli* were performed with an AmpliTest (Amplicon) in accordance with the manufacturer's instructions. These quantification kits apply TaqMan probes, and are specific to target bacterial genome sequences. The AmpliTest also provides internal control, allowing for the detection of potential inhibition of reactions. The 16S rRNA and *qacEΔ1* genes were quantified with a QUANTUM EvaGreen HRM Kit (SYNGEN) with the application of a non-specific dye. All the reactions for pathogenic genomes quantification were performed in technical triplicates, while 16S rRNA and *qacEΔ1* genes were quantified in technical duplicates. 16S rRNA gene was quantified to determine the quantity of overall bacteria in the sample of cultivated microflora. The quantities of other genes were then referred to the quantity of 16S rRNA genes to make the comparison of samples possible. In this way, the relative abundance of *Legionella* and *E. coli* could be determined.

The conditions for qPCRs are presented in Table 1. In each reaction, negative control was applied and the

Table 1 | Conditions for qPCRs

	16S rRNA	AmpliTests	qacEΔ1
Initial denaturation	95 °C, 15 min	95 °C, 5 min	95 °C, 15 min
Denaturation	95 °C, 15 sec, 40 cycles	95 °C, 30 sec, 45 cycles	95 °C, 15 sec, 40 cycles
Annealing	60 °C, 20 sec, 40 cycles	58 °C, 25 sec, 45 cycles	63 °C, 20 sec, 40 cycles
Elongation	72 °C, 20 sec, 40 cycles	–	72 °C, 20 sec, 40 cycles
Cooling	–	40 °C, 30 sec	–
Melt	72–95 °C	–	72–95 °C
Chemistry	EvaGreen	TaqMan probes with internal control	EvaGreen
Primers	As described in Huerta <i>et al.</i> (2013)	Supplied by manufacturer in kits	As described in Szekeres <i>et al.</i> (2018)
DNA per reaction (µL)	1	8	1
Each primer volume per reaction (µL)	0.5	Primers in commercial mastermix	1
Total volume per reaction (µL)	20	20	20

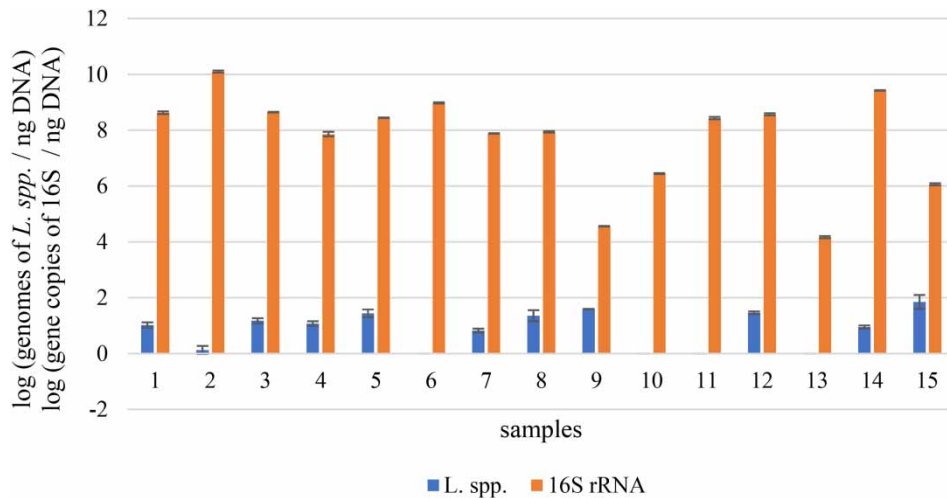


Figure 2 | Abundances of *Legionella* spp. genomes and 16S rRNA gene copy number in tested samples (in a logarithmic scale).

calculations of genomes or gene copy number were based on standard curves. For *Legionella* spp, *L. pneumophila* and *E. coli* genomes, standard curves were prepared as described in the previous study (Wolf-Baca & Siedlecka 2019), resulting in calculated genomes per reaction. For the purpose of 16S rRNA standard curve preparation, the genomic DNA ten-fold dilution of *E. coli* ATCC 25922 (BioMaxima) strain was used. In the case of *qacEA1* gene, the ten-fold dilution of purified PCR product was used.

