



Assessment of Bacterial Contamination in Ice Used in Drinking Water

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Abstract

This study aims to assess bacterial contamination in ice intended for use in drinking water and to examine factors associated with microbiological quality. A cross-sectional laboratory-based design is proposed in which ice samples are collected from selected food-service establishments, retail outlets, and/or institutional settings. Samples will be analyzed using validated microbiological methods for indicator organisms and, where laboratory capacity permits, selected pathogenic bacteria. Environmental and handling information will also be recorded, including ice source, storage conditions, scoop handling, container cleanliness, and water source. Descriptive statistics, correlation analysis, and multiple regression will be used to explore relationships between bacterial counts and handling or environmental factors. The study may provide evidence to support safer ice production, storage, and handling practices.

Keywords: Ice; drinking water; bacterial contamination; microbiological quality; coliform bacteria; *Escherichia coli*; food safety; water safety.

Introduction

Ice added to drinking water is generally expected to be microbiologically safe because it is consumed directly and often receives no further treatment before ingestion. Contamination may occur through the source water, inadequately cleaned ice-making equipment, contaminated storage bins, repeated hand or scoop contact, poor sanitation, or cross-contamination from the surrounding environment. Indicator organisms such as total coliforms and *Escherichia coli* can provide information about hygienic quality and possible fecal contamination. Assessing the microbiological quality of drinking ice can help identify weaknesses in production and handling practices and support targeted sanitation and food-safety interventions. The study therefore proposes laboratory testing of ice samples together with structured observation of relevant environmental and handling factors.

Research Method

Study design: A cross-sectional laboratory-based analytical study.

Research setting: Selected restaurants, cafés, food-service establishments, retail ice suppliers, and/or institutional facilities within the defined study area. The final geographic scope should be specified before sampling begins.

Study population: Ice intended for direct consumption or for addition to drinking water in eligible establishments.

Study sample: Approximately 100-200 independent ice samples are proposed, selected using a stratified sampling approach by establishment type and ice source where feasible. Only one clearly identified analytical sample should be linked to each sampling record.

Data Collection Tool and Data Analysis

Data collection will use a standardized sampling form documenting date, establishment type, ice source, storage method, apparent cleanliness of the bin or container, scoop availability and storage, handling practices, and relevant temperature or environmental observations. Laboratory testing should follow validated standard methods and the procedures approved by the participating microbiology laboratory. Outcomes may include aerobic bacterial counts and detection or enumeration of indicator organisms such as total coliforms and *E. coli*; testing for additional organisms should depend on the study protocol and laboratory capability.

Descriptive analysis will summarize detection frequencies and bacterial counts. Highly skewed colony-count data may require logarithmic transformation or non-parametric tests. Associations may be assessed using Spearman or Pearson correlation as appropriate. Multiple linear regression may be used for transformed continuous bacterial counts; logistic regression is preferable for binary contamination outcomes.

Inclusion and Exclusion Criteria

- Inclusion: Ice intended for human consumption or addition to drinking water; samples collected from eligible establishments during the defined study period; samples collected and transported according to the approved sampling protocol.
- Exclusion: Decorative or industrial ice not intended for consumption, samples with broken containers or compromised labels, samples with documented transport/storage protocol violations, duplicate samples not defined by the sampling plan, and samples with insufficient volume for required testing.

Procedure

- Obtain required institutional, laboratory, and site permissions.
- Train data collectors in aseptic sampling and standardized observation.
- Assign a unique code to each sample and complete the sampling form.

- Collect ice aseptically in sterile containers without unnecessary contact.
- Transport samples under the temperature and time conditions specified by the validated laboratory protocol.
- Allow the ice to melt under controlled laboratory conditions according to the selected standard method.
- Perform microbiological testing using validated methods and appropriate positive/negative quality controls.
- Record, verify, clean, and statistically analyze the laboratory and observational data.

Pilot Study

A pilot phase involving approximately 10-15 samples from establishments outside the final analytical sample will test the sampling form, labeling system, transport logistics, laboratory workflow, and data-entry procedures. The pilot will be used to correct operational problems before full-scale sampling.

Ethical Considerations

The study primarily evaluates environmental/food samples rather than human participants. Nevertheless, approval or exemption should be obtained from the relevant research ethics or institutional review body as required. Permission from participating establishments should be documented. Establishment identities should be coded and results reported in a manner that avoids unnecessary disclosure. Laboratory biosafety procedures must be followed for culture and handling of microorganisms.

Variables and Measures

- Primary dependent variables: Bacterial concentration/count and presence or absence of predefined indicator organisms.
- Potential microbiological indicators: aerobic plate count, total coliforms, and *E. coli*, according to the validated laboratory protocol.
- Independent variables: establishment type, ice source, storage method, visible container cleanliness, scoop handling, water source, sampling time, and selected environmental conditions.
- Binary contamination outcome: sample classified as meeting or not meeting the predefined microbiological criterion selected before analysis.
- Continuous outcome: bacterial count expressed in the unit required by the chosen standard method, with transformation if necessary for statistical modeling.

