Science of the Total Environment 838 (2022) 156381

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

Comparison of methods for the enumeration of coliphages in 100 mL water samples

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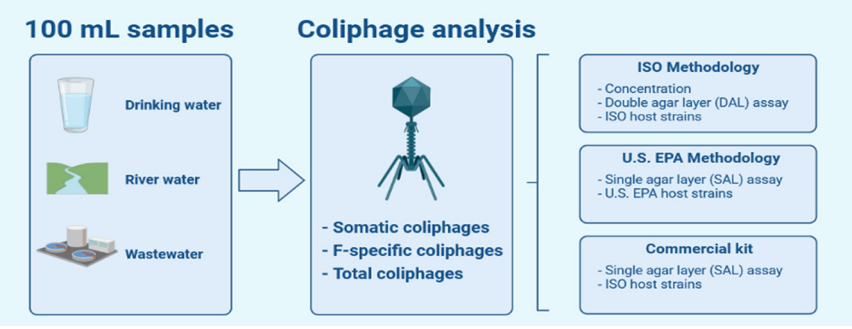
HIGHLIGHTS

- Somatic, F-specific and total coliphages were analysed in different water matrices.
- ISO and U.S. EPA methods and a commercial kit were compared for 100 mL samples.
- Environmental samples contain higher somatic than F-specific coliphage counts.
- In samples with more than 100 PFU, somatic coliphages were better detected by ISO.
- SAL assay using ISO host strains is useful for samples with less than 100 PFU.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Warish Ahmed

Keywords:
Coliphages
Single agar layer
Double agar layer
Water quality
Viral indicators

ABSTRACT

In the last decade coliphages have been included in many water quality regulations as viral faecal indicators. However, the standardised methods used to detect and quantify coliphages differ in bacterial host strains, culture media and techniques. In this comparative study, 100 mL samples of mineral drinking water, river water and wastewater were analysed with International Organization for Standardization (ISO) standard methods, with United States-Environmental Protection Agency (U.S. EPA) based methods as well as commercial kits combining a single agar layer (SAL) assay with ISO bacterial host strains. The three methods gave similar counts (p-value>0.05) for somatic and total coliphages in the matrices with less than 100 PFU/100 mL, whereas for F-specific coliphages, the U.S. EPA method provided statistically significant lower numbers (p-value<0.05) than the other two protocols, possibly because it uses a different bacterial host strain (*Escherichia coli* HS (pFamp) R vs. the ISO strain *Salmonella enterica* serovar Typhimurium WG49). In samples with more than 100 PFU/100 mL, the ISO method yielded higher counts of somatic coliphages than the other two protocols (p-value<0.05). As the three methods provided similar results in clean water, the approach combining a SAL assay with the ISO bacterial host strain could be a useful option for coliphage analysis in this type of sample, as it does not require a concentration step.

1. Introduction

Enteric bacteria have been successfully used as faecal indicator organisms (FIO) since the 19th century (Pipes et al., 1977). However, outbreaks of waterborne disease can have several etiological agents (bacteria, viruses, protozoa, etc.) and many have been associated with previously treated or bacteriologically safe water (Blanco et al., 2017; Giammanco et al., 2018; Mellou et al., 2014; Sinclair et al., 2009) used not only for drinking but

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http://dx.doi.org/10.1016/j.scitotenv.2022.156381
Received 11 March 2022; Received in revised form 27 May 2022; Accepted 27 May 2022
Available online 31 May 2022

also irrigation and bathing, among other purposes (Farrell et al., 2021). Compared to bacteria, viruses are smaller and more abundant; they also persist longer in the environment and are more resistant to treatments. For all these reasons, regulations and guidelines for water quality management should include viral indicators and establish upper limits according to water usage.

Coliphages are viruses that infect *Escherichia coli* and other closely related enteric bacteria. Their usefulness as viral indicators and model organisms to analyse and predict waterborne viral hazards is widely reported (Armon and Kott, 1996; IAWPRC Study Group on Health Related Water Microbiology, 1991; Jofre et al., 1995). Somatic coliphages (SOMCPH), which infect the host strain through the cell wall, and F-specific coliphages (FCPH), which infect through the F-pilus, are the main coliphage types associated with water quality monitoring (Jofre et al., 2016).

National and international water management directives and regulations are increasingly including the monitoring of both types as viral indicators (Blanch et al., 2020). Different characteristics distinguish these groups: while SOMCPH are typically found in higher concentrations compared to FCPH in ground-, surface and wastewaters (Jebri et al., 2015; Jofre et al., 2016), FCPH may be more resistant to some water treatments such as UV inactivation (Costán-Longares et al., 2008; Francy et al., 2012; Montemayor et al., 2008). Bacterial host strains have been developed to detect total coliphages (TCPH), namely somatic and F-specific at the same time (Guzmán et al., 2008).

