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# Evaluation of Hypochlorite Effectiveness as a Disinfectant Against Aerobic Bacteria

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**A B S T R A C T**

Hypochlorite compounds, including calcium hypochlorite ($Ca(OCl)_2$) and sodium hypochlorite (NaOCl), are widely recognized for their broad-spectrum antimicrobial properties. This study aimed to evaluate the effectiveness of hypochlorite as a disinfectant against aerobic bacteria, providing insights into its application in infection control and water treatment. The study employed the aerobic bacteria number test, also known as the total plate count method, to enumerate bacterial colonies before and after exposure to varying concentrations of hypochlorite solution. Water samples were collected from different sources, including an emergency room floor, a pharmaceutical installation, and a hospital inpatient room. Serial dilutions of the water samples were prepared and plated on nutrient agar, followed by incubation and colony counting to determine the bacterial load. The percentage reduction in bacterial numbers was calculated for each hypochlorite dose. The results demonstrated a significant reduction in bacterial populations following hypochlorite treatment. A dose of 30 mL/5 L (6 µL/mL) reduced the average number of bacteria by 83.29%, A dose of 60 mL/5 L (12 µL/mL) reduced the average number of bacteria by 98.60%, A dose of 120 mL/5 L (24 µL/mL) reduced the average number of bacteria by 99.84%, A dose of 240 mL/5L (48 µL/mL) reduced the average number of bacteria by 99.98%, A dose of 480 mL/5L (96 µL/mL) reduced the average number of bacteria by 100%. The extent of bacterial reduction was directly proportional to the hypochlorite dose, indicating a clear dose-response relationship. In conclusion, this study confirms the efficacy of hypochlorite as a disinfectant against aerobic bacteria. A hypochlorite dose of 480mL/5L (96 µL/mL) effectively achieved complete bacterial elimination under the tested conditions. The results support its use in various applications, including disinfection of surfaces and water purification.

## 1. Introduction

Disinfectants are chemical agents that play a critical role in infection control by reducing the number of viable microorganisms to a level that prevents disease transmission. These agents are essential in various settings, including healthcare facilities, households, and public spaces, where they help maintain hygiene and prevent the spread of infectious diseases. Disinfectants are crucial in breaking the chain of infection, which typically involves a susceptible host, a pathogen, and a mode of transmission. By targeting pathogens present on surfaces, equipment, or in water, disinfectants effectively reduce the risk of infection transmission. Among the various disinfectants available, hypochlorite compounds, such as sodium hypochlorite (NaOCl) and calcium hypochlorite ($Ca(OCl)_2$), have been widely used for decades due to their broad-spectrum antimicrobial activity, cost-effectiveness, and ease of use. These compounds are powerful oxidizing agents that disrupt the cellular processes of microorganisms, leading to their inactivation. The antimicrobial efficacy of hypochlorite compounds, coupled with their affordability and user-friendly nature, has made them a cornerstone of disinfection practices in various settings.[1-3]

Hypochlorite compounds exert their antimicrobial action through the release of hypochlorous acid (HOCl), a strong oxidizing agent that disrupts the cellular processes of microorganisms, leading to their inactivation. HOCl is a highly reactive molecule that can penetrate microbial cells and damage essential cellular components, such as proteins, DNA, and lipids. This damage disrupts critical cellular functions, ultimately leading to the death of the microorganism. Hypochlorite compounds are commonly employed in various settings, including healthcare facilities, households, and industrial processes, for the disinfection of surfaces, equipment, and water. In healthcare facilities, hypochlorite solutions are used to disinfect surfaces, medical equipment, and even skin to prevent the spread of healthcare-associated infections. In households, hypochlorite-based products are commonly used for cleaning and disinfecting kitchens, bathrooms, and other areas to maintain hygiene. Industrial processes also utilize hypochlorite compounds for water purification, ensuring the safety of drinking water and the control of microbial growth in various industrial settings. The efficacy of hypochlorite compounds against a wide range of microorganisms, including bacteria, viruses, and fungi, makes them a valuable tool in infection control and public health. This broad-spectrum activity is particularly important in settings where diverse microbial communities may be present, such as hospitals or water treatment facilities. By effectively targeting a wide range of microorganisms, hypochlorite compounds contribute to maintaining hygiene and preventing the spread of infectious diseases.[4-6]

The use of hypochlorite compounds has significant public health implications, as it helps to control the spread of infectious diseases in communities. By reducing the microbial load in public spaces, water supplies, and healthcare facilities, hypochlorite compounds contribute to creating healthier environments and reducing the burden of infectious diseases. The ability of hypochlorite compounds to inactivate a wide range of pathogens, including those responsible for waterborne and foodborne illnesses, makes them a crucial tool in protecting public health. In addition to their role in public health, hypochlorite compounds also have environmental implications. When used responsibly, hypochlorite compounds can help to disinfect wastewater and prevent the contamination of water bodies. However, it is essential to consider the potential environmental impact of hypochlorite use, as excessive use or improper disposal can lead to the release of harmful byproducts into the environment. Careful management of hypochlorite use and disposal is crucial to minimize any adverse environmental effects. Despite the widespread use of hypochlorite compounds, ongoing research is essential to further understand their efficacy, optimize their use, and minimize any potential risks. Research efforts focus on evaluating the effectiveness of hypochlorite compounds against emerging pathogens, determining the optimal concentrations and exposure times for various applications, and assessing the formation of disinfection byproducts under different conditions. This research is crucial in ensuring the safe and effective use of hypochlorite compounds in infection control and public health.[7-10] This study aimed to evaluate the effectiveness of hypochlorite as a disinfectant against aerobic bacteria using the total plate count method.

