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Peer Review

Review of: "Monitoring the Microbial Quality of Water"

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Reviewer Comments

General Comment

The manuscript provides a comprehensive overview of microbial water quality monitoring techniques, both culture-based, Optical density (turbidity) measurements, and molecular techniques, and offers an extensive discussion on environmental considerations and treatment methods. The inclusion of both conventional and advanced technologies adds depth, and the integration of current literature strengthens the report's validity. However, a few areas require clarification, restructuring, or expansion to enhance coherence and scientific rigor.

The Manuscript:

It is a **review manuscript**, not a research article.

The Title:

The current title is generally appropriate; however, to better reflect the comprehensive scope covering both detection methods and treatment strategies, a more descriptive title such as **"Comprehensive Approaches to Microbial Water Quality Assessment and Treatment: Culture-Based and Molecular Methods"** could enhance clarity and attract a wider audience.

Abstract Evaluation:

The abstract covers the main themes of the manuscript but could be improved by explicitly stating the key findings or recommendations. Including a brief mention of the limitations of current methods and the importance of emerging molecular techniques would add value. Also, a sentence summarizing the implications for water treatment practice would make it more comprehensive.

Part 1: Culture-Independent Molecular Methods

 doi.org/10.32388/34BG31

- The section effectively introduces various molecular techniques such as 16S rRNA sequencing, FISH, qPCR, and microfluidics.
- Accurate description of the primers used and the bacterial genera detected.
- Good linkage between fecal indicators and water quality.

In my opinion, some points are required for improvement:

1. **Scope Clarity**: While the methods are listed, the contextual differences in their application (e.g., surveillance, outbreak investigation, routine testing) could be clarified.
2. **Comparative Insight**: A brief comparative table or summary outlining the advantages and limitations of each method would help readers understand when to use which method.
3. **Grammar**: Some sentences are slightly fragmented or contain minor typographical errors (e.g., missing conjunctions or prepositions).

Part 2: Advanced Analytical Techniques

- This part introduces emerging technologies like stable isotope probing and flow cytometry, which are highly relevant for research-based water monitoring.
- The detail on SIP's use of dual isotopes is commendable and reflects current scientific practices.

In my opinion, some points are required for improvement:

1. **Flow of Information**: The explanation jumps quickly between techniques. Consider grouping or sequencing methods based on their application (e.g., cellular tracking, functional activity, population dynamics).
2. **Operational Feasibility**: Discussion on the practical feasibility of using these methods in resource-limited settings would add practical value.
3. **References Needed**: Some advanced techniques lack in-text citations or source validation.

Part 3: Culture-Based Detection Methods

- Provides a good explanation of traditional microbiological techniques such as MPN and membrane filtration.
- Incorporates standards and incubation conditions, enhancing scientific completeness.

In my opinion, some points are required for improvement:

1. **Integration with Part 1**: There is some redundancy between this and Part 1. Consider cross-referencing or integrating these sections to avoid duplication.
2. **Specificity of Media**: Mention of the specific types of selective media used for pathogens would improve technical clarity.
3. **Contextual Limitation**: A note on the time delays or accuracy concerns of culture-based methods would offer a balanced perspective.

Part 4: Environmental and Practical Factors in Assessment

- Recognizes key variables like sampling scale, environmental influence, and the expected use of water, which are often overlooked in purely technical reviews.
- Notes the economic and logistical implications of different sampling frequencies and methods.

In my opinion, some Points are required for Improvement:

1. **Scientific Rigor**: While comprehensive, this section reads more like guidance than analysis. It could benefit from examples or case studies.
2. **Terminology Clarification**: Terms like "curing of water" are vague and could be replaced with more accurate terms like "disinfection" or "treatment."
3. **Redundancy**: The "scale" discussion slightly overlaps with earlier parts on sampling frequency and should be consolidated.

Part 5: Water Treatment and Disinfection Methods

- Very well-developed section detailing chlorination, its mechanisms, limitations, and byproducts.
- Describes alternative treatment methods, including newer technologies like Sanosil and potassium permanganate.
- Scientific accuracy is high, especially in terms of virus resistance to chlorination and the chemical dynamics of THM and HAA formation.

In my opinion, some Points are required for Improvement:

1. **Structure**: This part is dense and could be better presented in sub-sections with bullet points or tables for chlorine types, viral resistance, and comparative effectiveness of treatments.
2. **Chemical Risk Clarity**: The discussion on THMs and HAAs is excellent but should link more explicitly to public health guidelines or WHO thresholds.

3. **References to WHO Standards**: Incorporating WHO or EPA chlorination limits and guidelines would strengthen this section's authority.

References:

The references cited are relevant and mostly recent, supporting the statements made throughout the text. To enhance the manuscript, I suggest including additional recent reviews or guideline documents from authoritative bodies such as the U.S. EPA's Water Quality Standards or recent journal reviews on qPCR applications in water microbiology.

Conclusion

This manuscript is an informative and timely contribution to the field of water microbiology. With some structural refinements, clearer sub-sectioning, and alignment of overlapping content, the document can be significantly improved. The reviewer encourages publication following moderate revision.

Plagiarism

Estimates are that about 2 billion people use contaminated drinking water, and about

502,000 die due to waterborne diarrheal infections yearly[2].

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alone kills more than those killed by malaria, AIDS, and measles combined[1].