Standardised methods (ISO and U.S. EPA) differ in the techniques and bacterial host strains used to detect and quantify coliphages. ISO-10705-2 (ISO, 2000a), a method for SOMCPH analysis, uses the double agar layer (DAL) plaque assay and *E. coli* C (ATCC 13706) and *E. coli* WG5 (ATCC 700078), whereas the ISO-10705-1 (ISO, 1995) method for FCPH enumeration is based on the DAL assay and *Salmonella enterica* serovar Typhimurium WG49 (NCTC 12484). Both ISO methods can be used with a maximum sample volume of 5 mL per plate and are also applicable in presence/absence tests, which may be adapted for quantitative analysis by the most probable number (MPN) method. Nevertheless, drinking and environmental water with very low loads of faecal pollution require the analysis of higher volumes. In addition, water regulations often stipulate routine analysis of microbiological parameters in 100 mL water samples, including coliphages (Ballesté et al., 2022). Some concentration procedures for coliphages have already been described (Sobsey et al., 1990), sequentially adapted (Sinton et al., 1996; Méndez et al., 2004) and validated according to ISO-10705-3 (ISO, 2003).

U.S. EPA methods 1601 and 1602 (U.S. EPA, 2001a, 2001b) consist of a presence/absence test and a Single Agar Layer (SAL) plaque assay using *E. coli* CN13 (ATCC 700609) and *E. coli* F_{amp}- *E. coli* HS (pFamp) R (ATCC 700891), from this point forward *E. coli* HS, as bacterial host strains to detect and/or quantify SOMCPH and FCPH, respectively. These procedures are for the analysis of 100 mL of groundwater. In addition, U.S. EPA method 1642 (U.S. EPA, 2018a) is used for the analysis of 100 mL of recreational and wastewater subjected to advanced treatments with a previous concentration by ultrafiltration of larger volumes (2 L) whereas U.S. EPA method 1643 is used for 100 mL of secondary wastewater (U.S. EPA, 2018b).

In the present study, the different methods for the enumeration of SOMCPH, FCPH and TCPH are compared in different water matrices. The aim was to obtain data that may be useful to improve coliphage analysis and facilitate its implementation in laboratory routines by reducing the number of procedural steps.

2. Materials and methods

2.1. Samples

A total of 50 samples were collected in May–June of 2021 from different sources as follows: 20 samples of mineral drinking water, 20 samples of freshwater (river water) and 10 samples of wastewater. Mineral drinking water bottles from different springs were purchased in a local retailer, whereas river water and wastewater samples were collected in 1 L sterile containers and transported to the laboratory at 4 °C where the analyses were performed within 24 h of collection.

Ten out of 20 mineral drinking water samples were spiked with a coliphage stock equivalent to 10 PFU of SOMCPH, FCPH and TCPH per 100 mL of sample. This stock was prepared with the secondary effluent of a wastewater treatment plant (WWTP) as a reference material according to the ISO method (ISO, 2000b).

Freshwater samples were collected in the lower course of the Llobregat river (Catalonia, Spain) and were classified in two levels of contamination: those with a lower coliphage concentration containing from 10 to 100 PFU/100 mL and a higher concentration of 100 to 1000 PFU/mL.

Wastewater samples were collected after secondary treatment at two different WWTPs located in the Barcelona Metropolitan Area (Catalonia, Spain) that treat the sewage for 2.8 and 1.7 million inhabitant-equivalents. Raw wastewater was not used in this research because of its extreme heterogeneity and unstable characteristics, which vary during a day.

In order to analyse samples with up to 10^3 PFU/100 mL, river and wastewater samples with higher concentration were autoclaved to use them as diluent for the same sample.

