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Molecular screening for protozoan parasites in bottled drinking water from different cities of Iran

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Abstract
Bottled water is widely considered a safe alternative to tap water. However, contamination with protozoan parasites such as *Cryptosporidium*, *Acanthamoeba*, and *Giardia* remains a public health concern, particularly because they are resistant to conventional disinfection methods. This study aimed to investigate the presence of these protozoa in commercially available bottled water brands across various cities in Iran. Between March and April 2024, bottled commercial drinking water from 42 different brands was purchased in various cities across Iran. For each brand, 250 mL of water was centrifuged, and genomic DNA was extracted from the resulting sediment. Samples were tested for *Cryptosporidium* spp., *Acanthamoeba* spp., and *Giardia* spp. DNA using conventional PCR. No DNA of the examined parasites was detected. While the absence of protozoan DNA in the tested bottled water is reassuring and generally indicates effective water sourcing, filtration, and packaging processes, it does not guarantee the water is completely free of all microorganisms, and the results should be interpreted with caution. The results suggest that, if present, parasites or their DNA occur at concentrations below the detection limit of the method used. Further testing with more sensitive methods, such as real-time PCR targeting different parasite genes and advanced water-concentration techniques, may be necessary to confirm their absence or detect low-level contamination.

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Introduction

Water is recognized as a key pathway for the spread of microorganisms (1, 2). Water sources can become contaminated with several parasites through human or animal fecal pollution (3). Among them, protozoa such as *Cryptosporidium*, *Giardia*, and *Acanthamoeba* are recognized as cosmopolitan waterborne pathogens (4). *Cryptosporidium* and *Giardia* are major causes of gastroenteritis and contribute significantly to the annual

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burden of this disease (5). *Acanthamoeba* has been identified in various environments, including water, soil, and milk (6, 7), and can cause lung and sinus infections, particularly in immunocompromised individuals (8). *Cryptosporidium* spp. infect a broad range of hosts, including mammals, birds, fish, and reptiles (9). Epidemiological studies conducted in Iran indicate that *C. parvum* is the predominant *Cryptosporidium* species, followed by *C. hominis* and *C. meleagridis* (10). Despite the well-established role of water as a major transmission route for *Cryptosporidium* spp. (2), a systematic assessment of this parasite in drinking water sources, particularly bottled drinking water, is still warranted.

To date, 14 species of the protozoan *Giardia* have been identified, with *Giardia duodenalis* and *Giardia enterica* accounting for most human giardiasis cases (11–13). These parasites are among the most common gastrointestinal protozoa worldwide, with a prevalence of 1–8% in developing countries and approximately 8.5% reported in Iran (14). As transmission occurs primarily via the fecal-oral route (15), water sources play a key role in its spread (16, 17). Reports of *Giardia* contamination in surface, drinking, and municipal water supplies in Iran and worldwide (18, 19) indicate a potential risk of bottled-water contamination and underscore the importance of monitoring these sources.

Acanthamoeba, with 22 identified genotypes (T1–T22), is the most common free-living protozoan in nature (20, 21). This amoeba has been detected in diverse environments, including seawater, freshwater lakes, swimming pools, hot springs, soil, dust, and air (22–25). In Iran, this parasite has been reported in water, soil, and milk, with genotype T4 being the dominant (7, 26). Because water is a major route of transmission (20), assessing drinking water sources, particularly bottled water, is essential.

Given the lack of data on the potential presence of *Cryptosporidium*, *Giardia,* and *Acanthamoeba* in commercial bottled water in Iran, this study aimed to investigate DNA of these parasites in bottled water collected from various cities across Iran using molecular techniques.

Materials and Methods

Study area and sample collection

Between March and April 2024, a total of 84 bottles of commercial bottled drinking water representing 42 different brands were purchased from stores in Tehran, Hamedan, and Mazandaran Provinces, Iran, Using onvenience (non-probability) sampling. The bottles were kept at room temperature and transferred to the Laboratory of Parasitology, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.

Sample processing and DNA isolation

As previously described (27), 250 mL from each bottle was transferred to 50 mL tubes, centrifuged at 10,000 × *g* for 20 min, and the resulting pellets were stored at -20 °C until DNA isolation. Approximately 250 mg of each pellet were used for DNA extraction with a commercial kit (MBST, Tehran, Iran) according to the manufacturer's instructions. Purified DNA was stored at -20 °C until molecular analyses.