## 2. Methods

This study employed a quantitative approach to evaluate the effectiveness of hypochlorite as a disinfectant against aerobic bacteria. The quantitative approach was chosen to provide objective and measurable data on the reduction of bacterial populations after exposure to hypochlorite. This approach allows for statistical analysis and the establishment of a clear dose-response relationship between hypochlorite concentration and bacterial reduction.

The study employed the total plate count method, a standard microbiological technique used to enumerate viable bacteria in a sample. This method is

widely used in microbiology for its accuracy and reliability in determining the number of viable bacteria in a given sample. The total plate count method involves serially diluting the sample and plating it on a nutrient agar medium, followed by incubation and counting the resulting bacterial colonies.

To assess the efficacy of hypochlorite in diverse environments, water samples were collected from three distinct locations: the floor of an emergency room, a pharmaceutical installation, and a hospital inpatient room. These locations were chosen to represent environments with varying levels of potential bacterial contamination. The emergency room floor is likely to have a high bacterial load due to the constant influx of patients and the potential for exposure to bodily fluids. The pharmaceutical installation represents a controlled environment where hygiene is crucial to prevent contamination of pharmaceutical products. The hospital inpatient room represents a more typical environment where bacterial contamination can occur from human activity and the presence of potentially infectious materials.

Sterile cotton swabs were used to collect samples from a $1m^2$ area of the floor in each location. The use of sterile cotton swabs ensures that no external bacteria are introduced into the sample, maintaining the integrity of the collected bacteria. The swabs were then immersed in 5 mL of Brain Heart Infusion (BHI) media for transport and initial processing. BHI media is a nutrient-rich medium that supports the growth of a wide range of bacteria, ensuring that the collected bacteria remain viable during transport and storage.

Petri dishes containing a 24-hour-old bacterial culture were used for the preparation of the bacterial suspension. The 24-hour-old culture provides an actively growing population of bacteria for the experiment. To each Petri dish, 2 mL of physiological NaCl diluent solution was added, followed by the addition of sterile glass beads. The physiological NaCl solution provides an isotonic environment for the bacteria, preventing osmotic shock and ensuring their viability. The sterile glass beads aid in the mechanical dislodging of bacteria from the culture medium, ensuring a complete and homogeneous collection of bacteria. The Petri dishes were then shaken vigorously to ensure the complete and homogeneous collection of bacteria. The resulting suspension was transferred to an Erlenmeyer flask. The turbidity of the suspension was compared to the McFarland I standard solution to estimate the bacterial population per mL, establishing the initial bacterial density before the addition of the disinfectant. The McFarland I standard solution provides a standardized reference for turbidity, allowing for a consistent estimation of bacterial density across different samples.

The total plate count method was employed to enumerate viable bacteria in the samples. This method is based on the principle that viable bacteria, when incubated on a suitable growth medium under appropriate conditions, will multiply and form visible colonies. Each colony is assumed to have originated from a single viable bacterium, allowing for the estimation of the number of viable bacteria in the original sample.

After collection, samples were homogenized in BHI media. Homogenization ensures an even distribution of bacteria throughout the sample, increasing the accuracy and reliability of the total plate count method. Six test tubes were prepared, each containing 9 mL of physiological NaCl diluent solution. 1 mL of the homogenized sample was added to the first tube, creating a $10^{-1}$ dilution. The tube was shaken thoroughly to ensure homogeneity. This process was repeated for subsequent dilutions, up to a $10^{-6}$ dilution. Serial dilution is crucial in the total plate count method as it reduces the bacterial concentration to a countable range, preventing overcrowding on the agar plates and ensuring accurate colony counting.

1 mL of each dilution was pipetted into a sterile Petri dish, in duplicate. Duplicate plating allows for greater accuracy and reduces the impact of potential errors or variations in a single plate. 12-15 mL of nutrient agar medium, which had been melted and maintained at 45-47°C, was poured into each Petri dish. The melted agar provides a suitable growth medium for the bacteria, and maintaining it at 45-

47°C prevents premature solidification while allowing for even mixing with the sample. The Petri dishes were gently swirled to ensure even mixing of the sample with the agar. A blank control was also prepared for each sample by mixing the dilution solution with the nutrient agar. The blank control serves as a baseline for comparison, ensuring that any observed bacterial growth is due to the sample and not contamination of the agar itself. After the agar solidified, the Petri dishes were inverted and incubated at 35-37°C for 24-48 hours. Incubation at 35-37°C provides optimal growth conditions for most common bacteria, allowing them to multiply and form visible colonies.