2.2. Enumeration of coliphages

SOMCPH and FCPH were quantified using three different methods: ISO, U.S. EPA-based method, and commercial kits that combine the SAL plaque assay of U.S. EPA with ISO methods (SAL/ISO) (Table 1). The analyses of SOMCPH and FCPH by the ISO method (ISO-SOM and ISO-F) were performed using the DAL plaque assay (ISO, 2000a, 1995) after concentration of the 100 mL water samples following a previously described method (Méndez et al., 2004), as recommended (ISO, 2003). The analysis of SOMCPH and FCPH by the U.S. EPA-based method (EPA-SOM and EPA-F) was performed using the BP1609 and BP1619 Kits (Bluephage S.L., Spain), respectively, based on these standardised methods which include a SAL assay and *E. coli* CN13 and *E. coli* HS as the respective bacterial host strains (U.S. EPA, 2001b) but slightly modified. The modification consists in the replacement of 10 mL of sterile water by 10 mL of additional bacterial working culture. For the SAL/ISO method, we used the commercial BP1604 and BP1614 Kits (Bluephage S.L., Spain) for SOMCPH and FCPH, respectively (SAL/ISO-SOM and SAL/ISO-F). These combine the U.S. EPA SAL assay with ISO media and bacterial host strains: Modified Scholten's Agar (MSA) and *E. coli* WG5 for SOMCPH analysis, and Tryptone-Yeast extract-Glucose Agar (TYGA) and *S. enterica* WG49 for FCPH (ISO, 2000a, 1995).

Two methods were used to quantify TCPH: ISO-T and SAL/ISO-T. In the former, the DAL plaque assay (Guzmán et al., 2008; ISO, 2000a) was applied after the concentration of 100 mL water samples (Méndez et al., 2004), as recommended (ISO, 2003). In the latter, the BP1641 Kit (Bluephage S.L., Spain) was used, which consists of the SAL plaque assay, the medium TYGA and bacterial host strain *E. coli* CB390, as previously described (Guzmán et al., 2008).

2.3. Statistical analysis

The normality distribution of the data was checked by Shapiro-Wilks test. Comparative analysis was performed by Student's *t*-test and the Wilcoxon test. Data analysis and plots were carried out using R Studio software v. 1.2.5001.

3. Results

Mineral drinking water was negative for SOMCPH, FCPH and TCPH by all the assessed methods, and coliphage numbers in spiked samples varied according to the method used (Fig. 1a). ISO-SOM and SAL/ISO-SOM results did not differ with statistical significance (p-value = 0.188), whereas EPA-SOM produced lower counts (p-value<0.05). Similarly, both ISO-T and SAL/ISO-T methods provided similar TCPH numbers, without statistically significant differences (p-value = 0.51). Regarding FCPH, higher counts

Table 1
Summary of the methods used in this study for the detection of coliphages in 100 mL samples. SAL: Single Agar Layer; DAL: Double Agar Layer.

Name	Method	Host strain	Coliphages detected	Technique	Water type
ISO-SOM	ISO 10705:3 + ISO 10705:2	*E. coli* WG5	Somatic coliphages	Concentration + DAL	All types of water expected to contain <3PFU/mL
SAL/ISO-SOM	BP1604	*E. coli* WG5	Somatic coliphages	SAL	Drinking and reclaimed water
EPA-SOM	USEPA1602/USEPA1642/USEPA1643	*E. coli* CN13	Somatic coliphages	SAL	Groundwater/treated water/recreational and wastewater after ultrafiltration
ISO-T	ISO 10705:3 + ISO 10705:2	*E. coli* CB390	Total coliphages	Concentration + DAL	All types of water expected to contain <3PFU/mL
SAL/ISO-T	BP1641	*E. coli* CB390	Total coliphages	SAL	Drinking and reclaimed water
ISO-F	ISO 10705:3 + ISO 10705:1	*S. enterica* WG49	F-specific coliphages	Concentration + DAL	All types of water expected to contain <3PFU/mL
SAL/ISO-F	BP1614	*S. enterica* WG49	F-specific coliphages	SAL	Drinking and reclaimed water
EPA-F	USEPA1602/USEPA1642/USEPA1643	*E. coli* HS	F-specific coliphages	SAL	Groundwater/treated water/recreational and wastewater after ultrafiltration

were obtained by SAL/ISO-F compared to ISO-F (p-value<0.05), whereas no statistically significant differences were observed between the results of SAL/ISO-F and EPA-F (p-value = 0.07) or ISO-F and EPA-F (p-value = 0.107). Despite the differences, the mineral drinking water samples were spiked with very low numbers (less than 10 PFU/100 mL) and the results obtained were all in the same logarithm unit.

River water samples were split in two groups (low and high faecal contamination) according to their theoretical concentration: those containing less than 100 PFU/100 mL (Fig. 1b) and those with more (Fig. 1c). In surface water with a low concentration, the results of the three assayed methods did not differ with statistical significance for SOMCPH and TCPH (p-value<0.05). However, FCPH counts by EPA-F were lower than those obtained by the ISO-F and SAL/ISO-F methods (p-values<0.05).