RESULTS

All qPCRs efficiencies were in a range of 0.88–1.09. The limits of quantification were 178 genome copies/reaction for *Legionella* spp. and *L. pneumophila*, and 1.59 genome copies/reaction for *E. coli*. In the case of the 16S rRNA and *qacEA1* gene, the limits of quantification were 16.9 and 184 gene copy number/reaction, respectively (Figure 2).

The abundance and prevalence of *Legionella* spp. were determined; however, no *L. pneumophila* target sequence was found in any tested samples. In the case of *E. coli*,

only one sample (number 13) gave a positive signal with calculated genome copy number of 0.13 genomes/ng DNA. Moreover, no *qacEA1* gene could be detected in any sample.

The results of calculations of genome copy number of *Legionella* spp. per ng of DNA in tested samples are presented in Table 2. In general, *Legionella* spp. were detected and quantified in 11/15 (73.3%) samples.

Legionella spp. was the most abundant in sample number 15, where it reached 71 copies of genome per ng of DNA. Further analysis of the data indicates that *Legionella* spp. prevalence was relevant only in samples 9 and 15, reaching 1.1×10^{-3} and 6.15×10^{-5} genomes of *Legionella* spp. per 16S gene copy number, respectively.

DISCUSSION

The objective of this study was to estimate the potential consumer health risk associated with the presence of pathogenic bacteria in non-boiled tap water samples using the qPCR approach. The usefulness of this method in *Legionella* quantification was proven a decade ago

Table 2 | Genome copy numbers of *Legionella* spp. in tested samples

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Genome copy number of <i>Legionella</i> spp. per ng of DNA in tested samples. The values are rounded to the nearest whole number	11	1	15	12	28	0	7	23	39	0	0	29	0	9	71

(Dusserre *et al.* 2008; Douterelo *et al.* 2014), but it is worth mentioning that promising methods for *L. pneumophila* detection other than qPCR include an immunodetection system described by Párraga-Niño *et al.* (2018) or Legio-Int/Quanti-Tray MPN described by Spies *et al.* (2018).

Among the tested pathogens, *L. pneumophila* was not found in any of the samples. This proves the good quality of Wrocław drinking water and treatment processes, and suggests a lowered risk for consumer health. Nevertheless, *L. pneumophila* is not the only factor causing legionellosis (Principe *et al.* 2017), and therefore such risk could not be fully excluded.

E. coli is also a risk to consumer health and is, one of the most important faecal contamination indicators (Coleman *et al.* 2011; Subba *et al.* 2013; Talukdar *et al.* 2013). It was detected in only one sample with no evidence of its viability. Moreover, even assuming that *E. coli* did not proliferate in broth, i.e. its quantity did not change during incubation, the calculated genome copy number/mL would be 9.10×10^{-3} (data not shown), which is far below the acceptable values specified in Polish law (Regulation 2017). The detection of *E. coli* in this sample may be the effect of the presence of residual DNA fragments with *E. coli*-specific sequence. As no live/dead treatment was performed before DNA extraction (Álvarez *et al.* 2013; Toplitsch *et al.* 2018), it is not excluded that DNA fragments detected in sample number 13 originated from dead *E. coli* cells. Nevertheless, these residual DNA fragments may suggest contamination, which has happened in the past.

The results indicate that *Legionella* spp. could still be present in microflora cultivated from tap water samples, even if no *Legionella*-enhancing supplement was added to the broth. However, based on this study, it could not be established whether the quantified cells were dead or alive.