Following incubation, the number of bacterial colonies on each plate was counted. Petri dishes with colony counts between 30-300 were selected for analysis. This range is considered statistically significant and provides a reliable estimate of the bacterial density in the original sample. If the colony count fell outside this range, appropriate adjustments were made according to standard microbiological practices to estimate the bacterial count. Adjustments may involve selecting plates with colony counts closer to the desired range or applying correction factors to account for overcrowding or excessive dilution.

Hypochlorite solutions were prepared at varying concentrations to assess the dose-response relationship; Dose 1: 30 mL/5L (6 µL/mL); Dose 2: 60 mL/5L (12 µL/mL); Dose 3: 120 mL/5L (24 µL/mL); Dose 4: 240 mL/5L (48 µL/mL); Dose 5: 480 mL/5L (96 µL/mL). These concentrations were prepared based on the conversion of 1 mL to 1000 µL. The varying concentrations allow for the investigation of the relationship between hypochlorite dose and bacterial reduction, providing insights into the optimal dose for effective disinfection.

The total plate count method was again utilized to determine the bacterial count after exposure to the hypochlorite solutions. This allows for a direct comparison of bacterial numbers before and after disinfection, providing a quantitative measure of hypochlorite effectiveness.

2 mL of the standardized bacterial suspension was added to a tube containing 2 mL of the test hypochlorite solution. The mixture was shaken or centrifuged to ensure homogeneity and then left undisturbed for 1 hour to allow for disinfection. Shaking or centrifugation ensures even contact between the bacteria and the hypochlorite solution, maximizing the disinfection process. The one-hour exposure time provides sufficient time for the hypochlorite to exert its antimicrobial action.

Similar to the initial total plate count, a series of six tubes containing 9 mL of physiological NaCl diluent solution were prepared. The disinfected bacterial suspension was serially diluted up to $10^{-6}$. Serial dilution is again performed to reduce the bacterial concentration to a countable range after disinfection. 1 mL of each dilution was plated in duplicate onto nutrient agar, as described previously. Duplicate plating ensures accuracy and reduces the impact of potential errors in colony counting.

The plates were incubated at 35-37°C for 24-48 hours, and the resulting colonies were counted using the colony forming unit (CFU) method to determine the number of bacteria per mL of sample. Incubation provides optimal growth conditions for any surviving bacteria, allowing them to form colonies for counting. The CFU method provides a standardized way of expressing the number of viable bacteria in the sample after disinfection.

The percentage reduction in bacterial numbers was calculated for each hypochlorite dose using the following formula; % Reduction = [(Initial CFU - Final CFU) / Initial CFU] * 100, where; Initial CFU = Colony forming units per milliliter (CFU/mL) in the water sample before hypochlorite treatment; Final CFU = CFU/mL in the water sample after hypochlorite treatment. This formula provides a quantitative measure of the effectiveness of each hypochlorite dose in reducing bacterial populations.

**3. Results and Discussion**

Table 1 presents the percentage reduction in bacterial numbers after exposure to varying

concentrations of hypochlorite solution in three different environments: an emergency room, a pharmaceutical room, and a hospital inpatient room. Across all three environments, there's a clear trend of increasing bacterial reduction with increasing hypochlorite concentrations. This demonstrates a strong dose-response relationship, indicating that higher concentrations of hypochlorite lead to greater disinfection efficacy. Even at the lowest concentration (6 µL/mL), hypochlorite achieves a substantial reduction in bacterial numbers, ranging from 81.83% to 84.33% across the three locations. This highlights the potent antimicrobial activity of hypochlorite even at low doses. At higher concentrations (24 µL/mL and above), hypochlorite achieves near-complete or complete elimination of bacteria (99.83% to 100% reduction). This indicates that hypochlorite is highly effective in disinfecting surfaces and reducing bacterial contamination to negligible levels. The percentage reduction in bacterial numbers is remarkably consistent across the three different environments, with very similar values observed for each hypochlorite dose. This suggests that hypochlorite's efficacy is not significantly affected by the specific environment or the initial bacterial load. The consistent results across different environments strengthen the generalizability of the findings, indicating that hypochlorite can be reliably used as a disinfectant in various settings. The P-values (between groups) are all greater than 0.05, indicating no statistically significant difference in the mean percentage reduction between the three environments for any given hypochlorite dose. This further supports the observation that hypochlorite's efficacy is consistent across different settings. The P-value (within groups) is 0.001, which is less than 0.05. This indicates a statistically significant difference in the mean percentage reduction within each environment across the different hypochlorite doses. This confirms the strong dose-response relationship observed, highlighting the importance of hypochlorite concentration in achieving effective disinfection.

