

BACTERIAL GROWTH ON THE SURFACE OF WATER CONTAINER: EVALUATING THE IMPACT OF HYGIENE PRACTICES

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Water container contamination —and can simple cleaning methods make a difference?”

INVESTIGATION

We would like to investigate the relationship between hygiene practices and bacterial growth on the surfaces of water containers. By comparing samples from containers cleaned under different conditions, we evaluate how the frequency and method of cleaning influence bacterial growth.

AIM

We aimed to determine the bacterial growth on the surface of the 15 water containers and estimate the different hygiene methods.

OBJECTIVE

- To determine bacterial growth on the surface of water containers.
- To compare bacterial contamination under different cleaning practices.
- To evaluate the effectiveness of common sanitation methods in reducing bacterial presence.

RESEARCH QUESTION

What is a suitable method for cleaning the surface area of a water container?

BACKGROUND RESEARCH

Water containers are commonly used in households and public areas to store drinking water in Mongolian rural areas. Despite their convenience, these containers can serve as breeding grounds for harmful microorganisms, especially when hygiene is neglected. This study aims to assess bacterial growth on the surfaces of such containers and examine how different hygiene practices impact microbial loading.

Last year, more than 41,051 infectious diseases were recorded in Mongolia, and 21,019 cases were in the Ulaanbaatar rural area. In 2023, more than 11,000 infectious diseases were recorded that were caused by hand, water-poor hygiene (1). Therefore, infectious disease and hygiene is closely connected with each other. Risks of contaminated water containers caused a lot of diseases, including gastrointestinal infections (diarrhea, vomiting), skin infections used for washing, and respiratory issues. In addition, there is a need to monitor the quality of water from deep wells in

rural areas, to study certain correlations, to prevent people from various infectious diseases, to protect the environment, to use water resources properly, and to provide citizens with accurate information on drinking water (2).

VARIABLES

Table 1.

Independent variable Washing water Cleaning tissue Cleaning alcohol Dishwashers	We used 4 types of sanitizing methods: 1. Washing water 2. Cleaning tissue 3. Cleaning alcohol 4. Dishwashers to determined bacterial growth
Dependent variable The amounts of microorganisms grown	
Controlled variable 1. Time for bacterial growth 2. Temperature for bacterial growth 3. Material of petri dish 4. Size of petri dish 5. Sterilizing equipment 6. Sterilizing chemicals	
Uncontrolled variable 1. The dirt and bacteria in the water container surface area 2. Condensation on the petri dishes	

APPARATUS AND MATERIALS

- | | |
|---|---|
| <ol style="list-style-type: none"> 1. Incubator 2. Autoclave 3. Petri dishes 4. Distilled water 5. Nutrient agar 6. Sanitizing alcohol 7. Forceps 8. Alcohol burner | <ol style="list-style-type: none"> 9. Beakers 10. 50 ml test tube with cap 11. Measuring cylinder 12. Disposable gloves 13. Weight scale 14. Parafilm paper 15. Scissors 16. 500 ml glass sample container 17. Sterilized cotton swabs |
|---|---|

METHOD AND PROCEDURE

Water containers were selected from various households water contained with signed agreements to use the scientific research case only.

Table 2.

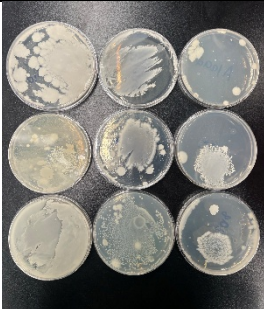
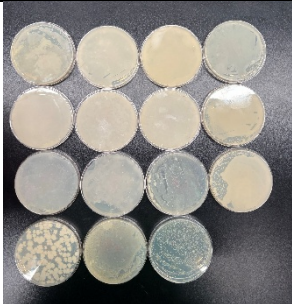
No	Procedure	Picture
1	Collecting sample from surface area of water container, water pipe after cleaned by water, cleaning tissue, cleaning alcohol, and dishwashers. Surface area: 10 cm x 10 cm using handmade card board	
2	Sample brought to lab to make experiments	
3	Using a pre-mixed nutrient agar powder containing peptone and other ingredients, dissolve the recommended amount (typically 23 grams per liter, but check the label) in 1 liter of distilled water, stirring thoroughly while heating until the mixture is fully dissolved. Then autoclave the solution at 120°C for 15 minutes to sterilize it. After cooling to 45-50°C, pour the agar into sterile petri dishes under aseptic conditions and let it solidify before using it for the bacteria growth experiment.	
4	Once the nutrient agar has solidified in the petri dishes, use a sterile inoculation loop to collect a small sample of the bacterial culture, ensuring to flame-sterilize the loop before and after use to maintain aseptic conditions. Gently streak the bacterial sample onto the agar surface in a zigzag pattern, or spread it evenly using a sterile spreader if aiming for a lawn of growth, then close the dish immediately to prevent contamination. Incubate the petri dish upside down at the appropriate temperature (e.g., 37°C for most bacteria) for 48 hours to allow bacterial colonies to grow, checking periodically for growth while maintaining sterile handling practices.	

