medical sciences forum

MDPI

Proceeding Paper

Microbiological Analysis of Borehole Water Quality †

Catarina Silva [1], Telma Costa [1], Nádia Silva [1], Sérgio Valério [2], Helena Barroso [2], Maria João Hilário [2,*] and Sara Alves [2]

[1] Egas Moniz School of Health & and Science, 2829-511 Almada, Portugal; catarinasilva96@hotmail.com (C.S.); telmafiliparc@gmail.com (T.C.); nadia.solene2001@gmail.com (N.S.)
[2] Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & and Science, 2829-511 Almada, Portugal; sfcvalerio@gmail.com (S.V.)
* Correspondence: mjhilario1973@gmail.com
† Presented at the 6th International Congress of CiiEM—Immediate and Future Challenges to Foster One Health, Almada, Portugal, 5–7 July 2023.

Abstract: Groundwater is often used as a primary resource by those who have boreholes inside their properties; however, this has caused concerns among health professionals. This water may contain microorganisms or substances that are harmful. The main objective of this study was to microbiologically analyse the quality of the water coming from boreholes in the village of Santo Ovídio, Setúbal, by searching for bacteria that indicate faecal contamination, —such as total and faecal coliforms, *Escherichia coli*, faecal Enterococci, *Clostridium* and *Pseudomonas aeruginosa*—and quantifying them via the membrane filtration method. The research method was a quantitative, simple, descriptive, level I study with a sample size of 20 participants. It was found that 60% of the samples contained at least one of these microorganisms.

Keywords: pathogenic microorganisms; water quality; faecal contamination; membrane filtration method

Citation: Silva, C.; Costa, T.; Silva, N.; Valério, S.; Barroso, H.; Hilário, M.J.; Alves, S. Microbiological Analysis of Borehole Water Quality. *Med. Sci. Forum* **2023**, *22*, 11. https://doi.org/10.3390/msf2023022011

Academic Editors: José Brito, Nuno Taveira and Ana I. Fernandes

Published: 9 August 2023

1. Introduction

The presence of local sanitation systems, human activity, urbanization, industrialization, and sewage makes groundwater vulnerable to contamination by organisms. Populations who have boreholes or wells often use groundwater as their main source of water. Many people assume water is safe to drink because it looks good and is crystal clear. However, this water may contain microorganisms or substances harmful to human health and be unsuitable for human consumption. The transmission of diseases through water mainly involves the faecal–oral route. To protect and preserve water quality, and therefore public health, it is essential to perform physicochemical, microbiological, and ecotoxicological analyses of water.

Escherichia coli (*E. coli*) and other faecal coliforms are used as the health parameters for monitoring water quality. Coliforms include all aerobic and facultative anaerobic organisms, as well as Gram-negative, non-sporulated, and rod-shaped bacteria, which ferment lactose with gas formation. Within the group of total coliforms, a subgroup of thermotolerant coliforms can be observed. These are the ones that indicate faecal pollution since they are restricted to the gastrointestinal tract. These are characterized by the presence of the enzymes ß-D-galactosidase and ß-glucuronidase and the ability to ferment lactose and mannitol with gas production, in a medium containing bile salts or other surfactants [1]. Within the thermotolerant coliforms, *E. coli* is the microorganism considered to be the best indicator of faecal contamination. The Chromogenic Coliform Agar medium is used for the isolation and identification of all these microorganisms, producing pink colonies for non-faecal coliforms and blue colonies for faecal coliforms [2]. Other bacteria, such as *Pseudomonas aeruginosa* (*P. aeruginosa*) and Enterococci can also be isolated in water. *P. aeruginosa* is Gram-negative aerobic that is non-sporulated and positive for catalase and

oxidase. In CN Agar medium, it produces greenish-coloured fluorescent colonies due to the production of the pigments, pyoverdine and pyocyanin [2,3]. Faecal Enterococci are non-spore-forming, facultative anaerobes and are negative for catalase and oxidase [4]. The selective medium used for its isolation is Slanetz and Bartley agar. This is based on the ability of this bacterium to reduce TTC (2,3,5-triphenyl tetrazolium chloride—colourless dye) to formazin, which leads to typical dark pink to red colonies [2,4]. Another Gram-positive bacterium present in the intestinal tract is *Clostridium perfringens* (*C. perfringens*). This bacillus is a strict anaerobe and is the most important sulfite-reducing bacteria within the genus *Clostridium*, forming heat-resistant spores. *C. perfrigens* does not multiply in aquatic environments, but its spores can survive in water for months and, consequently, its presence may indicate long-term faecal pollution [2,5]. The medium used to detect it is Tryptone–sulfite–cycloserine. The anaerobic sulfite-reducing spores of the bacterium can reduce the sulfite in the medium to sulphide, which in turn reacts with the ferric salt of the medium, thus producing black colonies [2,3].