Results

The following results are simulated examples only and are not actual laboratory findings:

Indicator	Simulated Result	Interpretation
Number of samples	150	Independent drinking-ice samples
Detectable total coliforms	18%	Suggests hygiene concerns in a subset of samples
Detectable <i>E. coli</i>	4%	Requires careful interpretation and confirmation using the validated method
Higher bacterial load with visibly unclean storage	Observed in simulated dataset	Potential handling/storage association
Samples from regularly sanitized machines	Lower median count in simulated dataset	Potential protective factor

Correlation Analysis and Multiple Regression Analysis

Correlation analysis (simulated): A positive association was observed between poorer storage-hygiene scores and log-transformed bacterial counts (Spearman $\rho=0.43$, $p<0.001$). More frequent documented cleaning was negatively associated with bacterial counts ($\rho=-0.36$, $p<0.001$). These values are illustrative only.

Multiple regression (simulated): In an illustrative model using log-transformed bacterial count as the dependent variable, poor storage-bin hygiene ($\beta=0.29$, $p=0.001$) and unsafe scoop handling ($\beta=0.22$, $p=0.008$) were associated with higher bacterial levels, while documented regular machine sanitation was associated with lower levels ($\beta=-0.25$, $p=0.003$). The illustrative adjusted R^2 was 0.34. If the final outcome is contamination present/absent, multiple logistic regression should be used instead.

Conclusion / Final Summary

The proposed study can provide a structured assessment of bacterial contamination in ice used for drinking water and identify handling and sanitation factors associated with poorer microbiological quality. Findings from an implemented study could support improvements in ice-machine cleaning, storage-bin sanitation, scoop handling, staff training, and routine monitoring. The numerical findings in this document are simulated and must be replaced with actual laboratory results before scientific reporting.

References

1. Food and Agriculture Organization of the United Nations, & World Health Organization. (2023). General principles of food hygiene (Codex Alimentarius Code of Practice No. CXC 1-1969). <https://doi.org/10.4060/cc6125en>
2. Gaglio, R., Francesca, N., Di Gerlando, R., Mahony, J., De Martino, S., Stucchi, C., Moschetti, G., & Settanni, L. (2017). Enteric bacteria of food ice and their survival in alcoholic beverages and soft drinks. *Food Microbiology*, 67, 17–22. <https://doi.org/10.1016/j.fm.2017.04.020>

3. Gerokomou, V., Voudarou, C., Vatopoulos, A., Velonakis, E., Rozos, G., Alexopoulos, A., Plessas, S., Stavropoulou, E., Bezirtzoglou, E., Demertzis, P. G., & Akrida-Demertzi, K. (2011). Physical, chemical and microbiological quality of ice used to cool drinks and foods in Greece and its public health implications. *Anaerobe*, 17(6), 351–353. <https://doi.org/10.1016/j.anaerobe.2011.06.005>
4. Hampikyan, H., Bingol, E. B., Cetin, O., & Colak, H. (2017). Microbiological quality of ice and ice machines used in food establishments. *Journal of Water and Health*, 15(3), 410–417. <https://doi.org/10.2166/wh.2017.159>
5. International Organization for Standardization. (1999). Water quality—Enumeration of culturable micro-organisms—Colony count by inoculation in a nutrient agar culture medium (ISO Standard No. 6222:1999). <https://www.iso.org/standard/28960.html>
6. International Organization for Standardization. (2006). Water quality—Sampling for microbiological analysis (ISO Standard No. 19458:2006). <https://www.iso.org/standard/33845.html>
7. International Organization for Standardization. (2014). Water quality—Enumeration of *Escherichia coli* and coliform bacteria—Part 1: Membrane filtration method for waters with low bacterial background flora (ISO Standard No. 9308-1:2014). <https://www.iso.org/standard/55832.html>
8. Lipps, W. C., Braun-Howland, E. B., & Baxter, T. E. (Eds.). (2023). *Standard methods for the examination of water and wastewater* (24th ed.). American Public Health Association, American Water Works Association, & Water Environment Federation.
9. Nichols, G., Gillespie, I., & De Louvois, J. (2000). The microbiological quality of ice used to cool drinks and ready-to-eat food from retail and catering premises in the United Kingdom. *Journal of Food Protection*, 63(1), 78–82. <https://doi.org/10.4315/0362-028X-63.1.78>
10. U.S. Food and Drug Administration. (2022). Food code 2022. <https://www.fda.gov/food/fda-food-code/food-code-2022>
11. World Health Organization. (2026). Guidelines for drinking-water quality: Fourth edition incorporating the first, second and third addenda. <https://www.who.int/publications/i/item/9789240121225>