SCIENTIFIC REPORTS

OPEN

Aptness of *Escherichia coli* host strain CB390 to detect total coliphages in Colombia

Received: 18 December 2018
Accepted: 14 June 2019
Published online: 25 June 2019

Claudia Campos[1], Javier Méndez[2], Camilo Venegas[1], Luisa Fernanda Riaño[1], Paula Castaño[1], Natalia Leiton[1] & Eliana Riaño[1]

Fecal bacteria have been used for more than a century as indicators of fecal contamination in water. In recent years, the monitoring of somatic and F-specific coliphages has been gradually included in guidelines and regulations as an additional parameter to reinforce water safety. The *Escherichia coli* host strain CB390 was tailored to detect both somatic and F-specific coliphages in a single test. The efficacy of this strain for bacteriophage detection, previously evaluated in Western Europe and North America, was assessed here for the first time in South America. The detection of somatic and F-specific coliphages by the strain CB390, as well as by standardized methods, was performed in drinking and river water and municipal and abattoir wastewaters. No statistical difference was found in the numbers of total coliphages detected by strain CB390 and the sum of somatic and F-specific coliphages determined separately by the standardized ISO methods. The data presented here provide further validation of the effectiveness of the host strain *E. coli* CB390 for the detection of total coliphages in waters in a single test and demonstrate its suitability for application in upper-middle income countries of the Americas (World Bank category).

The aptness of somatic and F-specific coliphages as indicators of viral and fecal contamination in water and food has been extensively studied, and the abundant scientific literature on this subject is covered in several reviews[1–5]. In the last few years, the monitoring of coliphages has been introduced in regulations or guidelines concerning the quality and management of waters for different uses[6–13], as well as biosolids[14,15] and food[16]. Relevant for the current study are the WHO guidelines about unplanned, unacknowledged or de facto potable water reuse[13], which refers to the long-standing and common practice of producing drinking water from water sources impacted by wastewater discharges. Few water production systems worldwide, especially those using surface water, fall outside of these WHO recommendations.

Existing guidelines recommend testing for either somatic or F-specific coliphages or both, and standardized methods are available for both groups[17–20]. In most types of water samples, somatic outnumber F-specific coliphages[21], whereas the opposite occurs in groundwater[22–24] and reclaimed UV-irradiated water[25,26]. However, many of the aforementioned rules do not specify whether to test for somatic or F-specific coliphages, which has resulted in a lack of clarity and uncertainty about how to implement coliphage analysis.

In this state of affairs, it could be advisable to assess the numbers of both phage types, using either the standardized methods to detect somatic and F-specific coliphages separately or a bacterial host strain capable of measuring both in a single test. Ideally, the amount of phages detected by such a host strain should be identical to the sum of values obtained by the standardized methods. Guzmán *et al.*[27] reported an *E. coli* strain CB390 tailored for the simultaneous detection of both somatic and F-specific coliphages, which functions as efficiently as the standardized ISO procedures (ISO 17050-1[17] and ISO 10705-2[18]) for separate analysis. In subsequent tests, phage numbers detected by the strain were comparable with those of US EPA methods 1601[19] and 1602[20]. Moreover, it has been successfully applied in different kinds of water samples in Spain and the USA[25,27–29]. In the current study, the suitability of CB390 to detect total coliphages was assessed in Colombia (South America), a country that differs from previously studied areas in income level (upper-medium according to the World Bank) and climate.

[1]Department of Microbiology. Faculty of Sciences, Pontificia Universidad Javeriana, Carrera 7 No. 40 – 62, Bogotá D.C., Colombia. [2]Department of Genetics, Microbiology and Statistics. Faculty of Biology, University of Barcelona, Av. Diagonal 643, 08028, Barcelona, Spain. Correspondence and requests for materials should be addressed to J.M. (email: jmendez@ub.edu)