SAFETY PROCEDURE

1. Personal Protective Equipment (PPE): Wear a lab coat, disposable gloves, safety goggles, and a face mask if needed; remove PPE carefully to avoid contamination.
2. Aseptic Technique: Use sterile tools, flame-sterilize inoculation loops, and keep culture containers closed to prevent contamination and spills.
3. Workspace Preparation: Sterilize equipment and disinfect work surfaces with 70% ethanol before and after experiments.
4. Waste Disposal: Autoclave all biological waste (e.g., petri dishes, pipettes) and dispose of sharps and chemical waste per local regulations.
5. Emergency Response: For spills, cover with disinfectant-soaked absorbent, clean after 10-15 minutes, and autoclave waste; wash exposed skin immediately and report incidents.

RESULT AND DISCUSSION

Table 1. Series of samples from different spot

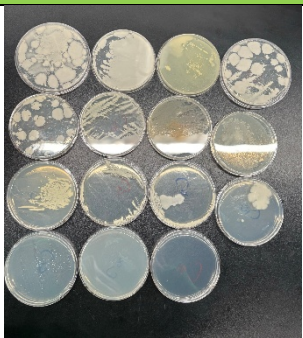
Series 1	Series 2	Series 3	Series 4
			
Sample from water it self	Water pipe from water well	The mouth of a water container	Sample from outside wall of container

The table 1 shows four series of petri dishes containing bacterial cultures, each representing samples from different water sources or surfaces. Series 1 (Sample from water itself): This petri dish shows no visible bacterial colonies. This suggests that the water sample itself has very low or no bacterial contamination, possibly due to it being treated, filtered, or naturally clean. Series 2 (Water pipe from water well): This dish has several distinct bacterial colonies. This indicates moderate bacterial contamination, likely because the water pipe from the well may harbor bacteria due to biofilm formation or stagnant water, which provides a suitable environment for bacterial growth. Series 3 (The mouth of a water container): These dishes show a higher number of bacterial colonies compared to Series 2. The mouth of a water container is often exposed to air, human contact, or environmental contaminants, creating a favorable environment for bacterial growth and leading to increased contamination. Series 4 (Sample from outside wall of container): These dishes show the highest number of bacterial colonies, indicating heavy contamination. The outside wall of the container is exposed to the environment, dust, and potential human handling, making it a hotspot for bacterial accumulation.

Reasoning: The varying levels of bacterial growth across the series reflect the different exposure levels to contaminants. Water itself (Series 1) is typically the cleanest if treated or sourced properly. Pipes (Series 2) can harbor some bacteria due to internal conditions. The mouth of a container (Series 3) and its outer wall (Series 4) are more prone to contamination due to frequent contact with external factors like hands, air, or surfaces, leading to higher bacterial presence.

Afterward, we tested and searched for the most effective cleaning materials to clean the container and minimize contamination.

Table 2. Series of samples using different cleaning methods

Series 5	Series 6	Series 7
		
After cleaning water container with wet tissue.	After cleaning water container with sanitization alcohol.	After cleaning water container with soap.

The experimental results presented in the petri dish series—Series 5, Series 6, and Series 7—demonstrate the efficacy of three distinct cleaning methods (wet tissue, sanitization alcohol, and soap, respectively) in reducing bacterial contamination on a water container. The observed bacterial growth in each series provides insight into the antimicrobial and mechanical properties of these methods. Below is a detailed scientific analysis of the results, including a comparative evaluation and mechanistic reasoning.

Series 5: The petri dishes in Series 5 exhibit a high density of bacterial colonies following cleaning of the water container with a wet tissue. Wet tissues typically consist of water, mild surfactants, and occasionally low concentrations of disinfectants. Their primary function is to mechanically remove visible debris rather than to exert a strong antimicrobial effect. The substantial bacterial growth suggests that this method fails to significantly reduce the viable bacterial population. Wet tissues lack potent antimicrobial agents, allowing surviving bacteria to proliferate in the nutrient-rich environment of the petri dish. Residual moisture left by the wet tissue may facilitate bacterial survival or growth by providing a hydrated environment conducive to cellular processes. The wiping action might redistribute bacteria across the surface rather than effectively remove or kill them.

Series 6: Series 6 shows minimal bacterial growth, with only a few scattered colonies visible on the petri dishes after cleaning with sanitization alcohol. Sanitization alcohol, likely composed of isopropyl alcohol or ethanol (typically 60–70% concentration), is a well-established disinfectant with robust antimicrobial properties. The sparse colony formation indicates a high level of efficacy in reducing bacterial contamination. This can be explained by: Alcohol disrupts bacterial cell membranes by solubilizing lipids and denatures intracellular proteins, leading to rapid cell death. This bactericidal effect

targets a broad spectrum of microorganisms, including vegetative bacteria. The minimal growth suggests that the majority of bacteria were killed, with only a small fraction surviving—possibly due to physical inaccessibility (e.g., within crevices) or intrinsic resistance to alcohol. Alcohol evaporates quickly, leaving no residual moisture to support bacterial regrowth, unlike wet tissues.