In surface water with a high concentration of coliphages, higher SOMCPH counts were obtained by ISO-SOM than the two methods using SAL (p-values<0.05), whose results did not differ (p-value = 0.19). This trend was not repeated for TCPH, as ISO-T and SAL/ISO-T gave similar results (p-value = 0.79). FCPH counts could not be compared as the concentrations were below 100 PFU/100 mL in all the samples.

In secondary effluent wastewater, counts differed depending on the method (Fig. 1d). SOMCPH recovery was higher by ISO-SOM than the methods using a SAL assay, with statistically significant differences between ISO-SOM and SAL/ISO-SOM (p-value<0.05). In contrast, no such differences were found for TCPH between the ISO-T and SAL/ISO-T methods. FCPH counts by ISO-F and SAL/ISO-F were similar (p-value = 0.19) and higher compared to the EPA-F method (p-values<0.05).

When comparing the overall performance of coliphage quantification methods in low-concentration samples (below 100 PFU/100 mL), no differences were found between the ISO, U.S. EPA, and SAL/ISO methods for SOMCPH and TCPH, but FCPH counts were higher by ISO and SAL/ISO than the U.S. EPA method. In high concentration samples, the ISO method yielded higher SOMCPH and TCPH counts than the methods using a SAL assay; the differences were statistically significant for SOMCPH with regard to SAL/ISO and U.S. EPA (p-values<0.05), and for TCPH with regard to SAL/ISO (p-value<0.05).

Finally, taking into account all the different sample types and the numbers obtained with the ISO method as a reference, ratios between the methods were calculated (Fig. 2). For all methods, the ratio is near to one, meaning they yielded very similar results. However, the variability of U.S. EPA methods for both somatic and F-specific coliphages was higher as determined by the proportions between U.S. EPA methods and ISO methods (Fig. 2). On the other hand, the ratios between the different methods show that there is no variability between the results obtained by the studied SAL/ISO methods and the ISO methods for both somatic and F-specific coliphages.

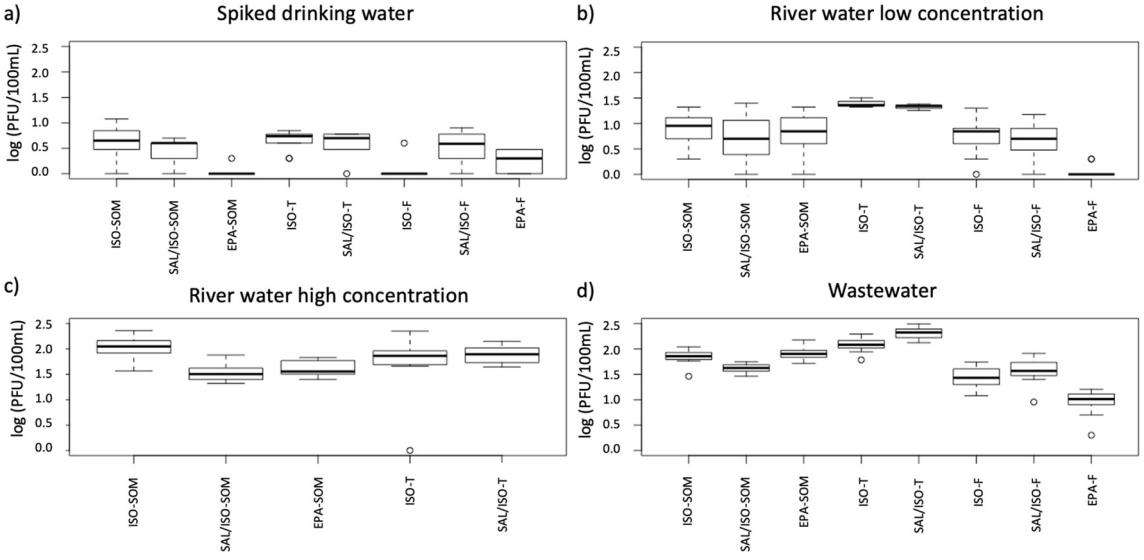

Fig. 1. Boxplot of the concentrations of somatic coliphages (SOM), F-specific coliphages (F) and total coliphages (T) obtained using ISO, SAL/ISO and U.S. EPA methods in: a) spiked drinking water, b) river water with low coliphage concentrations, c) river water with high coliphage concentrations and d) wastewater samples.

