



# Minimal inhibitory concentration (MIC) of sodium hypochlorit against bacterial isolate from equipment in the microbiology laboratory

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ARTICLE INFO	ABSTRACT
<p><b>Article history:</b></p> <p>Received Apr 25, 2024 Revised May 27, 2024 Accepted May 29, 2024</p>	<p>Microbiology laboratory equipment is susceptible to bacterial contamination and therefore has the potential to become a medium for the spread of disease and infection. The existence of a less than sterile microbiological analysis process in the STIKes Karsa Husada Garut Microbiology Laboratory causes bacterial contamination of laboratory equipment such as bacterial analysis that does not comply with standards or the use of less than sterile equipment. To control the spread of contaminants, a decontamination process is required using a chemical disinfectant, namely sodium hypochlorite. Measuring the effectiveness of a disinfectant by looking at or can be determined by the Minimum Inhibitory Concentration (MIC). The aim of this research was to determine the effectiveness of sodium hypochlorite disinfectant in inhibiting the growth of microorganisms in bacterial isolates from the STIKes Karsa Husada Garut Microbiology Laboratory equipment. The data analysis used was analyzed descriptively. The results of this research are the Minimum Inhibitory Concentration (MIC) of sodium hypochlorite disinfectant on bacterial isolates from the STIKes Karsa Husada Garut Microbiology Laboratory equipment at a concentration of 4%. So it can be concluded that sodium hypochlorite disinfectant with a concentration of 4% is effective in inhibiting the growth of microorganisms.</p>
<p><b>Keywords:</b></p> <p>Disinfectant; Microbiology Laboratory; Minimum Inhibitory Concentration; Sodium Hypochlorite.</p>	

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## 1. Introduction

The microbiology laboratory is one of the facilities used for microbiological analysis, including bacteria and fungi (Ramdhani and Supriyatna, 2023). Likewise, the microbiology laboratory in the D-III Health Analyst study program at STIKes Karsa Husada Garut is a laboratory used in lectures and practicum facilities. In a microbiology laboratory there are several tools, namely incubators, ovens, autoclaves, refrigerators, and also microscopes (Chauhan and Jindal, 2020). This microbiology laboratory equipment is susceptible to bacterial contamination, making it a potential medium for spreading disease and

infection (Abatenh, Gizaw and Tsegaye, 2018). Therefore, to control the spread of contaminants, a decontamination process is needed.

The decontamination process is an important step to remove or reduce contamination from surfaces, equipment, or the environment. Decontamination can be carried out using various methods which are grouped into chemical, physical and mechanical methods. The selection of the appropriate method depends greatly on the specific situation and conditions, such as the type of tool or surface being contaminated, the type of contaminant, and the level of decontamination required (Zahid, 2014). Chemical methods involve the use of chemicals to kill or deactivate microorganisms, one of which is disinfectants (Emdiyono and Triyantoro, 2018).

Disinfectants are chemicals used to kill or inactivate microorganisms on the surface of inanimate objects. Choosing the right disinfectant depends on the type of microorganisms you want to eliminate and the type of surface you want to clean (Gebel *et al.*, 2013). The use of alcohol such as ethanol and isopropanol is effective against bacteria and viruses (Gebel *et al.*, 2013). Decontamination hydrogen peroxide is often used in solution or vapor form (Ayub *et al.*, 2024). Quaternary ammonium compounds used in many household and industrial cleaning products (Osimitz and Droege, 2021). Decontamination with chlorine and chlorine compounds are commonly used in the form of bleach solutions (de Andrade and Pereira, 2020).

Sodium hypochlorite with the chemical formula NaOCl is a solution that is generally used as a disinfectant. This disinfectant is often used because it is relatively cheap and easy to obtain. Sodium hypochlorite has been proven to kill various viruses such as hepatitis viruses, SARS, HIV, and can also kill gram-positive and gram-negative bacteria (Artasensi, Mazzotta and Fumagalli, 2021). The concentrations of sodium hypochlorite solution commonly used are 0.5%, 1%, 2.5% and 5.25% (Maneea *et al.*, 2023). For an acceptable level of cytotoxicity, a concentration of 0.5% is recommended, but this requires at least 30 minutes to inhibit bacterial growth (Pirela, Maggiolo and Yévenes, 2020).