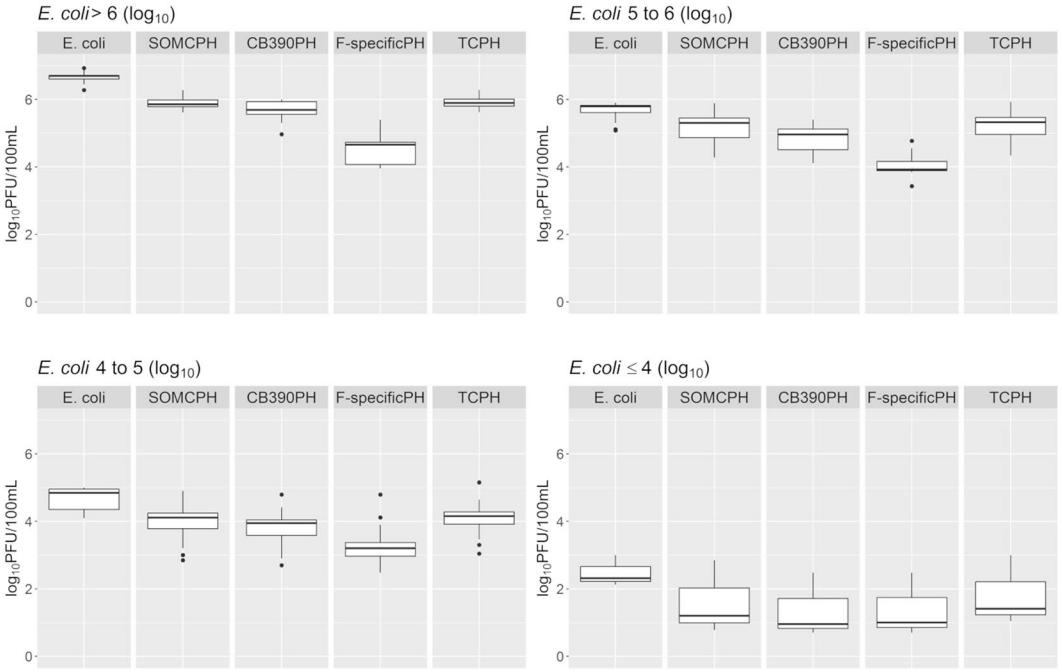

Figure 1. Boxplots of bacteriophage counts grouped by concentration of *E. coli* in the analyzed water samples. SOMCPH, CB390PH, F-specificPH and TCPH refer, respectively, to somatic coliphages, bacteriophages detected using the strain CB390, F-specific coliphages and total coliphages enumerated according to the ISO standard methods 10705-2 and 10705-1.

Thus, concentrations of *E. coli*, and somatic and F-specific coliphages detected by the *E. coli* strain CB390 were determined in an array of samples consisting of urban and abattoir wastewaters, river water and tap water. Considering the worldwide applicability of WHO guidelines for potable water reuse (13), the data provided here is relevant and useful.

Materials and Methods

Samples. One hundred and ninety-eight water samples with different levels of fecal contamination, representing diverse settings, were collected and analyzed over a 2-year period. Somatic and F-specific coliphages, bacteriophages infecting *E. coli* strain CB390 and *E. coli*, were analyzed from 48 samples of urban sewage, 84 samples of the Bogotá River collected in different sectors of the river with very different fecal contaminant loads, and 46 tap water samples of the Bogotá network. Regarding sewage samples, 18 of them were influent sewage of wastewater treatment plants and 30 were secondary effluents. Samples of drinking water were collected from faucets inside private homes throughout a marginal neighborhood, Ciudad Bolívar, occupied by citizens displaced by the political violence of the last decades. Inhabitants from this district normally use small homemade tanks for water storage and take water from taps that are fitted with gadgets to prevent water splashing. In addition, *E. coli*, somatic coliphages and bacteriophages infecting strain CB390 were analyzed in 20 samples of raw wastewater of abattoirs slaughtering porcine and bovine specimens.

Sample processing. Before the phage analysis, wastewater, secondary effluents and river water samples were decontaminated by filtration through 0.22 µm diameter pore-size Millex-GP membranes, which are low-protein-binding polyethersulfone membranes (Millipore Corporation, Bedford, MA, USA).

The bacteriophages from samples corresponding to a section of the river with low fecal contamination and drinking water from the distribution network were concentrated from 1 liter samples in accordance with Méndez *et al.*[30]. Briefly, phages in 1000 mL of water samples were concentrated by adsorption to 0.22 µm pore-size cellulose ester membrane filters (GSWP; Millipore Corp., Bedford, MA), followed by elution with a solution of 1% beef extract, 0.05 M NaCl, and 3% Tween 80, and ultra-sonication.