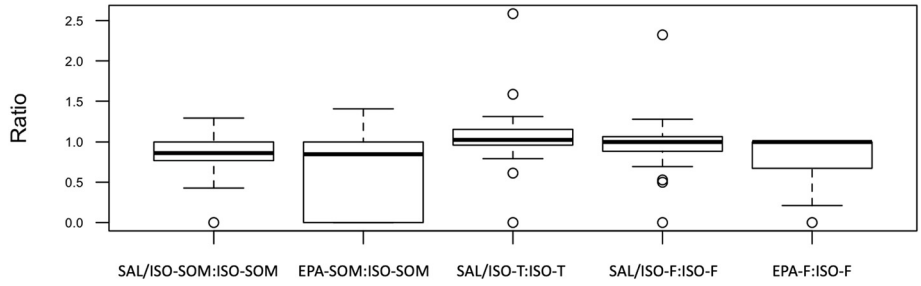

Fig. 2. Ratios between the SAL/ISO and U.S.EPA methods and ISO methods for somatic coliphages (SOM), total coliphages (T) and F-specific coliphages (F).

4. Discussion

Coliphages have been included in many water quality regulations and guidelines in recent years, although the standardised methods used for their determination differ in bacterial host strains, media, and technique. We here compare and discuss the coliphage enumeration results obtained by three different methods, including standardised methods and commercialized kits, in 100 mL samples of different types of water.

There were no detectable levels of endogenous coliphages in unamended mineral drinking water. When the samples were spiked, different SOMCPH, FCPH and TCPH counts were obtained according to the method used. Despite the statistical differences, the samples were spiked with 10 PFU/100 mL, which is a very low concentration, and the counts were all in the same logarithm, suggesting that the methods used in this study could be indifferently adopted to analyse this type of water. In this study, all SOMCPH analyses by the ISO method were performed with the *E. coli* WG5 strain. Although the ISO method prescribes the use of *E. coli* strain C to detect SOMCPH in clean water and *E. coli* WG5 for polluted water, it has been demonstrated that nalidixic acid can inhibit the growth of Gram negative bacteria (Crumplin and Smith, 1975), whereas *E. coli* WG5 is a nalidixic acid-resistant strain. Therefore, from a water quality monitoring perspective, the use of *E. coli* WG5 is preferable, as it entails a lower risk of interference in the plaque assays from sample microbiota, including that of drinking water.

The standardised methods can provide an early reading for SOMCPH after 6–7 h, which includes bacterial culture growth, experimentation, and 4 h of incubation, but overnight incubation is advisable to ensure the detection and quantification of all the coliphages in the samples, especially when their concentrations are low (Mendez et al., 2017). The inclusion of coliphages in water quality monitoring and risk management has stimulated research on ways to shorten the standardised protocols for coliphage enumeration in number of steps, time of procedure and time required to obtain the results. The emerging rapid methods, some of them with commercial application, are mainly based on non-agar supports to improve plaque display or an enzymatic reaction produced by phage-induced bacterial lysis (Blanch et al., 2020). Although still not included in official regulations, the implementation of these new techniques could revolutionize water management in the near future.

Our analysis of environmental water samples confirmed that SOMCPH outnumber FCPH in concentration by about one logarithm. This trend has been observed in previous studies around the world using DAL and SAL assays (Dias et al., 2018; Dungeni et al., 2010; Lucena et al., 2003; Rezaeinejad et al., 2014). A meta-analysis of studies on coliphage density in different types of environmental water found that SOMCPH counts are usually significantly higher than those of FCPH in surface water, with a few exceptions in North America (Nappier et al., 2019) that could be attributed to differences in the technique, bacterial host strains and sample volume used in the analysis.

In river water with low coliphage concentrations, the EPA-F method provided significantly lower counts of FCPH compared to ISO-F and SAL/ISO-F, in disagreement with other studies (Sobsey et al., 2004). The same trend was observed in wastewater, suggesting that the *E. coli* HS bacterial host strain (U.S. EPA) appeared to be less receptive to coliphage infection compared to *S. enterica* serovar Typhimurium WG49 (ISO). Another relevant factor is that the plaques formed by *E. coli* HS are more difficult to detect with the naked eye, making their count more challenging for an inexperienced technician.

In river water samples with coliphage concentrations below 100 PFU/100 mL, SOMCPH counts were similar by the three tested methods. This suggests that the SAL/ISO technique is also a suitable new option for the analysis of 100 mL samples, with the advantage that it does not require the concentration step of the ISO 10705-3 method before enumeration. After the concentration, which is performed by membrane filtration, coliphage recovery can be influenced by various factors such as sample turbidity, coliphage type and virus adsorption to the membrane (Méndez et al., 2004).