Table 1. Percentage reduction in bacterial number: study in the emergency room, pharmaceutical room, and hospital inpatient room.

| **Hypochlorite Dose (µL/mL)** | **Emergency Room Mean (SD) (%)** | **Pharmaceutical Room Mean (SD) (%)** | **Inpatient Room Mean (SD) (%)** | **P-value (between groups)** | **P-value (within groups)** |
|---|---|---|---|---|---|
| 6 | 83.73 (2.11) | 84.33 (1.22) | 81.83 (1.55) | 435 | 0.001 |
| 12 | 99.0 (0.12) | 98.20 (0.88) | 98.6 (0.21) | 112 | 0.001 |
| 24 | 99.83 (0.05) | 99.84 (0.04) | 99.84 (0.03) | 921 | 0.001 |
| 48 | 99.98 (0.01) | 99.99 (0.01) | 99.99 (0.01) | 387 | 0.001 |
| 96 | 100 (0) | 100 (0) | 100 (0) | 1.000 | 0.001 |

Figure 1 visually represents the percentage reduction in bacterial numbers achieved by different hypochlorite doses in three distinct environments: an emergency room, an inpatient room, and a pharmaceutical room. The graph clearly illustrates the relationship between hypochlorite concentration and its effectiveness as a disinfectant. The graph showcases a clear dose-response relationship. As the hypochlorite dose increases, the percentage reduction in bacterial numbers also increases. This pattern is consistent across all three environments, demonstrating that higher hypochlorite concentrations result in more effective bacterial elimination. Even at the lowest hypochlorite dose (approximately 6 µL/mL), a substantial reduction in bacteria is observed in all three environments. This highlights the potent antimicrobial activity of hypochlorite, even at low concentrations. At higher

hypochlorite doses (24 µL/mL and above), the percentage reduction in bacterial numbers reaches close to 100% in all three environments. This indicates that hypochlorite is highly effective in eliminating bacteria from surfaces and achieving a high level of disinfection. The graph demonstrates a similar trend of bacterial reduction across the emergency room, inpatient room, and pharmaceutical room. This suggests that hypochlorite's efficacy is not significantly influenced by the specific environment or the initial bacterial load. This consistency reinforces the versatility of hypochlorite as a disinfectant in various settings.

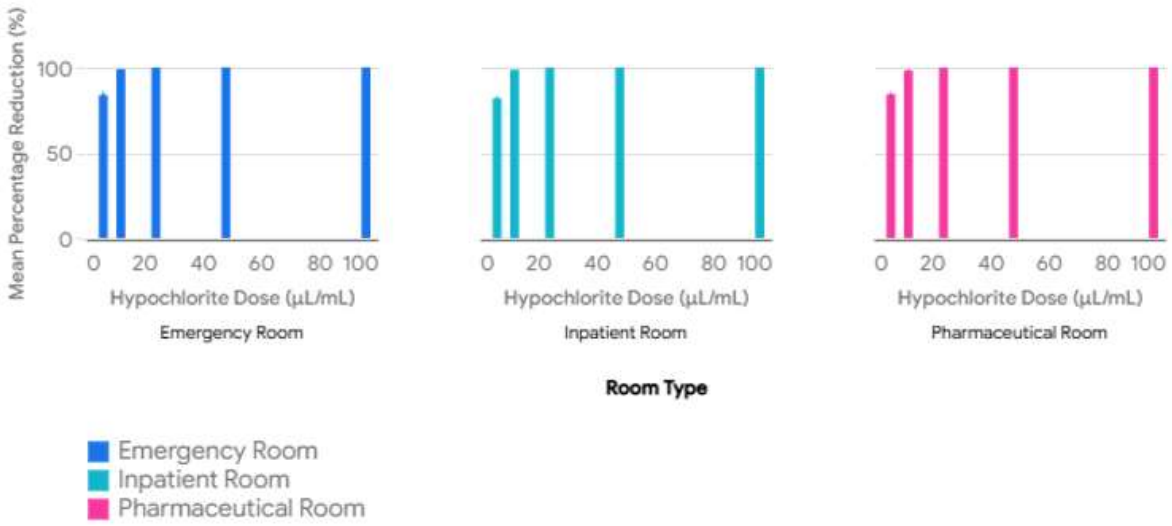


Figure 1. Comparison reduction bacterial number in emergency room, pharmaceutical room, and hospital inpatient room. The extent of bacterial reduction was directly proportional to the hypochlorite dose, indicating a clear dose-response relationship.

This study aimed to rigorously evaluate the effectiveness of hypochlorite as a disinfectant against aerobic bacteria, and the results unequivocally confirm its robust antimicrobial activity. The study employed the total plate count method, a standard microbiological technique used to enumerate viable bacteria in a sample. Water samples were collected from three distinct locations, the floor of an emergency room, a pharmaceutical installation, and a hospital inpatient room. These locations were chosen to represent environments with varying levels of potential bacterial contamination, allowing us to assess the efficacy of hypochlorite in diverse settings. The emergency room floor is likely to have a high bacterial load due to the constant influx of patients and the potential for exposure to bodily fluids. The pharmaceutical installation represents a controlled environment where hygiene is crucial to prevent contamination of pharmaceutical products. The hospital inpatient room represents a more typical environment where bacterial contamination can occur from human activity and the presence of potentially infectious materials. Sterile cotton swabs were used to collect samples from a $1m^2$ area of the floor in each location. The use of sterile cotton swabs ensures that no external bacteria are introduced into the sample, maintaining the integrity of the collected bacteria. The swabs were then immersed in 5 mL of Brain Heart Infusion (BHI) media for transport and initial processing. BHI media is a nutrient-rich medium that supports the growth of a wide range of bacteria, ensuring that the collected bacteria remain viable during transport and storage. Petri dishes containing a 24-hour-old bacterial culture were used for the