Bacteria enumeration. *E. coli* were enumerated by membrane filtration using Chromocult coliform agar (Merck, Darmstadt, Germany) supplemented with antibiotics (*E. coli*/coliform selective supplement; Merck). Dark-blue/purple colonies were presumed to be *E. coli* and confirmed by the addition of Kovac's reagent[31].

Bacteriophage enumeration. Somatic coliphages were quantified by enumerating plaque forming units (PFUs) in the *E. coli* host strain WG5 according to the ISO 10705-2 standard method[18]. F-specific coliphages were quantified by enumerating PFUs in strain WG49 of *Salmonella enterica* serovar *typhimurium* according to the ISO 10705-1 standard method[17]. To quantify somatic and F-specific coliphages in a single assay, host strain CB390 was grown in MSB broth with 100 µg per ml of ampicillin. TYG agar and TGY semisolid agar[17] supplemented with

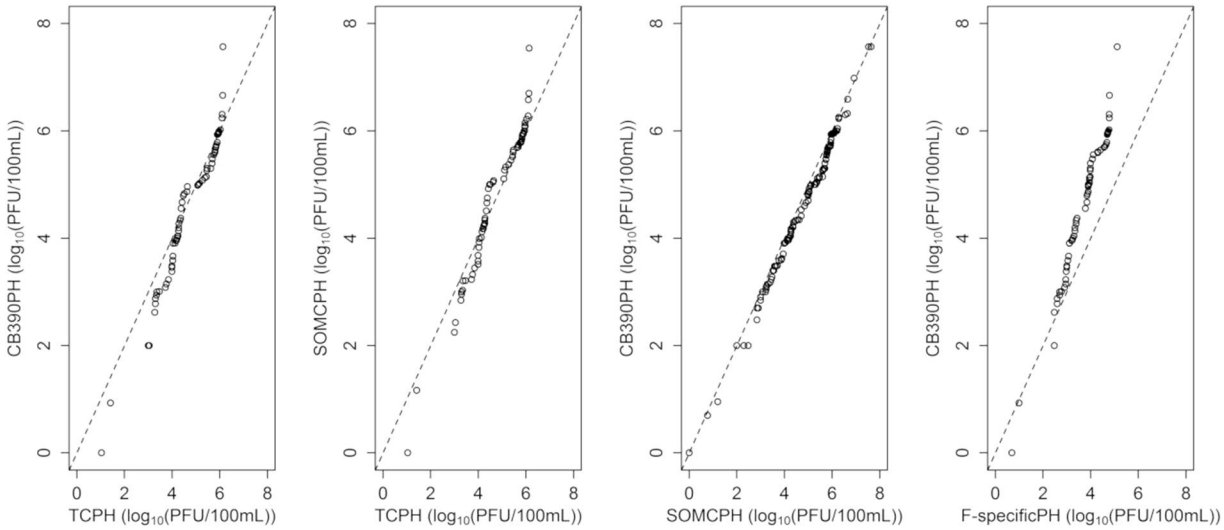

Figure 2. QQ-plot of bacteriophage counts. SOMCPH, CB390PH, F-specificPH and TCPH refer, respectively, to somatic coliphages, bacteriophages detected using the strain CB390, F-specific coliphages and total coliphages enumerated according to the ISO standard methods 10705-2 and 10705-1.

	E. coli	SOMCPH	CB390PH	F-specificPH
Number of samples	44	36	44	44
% positive per litre	ND	0	4.5	6.6
% positive per 100 ml	13	ND	ND	ND
NMP per 100 ml	0.15	<0.0028	0.0041	0.0071

Table 1. Levels of *E. coli* and coliphages in drinking water. SOMCPH, CB390PH and F-specificPH state, respectively, for somatic coliphages, bacteriophages detected using the strain CB390 and F-specific coliphages.

ampicillin (100 g ml^{-1}), Ca^{2+}, and Mg^{2+}, as in the ISO 10705-2[18] standard procedure, were used for the lower and upper layers in the double-agar-layer test, as indicated in Guzmán *et al.*[27].