In wastewater samples, the lower counts obtained by techniques using a SAL rather than DAL assay are consistent with the scant data available in the literature (Méndez et al., 2020; Mooijman et al., 2001). When describing the SAL procedure in the late 1980s, Grabow and Coubrough compared different media and host bacterial strains, but recognised the difficulty of conducting a comparative study of SAL and DAL assays in 100 mL samples due to the highly variable recoveries produced by each technique (Grabow and Coubrough, 1986). In SAL assays with samples with high coliphage concentrations, a possible explanation is coliphage adsorption to the surface of the Petri dish, which varies according to the material used. This is not the case in DAL assays, where the host bacteria and sample are in contact with an agar layer. Additionally, the DAL assay is limited to only 1 mL sample volumes and requires significant larger numbers of replicate plates to process volumes at or near 100 mL.

Based on the overall results, the use of the SAL/ISO method, which combines the SAL assay with ISO host bacterial strains, could facilitate the analysis of somatic coliphages, F-specific coliphages and total coliphages in large volume water samples. Conversely, in volumes of up to 10 mL, the DAL technique could provide better recoveries.

5. Conclusions

- Sample analyses confirmed that somatic coliphages are present in higher concentrations than F-specific coliphages in environmental water.
- The combination of a SAL assay with the ISO bacterial host strain provided similar results to the ISO method in 100 mL samples when the somatic coliphage concentration was below 100 PFU/100 mL; the method using the SAL assay could therefore substitute the ISO method for the analysis of clean samples and thus avoid the concentration step.
- The method combining a SAL assay and the ISO bacterial host strain and the ISO method yielded higher F-specific coliphage counts than the U.S. EPA method, suggesting that *S. enterica* serovar Typhimurium WG49 counts better than *E. coli* HS.

CRediT authorship contribution statement

Miriam Pascual-Benito: Investigation, Methodology, Writing – original draft, Conceptualization, Formal analysis. **Ariadna Jorba-Plassa**: Investigation, Methodology. **Raquel Casas-Mangas**: Investigation,

Methodology. **Anicet R. Blanch**: Funding acquisition, Project administration, Supervision, Writing - review & editing, Conceptualization. **Julia Martín-Díaz**: Investigation, Methodology, Formal analysis, Supervision, Writing - review & editing, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Julia Martín-Díaz reports financial support was provided by Bluephage, S.L. Miriam Pascual-Benito reports financial support was provided by Bluephage, S.L. Ariadna Jorba-Plassa reports financial support was provided by Bluephage, S.L.

Acknowledgements

We are grateful for the comments and suggestions for the study and writing of the manuscript by Prof. Juan Jofre and Prof. Francisco Lucena from the University of Barcelona.

Funding

This study has been partially supported by Bluephage S.L. and by the Fundació Bosch i Gimpera of the University of Barcelona (FBG 311025 project).

References

Armon, R., Kott, Y., 1996. Bacteriophages as indicators of pollution. Crit. Rev. Environ. Sci. Technol. 26, 299–335. https://doi.org/10.1080/10643389609388494.

Ballesté, E., Blanch, A.R., Muniesa, M., García-Aljaro, C., Rodríguez-Rubio, L., Martín-Díaz, J., Pascual-Benito, M., Jofre, J., 2022. Bacteriophages in sewage: abundance, roles, and applications. FEMS Microbes 3, 1–12. https://doi.org/10.1093/femsmc/xtac009.

Blanch, A.R., Lucena, F., Muniesa, M., Jofre, J., 2020. Fast and easy methods for the detection of coliphages. J. Microbiol. Methods 173, 105940. https://doi.org/10.1016/j.mimet.2020.105940.

Blanco, A., Guix, S., Fuster, N., Fuentes, C., Bartolomé, R., Cornejo, T., Pintó, R.M., Bosch, A., 2017. Norovirus in bottled water associated with gastroenteritis outbreak, Spain, 2016. Emerg. Infect. Dis. 23, 1531–1534. https://doi.org/10.3201/eid2309.161489.

Costán-Longares, A., Montemayor, M., Payán, A., Méndez, J., Jofre, J., Mujeriego, R., Lucena, F., 2008. Microbial indicators and pathogens: removal, relationships and predictive capabilities in water reclamation facilities. Water Res. 42, 4439–4448. https://doi.org/10.1016/j.watres.2008.07.037.

Crumplin, G.C., Smith, J.T., 1975. Nalidixic acid: an antibacterial paradox. Antimicrob. Agents Chemother. 8, 251–261. https://doi.org/10.1128/AAC.8.3.251.