PCR assay

Specimens were tested for *Cryptosporidium*, *Acanthamoeba*, and *Giardia* by conventional PCR assay (Table 1). PCR reactions were prepared in a final volume of 25 µL using 2× Red PCR Master Mix (Ampliqon, Denmark) and 10 pmol of each primer. For *Giardia*, 2 µL of DNA template and, for *Acanthamoeba*, 5 µL of DNA template were added to the reaction mixture. For *Cryptosporidium*, the first-round nested PCR contained 2 µL of DNA template, while 0.5 µL of the first-round PCR product served as the template for the second round. Positive controls consisted of previously confirmed *Cryptosporidium*- and *Acanthamoeba*-positive spring water samples, as described by Sazmand et al. (28), and DNA extracted from the stool of a *Giardia*-positive patient. Positive and negative controls were included in all thermocycling reactions. The PCR products were analyzed by electrophoresis in a 1% agarose gel (SinaClon, Tehran, Iran). Electrophoresis was performed

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at 120 V for 10 minutes, then at 110 V for 35 minutes, and bands were visualized under UV light with a transilluminator (Vilber Lourmat, France).

Table 1. Primers, target genes, and PCR conditions used in this study.

Parasite	Target	Primers (5' to 3')	Product (bp)	Reference
Giardia	*gdh*	F1 GGGCAAGTCCGACAACGA	261	(29)
		R1 GCACATCTCCTCCAGGAAGTAGAC		
Cryptosporidium	18SrRNA	Nested PCR		(30, 31)
		Nest 1	733	
		SHP1 ACCTATCAGCTTTAGACGGTAGGGTAT		
		SHP2 TCTCATAAGGTGCTGAAGGAGTAAGG		
		Nest 2		
		SHP3 ACAGGGAGGTAGTGACAAGAAATAACA		
		SSU-R3 AAGGAGTAAGGAACAACCTCCA	611	
Acanthamoeba	*18S rRNA*	JDP1: GGCCCAGATCGTTACCGTGAA	475	(32)
		JDP2: TCTCACAAGCTGCTAGGGAGTCA		

Results

None of the samples tested positive for any targeted protozoa, indicating no detectable contamination in the brands examined.

Discussion

In this study, various bottled water samples were collected and analyzed, and all tested negative for the target parasites. Our findings may reflect the effectiveness of treatment and filtration processes used in bottled water production. However, previous studies in Iran have reported contamination of surface and drinking water with protozoan parasites, including *Giardia*, *Cryptosporidium,* and *Acanthamoeba* (28, 33–35). Although the latter studies did not specifically investigate bottled water, the presence of these parasites in spring and well waters, which are among the source waters used to produce the bottled water examined in the present study, indicates a potential risk of their introduction into bottled water if filtration and treatment processes are inadequate. Therefore, continuous monitoring of bottled water production using up-to-date detection tools and methods is essential to ensure water safety.

Although numerous studies have reported *Cryptosporidium* in surface and groundwater in Iran and other regions worldwide (18, 28, 36–38), data on bottled water contamination remain limited. A study in Brazil detected this parasite in bottled water using immunofluorescence and reported contamination levels sufficient to cause infection (39). In contrast, a similar investigation in Saudi Arabia found no *Cryptosporidium* in bottled water samples (40). These discrepancies may reflect temporal differences between studies, improvements in water filtration and treatment methods over time, and regional variations in source-water contamination. Given that even a single detected oocyst may serve as a warning sign of cryptosporidiosis (39), continuous monitoring of drinking and bottled water is essential.

Giardia cysts have been reported in various water sources, including surface and drinking water, worldwide and in Iran (19, 41). Previously, the presence of this protozoan in bottled water in Russia and Bulgaria was reported using immunofluorescence techniques (42). In contrast, a study in Brazil that examined commercially available bottled mineral water, similar to our study, did not detect *Giardia* (39). This discrepancy may reflect regional variation and differences in filtration and treatment practices within the bottled water industry. In light of the documented presence of this protozoan in diverse water sources throughout Iran (2), there is a clear risk

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of its entry into bottled water; therefore, continuous monitoring of bottled water using sensitive detection methods, such as qPCR, is essential.