The prevalence of *Legionella* spp. genomes in microflora cultivated from tap water samples tested in this study was generally low. It is worth mentioning that viable but non-culturable *Legionella* cells may be predominant in tap water samples (Dietersdorfer *et al.* 2018). This may explain the low level of *Legionella* spp. prevalence observed in this study. Other bacteria present in a given sample might proliferate, while *Legionella* spp. remain at the same level during a short incubation time. Contrary results indicate very high contamination of *Legionella* in Australian tap

water, mostly in samples collected in the summer season (Whiley *et al.* 2014). Because the sampling campaign performed in this study was undertaken only once at the turn of spring and summer, the seasonal variation of contamination by pathogens could not be excluded.

Despite its low levels, *Legionella* spp. was frequently detected and quantified in this study. Similar results were obtained by Liu *et al.* (2019), who found *Legionella* spp. in 100% samples, while the presence of *L. pneumophila* was confirmed only in 9.1% of all samples.

Waak *et al.* (2018) tested the presence of *Legionella* spp. in water-main biofilms (scraped from pipes) and tap water samples from two distribution systems: a chloramine-containing system in the United States and a system in Norway that does not maintain a residual disinfectant. They were able to detect *Legionella* spp. in 43% of biofilm samples from the Norwegian system with no residual disinfectant, with a maximum concentration of 7.8×10^4 gene copies cm^{-2} . *Legionella* spp. was not detected in biofilm samples from the US chloraminated system. In the case of tap water samples, *Legionella* spp. was detected in 26% and 64% of tested samples from the US and Norwegian system, respectively, with a maximum concentration of $2.0 \times 10^3/\text{L}$ for chloraminated system, and $2.8 \times 10^3/\text{L}$ for the system with no residual disinfectant. The results obtained in this study are higher than those presented by Waak *et al.* (2018) as 73.3% of tested tap water samples were *Legionella* spp. positive. Waak *et al.* also detected a *L. pneumophila* gene marker in 3% and 9% of tested biofilm samples from the US and Norwegian system, respectively, while tap water samples from both sampling regions were free from *L. pneumophila*, which is in accordance with results presented in this paper. *L. pneumophila* serogroup 1 gene marker was not found in any of the tested samples of water-main biofilms or tap water in the US and Norwegian systems (Waak *et al.* 2018).

Legionella is often tested only in hot tap water installations (Wolf-Baca & Siedlecka 2019); indeed, Polish law demands *Legionella* monitoring only in hot water samples (Regulation 2017). However, its proliferation in heating units is initiated by the presence of these bacteria in the water distribution system. There is therefore a risk that in point-of-use faucets with considerable *Legionella* spp. prevalence, much more *Legionella* cells may be found in hot water samples. Intake of aerosols with *Legionella*

during the use of hot tap water (for example, for baths and other domestic purposes) is known to pose a potential risk of legionellosis (Wong *et al.* 2006). Detection of the *Legionella* spp. sequence targets in drinking water samples should therefore not be neglected. Replacement of chlorine with monochloramine was suggested as a method for improved removal of *Legionella* from hot tap water (Flannery *et al.* 2006; Moore *et al.* 2006). The importance of the presence of residual disinfectant in the drinking water distribution system was confirmed in the study by Waak *et al.* (2018). The US chloraminated drinking water distribution system was shown to be less contaminated with *Legionella* and overall bacteria (in the case of water-main biofilm samples from pipes and from tap water samples) than the Norwegian system with no residual disinfectant (Waak *et al.* 2018).

As mentioned in the Introduction, the *qacEΔ1* gene was used as a representative gene of class 1 integrons, conferring resistance of bacteria (Stalder *et al.* 2014; Szekeres *et al.* 2018). Its lack in cultivated microflora may therefore lead to the conclusion that the overall resistance in bacteria collected from tap water samples is low. Constant chlorination of the Wrocław drinking water distribution system and the assurance of appropriate levels of residual disinfectant along the entire pipeline network should therefore be sufficient in prevention the proliferation of bacterial pathogens in consumers' tap water. However, the disinfection processes may also lead to the formation of disinfection by-products, which could be considered as another threat to consumer health (Pogorzelec & Piekarska 2018). Moreover, it is not excluded that bacteria susceptible to quaternary ammonium compounds can be resistant to chlorine compounds. The resistance of bacteria dwelling in tap water to chlorine and its compounds should therefore be tested more in-depth in future studies.