The effectiveness of using disinfectants in the decontamination process can be seen from the presence and number of microorganisms that grow after the decontamination process. Measuring the effectiveness of disinfectants by looking at or can be determined by the Minimum Inhibitory Concentration (MIC) (Selam *et al.*, 2022). Determination of concentration using the dilution method. Minimum Inhibitory Concentration shows the smallest concentration of disinfectant that is able to inhibit the growth of microorganisms after 18-24 hours after the incubation period. Determining the MIC is easy to do because you can see whether or not bacteria are growing by simply looking at whether or not a solution is cloudy after isolating the bacteria (Chikezie, 2017).

The use of laboratories by many students and the microbiological analysis process that is less than sterile can cause bacterial contamination of laboratory equipment such as bacterial analysis that does not comply with standards or the use of equipment that is less than sterile. Based on the background explanation, to find out what the Minimum Inhibitory Concentration (MIC) of Sodium Hypochlorite Disinfectant is in Bacterial Isolates from Microbiology Laboratory Equipment, especially at STIKes Karsa Husada Garut.

## 2. Methods

### 1. Tools and material

The tools used in this research were oven, autoclave, microscope, incubator, micropipette, beaker, measuring cup, small and large test tubes, measuring pipette, bullf, tube rack, spatula, stir bar, sterile cotton swab, sterile yellow tip, sterile blue tip. The materials used in this research were Sodium Hypochlorite (NaOCl), Nutrient Broth (NB), Muller Hinton Broth (MHB), sterile 0.85% physiological NaCl, McFarland 0.5, distilled water.

### 2. Preparation of bacterial media

Nutrient Broth as a medium for bacterial isolation was made with a composition of 1.3 gr/ml and put into test tubes of 5 ml each. The Muller Hinton Broth medium used as a medium for adding serial dilutions and bacterial inoculation was made with a composition of 2.1 gr/ml and then 1 ml was added to each tube. The media was sterilized in an autoclave at 121°C 1 atm for 15 minutes

### 3. Isolation of bacteria from microbiology laboratory equipment

After the bacterial isolation media is made, then samples of bacterial contamination are taken from the microbiology laboratory equipment which is carried out by swabbing using a sterile swab on each piece of equipment. For equipment such as incubators, refrigerators and autoclaves, swabs are applied to each section, while for microscopes swabs are carried out on the microscope arm from base to tip. Tool swabs are carried out once on each microbiology laboratory equipment. The bacterial swab was placed in Nutrient Broth media for isolation and then incubated at 37°C for 24 hours.

### 4. Bacterial preparation

Bakteri yang sudah terisolasi di media Nutrient Broth kemudian dilakukan preparasi bakteri sesuai standar kekeruhan 0,5 McFarland. Bandingkan standar kekeruhan 0,5 McFarland dengan suspensi bakteri, apabila belum terdapat kesamaan dengan standar, tambahkan NaCl fisiologis sampai suspensi bakteri sama dengan standar 0,5 McFarland.

### 5. Preparation of sodium hypochlorite solution

A stock of sodium hypochlorite solution with a concentration of 16% is made by diluting 0.8 ml of sodium hypochlorite solution then adding 4.2 ml of sterilized distilled water and then homogenizing. To make serial dilutions, prepare 8 tubes containing 1 ml Muller Hinton Broth, then in tube 1 put 1 ml of 16% disinfectant made from a mixture of 0.8 ml sodium hypochlorite with 4.2 ml sterile distilled water, then from tube 1 transfer 1 ml into tube 2, from tube 2 transfer 1 ml to tube 3, transfer to tube 8. So you get serial dilutions of 0.125%, 0.25%, 0.5%, 1%, 2%, 4% and 8% .

### 6. Minimum inhibition concentration (MIC) determination

After serial dilutions of sodium hypochlorite are made, MIC testing is carried out by adding 10 µl of bacteria ( $1.5 \times 10^8$  CFU) to each serial dilution. After the bacteria were added, they were incubated at 37°C for 24 hours. After that, the results are observed to see whether the solution is cloudy or not.