Dias, E., Ebdon, J., Taylor, H., 2018. The application of bacteriophages as novel indicators of viral pathogens in wastewater treatment systems. Water Res. 129, 172–179. https://doi.org/10.1016/j.watres.2017.11.022.

Dungeni, M., van Der Merwe, R.R., Momba, M.N.B., 2010. Abundance of pathogenic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in the Gauteng Province, South Africa. Water SA 36, 607–614. https://doi.org/10.4314/wsa.v36i5.61994.

Farrell, M.L., Joyce, A., Duane, S., Fitzhenry, K., Hooban, B., Burke, L.P., Morris, D., 2021. Evaluating the potential for exposure to organisms of public health concern in naturally occurring bathing waters in Europe: a scoping review. Water Res. 206. https://doi.org/10.1016/j.watres.2021.117711.

Francy, D.S., Stelzer, E.A., Bushon, R.N., Brady, A.M.G., Williston, A.G., Riddell, K.R., Borchardt, M.A., Spencer, S.K., Gellner, T.M., 2012. Comparative effectiveness of membrane bioreactors, conventional secondary treatment, and chlorine and UV disinfection to remove microorganisms from municipal wastewaters. Water Res. 46, 4164–4178. https://doi.org/10.1016/j.watres.2012.04.044.

Giammanco, G.M., Bonura, F., Urone, N., Purpari, G., Cuccia, M., Pepe, A., Li Muli, S., Cappa, V., Saglimbene, C., Mandolfo, G., Marino, A., Guercio, A., Di Bartolo, I., De Grazia, S., 2018. Waterborne norovirus outbreak at a seaside resort likely originating from municipal water distribution system failure. Epidemiol. Infect. 146, 879–887. https://doi.org/10.1017/S095026881800081X.

Grabow, W.O.K., Coubrough, P., 1986. Practical direct plaque assay for coliphages in 100-ml samples of drinking water. Appl. Environ. Microbiol. 52, 430–433. https://doi.org/10.1128/aem.52.3.430-433.1986.

Guzmán, C., Mocé-Llivina, L., Lucena, F., Jofre, J., 2008. Evaluation of Escherichia coli host strain CB390 for simultaneous detection of somatic and F-specific coliphages. Appl. Environ. Microbiol. 74, 531–534. https://doi.org/10.1128/AEM.01710-07.

IAWPRC Study Group on Health Related Water Microbiology, 1991. Review paper bacteriophages as model viruses in water quality control. Water Res. 25, 529–545.

ISO, 1995. International Standard ISO 10705-1 :1995. Water Quality - Detection and Enumeration of Bacteriophages. Part 1: Enumeration of F-specific RNA Bacteriophages.

ISO, 2000. International Standard ISO 10705-2:2000. Water Quality - Detection and Enumeration of Bacteriophages. Part 2: Enumeration of Somatic Coliphages.

ISO, 2000. International Standard ISO 7899-2 Water quality - Detection and Enumeration of Intestinal Enterococci - Part 2: Membrane Filtration Method.

ISO, 2003. International Standard ISO 10705-3:2003 Water Quality- Validation of Methods for Concentration of Bacteriophages From Water.

Jebri, S., Muniesa, M., Jofre, J., 2015. General and host-associated bacteriophage indicators of fecal pollution. In: Rose, J.B., Jiménez-Cisneros, B. (Eds.), Global Water Pathogens Project. Http://Www.Waterpathogens.Org (A.Farnleitner, and A. Blanch (Eds) Part 2 Indicators and Microbial Source Tracking Markers).

Jofre, J., Olle, E., Ribas, F., Vidal, A., Lucena, F., 1995. Potential usefulness of bacteriophages that infect Bacteroides fragilis as model organisms for monitoring virus removal in drinking water treatment plants. Appl. Environ. Microbiol. 61, 3227–3231. https://doi.org/10.1128/aem.61.9.3227-3231.1995.

Jofre, J., Lucena, F., Blanch, A.R., Muniesa, M., 2016. Coliphages as model organisms in the characterization and management of water resources. Water 8. https://doi.org/10.3390/w8050199.

Lucena, F., Méndez, X., Morón, A., Calderón, E., Campos, C., Guerrero, A., Cárdenas, M., Gantzer, C., Shwartzbrood, L., Skraber, S., Jofre, J., 2003. Occurrence and densities of bacteriophages proposed as indicators and bacterial indicators in river waters from Europe and South America. J. Appl. Microbiol. 94, 808–815. https://doi.org/10.1046/j.1365-2672.2003.01812.x.