Data treatment and statistics. The sum of the counts of somatic and F-specific coliphages was considered as total coliphages. To avoid dispersion due to the very different levels of fecal pollution of the tested waters, especially the river samples, the box-plots were calculated associating samples according to *E. coli* concentration intervals rather than by water origin.

Statistical analysis was carried out using R software package version 3.5.1[32] and RStudio version 1.465[33]. Raw data were plotted as box-plots; the calculations performed to generate the box-plot graphs included quartiles and outlier values for each group of samples. Additionally, the data were plotted as quantile-quantile plots (qq-plots). Outlier values were determined according to Tukey's method and were omitted from the statistical comparison tests.

Since not all data were normally distributed, the Wilcoxon rank sum test with continuity correction, the Kolmogorov-Smirnoff test and a two-sample Anderson-Darling test[34] were carried out to compare the series of data. Whenever it is mentioned that differences were statistically significant or not, this is true for all three tests.

Values of coliphages and *E. coli* concentrations in the drinking water, where the positive detection frequency was very low, were estimated by applying Thomas' formula for the calculation of the most probable number for long series of data[35].

Results and Discussion

Figures 1 and 2 show the counts of the different indicators studied in urban and abattoir wastewaters and surface waters, indicating magnitude, order of abundance and the proportions among them, as described in the related literature[21]. These results are of interest, since there is a lack of comparative data for total coliphages detected by strain CB390 or other coliphages in the Americas.

The values obtained for drinking water are shown in Table 1. Worthy of mention, although out of the main scope of the current work, are the low values of fecal contamination found in drinking water samples, which is a positive result for a marginal neighborhood like the one studied here. The predominance of *E. coli* over the coliphages points to a source of contamination in the neighborhood, perhaps in the depots or due to cross-contamination, since water disinfection leads to higher proportions of coliphages than *E. coli*[36]. Unfortunately, the values of coliphages were too low to derive significant conclusions regarding the aptness of strain CB390 for counting total coliphages.

No statistical difference ($p > 0.05$) could be appreciated when the values of somatic coliphages, strain CB390 and the total coliphages were compared. Only the values of F-specific coliphages were significantly lower ($p < 0.05$) than the others. A comparison of the different bacteriophage counts in the waste and surface waters, sample-by-sample, is visualized in Fig. 2, as in other studies on somatic and F-specific coliphages in similar waters[5,21]. As described elsewhere[25,27,28], the inclusion of F-specific phages to obtain total coliphages did not result in significantly higher values ($p > 0.05$) than those of somatic coliphages in the kinds of water tested. Additionally, the counts obtained by strain CB390, though slightly lower, are not significantly different ($p > 0.05$) from those obtained by the ISO procedure for somatic coliphages and total coliphages. This has also been observed in Europe[25,27] and the USA when using US EPA methods to detect somatic and F-specific coliphages[28,29]. Therefore, these data reinforce the conclusion that the *E. coli* host strain CB390 is effective for the simultaneous detection of the total number of coliphages in different types of fecally contaminated water. Moreover, it is suitable for application in South American countries with upper-middle incomes.