### 7. Data analysis

The data obtained from this research was analyzed descriptively by looking at what concentration bacteria could not grow as indicated by whether the solution was cloudy or not.

## 3. Results and Discussion

The results of research data obtained from testing the minimum inhibitory concentration of sodium hypochlorite disinfectant on bacterial isolates from Microbiology Laboratory equipment can be seen at Figure 1 and Presentation of Minimum Inhibitory Concentration (MIC) data is represented in Table 1.

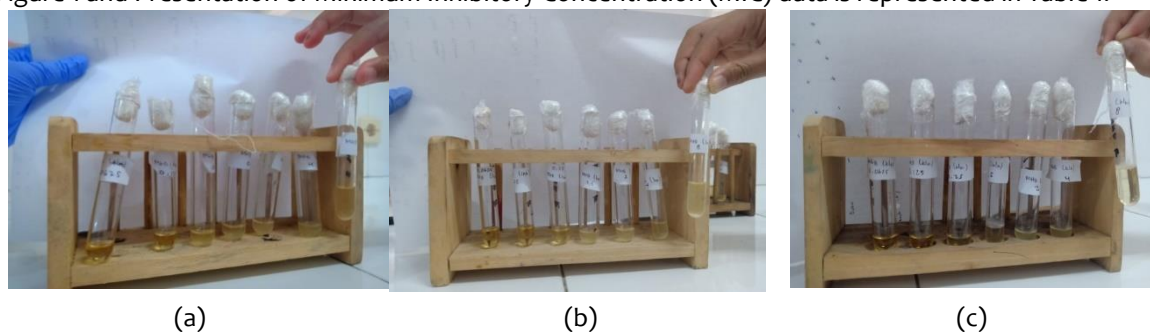


Figure 1. Bacterial growth results isolated from (a) autoclave (b) incubator (c) refrigerator incubated in media with a series of concentrations of sodium hypochlorite

Table 1. Results of Minimum Inhibitory Concentration of Sodium Hypochlorite Disinfectant on Bacterial Isolates from Microbiology Laboratory Equipment

No	Bacterial isolate from	Bacterial growth at NaOCl concentration							MIC
		8%	4%	2%	1%	0,5%	0,25%	0,125%	
1	Autoclave	-	-	+	+	+	+	+	4%

Mamay, *Minimal inhibitory concentration (MIC) of sodium hypochlorit against bacterial isolate from equipment in the microbiology laboratory*

2	Incubator	-	-	-	+	+	+	+	2%
3	Refrigerator	-	-	-	+	+	+	+	2%
4	Microscope	-	-	+	+	+	+	+	4%
5	Oven	-	-	-	+	+	+	+	2%

Information :

(+): There is turbidity in the liquid media indicating bacterial growth

(-): There is clarity in the liquid media indicating there is no bacterial growth

The parameters used in this research are determining the Minimum Inhibitory Concentration (MIC). The test consists of observing the level of turbidity to determine the MIC value of sodium hypochlorite disinfectant on bacterial isolates from Microbiology Laboratory equipment with an incubation period of 24 hours at a temperature of 37°C (Geraldés *et al.*, 2021). Based on theory, the minimum inhibitory concentration test parameter is the lowest concentration that can still inhibit the growth of microorganisms based on turbidity (presence of bacterial growth) and clarity (no bacterial growth), which can be seen after incubation for 18-24 hours at 37°C. The MIC value is determined by observing the smallest level that is still clear which indicates the absence of bacterial growth (Umoren *et al.*, 2022).

Seen from table 4.1, the results of research on determining MIC values show that after incubation for 1x24 hours at concentrations of 1%, 0.5%, 0.25% and 0.125% there is turbidity in the test tube which shows that there is bacterial growth in the MHB media containing sodium hypochlorite disinfectant, whereas at concentrations of 8%, 4% and 2% there was no turbidity in the test tube. This shows that the MIC value of sodium hypochlorite disinfectant is 4% which was obtained by observing the smallest level that was still clear from the bacterial suspension that had been incubated at 37°C for 24 hours. However, for tubes originating from autoclaved and microscoped bacterial isolate samples at a concentration of 2%, there was turbidity which indicated that bacteria could still grow at that concentration. Differences in MICs in each laboratory tool are possible due to differences in the types of bacteria isolated on each tool. The value of the disinfectant concentration in inhibiting bacteria cannot determine whether the bacteria are classified as resistant, moderate, or susceptible to this disinfectant. Many researchers consider bacteria resistant when their MIC value is at least two to four times higher than that of more susceptible bacteria (Rodríguez-Melcón *et al.*, 2022).