Globally, multiple reports have documented contamination of bottled water with *Acanthamoeba* (43, 44). For instance, a study using a technique similar to that employed in the present study identified genotypes T4, T5, and T11 in bottled water in Brazil (45). In Iran, *Acanthamoeba* has been detected in water sources used for bottled water production, including wells and surface water (25, 33), with reported genotypes T2, T3, T4, T5, T11, T13, and T15, among which T4 is the most prevalent (46). *Acanthamoeba* can promote both amoebic and bacterial resistance, facilitating the proliferation of bacteria such as *Pseudomonas aeruginosa* (47), *Vibrio cholerae* (48), and other pathogenic bacteria (49), thereby increasing the risk of human contamination. Overall, *Acanthamoeba* appears to be more frequently detected in water sources, possibly due to higher nutrient availability (20). Considering that this amoeba poses a significant risk for immunocompromised individuals (50), Continuous monitoring of water sources, including bottled water, and the use of larger water volumes and techniques such as membrane filtration or capsule filtration followed by elution are strongly recommended.

Conclusion

To our knowledge, this is the first study molecularly screenprotozoan parasites in bottled drinking water in Iran, although several previous studies have examined its chemical and microbiological quality parameters. Given that previous studies on bottled water samples have reported contamination with various pathogens, including *Escherichia coli*, *Salmonella* species, *Vibrio cholerae*, and *Pseudomonas aeruginosa* (51–53), continued monitoring of bottled water quality is recommended. Further using larger water volumes and more sensitive methods, such as real-time PCR targeting different parasite genes and advanced water-concentration techniques, may be needed to detect any parasites potentially present at low levels.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical approval

Not applicable.

Artificial Intelligence Statement

During the preparation of this work, Grammarly was used to improve readability and to correct grammatical, punctuation, and typographical errors. After using this tool, the authors reviewed and edited all content as needed and take full responsibility for the publication's content.

References

1. Reynolds KA, Mena KD, Gerba CP. Risk of waterborne illness via drinking water in the United States. Rev Environ Contam Toxicol 2008:117-58. https://doi.org/10.1007/978-0-387-71724-1_4
2. Hoseinzadeh E, Rostamian A, Razaghi M, Wei C. Waterborne transmission of protozoan parasites: A review of water resources in Iran-An update 2020. Desalin Water Treat. 2021;213:91-105. https://doi.org/10.5004/dwt.2021.26678
3. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol. 2010;124(1):80-9. https://doi.org/10.1016/j.exppara.2009.03.018
4. Egan S, Barbosa AD, Feng Y, Xiao L, Ryan U. Critters and contamination: zoonotic protozoans in urban rodents and water quality. Water Res. 2024;251:121165. https://doi.org/10.1016/j.watres.2024.121165