References

1. IAWPRC Study group on health related water microbiology. Bacteriophages as model viruses in water quality control. *Water Res.* **25**, 529–545 (1991).
2. Armon, R. & Kott, Y. Bacteriophages as indicators of pollution. *Crit. Rev. Environ. Sci. Technol.* **26**, 299–335 (1996).
3. Grabow, W. Bacteriophages: update on application as models for viruses in water. *Water SA* **27**(2), 251–268 (2001).
4. Jofre, J. Human Viruses in Water: Perspectives in Medical Virology, In Bosch, A (ed.), *Human Viruses in Water: Perspectives in Medical Virology*. Elsevier, London. ISBN: 9780080553276 (2007).
5. Jofre, J., Lucena, F., Blanch, A. & R. Muniesa, M. Review. Coliphages as Model Organisms in the Characterization and Management of Water Resources. *Water* **8**(5), 199, https://doi.org/10.3390/w8050199 (2016).
6. Queensland Government. Water quality guidelines for recycled water schemes. Queensland Water Supply Regulator, Water Supply and Sewerage Services, Department of Energy and Water Supply. Australia, https://www.dews.qld.gov.au/__data/assets/pdf_file/0019/45172/water-quality-guidelines.pdf (2008).
7. North Carolina Administration. North Carolina Administrative Code 15A NCAC 20U. North Carolina Department of Environmental Quality. Raleigh, USA, http://reports.oah.state.nc.us/ncac/title%2015a%20-%20environmental%20quality/chapter%2002%20-%20environmental%20management/subchapter%20u/subchapter%20u%20rules.pdf (2011).
8. NHMRC, NRMMC, 2011. Australian Drinking Water Guidelines 6. National Water Quality Management Strategy. National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia, Canberra. ISBN Online: 1864965118, https://nhmrc.gov.au/sites/default/files/documents/NHMRC%20ADWG%206%20-%20Version%203.5%20-%20Proof%203.pdf (2011).
9. U.S. EPA. National Primary Drinking Water Regulations: Ground Water Rule. Final Rule; 40 CFR Parts 9, 141 and 142. Federal register, vol. 71 FR 65573, https://www.federalregister.gov/documents/2006/11/08/06-8763/national-primary-drinking-water-regulations-ground-water-rule (2006).
10. U.S. EPA. Review of coliphages as possible indicators of fecal contamination for ambient water quality. EPA 820-R-15-098, https://www.epa.gov/sites/production/files/2015-10/documents/coliphages-literature-review-report-2015.pdf (2015).
11. European Commission. Europe Drinking Water DRAFT: Revision of Annex I of the Council Directive on the Quality of Water intended for Human consumption (Drinking Water Directive). Background paper on microbiologically safe water and microbiological parameters, https://circabc.europa.eu/sd/a/27a90048-2541-4e10-ae06-a323513db584/02%20-%20DWD_background%20paper_microbiology_20160915.pdf (2016).
12. Alcalde-Sanz, L. & Gawlik, B. M. Minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge - Towards a legal instrument on water reuse at EU level, EUR 28962 EN, Publications Office of the European Union, Luxembourg, ISBN 978-92-79-77175-0, https://doi.org/10.2760/804116, JRC 109291 (2017).
13. World Health Organization. Potable reuse: Guidance for producing safe drinking-water. Geneva. Licence: CC BY-NC-SA 3.0 IGO, http://www.who.int/iris/handle/10665/258715 (2017).
14. República de Colombia. Decreto no 1287. Criterios para el uso de biosólidos generados en plantas de tratamiento de aguas residuales municipales. Gobierno de Colombia. Ministerio de Vivienda Ciudad y Territorio. Bogotá, http://www.minvivienda.gov.co/Decretos%20Vivienda/1287%20-%202014.pdf (2014).
15. Western Australian Government. Western Australia Guidelines for Biosolids Management. Department of Environmental Conservation. Perth. Australia. ISBN [online]: 1 921094 21 4, https://www.der.wa.gov.au/images/documents/our-services/approvals-and-licences/western-australian-guidelines-for-biosolids-management-dec-2012.pdf (2012).
16. National Shellfish Sanitation Program (NSSP). Guide for the Control of Molluscan Shellfish 2017 Revision. From the U.S. Food and Drug Administration website, http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm (2017).
17. International Organization for Standardization. Water quality. Detection and enumeration of bacteriophages. 1. Enumeration of F-specific RNA bacteriophages. ISO 10705-1. International Organization for Standardization, Geneva, Switzerland (1995).
18. International Organization for Standardization. Water quality: Detection and enumeration of bacteriophages. 2. Enumeration of somatic coliphages. ISO 10705-2. International Organization for Standardization, Geneva, Switzerland (2000).
19. U.S. EPA. Method 1601: Detection of Male-specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure. EPA 821-R-01-030 Office of Water, Engineering and Analysis Division, Washington, DC, https://www.epa.gov/sites/production/files/2015-12/documents/method_1601_2001.pdf (2001).
20. U.S. EPA. Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure. EPA 821-R-01-029. Office of Water, Engineering and Analysis Division, Washington, DC. https://www.epa.gov/sites/production/files/2015-12/documents/method_1602_2001.pdf (2001).
21. Jebri, S., Muniesa, M., Jofre, J. 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 (Farnleitner, A. & Blanch, A. (eds) Part 2 Indicators and Microbial Source Tracking Markers), http://www.waterpathogens.org/book/coliphage Michigan State University, E. Lansing, MI, UNESCO (2017).
22. Lucena, F. *et al*. Occurrence of bacterial indicators and bacteriophages infecting enteric bacteria in groundwater in different geographical areas. *J. Appl. Microbiol.* **101**, 96–102, https://doi.org/10.1111/j.1365-2672.2006.02907.x (2006).
23. Abbaszadegan, M., Lechevallier, M. & Gerba, C. Occurrence of Viruses in US groundwaters. *J. Am. Water Work. Assoc.* **95**(9), 107–102, https://doi.org/10.1139/f94-052 (2003).
24. Armon, R., Araujo, R., Kott, Y., Lucena, F. & Jofre, J. Bacteriophages of enteric bacteria in drinking water, comparison of their distribution in two countries. *J. Appl. Microbiol.* **83**, 627–633 (1997).
25. Agulló-Barceló, M. *et al*. Simultaneous detection of somatic and F-specific coliphages in different settings by Escherichia coli strain CB390. *FEMS Microbiol. Lett.* **363**(17), https://doi.org/10.1093/femsle/fnw180 (2016).
26. Payán A. Bacteriófagos como modelo en el origen de la contaminación fecal y en aguas regeneradas. PhD. thesis. University of Barcelona, Barcelona, Spain (2006).