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Diarrhoea stands 2nd in killing children after pneumonia, and it

https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(13)60222-6/abstract

hydrophila in mares at a thoroughbred stable led to infertility and abortions in mares[7]

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Culturing means facilitating microbes by providing a nutrient solution and growing

them in the appropriate environment at an optimal temperature.

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The test should also be good enough to be integrated into education programs to

mobilize stakeholders.

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In drinking water, no Giardia lamblia, Cryptosporidium, Legionella, and enteric viruses are detectable in a 100 mL water sample.

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Besides, sulphite- reducing Clostridia spore count using the roll tubes, heterotrophic bacterial count as an indicator of total bacterial load, is done through spread agar methods.

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Mark Sobsey, of the University of North Carolina at Chapel Hill, outlined the ideal characteristics of a microbial test for water quality monitoring on a limited budget:

https://blogs.worldbank.org/en/water/how-test-water-quality-low-cost-low-tech-options-microbial-testing

It should be portable, low-skill, self-contained, lab-free, and electricity-free.

https://blogs.worldbank.org/en/water/how-test-water-quality-low-cost-low-tech-options-microbial-testing

Simple Tests: Samples are tested in tubes, bags, or plates with a nutrient solution, and positive growth is indicated by a color change of the indicator.

https://blogs.worldbank.org/en/water/how-test-water-quality-low-cost-low-tech-options-microbial-testing

This "holy grail" test does not yet exist.

https://blogs.worldbank.org/en/water/how-test-water-quality-low-cost-low-tech-options-microbial-testing

It should be available globally at a cost of less than $0.10 (USD) per test, and it should be easy to interface with data reporting and communications technologies.

https://blogs.worldbank.org/en/water/how-test-water-quality-low-cost-low-tech-options-microbial-testing

It can also measure the fraction of degradation directly caused by microbial activity during bioremediation.

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Thus, a practical solution is to test for an “indicator organism,” which may act as a sign of fecal contamination, like E. coli.

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The PA test is suitable for screening in situations where the chances of microbial contamination are minimal, such as deep groundwater.

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The changes in color of the media due to the reduction of the dye indicate the presence of microbes, indicating microbial contamination.

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You can convert the number of positives to a statistical estimate of bacterial concentration using MPN tables[8].

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The positives are indicated by a color change in the media.

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Bacteroidetes (including genera of Bacteroides, Parabacteroides, Prevotella, and Alistipes), and Bacteroides (B.

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The lter membranes are used for the extraction of DNA.

https://sciencecity.unionstation.org/2020/04/29/extracting-dna

c. Flow cytometry: This method offers rapid and accurate counting of bacteria using a uorescent dye

https://www.researchgate.net/publication/391806204_Monitoring_Microbial_Quality_of_Water

The membrane retains almost all the bacteria in the sample as the water passes through it.

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However, these tests are a bit labour-intensive and time-consuming.

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electron receptors in their metabolic pathways), a metallic sheen, slimy coatings that appear yellow, brown, red, and or black, and brown foam on the surface or water colour.

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faecalis and protects them from the detrimental effect of chlorination/ disinfection.

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Factors necessary to be considered while assessing water quality:

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continuous sampling, regular and irregular sampling) can affect the outcome of the testing.

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They are often used for removing heavy metals and sulphate from acid rock drainage and other wastewater.

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d. Counting sulphate reducing bacteria in water: Sulphate-reducing bacteria (SRB) belong to a diverse

https://www.researchgate.net/publication/391806204_Monitoring_Microbial_Quality_of_Water

Chlorination deactivates microorganisms through damaging the cell wall, altering the permeability of the cell, altering the cell protoplasm, inhibiting enzyme activity so it'sunable to use its food to produce

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energy, and inhibiting cell reproduction due to its strong oxidizing potential.

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The cyclops carrying embryos of Dracunculus medinensis (cause of dracunculiasis or

Guinea-worm disease) are common in open wells, harvested rain water, and water from water reservoirs used as the

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Chlorination can be done at several stages of the water treatment process.

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An ideal method of water disinfection/treatment should not leave any noxious (hazardous to health) byproducts behind after treatment.

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and run-off water accumulated in ponds, lakes, and other surface water reservoirs used as a source of drinking water).

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THM is a carcinogen and should not be present in water beyond 0.1 ppm levels.

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However, if excess is added, it causes pink discoloration of water and an increase in manganese concentration; the permitted limit for manganese in water is 0.05 ppm.

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used at a 1-3 ppm level; for open well water, it is used at a 1000-2000 ppm level.

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It can remove impurities like off-taste and odor-causing substances, limit the amount of THM and HAA formation (byproducts of halogenation), as well as soluble manganese and iron.

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To ensure freedom from causal agents of amoebiasis, giardiasis, and cryptosporidiosis, microscopic examination after concentration is recommended.

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Even chlorination produces trihalomethane (THM) and haloacetic acid (HAA) if the

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water has higher values of organic contaminants like humic acid and fulvic acid (mainly present in rainwater harvesters

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Sanosil, a technology of water treatment, uses a combination of hydrogen peroxide and silver to disinfect water.

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Pre-chlorination is done almost immediately after the water enters the treatment facility, where the chlorine is added directly to the raw water to eliminate aquatic ora and fauna.

https://chunkewatertreatment.com/chlorination-in-water-treatment-the-essential-guide

Declarations

Potential competing interests: No potential competing interests to declare.