The results of the research above are classified as results of large concentrations because the bacteria contained in the media are a mixture of bacteria whose species are not yet known so the minimum inhibitory value is large. This is different from research on the minimum inhibition of NaOCl disinfectant on known bacteria, *Pseudomonas aeruginosa* which have an MIC of 0.2% (Köhler *et al.*, 2018). Apart from that, there is also research on the minimum inhibitory concentration on *Escherichia coli* with an MIC value of 0.75% (Abdullah Yahya Al-Beshari *et al.*, 2018) and on *A. calcoaceticus* and *S. maltophilia* with an MIC value of 0.0125% and 0.0175% (Gomes, Simões and Simões, 2016). The minimum inhibitory concentration value will be large if the bacterial species is not yet known.

Based on the research results above, it shows that low levels of sodium hypochlorite disinfectant are effective in inhibiting bacterial growth. This is because the MIC test is a test parameter determined with the lowest concentration value that inhibits the growth of microorganisms. Sodium hypochlorite has been proven to be used as a bacterial disinfectant because it has a working mechanism of killing microorganisms by oxidizing the peptide bonds in bacterial cell membranes and denaturing proteins so that the metabolic function of bacterial cells is disrupted (Utami, Mulyawati and Soebandi, 2016).

## Discussions

The results of the study demonstrate that sodium hypochlorite is effective at inhibiting bacterial growth at varying concentrations, depending on the source of the bacterial isolates. The MIC values for sodium hypochlorite disinfectant varied between 2% and 4% across different laboratory equipment. Specifically, bacterial isolates from the autoclave and microscope showed an MIC of 4%, while those from the incubator, refrigerator, and oven had an MIC of 2%. This variability in MIC values suggests that the

bacterial populations present on different equipment may consist of different species with varying susceptibility to sodium hypochlorite. The observed MIC values are significantly higher than those reported for known bacterial species such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter calcoaceticus*, and *Stenotrophomonas maltophilia*, which indicates that the unknown bacterial mixtures in this study exhibit a higher resistance to sodium hypochlorite.

The relatively high MIC values in this research can be attributed to the complexity and diversity of the bacterial isolates, which are not identified to the species level. This contrasts with studies where specific bacterial species with lower MIC values are known, highlighting the influence of bacterial identity on disinfectant efficacy. Despite the higher concentrations required, the study confirms the effectiveness of sodium hypochlorite as a disinfectant, aligning with its known mechanism of action that involves the oxidation of peptide bonds and protein denaturation in bacterial cell membranes, ultimately disrupting bacterial metabolic functions. These findings underscore the importance of understanding the microbial composition on laboratory equipment to tailor disinfection strategies effectively and ensure optimal antimicrobial efficacy.

#### 4. Conclusion

This research demonstrates the effectiveness of sodium hypochlorite as a disinfectant for bacterial isolates from microbiology laboratory equipment, with MIC values ranging from 2% to 4%. However, there are several limitations to this study. The bacterial isolates were not identified to the species level, which may have contributed to the relatively high MIC values observed. This lack of bacterial identification limits the ability to generalise the findings to specific bacterial species and complicates comparisons with other studies where bacteria were identified and exhibited lower MIC values. Additionally, the study was conducted under controlled laboratory conditions, which may not fully replicate the diverse and variable conditions found in real-world settings. Future research should aim to identify the bacterial species present on laboratory equipment to provide a more detailed understanding of the microbial populations and their susceptibilities to sodium hypochlorite. This would enable more precise determination of MIC values and facilitate comparisons with other studies. Furthermore, expanding the study to include a broader range of disinfectants and testing under varied environmental conditions would provide a more comprehensive assessment of disinfectant efficacy. Implementing these suggestions will enhance the reliability and applicability of the findings, contributing to improved disinfection strategies in microbiology laboratories.

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