27. Guzmán, C., Mocé-Llivina, L., Lucena, F. & Jofre, J. 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 (2008).
28. Bailey, E. S., Price, M., Casanova, L. M. & Sobsey, M. D. *E. coli* CB390: An alternative *E. coli* host for simultaneous detection of somatic and F+ coliphage viruses in reclaimed and other waters. *J. Virol. Methods* **250**, 25–28, https://doi.org/10.1016/j.jviromet.2017.09.016 (2017).
29. Bailey, E. S., Casanova, L. M., Simmons, O. D. & Sobsey, M. D. Tertiary treatment and dual disinfection to improve microbial quality of reclaimed water for potable and non-potable reuse: A case study of facilities in North Carolina. *Sci. Total Environ.* **630**, 379–388, https://doi.org/10.1016/j.scitotenv.2018.02.239 (2018).
30. Méndez, J. *et al*. Standardised evaluation of the performance of a simple membrane filtration-elution method to concentrate bacteriophages from drinking water. *J. Virol. Methods* **117**, 19–25, https://doi.org/10.1016/j.jviromet.2003.11.013 (2004).
31. International Organization for Standardization. ISO 9308-1: Detection and enumeration of *Escherichia coli* and coliform bacteria-part 1: Membrane filtration method for water with low bacteria background flora, Geneva, Switzeland (2014).
32. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/ (2018).
33. RStudio Team. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, http://www.rstudio.com/ (2016).
34. Scholz & Zhu A. kSamples: K-Sample Rank Tests and their Combinations. R package version 1.2-8, https://CRAN.R-project.org/package=kSamples (2018).
35. Man, J. C. The probability of most probable numbers. *Eur. J. Appl. Microbiol.* **1**, 67–78 (1975).
36. Méndez, J. *et al*. Assessment of drinking water quality using indicator bacteria and bacteriophages. *J. Water Health* **2**, 201–214, https://doi.org/10.1016/S0168-583X(01)01211-3 (2004).

Acknowledgements

Pontificia Universidad Javeriana supported the present work, through the project "Evaluation of strain *E. coli* CB390 for the counting of somatic coliphages and F-specific phages as a tool to evaluate contamination of viral origin in drinking and wastewater". The authors thank Joan Jofre for the donation of strain *E coli* CB390 and suggestions in the research and preparation of the publication and the program Vidas Móviles for helping in the collection of drinking water samples.

Author Contributions

All authors contributed to all features of the paper. C.C. conceived the idea for the research, and also contributed to the development of the project by obtaining economic resources and to the sampling campaign. J.M. performed the statistical analysis and contributed in the preparation of the manuscript. C.V. contributed to the sampling campaign of all water sources analyzed. L.F.R., P.C., N.L. and E.R. were part of the laboratory technician team and they performed the analysis of drinking water, Bogotá River, domestic and abattoirs wastewater samples.

Additional Information

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.