Corrected Proof

5. Sazmand A, Rasooli A, Nouri M, Hamidinejat H, Hekmatimoghaddam S. Prevalence of *Cryptosporidium* spp. in camels and involved people in Yazd Province, Iran. Iran J Parasitol. 2012;7(1):80-4.
6. Wopereis DB, Bazzo ML, de Macedo JP, Casara F, Golfeto L, Venancio E. Free-living amoebae and their relationship to air quality in hospital environments: characterization of *Acanthamoeba* spp. obtained from air-conditioning systems. Parasitology. 2020;147(7):782-90. https://doi.org/10.1017/s0031182020000487
7. Bahrami S, Avarseji AA, Zarei M, Haji Hajikolaei MR. First report of isolation and characterization of *Acanthamoeba* spp. from the milk used for calf feeding. Acta Parasitol. 2024;69(1):609-615. https://doi.org/10.1007/s11686-024-00805-2
8. Kalra SK, Sharma P, Shyam K, Tejan N, Ghoshal U. *Acanthamoeba* and its pathogenic role in granulomatous amebic encephalitis. Exp Parasitol. 2020;208:107788. https://doi.org/10.1016/j.exppara.2019.107788
9. Firoozi Z, Sazmand A, Zahedi A, Astani A, Fattahi-Bafghi A, Kiani-Salmi N, et al. Prevalence and genotyping identification of *Cryptosporidium* in adult ruminants in central Iran. Parasit Vectors. 2019;12(1):510. https://doi.org/10.1186/s13071-019-3759-2
10. Ghafari R, Rafiei A, Tavalla M, Choghakabodi PM, Nashibi R, Rafiei R. Prevalence of *Cryptosporidium* species isolated from HIV/AIDS patients in southwest of Iran. Comp immunol Microbiol Infect Dis. 2018;56:39-44. https://doi.org/10.1016/j.cimid.2017.12.002
11. Bullumulla S, Xiao L, Feng Y, Ash A, Ryan U, Barbosa AD. Update on transmission of zoonotic *Giardia* in cattle. Trends Parasitol. 2025; 41(3):210-21. https://doi.org/10.1016/j.pt.2025.01.003
12. Zahedi A, Field D, Ryan U. Molecular typing of *Giardia duodenalis* in humans in Queensland–first report of Assemblage E. Parasitology. 2017;144(9):1154-61. https://doi.org/10.1017/s0031182017000439
13. Wielinga C, Williams A, Monis P, Thompson RA. Proposed taxonomic revision of *Giardia duodenalis*. Infect Genet Evol. 2023;111:105430. https://doi.org/10.1016/j.meegid.2023.105430
14. Asghari A, Mahdavi F, Karimi K, Mohammadi MR, Shamsi L, Asgari Q, et al. Molecular epidemiology and multilocus genotyping of *Giardia duodenalis* in individuals attending major public hospitals in Shiraz, southwestern Iran: A public health concern. Parasite Epidemiol Control. 2024;25:e00354. https://doi.org/10.1016/j.parepi.2024.e00354
15. Ryan UM, Feng Y, Fayer R, Xiao L. Taxonomy and molecular epidemiology of *Cryptosporidium* and *Giardia*–a 50 year perspective (1971–2021). Int J Parasitol. 2021;51(13-14):1099-119. https://doi.org/10.1016/j.ijpara.2021.08.007
16. Dixon BR. *Giardia duodenalis* in humans and animals–Transmission and disease. Res Vet Sci. 2021;135:283-289. https://doi.org/10.1016/j.rvsc.2020.09.034
17. Zahedi A, Ryan U, Rawlings V, Greay T, Hancock S, Bruce M, et al. *Cryptosporidium* and *Giardia* in dam water on sheep farms–An important source of transmission? Vet Parasitol. 2020;288:109281. https://doi.org/10.1016/j.vetpar.2020.109281
18. Mahmoudi M, Ashrafi K, Abedinzadeh H, Tahvildar-Bideruni F, Haghighi A, Bandehpour M, et al. Development of sensitive detection of *Cryptosporidium* and *Giardia* from surface water in Iran. Iran J Parasitol. 2011;6(3):43-51.
19. Moreira N, Bondelind M. Safe drinking water and waterborne outbreaks. J Water Health. 2017;15(1):83-96. https://doi.org/10.2166/wh.2016.103
20. Siddiqui R, Khan NA. Biology and pathogenesis of *Acanthamoeba*. Parasit Vectors. 2012;5:6. https://doi.org/10.1186/1756-3305-5-6
21. Fuerst PA, Booton GC. Species, sequence types and alleles: dissecting genetic variation in *Acanthamoeba*. Pathogens. 2020;9(7):534. https://doi.org/10.3390/pathogens9070534
22. Badirzadeh A, Niyyati M, Babaei Z, Amini H, Badirzadeh H, Rezaeian M. Isolation of free-living amoebae from sarein hot springs in Ardebil province, Iran. Iran J Parasitol. 2011;6(2):1-8.
23. Chaúque BJM, da Silva TCB, Dos Santos DL, Benitez GB, Chaúque LGH, Benetti AD, et al. Global prevalence of free-living amoebae in solid matrices - A systematic review with meta-analysis. Acta Trop. 2023;247:107006. https://doi.org/10.1016/j.actatropica.2023.107006
24. Chaúque BJM, Dos Santos DL, Anvari D, Rott MB. Prevalence of free-living amoebae in swimming pools and recreational waters, a systematic review and meta-analysis. Parasitol Res. 2022;121(11):3033-50. https://doi.org/10.1007/s00436-022-07631-3