Series 7: The petri dishes in Series 7 display moderate bacterial growth, with a colony count greater than Series 6 but less than Series 5, following cleaning with soap. Soap, typically a mixture of surfactants such as sodium lauryl sulfate or fatty acid salts, functions primarily through mechanical removal rather than direct killing of bacteria. The intermediate level of growth reflects a partial reduction in bacterial load, explained by: Soap reduces surface tension and emulsifies lipids and organic matter, dislodging bacteria from the surface and allowing their removal during rinsing. This physical action decreases the number of viable bacteria transferred to the petri dish. Specifically formulated as an antibacterial soap (e.g., containing triclosan), soap does not inherently kill bacteria. Surviving bacteria, not removed during washing, can grow in the culture. If rinsing is incomplete, trace organic material or soap residue might provide nutrients or a neutral environment for bacterial growth, though less so than wet tissue moisture.

Comparative Analysis of Cleaning Efficacy

The differences in bacterial growth across the three series allow for a direct comparison of the cleaning methods' effectiveness:

Series 5 (Wet Tissue) vs. Series 6 (Sanitization Alcohol): Series 5 shows significantly higher bacterial growth than Series 6. Alcohol's bactericidal properties far exceed the wet tissue's mild cleaning action. While alcohol actively kills bacteria through biochemical disruption, the wet tissue relies on limited mechanical removal and lacks sufficient antimicrobial agents, resulting in a higher surviving bacterial population.

Series 5 (Wet Tissue) vs. Series 7 (Soap): Series 5 exhibits more bacterial growth than Series 7. Soap outperforms the wet tissue due to its superior ability to emulsify and physically remove bacteria from the surface. The wet tissue's less effective surfactants and potential to leave moisture behind make it less capable of reducing bacterial load compared to soap's cleansing action.

Series 6 (Sanitization Alcohol) vs. Series 7 (Soap): Series 6 demonstrates less bacterial growth than Series 7. Alcohol's direct antimicrobial activity surpasses soap's primarily mechanical removal mechanism. While soap reduces bacterial presence through washing, alcohol kills bacteria outright, leading to fewer viable cells capable of forming colonies.

Overall Efficacy Ranking and Scientific Rationale: Based on the observed bacterial growth, the cleaning methods can be ranked by their effectiveness in reducing bacterial contamination as follows: sanitization alcohol > soap > wet tissue.

Sanitization Alcohol (Series 6): Most effective due to its rapid, broad-spectrum bactericidal action, disrupting cellular integrity and minimizing viable bacteria.

Soap (Series 7): Moderately effective, relying on emulsification and physical removal to decrease bacterial load, though it lacks the killing power of alcohol.

Wet Tissue (Series 5): Least effective, with minimal antimicrobial activity and potential to leave conditions favorable for bacterial survival or redistribution.

This ranking aligns with the established properties of each method: alcohol's disinfectant capabilities, soap's surfactant-based cleansing, and the wet tissue's limited utility for microbial control. These results underscore the importance of selecting a cleaning method based on its intended purpose—disinfection versus physical cleaning—and the target level of bacterial reduction required.

CONCLUSION

Based on the bacterial growth observed in the petri dishes, the effectiveness of different cleaning methods in reducing bacterial contamination on surfaces, such as a water container, varies significantly. The results clearly demonstrate that sanitization alcohol is the most effective, producing minimal bacterial colonies. This high efficacy can be attributed to alcohol's strong bactericidal properties, which disrupt bacterial cell membranes and denature proteins, effectively killing the bacteria. Soap, while moderately effective, reduces bacterial load primarily through mechanical removal via emulsification and rinsing, making it less potent than alcohol at eliminating bacteria entirely. In contrast, the wet tissue showed the least effectiveness, likely due to its lack of sufficient antimicrobial agents and the potential for residual moisture to support bacterial survival rather than reduce it. These findings highlight the importance of choosing an appropriate cleaning method based on the specific need for microbial control. For situations requiring thorough disinfection—such as sterilizing surfaces—sanitization alcohol emerges as the superior option. Soap remains a practical choice for routine cleaning where complete bacterial elimination is not essential. However, wet tissues should be avoided when the goal is to reduce bacterial contamination, given their limited impact. Practically, these results support the use of alcohol-based sanitizers in settings where hygiene is critical, such as healthcare facilities or food preparation areas. By leveraging the strengths of each cleaning method, bacterial contamination can be minimized effectively, ensuring safer and cleaner environments.

REFERENCES

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