Future studies should also investigate the culture methods for enumeration of pathogenic bacteria, including, for example, the heterotrophic plate count method on selective agar media for *Legionella* and *Escherichia*.

CONCLUSIONS

This study investigates the presence of *Legionella* and *Escherichia coli* in the public water supply system of

Wrocław, based on the results of qPCR analyses of 15 samples collected from the entire network. The prevalence of *Legionella* spp. cells in microbiome of tap water samples collected from 15 sampling points of drinking water distribution system in Wrocław was generally low. Only in two points samples (9 and 15) was the quantity of *Legionella* spp. in overall bacteria load increased. Neither *L. pneumophila* nor *E. coli* (except sampling point number 13) were detected. These data suggest the sufficient quality of consumer tap water in Wrocław. The *qacEΔ1* gene, conferring resistance to quaternary ammonium compounds in class 1 integrons, was not detected in the samples, suggesting low resistance of cultivated bacteria. Nevertheless, the presence of *Legionella* spp. in drinking water distribution systems may lead to their proliferation in heating installations, posing a health risk to tap water consumers.

REFERENCES

- Álvarez, G., González, M., Isabal, S., Blanc, V. & León, R. 2013 Method to quantify live and dead cells in multi-species oral biofilm by real-time PCR with propidium monoazide. *AMB Express* **3**, 1–8.
- Ashbolt, N. J. 2015 Microbial contamination of drinking water and human health from community water systems. *Current Environmental Health Reports* **2**, 95–106.
- Banach, A., Ciesielski, S., Bacza, T., Pieczykolan, M. & Ziembinska-Buczyńska, A. 2019 Microbial community composition and methanogens' biodiversity during a temperature shift in a methane fermentation chamber. *Environmental Technology* **40** (24), 3252–3263.
- Coleman, B. L., Salvadori, M. I., McGeer, A. J., Sibley, K. A., Numann, N. F., Bondy, S. J., Gutmanis, I. A., McEwen, S. A., LaVoie, M., Strong, D., Johnson, I., Jamieson, F. B. & Louie, M. 2012 The role of drinking water in the transmission of antimicrobial-resistant *E. coli*. *Epidemiology and Infection* **140**, 633–642.
- Czarkowski, M. P., Cielebąk, E., Kondej, B. & Sadlocha, A. 2019 *Infectious Diseases and Poisonings in Poland in 2018*. Report of National Institute of Public Health – National Institute of Hygiene Department of Epidemiology and Surveillance of Infectious Diseases. National Institute of Public Health, Warsaw, Poland.
- Dietersdorfer, E., Kirschner, A., Schrammel, B., Ohradanova-Repic, A., Stockinger, H., Sommer, R., Walochnik, J. & Cervero-Aragó, S. 2018 Starved viable but non-culturable (VBNC) *Legionella* strains can infect and replicate in amoebae and human macrophages. *Water Research* **141**, 428–438.
- Douterelo, I., Boxall, J. B., Deines, P., Sekar, R., Fish, K. E. & Biggs, C. A. 2014 Methodological approaches for studying the