The International Organization for Standardization is responsible for establishing international standards known as ISO standards for the purpose of improving quality. There is an ISO standard for each surveyed microorganism. The method of choice for the research of each microorganism is the membrane filtration method. To confirm the presence of specific microorganisms in each medium, biochemical confirmation tests were performed, such as the use of Hajna Kliger medium, as well as catalase and oxidase tests. The Hajna Kliger medium is used for the confirmation and identification of *Enterobacteriaceae*. This medium allows can differentiate between dextrose- lactose and/or sucrose-fermenting bacteria and detect hydrogen sulfide and gas production. The catalase test is used to evaluate the presence of the enzyme, catalase [6]. Catalase is present in most aerobic and facultative anaerobic bacteria. The presence of this enzyme makes it possible to differentiate microorganisms of the genus *Staphylococcus* spp. (catalase-positive) from other non-catalase-producing Gram-positive cocci. The oxidase test allows the presence of the cytochrome, oxidase enzyme, to be determined. The oxidase test is extremely important for differentiating between *Enterobacteriaceae* and *Pseudomonas* [2,4,7].

2. Materials and Methods

The main objective of the present study was to microbiologically analyse the quality of water coming from boreholes in the locality of Santo Ovídio, Setúbal. This was carried out through a microbiological analysis of 20 water samples from boreholes belonging to study participants, who were previously informed about the study via leaflets. As a procedure, samples were collected in sterile containers and kept refrigerated until they reached the laboratory. Microbiological analysis was performed within 24 h of collection. Then, 100 mL was filtered for each analysis/quantification. Filtration was carried out through a membrane filter using a vacuum pump. After filtration, the filters were transferred to the appropriate culture medium. Subsequently, additional confirmation tests were performed. According to the variables, the sample, and the objectives of the study, no statistical tests were used; only a simple statistical analysis was performed using SPSS. The results are organized in tables and circle graphs.

3. Results

The obtained results are shown in Table 1. Figure 1 shows a pie chart with the total number of samples that tested positive for at least one of the investigated bacteria. These samples (60%of the total) were considered contaminated.

Table 2 shows the percentages of all microorganisms found in the tested samples. As there were poly-contaminated samples, the total number of positives in the study represented in this table is higher than 20 (total number of samples).

Table 1. The number of colonies present in each sample.

Samples /Bacteria	Total Coliforms	Faecal Coliforms (*E. coli*)	*Intestinal Enterococci*	*Pseudomonas aeruginosa*	*Clostridium* Sulfite-Reducing
SO1	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL
SO2	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL
SO3	1 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL
SO4	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	19 UFC/100 mL
SO5	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	1 UFC/100 mL
SO6	12 UFC/100 mL	2 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL
SO7	>100 UFC/100 mL	6 UFC/100 mL	18 UFC/100 mL	5 UFC/200mL	1 UFC/100 mL
SO8	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL
SO9	>100 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	100 UFC/100 mL
SO10	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL
SO11	>100 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	40 UFC/100 mL
SO12	>100 UFC/100 mL	68 UFC/100 mL	2 UFC/100 mL	0 UFC/200mL	>100 UFC/mL
SO13	101 UFC/100 mL	1 UFC/100 mL	1 UFC/100 mL	0 UFC/200mL	40 UFC/100 mL
SO14	>100 UFC/100 mL	15 UFC/100 mL	13 UFC/100 mL	104 UFC/200mL	20 UFC/100 mL
SO15	30 UFC/100 mL	1 UFC/100 mL	1 UFC/100 mL	0 UFC/200mL	15 UFC/100 mL
SO16	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	>15 UFC/mL
SO17	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL
SO18	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL
SO19	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL
SO20	0 UFC/100 mL	0 UFC/100 mL	0 UFC/100 mL	0 UFC/200mL	0 UFC/100 mL

Table 2. Percentages of all microorganisms found in the tested samples.