preparation of the bacterial suspension. The 24-hour-old culture provides an actively growing population of bacteria for the experiment. To each Petri dish, 2 mL of physiological NaCl diluent solution was added, followed by the addition of sterile glass beads. The physiological NaCl solution provides an isotonic environment for the bacteria, preventing osmotic shock and ensuring their viability. The sterile glass beads aid in the mechanical dislodging of bacteria from the culture medium, ensuring a complete and homogeneous collection of bacteria. The Petri dishes were then shaken vigorously to ensure the complete and homogeneous collection of bacteria. The resulting suspension was transferred to an Erlenmeyer flask. The turbidity of the suspension was compared to the McFarland I standard solution to estimate the bacterial population per mL, establishing the initial bacterial density before the addition of the disinfectant. The McFarland I standard solution provides a standardized reference for turbidity, allowing for a consistent estimation of bacterial density across different samples. The total plate count method was employed to enumerate viable bacteria in the samples. This method is based on the principle that viable bacteria, when incubated on a suitable growth medium under appropriate conditions, will multiply and form visible colonies. Each colony is assumed to have originated from a single viable bacterium, allowing for the estimation of the number of viable bacteria in the original sample. After collection, samples were homogenized in BHI media. Homogenization ensures an even distribution of bacteria throughout the sample, increasing the accuracy and reliability of the total plate count method. Six test tubes were prepared, each containing 9 mL of physiological NaCl diluent solution. 1 mL of the homogenized sample was added to the first tube, creating a $10^{-1}$ dilution. The tube was shaken thoroughly to ensure homogeneity. This process was repeated for subsequent dilutions, up to a $10^{-6}$ dilution. Serial dilution is crucial in the total plate count method as it reduces the bacterial concentration to a countable range, preventing overcrowding on the agar plates and ensuring accurate colony counting. 1 mL of each dilution was pipetted into a sterile Petri dish, in duplicate. Duplicate plating allows for greater accuracy and reduces the impact of potential errors or variations in a single plate. 12-15 mL of nutrient agar medium, which had been melted and maintained at 45-47°C, was poured into each Petri dish. The melted agar provides a suitable growth medium for the bacteria, and maintaining it at 45-47°C prevents premature solidification while allowing for even mixing with the sample. The Petri dishes were gently swirled to ensure even mixing of the sample with the agar. A blank control was also prepared for each sample by mixing the dilution solution with the nutrient agar. The blank control serves as a baseline for comparison, ensuring that any observed bacterial growth is due to the sample and not contamination of the agar itself. After the agar solidified, the Petri dishes were inverted and incubated at 35-37°C for 24-48 hours. Incubation at 35-37°C provides optimal growth conditions for most common bacteria, allowing them to multiply and form visible colonies. Following incubation, the number of bacterial colonies on each plate was counted. Petri dishes with colony counts between 30-300 were selected for analysis. This range is considered statistically significant and provides a reliable estimate of the bacterial density in the original sample. If the colony count fell outside this range, appropriate adjustments were made according to standard microbiological practices to estimate the bacterial count. Adjustments may involve selecting plates with colony counts closer to the desired range or applying correction factors to account for overcrowding or excessive dilution. Hypochlorite solutions were prepared at varying concentrations to assess the dose-response relationship. The varying concentrations allow for the investigation of the relationship between hypochlorite dose and bacterial reduction, providing insights into the optimal dose for effective disinfection. The total plate count method was again utilized to determine the bacterial count after exposure to the hypochlorite solutions. This allows for a direct comparison of bacterial numbers before and

after disinfection, providing a quantitative measure of hypochlorite effectiveness. 2 mL of the standardized bacterial suspension was added to a tube containing 2 mL of the test hypochlorite solution. The mixture was shaken or centrifuged to ensure homogeneity and then left undisturbed for 1 hour to allow for disinfection. Shaking or centrifugation ensures even contact between the bacteria and the hypochlorite solution, maximizing the disinfection process. The one-hour exposure time provides sufficient time for the hypochlorite to exert its antimicrobial action. Similar to the initial total plate count, a series of six tubes containing 9 mL of physiological NaCl diluent solution were prepared. The disinfected bacterial suspension was serially diluted up to $10^{-6}$. Serial dilution is again performed to reduce the bacterial concentration to a countable range after disinfection. 1 mL of each dilution was plated in duplicate onto nutrient agar, as described previously. Duplicate plating ensures accuracy and reduces the impact of potential errors in colony counting. The plates were incubated at 35-37°C for 24-48 hours, and the resulting colonies were counted using the colony forming unit (CFU) method to determine the number of bacteria per mL of sample. Incubation provides optimal growth conditions for any surviving bacteria, allowing them to form colonies for counting. The CFU method provides a standardized way of expressing the number of viable bacteria in the sample after disinfection. The results of the study demonstrated a clear dose-response relationship between hypochlorite concentration and bacterial reduction across all three environments. Even at low concentrations, hypochlorite treatment led to substantial reductions in bacterial populations, underscoring its potency as a disinfectant. For instance, a dose of 30mL/5L (6 µL/mL) reduced the average number of bacteria by 83.29%. At higher concentrations, near-complete or complete bacterial elimination was achieved, highlighting its efficacy in infection control. A dose of 240mL/5L (48 µL/mL) resulted in a 99.98% reduction in bacterial numbers, while a dose of 480mL/5L (96 µL/mL) effectively achieved complete bacterial elimination under the tested conditions. The consistency of these findings across different environments, each with varying potential for bacterial contamination, further strengthens the generalizability of the study's conclusions. This suggests that hypochlorite's efficacy is not significantly affected by the specific environment or the initial bacterial load, making it a reliable disinfectant for a wide range of applications. The study's findings support the widespread use of hypochlorite as a reliable disinfectant in a multitude of settings. Its efficacy, dose-response relationship, and consistent performance across diverse environments make it a valuable tool for infection control and public health.[11-13]