Corrected Proof

25. Ghasemi AA, Akhlaghi E, Babaei Z, Zia-Ali N, Rostami S, Borhani M, et al. Molecular identification of potentially pathogenic free-living amoebae in environmental samples from urban areas of Kerman, Southeastern Iran. Parasite Epidemiol Control. 2026;32:e00476. https://doi.org/10.1016/j.parepi.2026.e00476
26. Kialashaki E, Daryani A, Sharif M, Gholami S, Dodangeh S, Moghddam YD, et al. *Acanthamoeba* spp. from water and soil sources in Iran: a systematic review and meta-analysis. Ann Parasitol. 2018;64(4):285–97. https://doi.org/10.17420/ap6404.163
27. Zahedi A, Gofton AW, Greay T, Monis P, Oskam C, Ball A, et al. Profiling the diversity of *Cryptosporidium* species and genotypes in wastewater treatment plants in Australia using next generation sequencing. Sci Total Environ. 2018;644:635-48. https://doi.org/10.1016/j.scitotenv.2018.07.024
28. Sazmand A, Moradi L, Ghadirian N, Rafiei P, Edrissian M, Sadeghi R, et al. Natural does not always mean safe: first molecular detection of *Cryptosporidium* and *Acanthamoeba* in Iranian spring waters. Sci Rep. 2026. https://doi.org/10.1038/s41598-026-71770-1
29. Yang R, Jacobson C, Gardner G, Carmichael I, Campbell AJ, Ryan U. Development of a quantitative PCR (qPCR) for *Giardia* and analysis of the prevalence, cyst shedding and genotypes of *Giardia* present in sheep across four states in Australia. Exp Parasitol. 2014;137:46-52. https://doi.org/10.1016/j.exppara.2013.12.004
30. Silva SO, Richtzenhain LJ, Barros IN, Gomes AM, Silva AV, Kozerski ND, et al. A new set of primers directed to 18S rRNA gene for molecular identification of *Cryptosporidium* spp. and their performance in the detection and differentiation of oocysts shed by synanthropic rodents. Exp Parasitol. 2013;135(3):551-7. https://doi.org/10.1016/j.exppara.2013.09.003
31. Zahedi A, Phasey J, Boland T, Ryan U. First report of *Cryptosporidium* species in farmed and wild buffalo from the Northern Territory, Australia. Parasitol Res. 2016;115(3):1349-53. https://doi.org/10.1007/s00436-016-4901-0
32. Schroeder JM, Booton GC, Hay J, Niszl IA, Seal DV, Markus MB, et al. Use of subgenic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of *Acanthamoebae* from humans with keratitis and from sewage sludge. J Clin Microbiol. 2001;39(5):1903-11. https://doi.org/10.1128/jcm.39.5.1903-1911.2001
33. Karimi A, Kheirandish F, Mamaghani AJ, Taghipour N, Mousavi SF, Aghajani A, et al. Identification and genotyping of *Acanthamoeba* spp. in the water resources of western Iran. Parasite Epidemiol Control. 2023;22:e00308. https://doi.org/10.1016/j.parepi.2023.e00308
34. Sharafi R, Aghapour AA, Aminpour A. Prevalence and molecular analysis of *Cryptosporidium* spp. collected from surface water. Environ Monit Assess. 2023;195(4):499. https://doi.org/10.1007/s10661-023-11097-w
35. Javanmard E, Niyyati M, Taghipour A, Fatemi M, Mirjalali H, Karanis P. Isolation and identification of potentially pathogenic free-living amoeba in drinking, surface, and stagnant water sources from Alborz Province, Iran. J Water Health. 2022;20(4):620-9. https://doi.org/10.2166/wh.2022.229
36. Kamrani F, Nouri SM, Heidari A, Dehghanifard E, Miahipour A, Sezavar M. The Occurrence of *Cryptosporidium* and *Giardia* parasites in drinking water resources of Alborz province, the Central Part of Iran in 2018. Int J Enteric Pathog. 2021;9(1):26-30. https://doi.org/10.34172/ijep.2021.06
37. Ligda P, Claerebout E, Kostopoulou D, Zdragas A, Casaert S, Robertson LJ, et al. *Cryptosporidium* and *Giardia* in surface water and drinking water: Animal sources and towards the use of a machine-learning approach as a tool for predicting contamination. Environ Pollut. 2020;264:114766. https://doi.org/10.1016/j.envpol.2020.114766
38. Medema G, Teunis P, Blokker M, Deere D, Davison A, Charles P, et al. WHO guidelines for drinking water quality: *Cryptosporidium*. WHO, New York. 2006;138.
39. Franco R, Cantusio Neto R. Occurrence of cryptosporidial oocysts and *Giardia* cysts in bottled mineral water commercialized in the city of Campinas, State of Sao Paulo, Brazil. Mem Inst Oswaldo Cruz. 2002;97:205-7. https://doi.org/10.1590/s0074-02762002000200012
40. Hawash Y, Ghonaim M, Hussein Y, Alhazmi A, Alturkistani A. Identification of *Giardia lamblia* and the human infectious-species of *Cryptosporidium* in drinking water resources in Western Saudi Arabia by nested-PCR assays. Trop Biomed. 2015;32(2):216-24.
41. Saeedi E, Joneidi Jafari N, Salehzadeh A. Detection of *Giardia lamblia* cysts in surface waters of rasht city, Iran. J Med Microbiol Infect Dis. 2018;6(1):8-12. http://dx.doi.org/10.29252/JoMMID.6.1.8