- microbial ecology of drinking water distribution systems. *Water Research* **65**, 134–156.
- Douterelo, I., Jackson, M., Solomon, C. & Boxall, J. 2016 Microbial analysis of in situ biofilm formation in drinking water distribution systems: implications for monitoring and control of drinking water quality. *Applied Microbiology and Biotechnology* **100**, 3301–3311.
- Dusserre, E., Ginevra, C., Hallier-Soulier, S., Vandenesch, F., Festoc, G., Etienne, J., Jarraud, S. & Molmeret, M. 2008 A PCR-based method for monitoring *Legionella pneumophila* in water samples detects viable but noncultivable legionellae that can recover their cultivability. *Applied and Environmental Microbiology* **74**, 4817–4824.
- El-Chakhtoura, J., Prest, E., Saikaly, P., van Loosdrecht, M., Hammes, F. & Vrouwenvelder, H. 2015 Dynamics of bacterial communities before and after distribution in a full-scale drinking water network. *Water Research* **74**, 180–190.
- Falkinham, J., Pruden, A. & Edwards, M. 2015 Opportunistic premise plumbing pathogens: increasingly important pathogens in drinking water. *Pathogens* **4**, 373–386.
- Flannery, B., Gelling, L. B., Vugia, D. J., Weintraub, J. M., Salerno, J. J., Conroy, M. J., Stevens, V. A., Rose, C. E., Moore, M. R., Fields, B. S. & Besser, R. E. 2006 Reducing *Legionella* colonization of water systems with monochloramine. *Emerging Infectious Diseases* **12**, 588–596.
- Gyles, C. & Boerlin, P. 2014 Horizontally transferred genetic elements and their role in pathogenesis of bacterial disease. *Veterinary Pathology* **51**, 328–340.
- Huerta, B., Marti, E., Gros, M., López, P., Pompêo, M., Armengol, J., Barceló, D., Balcázar, J. L., Rodríguez-Mozaz, S. & Marcé, R. 2013 Exploring the links between antibiotic occurrence, antibiotic resistance, and bacterial communities in water supply reservoirs. *Science of the Total Environment* **456–457**, 161–170.
- Khan, S., Beattie, T. K. & Knapp, C. W. 2016 Relationship between antibiotic- and disinfectant-resistance profiles in bacteria harvested from tap water. *Chemosphere* **152**, 132–141.
- Liu, L., Xing, X., Hu, C. & Wang, H. 2019 One-year survey of opportunistic premise plumbing pathogens and free-living amoebae in the tap-water of one northern city of China. *Journal of Environmental Sciences* **77**, 20–31.
- Moore, M. R., Fields, B., Lucas, C., Besser, R. E., Pryor, M. & Phelan, M. 2006 Introduction of monochloramine into a municipal water system: impact on colonization of buildings by *Legionella* spp. *Applied and Environmental Microbiology* **72**, 378–383.
- Párraga-Niño, N., Quero, S., Ventós-Alfonso, A., Uria, N., Castillo-Fernandez, O., Ezenarro, J. J., Muñoz, F. X., Garcia-Nuñez, M. & Sabrià, M. 2018 New system for the detection of *Legionella pneumophila* in water samples. *Talanta* **189**, 324–331.
- Paruch, A. M. & Mæhlum, T. 2012 Specific features of *Escherichia coli* that distinguish it from coliform and thermotolerant coliform bacteria and define it as the most accurate indicator of faecal contamination in the environment. *Ecological Indicators* **23**, 140–142.
- Pereira, R. P. A., Peplies, J., Brettar, I. & Höfle, M. G. 2017 Development of a genus-specific next generation sequencing approach for sensitive and quantitative determination of the *Legionella* microbiome in freshwater systems. *BMC Microbiology* **17**, 1–14.
- Pogorzelec, M. & Piekarska, K. 2018 Application of semipermeable membrane devices for long-term monitoring of polycyclic aromatic hydrocarbons at various stages of drinking water treatment. *Science of The Total Environment* **631–632**, 1431–1439.
- Principe, L., Tomao, P. & Visca, P. 2017 Legionellosis in the occupational setting. *Environmental Research* **152**, 485–495.
- Regulation of the Minister of Health of 7 December 2017 regarding the quality of water for human consumption.
- Rudi, K., Berg, F., Gaustad, E., Tannes, T. & Vatn, M. 2010 Ratios between *Alpha*-, *Beta*- and *Gamma*-proteobacteria in tap water determined by the ProteoQuant assay. *Letters in Applied Microbiology* **50**, 1–6.
- September, S. M., Els, F. A., Venter, S. N. & Brozel, V. S. 2007 Prevalence of bacterial pathogens in biofilms of drinking water distribution systems. *Journal of Water and Health* **5** (2), 219–227.
- Spies, K., Pleischl, S., Lange, B., Langer, B., Hübner, I., Jurzik, L., Luden, K. & Exner, M. 2018 Comparison of the Legiolert™/ Quanti-Tray® MPN test for the enumeration of *Legionella pneumophila* from potable water samples with the German regulatory requirements methods ISO 11731-2 and ISO 11731. *International Journal of Hygiene and Environmental Health* **221**, 1047–1053.
- Stalder, T., Barraud, O., Jove, T., Casellas, M., Gaschet, M., Dagot, C. & Ploy, M.-C. 2014 Quantitative and qualitative impact of hospital effluent on dissemination of the integron pool. *ISME Journal* **8** (4), 768–777.
- Stypulkowska-Misiurewicz, H. & Czerwiński, M. 2016 Legionellosis in Poland in 2014. *Epidemiological Chronicle* **70** (20), 203–207.
- Subba, P., Joshi, D. R. & Bhatta, D. R. 2013 Antibiotic resistance pattern and plasmid profiling of thermotolerant *Escherichia coli* isolates in drinking water. *Journal of Nepal Health Research Council* **11** (23), 44–48.
- Szekeres, E., Chiriac, C. M., Baricz, A., Szóke-Nagy, T., Lung, I., Soran, M. L., Rudi, K., Dragos, N. & Coman, C. 2018 Investigating antibiotics, antibiotic resistance genes, and microbial contaminants in groundwater in relation to the proximity of urban areas. *Environmental Pollution* **236**, 734–744.
- Talukdar, P. K., Rahman, M., Rahman, M., Nabi, A., Islam, Z., Hoque, M. M., Endtz, H. P. & Islam, M. A. 2013 Antimicrobial resistance, virulence factors and genetic diversity of *Escherichia coli* isolates from household water supply in Dhaka, Bangladesh. *PLoS ONE* **8**, 1–8.
- Toplitsch, D., Platzer, S., Pfeifer, B., Hautz, J., Mascher, F. & Kittinger, C. 2018 *Legionella* detection in environmental