The observation of a clear dose-response relationship between hypochlorite concentration and bacterial reduction is a crucial finding of this study, offering valuable insights into the dynamics of hypochlorite's antimicrobial action. This direct proportionality aligns with the current understanding of hypochlorite's mechanism of action, primarily mediated by hypochlorous acid (HOCl), the active component generated from hypochlorite solutions. Hypochlorite solutions, when in water, undergo a chemical equilibrium reaction that results in the formation of hypochlorous acid (HOCl) and hypochlorite ions ($OCl^-$). The relative proportions of HOCl and $OCl^-$ depend on the pH of the solution. At lower pH values, HOCl predominates, while at higher pH values, $OCl^-$ is the major species. HOCl is the more potent antimicrobial agent of the two. HOCl is a potent oxidizing agent that disrupts critical cellular processes within bacteria, ultimately leading to their inactivation. HOCl readily reacts with various cellular components, including proteins, DNA, and lipids, causing irreversible damage. This oxidative damage disrupts essential cellular functions, such as enzyme activity, DNA replication, and membrane integrity, leading to bacterial cell death. HOCl can oxidize amino acids, particularly those containing sulfur (cysteine and methionine) and aromatic rings (tyrosine and tryptophan). This oxidation can lead to protein misfolding, aggregation, and loss of function,

disrupting essential enzymatic activities and cellular processes. HOCl can react with DNA bases, causing damage such as base modifications, strand breaks, and cross-linking. This DNA damage can interfere with DNA replication and transcription, ultimately leading to cell death. HOCl can oxidize unsaturated fatty acids in cell membranes, leading to lipid peroxidation and disruption of membrane integrity. This can cause leakage of cytoplasmic contents, loss of membrane potential, and ultimately cell death. HOCl can directly attack the bacterial cell membrane, compromising its integrity and causing leakage of cytoplasmic contents. This disruption of the cell membrane further contributes to the loss of essential cellular components and ultimately cell death. HOCl can react with phospholipids, the main components of cell membranes, causing their oxidation and degradation. This can lead to the formation of pores in the membrane, compromising its integrity and allowing the leakage of cytoplasmic contents. HOCl can also disrupt the electrochemical gradient across the cell membrane, which is essential for many cellular processes, including energy production and nutrient transport. This disruption can further contribute to cell death. HOCl can also interfere with DNA synthesis by reacting with DNA bases and causing DNA strand breaks. This inhibition of DNA replication prevents bacterial cell division and proliferation, contributing to the overall bactericidal effect. HOCl can react with DNA bases, particularly guanine, causing base modifications and strand breaks. These DNA lesions can stall DNA replication and trigger DNA repair mechanisms. If the DNA damage is extensive and overwhelms the repair capacity of the cell, it can lead to cell death. The dose-response relationship observed in this study indicates that the higher the concentration of HOCl, the greater the extent of disruption to bacterial cellular processes and the more pronounced the bactericidal effect. The dose-response relationship underscores the importance of careful dose optimization in various settings. While higher doses may lead to more complete bacterial elimination, it's crucial to consider factors such as the initial bacterial load, the presence of organic matter that may interfere with hypochlorite's action, and the potential for the formation of harmful disinfection byproducts at excessive doses. The higher the initial bacterial load, the higher the hypochlorite concentration required to achieve effective disinfection. Organic matter can react with HOCl, reducing its availability for bacterial inactivation. Therefore, higher hypochlorite doses may be needed in the presence of organic matter to achieve the desired level of disinfection. Excessive hypochlorite doses can lead to the formation of disinfection byproducts (DBPs), some of which are potentially harmful to human health. Therefore, it's important to optimize the hypochlorite dose to minimize DBP formation while ensuring effective disinfection. Overdosing of hypochlorite can lead to the formation of potentially harmful disinfection by-products, while underdosing may not achieve the desired level of disinfection. Therefore, careful dose optimization is crucial to balance efficacy and safety in disinfection practices. Exposure to high levels of hypochlorite can cause irritation to the skin, eyes, and respiratory tract. Ingestion of hypochlorite solutions can cause gastrointestinal irritation and other health problems. Therefore, it's important to use hypochlorite solutions safely and according to the manufacturer's instructions. Hypochlorite solutions can be corrosive to certain materials, such as metals and some plastics. Therefore, it's important to choose hypochlorite-compatible materials for disinfection purposes and to avoid prolonged contact with sensitive materials. Excessive hypochlorite levels can have negative environmental impacts, such as the formation of disinfection byproducts (DBPs) in water and toxicity to aquatic life. Understanding the dose-response relationship helps in minimizing hypochlorite consumption while ensuring effective disinfection, contributing to more sustainable and environmentally responsible practices. Hypochlorite can react with organic matter in water to form DBPs, some of which are potentially harmful to human health. To minimize DBP formation, it's important to optimize hypochlorite doses and to remove organic matter from water before