Mellou, K., Katsioulis, A., Potamiti-Komi, M., Pournaras, S., Kyritsi, M., Katsiaflaka, A., Kallimani, A., Kokkinos, P., Petinaki, E., Sideroglou, T., Georgakopoulou, T., Vantarakis, A., Hadjichristodoulou, C., 2014. A large waterborne gastroenteritis outbreak in central Greece, March 2012: challenges for the investigation and management. Epidemiol. Infect. 142, 40–50. https://doi.org/10.1017/S0950268813000939.

Méndez, J., Audicana, A., Isern, A., Llaneza, J., Moreno, B., Jofre, J., Lucena, F., 2004. Standardised Evaluation of the Performance of a Simple Membrane Filtration-elution Method to Concentrate Bacteriophages From Drinking Water. 117, pp. 19–25. https://doi.org/10.1016/j.jviromet.2003.11.013.

Mendez, J., Monleon-Getino, A., Jofre, J., Lucena, F., 2017. Use of non-linear mixed-effects modelling and regression analysis to predict the number of somatic coliphages by plaque enumeration after 3 hours of incubation. J. Water Health 15, 706–717. https://doi.org/10.2166/wh.2017.067.

Méndez, J., Toribio-Avedillo, D., Mangas-Casas, R., Martínez-González, J., 2020. Bluephage, a method for efficient detection of somatic coliphages in one hundred milliliter water samples. Sci. Rep. 10, 1–6. https://doi.org/10.1038/s41598-020-60071-w.

Montemayor, M., Costan, A., Lucena, F., Jofre, J., Muñoz, J., Dalmau, E., Mujeriego, R., Sala, L., 2008. The combined performance of UV light and chlorine during reclaimed water disinfection. Water Sci. Technol. 57, 935–940. https://doi.org/10.2166/wst.2008.206.

Mooijman, K.A., Bahar, M., Contreras, N., Havelaar, A.H., 2001. Optimisation of the ISO-method on enumeration of somatic coliphages (draft ISO 10705–2). Water Sci. Technol. 43, 205–208. https://doi.org/10.2166/wst.2001.0739.

Nappier, S.P., Hong, T., Ichida, A., Goldstone, A., Eftim, S.E., 2019. Occurrence of coliphage in raw wastewater and in ambient water: a meta-analysis. Water Res. 153, 263–273. https://doi.org/10.1016/j.watres.2018.12.058.

Pipes, W.O., Ward, P., Ahn, S.H., 1977. Frequency distributions for coliform bacteria in water. J. / Am. Water Work. Assoc. 69, 664–668. https://doi.org/10.1002/j.1551-8833.1977.tb06847.x.

Rezaeinejad, S., Vergara, G.G.R.V., Woo, C.H., Lim, T.T., Sobsey, M.D., Gin, K.Y.H., 2014. Surveillance of enteric viruses and coliphages in a tropical urban catchment. Water Res. 58, 122–131. https://doi.org/10.1016/j.watres.2014.03.051.

Sinclair, R.G., Jones, E.L., Gerba, C.P., 2009. Viruses in recreational water-borne disease outbreaks: a review. J. Appl. Microbiol. 107, 1769–1780. https://doi.org/10.1111/j.1365-2672.2009.04367.x.

Sinton, L.W., Finlay, R.K., Reid, A.J., 1996. A simple membrane filtration-elution method for the enumeration of F- RNA, F-DNA and somatic coliphages in 100-ml water samples. J. Microbiol. Methods 25, 257–269. https://doi.org/10.1016/0167-7012(95)00100-X.

Sobsey, M.D., Schwab, K.J., Handzel, T.R., 1990. Simple membrane filter method to concentrate and enumerate male-specific RNA coliphages. Res. Technol. 82, 52–59. https://doi.org/10.1002/j.1551-8833.1990.tb07020.x.

Sobsey, M.D., Yates, M.V., Hsu, F.C., Lovelace, G., Battigelli, D., Margolin, A., Pillai, S.D., Nwachuku, N., 2004. Development and evaluation of methods to detect coliphages in large volumes of water. Water Sci. Technol. 50, 211–217. https://doi.org/10.2166/wst.2004.0056.

U.S. EPA, 2001. Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure 40.

U.S. EPA, 2001. Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure 38.

U.S. EPA, 2018. Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure 1–40.

U.S. EPA, 2018. Method 1643: Male-specific (F+) and Somatic Coliphage in Secondary (No Disinfection) Wastewater by the Single Agar Layer (SAL) Procedure 1–40.