- samples as an example for successful implementation of qPCR. *Water* **10** (8), 1012.
- Vásquez-García, A., Oliveira, A. P. S. C. d., Mejia-Ballesteros, J. E., Godoy, S. H. S. d., Barbieri, E., Sousa, R. L. M. d. & Fernandes, A. M. 2019 *Escherichia coli* detection and identification in shellfish from southeastern Brazil. *Aquaculture* **504**, 158–163.
- Waak, M. B., LaPara, T. M., Hallé, C. & Hozalski, R. M. 2018 Occurrence of *Legionella* spp. in water-main biofilms from two drinking water distribution systems. *Environmental Science & Technology* **52**, 7630–7639.
- Whiley, H., Keegan, A., Fallowfield, H. & Bentham, R. 2014 Detection of *Legionella*, *L. pneumophila* and *Mycobacterium avium* complex (MAC) along potable water distribution pipelines. *International Journal of Environmental Research and Public Health* **11**, 7393–7405.
- WHO 2007 *Legionella and the Prevention of Legionellosis*. WHO, Geneva, Switzerland.
- WHO 2017 *Guidelines for Drinking-Water Quality*. WHO, Geneva, Switzerland.
- Wolf-Baca, M. & Siedlecka, A. 2019 Detection of pathogenic bacteria in hot tap water using the qPCR method: preliminary research. *SN Applied Sciences* **1**, 840.
- Wong, S., Pabbaraju, K., Burk, V. F., Broukhanski, G. C., Fox, J., Louie, T., Mah, M. W., Bernard, K. & Tilley, P. A. G. 2006 Use of sequence-based typing for investigation of a case of nosocomial legionellosis. *Journal of Medical Microbiology* **55**, 1707–1710.

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