Corrected Proof

42. Karanis P, Sotiriadou I, Kartashev V, Kourenti C, Tsvetkova N, Stojanova K. Occurrence of *Giardia* and *Cryptosporidium* in water supplies of Russia and Bulgaria. Environ Res. 2006;102(3):260-71. https://doi.org/10.1016/j.envres.2006.05.005
43. Rivera F, Galván M, Robles E, Leal P, González L, Lacy AM. Bottled mineral waters polluted by protozoa in Mexico. J Protozool. 1981;28(1):54-6. https://doi.org/10.1111/j.1550-7408.1981.tb02803.x
44. Salazar HC, Moura H, Ramos RT. Isolamento de amebas de vida livre a partir de água mineral engarrafada. Rev Saude Publica. 1982;16:261-7. https://doi.org/10.1590/s0034-89101982000500001
45. Maschio VJ, Chies F, Carlesso AM, Carvalho A, Rosa SP, Van Der Sand ST, et al. *Acanthamoeba* T4, T5 and T11 isolated from mineral water bottles in southern Brazil. Curr Microbiol. 2015;70(1):6-9. https://doi.org/10.1007/s00284-014-0676-7
46. Motavallihaghi M, Karami M, Rahmati K, Ameri P, Etemadifar F, Maghsood AH. Distribution of *Acanthamoeba* genotypes in Iran: a systematic review and meta-analysis. South Asia J Parasitol. 2019;3:1-10.
47. Khan NA, Siddiqui R. Predator vs aliens: bacteria interactions with *Acanthamoeba*. Parasitology. 2014;141(7):869-74. https://doi.org/10.1017/s003118201300231x
48. Abd H, Weintraub A, Sandström G. Intracellular survival and replication of *Vibrio cholerae* O139 in aquatic free- living amoebae. Environ Microbiol. 2005;7(7):1003-8. https://doi.org/10.1111/j.1462-2920.2005.00771.x
49. Greub G, Raoult D. Microorganisms resistant to free-living amoebae. Clin Microbiol Rev. 2004;17(2):413-33. https://doi.org/10.1128/cmr.17.2.413-433.2004
50. Marciano-Cabral F, Cabral G. *Acanthamoeba* spp. as agents of disease in humans. Clin Microbiol Rev. 2003;16(2):273-307. https://doi.org/10.1128/cmr.16.2.273-307.2003
51. Bonyadian M, Moshtaghi H, Nadi H. PCR detection of *Vibrio cholerae*, *Escherichia coli*, and *Salmonella* sp. from bottled drinking water in Iran. J Infect Dev Ctries. 2018;12(09):700-5. https://doi.org/10.3855/jidc.10160
52. Kouchesfahani MM, Alimohammadi M, Nodehi RN, Aslani H, Rezaie S, Asadian S. *Pseudomonas aeruginosa* and heterotrophic bacteria count in bottled waters in Iran. Iran J Public Health. 2015;44(11):1514-19.
53. Schalli M, Platzer S, Haas D, Reinthaler FF. The behaviour of *Escherichia coli* and *Pseudomonas aeruginosa* in bottled mineral water. Heliyon. 2023;9(11). https://doi.org/10.1016/j.heliyon.2023.e21634

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