disinfection. Hypochlorite can be toxic to aquatic life, particularly at high concentrations. It's important to prevent the release of hypochlorite into natural water bodies and to ensure that wastewater containing hypochlorite is properly treated before discharge.[14-16]

The findings of this study are consistent with a wealth of previous research demonstrating the efficacy of hypochlorite against a broad spectrum of microorganisms. Numerous studies have documented its effectiveness against bacteria, viruses, fungi, and protozoa, supporting its widespread use in healthcare, household, and industrial settings. A meta-analysis of 29 studies evaluating the efficacy of hypochlorite against various pathogens in healthcare settings found that hypochlorite solutions at concentrations ranging from 0.05% to 0.5% achieved significant reductions in microbial load on surfaces. The study concluded that hypochlorite is a highly effective disinfectant for use in hospitals and other healthcare facilities. Another study published in the International Journal of Food Microbiology investigated the use of hypochlorite in the food industry. The researchers found that hypochlorite effectively reduced bacterial contamination on various food contact surfaces, including stainless steel, plastic, and rubber. They concluded that hypochlorite is a valuable tool for ensuring food safety and preventing foodborne illnesses. Hypochlorite's efficacy against a wide range of microorganisms, including bacteria, viruses, and fungi, makes it a valuable tool in infection control and public health. This broad-spectrum activity is particularly important in settings where diverse microbial communities may be present, such as hospitals or water treatment facilities. By effectively targeting a wide range of microorganisms, hypochlorite compounds contribute to maintaining hygiene and preventing the spread of infectious diseases. Hypochlorite is effective against a wide range of bacteria, including both Gram-positive and Gram-negative bacteria. Studies have shown that hypochlorite can inactivate bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* spp., which are common causes of infections. Hypochlorite is also effective against many viruses, including enveloped and non-enveloped viruses. Studies have demonstrated its efficacy against viruses such as influenza virus, norovirus, and adenovirus, which are responsible for respiratory, gastrointestinal, and other infections. Hypochlorite has been shown to be effective against various fungi, including *Candida albicans*, *Aspergillus niger*, and *Trichophyton mentagrophytes*, which can cause skin infections, respiratory infections, and other health problems. Hypochlorite is also effective against some protozoa, such as *Cryptosporidium parvum* and *Giardia lamblia*, which are common causes of waterborne diseases. Hypochlorite has been widely used in healthcare settings for decades due to its broad-spectrum antimicrobial activity, cost-effectiveness, and ease of use. These compounds are powerful oxidizing agents that disrupt the cellular processes of microorganisms, leading to their inactivation. Hypochlorite solutions are commonly used to disinfect surfaces in healthcare facilities, such as floors, walls, countertops, and medical equipment. This helps to prevent the spread of healthcare-associated infections (HAIs) by reducing the microbial load on environmental surfaces. Hypochlorite solutions are also used to disinfect medical equipment, such as endoscopes, surgical instruments, and respiratory therapy equipment. This helps to ensure that medical equipment is free of pathogens and safe for patient use. Hypochlorite solutions can be used for skin disinfection, such as hand hygiene and pre-operative skin preparation. This helps to reduce the risk of infection transmission from healthcare providers to patients and vice versa. In addition to its use in healthcare settings, hypochlorite is also widely used in household and industrial settings for disinfection and water purification purposes. Hypochlorite-based products are commonly used in households for cleaning and disinfecting kitchens, bathrooms, and other areas. This helps to maintain hygiene and prevent the spread of infectious diseases within households. Hypochlorite compounds are used in various industrial processes, such as water

purification, wastewater treatment, and food processing. In water treatment, hypochlorite is used to disinfect drinking water and ensure its safety for human consumption. In wastewater treatment, hypochlorite helps to reduce the microbial load and prevent the contamination of water bodies. In food processing, hypochlorite is used to disinfect food contact surfaces and prevent foodborne illnesses. Hypochlorite is often compared to other disinfectants, such as alcohols, quaternary ammonium compounds, and hydrogen peroxide. Each disinfectant has its own advantages and disadvantages, and the choice of disinfectant depends on the specific application and the types of microorganisms that need to be inactivated. Alcohols, such as ethanol and isopropanol, are effective against a wide range of bacteria and some viruses, but they have limited activity against spores and some non-enveloped viruses. Alcohols are also flammable and can be damaging to some materials. Quaternary ammonium compounds (QACs) are effective against a broad spectrum of microorganisms, including bacteria, fungi, and some viruses. However, QACs have limited activity against some non-enveloped viruses and bacterial spores. Hydrogen peroxide is a broad-spectrum disinfectant that is effective against bacteria, viruses, fungi, and spores. However, hydrogen peroxide can be corrosive to some materials and is less stable than hypochlorite. Hypochlorite, with its broad-spectrum activity, cost-effectiveness, and ease of use, remains a popular choice for disinfection in various settings. However, it's important to consider its limitations, such as its potential to form DBPs and its corrosiveness to some materials.[17,18]