		Positive	
		N	Percentage
Bacterium	Total Coliforms	9	28.1%
	Faecal Coliforms/*E. coli*	6	18.8%
	Enterococci	5	15.6%
	Pseudomonas	2	6.3%
	Sulphite Reducing *Clostridium*	10	31.3%
	Total positive samples in the study	32	100.0%

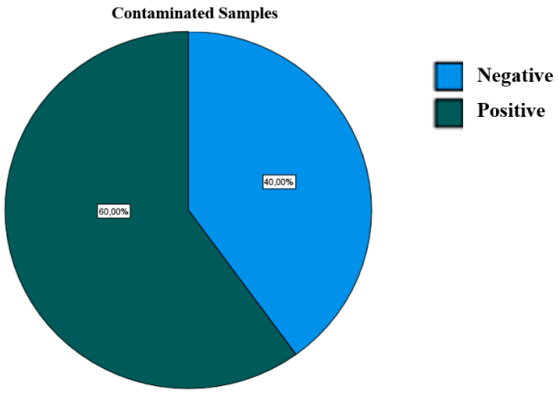

Figure 1. Percentages of contaminated samples vs. non-contaminated samples.

4. Discussion

The objective of this study was to access if the water derived from boreholes was of good microbial quality. Contamination by bacteria was observed in 60% of the samples. The analysis of the positive samples revealed the presence of different bacteria: total coliforms (28.1%), *E. coli* (18.8%), *Enterococci* (15.6%), *Pseudomonas* (6.3%), and *Clostridium* (31.3%) were the most common. All of these results were confirmed by biochemical tests and validated

by controls. The study confirms that some waters are highly contaminated, presenting all the bacteria investigated as observed in SO07 and SO14. Only SO19 did not present any microorganisms; however, we cannot confirm good microbiological water quality, since not all microorganisms were investigated. All samples that were positive for all investigated bacteria ended up exceeding the bacterial limits permitted by law, which states that these microorganisms should not be present in groundwater.

5. Conclusions

Considering that residents are senior citizens (a more vulnerable population) and the safety of groundwater was not confirmed, consuming this contaminated water could increase health problems. An alert was given to the population in danger of consuming water from untreated boreholes, and they were informed of all the obtained results. With this study, we hope to provide a starting point for future studies to address this topic in more locations and with a larger sample, contributing to the safer use of groundwater.

Author Contributions: Conceptualization, C.S.; methodology, C.S. and T.C.; software, S.V.; validation, C.S., T.C. and H.B.; formal analysis, C.S. and T.C.; investigation, C.S. and T.C.; resources, C.S., T.C. and H.B.; data curation, C.S. and T.C.; writing—original draft preparation, T.C.; writing—review and editing, C.S., T.C., N.S., H.B. and S.V.; visualization, C.S., T.C., N.S. and H.B.; supervision, S.V., S.A., M.J.H. and H.B.; project administration, S.V. and S.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding, but all the required materials were provided by Egas Moniz School of Health & Science.

Institutional Review Board Statement: The study was conducted in accordance and approved by the Institutional Review Board (or Ethics Committee) of Egas Moniz School of Health & Science (protocol code MP.EM.PE.18_02 and date of 7 June 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is available in Egas Moniz School of Health and Science, but cannot be accessed due to privacy and ethical restrictions.

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

References

1. Souto, J.P.; Sarmento Lira, A.G.; Figueira, J.d.; Nascimento da Silva, A.; Silva, E.S. Poluição Fecal da Água: Microrganismos Indicadores. In Proceedings of the VI Congresso Brasileiro de Gestão Ambiental, Porto Alegre, RS, Brazil, 23–26 November 2015.
2. Alves Cunha, M.C. Execução de Ensaios Microbiológicos nas Áreas Alimentar, Ambiental e Técnica em Contexto Empresarial. Master's Dissertation, Faculdade de Ciências da Universidade do Porto, Porto, Portugal, 2017.
3. Mendes, B.; Santos Oliveira, J. *Qualidade da Água para Consumo Humano*, 1st ed.; Lidel: Lisboa, Portugal, 2004.
4. Valente Bernardo, M.M. Comparação dos Métodos Aplicados na Deteção de Bactérias Coliformes, *Escherichia coli* e *Enterococcus* sp. em Águas para Fins Recriativos. Master's Dissertation, Universidade do Algarve, Faro, Portugal, 2007.
5. Quintela, S.D. Qualidade da Água para Consumo Humano: Riscos Associados à Presença de Biofilmes em Torneiras de Espaços Escolares. Master's Dissertation, Escola Superior de Tecnologia da Saúde do Porto, Porto, Portugal, 2016.
6. Barroso, H.; Taveira, N.; Meliço-Silvestre, A. *Microbiologia Médica*; Lidel: Lisboa, Portugal, 2014.
7. Rosa Candeias, G.M. Qualidade Microbiológica do Gelo Usado em Estabelecimentos de Restauração e de Bebidas. Master's Dissertation, Instituto Superior de Ciências da Saúde Egas Moniz, Almada, Portugal, 2014.