The consistent efficacy of hypochlorite observed across the three different environments in this study is a noteworthy finding. It suggests that hypochlorite's antimicrobial activity is not significantly influenced by the specific environment or the initial bacterial load. This versatility is a significant advantage, making hypochlorite a reliable disinfectant for a wide range of applications. The three environments selected for this study represent a diverse range of settings with varying levels of potential bacterial contamination. The emergency room floor is likely to have a high bacterial load due to the constant influx of patients and the potential for exposure to bodily fluids. The pharmaceutical installation represents a controlled environment where hygiene is paramount to prevent contamination of pharmaceutical products. The hospital inpatient room represents a more typical environment where bacterial contamination can occur from human activity and the presence of potentially infectious materials. Despite the differences in these environments, the study found that hypochlorite effectively reduced bacterial populations across all three settings. This suggests that hypochlorite's efficacy is not significantly affected by the specific environment or the initial bacterial load. This is an important finding because it means that hypochlorite can be used as a reliable disinfectant in a variety of settings, regardless of the level of contamination. In the emergency room, with its high potential for bacterial contamination from bodily fluids and the constant influx of patients, hypochlorite effectively reduced bacterial populations, contributing to infection control in this critical healthcare setting. The emergency room is a high-risk environment for the transmission of infections due to the presence of patients with a variety of illnesses, including those with compromised immune systems. Hypochlorite's ability to effectively reduce bacterial populations in this setting makes it an important tool for infection control. In the pharmaceutical installation, where hygiene is paramount to prevent product contamination, hypochlorite's efficacy provides assurance in maintaining the sterility of the environment. The pharmaceutical industry has strict regulations regarding hygiene and sterility to ensure the safety and efficacy of pharmaceutical products. Hypochlorite's ability to effectively eliminate bacteria in this setting makes it a valuable tool for maintaining the sterility of the environment and preventing product contamination. In the hospital inpatient room, a more typical setting with moderate bacterial presence, hypochlorite's consistent performance highlights its

suitability for routine disinfection practices. Hospital inpatient rooms are regularly cleaned and disinfected to prevent the spread of healthcare-associated infections (HAIs). Hypochlorite's consistent efficacy in reducing bacterial populations in this setting makes it a suitable choice for routine disinfection practices. The consistent efficacy of hypochlorite across different environments highlights its versatility and reliability as a disinfectant. This is particularly important in settings where the level of bacterial contamination may vary, such as healthcare facilities, schools, and public spaces. Hypochlorite can be used with confidence in these settings, knowing that it will effectively reduce bacterial populations regardless of the specific environment or the initial bacterial load. The consistent efficacy of hypochlorite across different environments has important public health implications. By effectively reducing bacterial contamination in public spaces, water supplies, and healthcare facilities, hypochlorite compounds contribute to creating healthier environments and reducing the burden of infectious diseases. The ability of hypochlorite compounds to inactivate a wide range of pathogens, including those responsible for waterborne and foodborne illnesses, makes them a crucial tool in protecting public health.[19,20]

## 4. Conclusion

This study rigorously evaluated the effectiveness of hypochlorite as a disinfectant against aerobic bacteria using the total plate count method. Water samples were collected from three distinct locations, an emergency room floor, a pharmaceutical installation, and a hospital inpatient room. These locations were chosen to represent environments with varying levels of potential bacterial contamination. The results of the study demonstrated a clear dose-response relationship between hypochlorite concentration and bacterial reduction across all three environments. Even at low concentrations, hypochlorite treatment led to substantial reductions in bacterial populations, underscoring its potency as a disinfectant. For instance, a dose of 30mL/5L (6 µL/mL) reduced the average number of bacteria by 83.29%. At higher concentrations, near-complete or complete bacterial elimination was achieved, highlighting its efficacy in infection control. A dose of 240mL/5L (48 µL/mL) resulted in a 99.98% reduction in bacterial numbers, while a dose of 480mL/5L (96 µL/mL) effectively achieved complete bacterial elimination under the tested conditions. The consistency of these findings across different environments, each with varying potential for bacterial contamination, further strengthens the generalizability of the study's conclusions. This study confirms the efficacy of hypochlorite as a disinfectant against aerobic bacteria. The results support its use in various applications, including disinfection of surfaces and water purification. A hypochlorite dose of 480mL/5L (96 µL/mL) effectively achieved complete bacterial elimination under the tested conditions.

## 5. References


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