Original Article

Pre-Clinical Laboratory Evaluation of Chlorhexidine for Disinfection of Semi-Critical Respiratory Equipment in Nursing Practice

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ABSTRACT

Background: Healthcare-associated infections (HAIs) remain a persistent challenge in nursing practice, particularly in the reprocessing of semi-critical respiratory equipment. Although chlorhexidine is widely used as an antiseptic, evidence regarding its pre-clinical disinfectant performance, physicochemical suitability, and waste safety within nurse-led device reprocessing workflows remains limited. This gap is especially relevant in settings where reusable respiratory devices are routinely handled by nurses.
Methods: This study employed a pre-clinical experimental laboratory design. The independent variable was chlorhexidine concentration, while dependent variables included antimicrobial efficacy (phenol coefficient), physicochemical parameters (pH, specific gravity, viscosity), and acute toxicity. Antimicrobial testing was conducted against Staphylococcus aureus and Pseudomonas aeruginosa. Physicochemical assessments followed standardized laboratory procedures, and acute toxicity was evaluated using Danio rerio (zebrafish) larvae to inform waste disposal safety. Expert validation was conducted using the Content Validity Index (CVI). Descriptive and endpoint-based analyses were applied without inferential statistics.
Results: Chlorhexidine demonstrated strong bactericidal activity against both test organisms, with phenol coefficient values indicating effective disinfectant performance. Physicochemical characteristics remained within acceptable ranges for semi-critical respiratory device reprocessing. Toxicity assessment showed dose-dependent larval mortality, indicating the need for dilution before waste discharge. Expert validation identified chlorhexidine 7.5% as the most clinically relevant concentration for nurse-led practice. No p-values were applied due to the endpoint-based experimental design.
Conclusion: Chlorhexidine shows effective pre-clinical disinfectant potential for semi-critical respiratory equipment within controlled laboratory conditions. Its application in nursing practice should be accompanied by standardized concentration control and mandatory dilution before disposal to mitigate environmental toxicity. Further clinical and field-based validation is recommended.

Keywords: Equipment Contamination; Device-Related Infections; Disinfection; Infection Control, Nursing

Implications for Practice:

- Chlorhexidine can be adopted in nurse-led disinfection protocols for reusable semi-critical respiratory equipment to reduce microbial contamination and HAI risk.
- Safe disposal requires standardized dilution practices, which should be embedded into nursing workflows and environmental safety training.
- Evidence from this study can inform nursing-driven infection control policies, including equipment reprocessing guidelines and institutional waste-handling standards.

Introduction

Healthcare-associated infections (HAIs) remain among the most preventable yet persistent threats to patient safety worldwide, contributing substantially to increased mortality, prolonged hospital length of stay, and escalating healthcare costs (Gidey et al., 2023; Raoofi et al., 2023). Device-associated infections, particularly those involving semi-critical respiratory equipment such as tracheostomy tubes, oxygen interfaces, and nebulizer components, represent a major source of nosocomial pneumonia and cross-transmission of opportunistic pathogens (Garvey, 2024; Rutala & Weber, 2019). In low- and middle-income countries, where reusable respiratory devices are frequently reprocessed manually by nurses, variability in disinfection practices further amplifies infection risk (Allegranzi et al., 2011; World Health Organization, 2016). In these settings, respiratory equipment is frequently reprocessed by nurses using variable disinfection practices, further increasing infection risk and underscoring the relevance of applied nursing workflows (Quinn et al., 2015; Sarani et al., 2020).

Chlorhexidine is a broad-spectrum antiseptic extensively adopted in clinical care, yet evidence regarding its applied use on reusable semi-critical respiratory equipment is limited, inconsistent, and at times contradictory. While some studies report significant microbial reduction on medical devices and equipment (Biswas et al., 2019; Hutauruk et al., 2021), others highlight toxicity concerns and insufficient evaluation in real equipment reprocessing and disposal pathways (Faria et al., 2019; Zhang et al., 2024). Prior research has predominantly focused on body surface antisepsis or oral decontamination, rather than direct equipment reprocessing led by nurses, limiting translation into nurse-driven disinfection policies for reusable respiratory tools (Rutala & Weber, 2019). Furthermore, conflicting evidence on safety thresholds, environmental toxicity, and waste dilution practices underscores the need for standardized applied evaluation contextualized in clinical nursing practice.

This study is guided by Orem's Self-Care Deficit Nursing Theory (SCDNT), which emphasizes nurses' compensatory role in maintaining airway hygiene and preventing infection when patients are unable to perform self-care independently (Goes et al., 2020; Yip, 2021). The reprocessing of reusable semi-critical respiratory equipment represents a nurse-compensatory self-care system, in which inadequate disinfection may directly increase the risk of device-related healthcare-associated infections (HAIs), particularly among tracheostomy-dependent patients who rely heavily on properly maintained airway devices.

Previous laboratory-based studies evaluating disinfectants have predominantly focused on bactericidal efficacy (Ariningpraja et al., 2024; Novianty et al., 2025), with limited consideration of physicochemical characteristics that influence clinical usability, such as pH, viscosity, and solution stability. These parameters are essential for ensuring compatibility with respiratory equipment and feasibility within routine nursing workflows. In addition, issues related to disinfectant dilution and disposal safety are

rarely integrated into laboratory evaluations, despite their practical importance for environmental safety and nurse-led infection prevention practices.

Consequently, there remains a need for a comprehensive pre-clinical evaluation that maintains established antimicrobial testing approaches while extending assessment to include physicochemical suitability and dilution-related safety considerations. Integrating these elements may generate more applicable evidence to support nurse-led decision-making in the reprocessing of reusable semi-critical respiratory equipment.

Therefore, this study aimed to evaluate the antimicrobial efficacy, physicochemical characteristics, and disposal safety of chlorhexidine as a disinfectant for reusable semi-critical respiratory equipment, to support evidence-based infection prevention in nursing practice.

Methods

Study Design

This study employed an experimental, pre-clinical applied evaluation design to assess the antimicrobial performance, physicochemical suitability, and disposal safety of chlorhexidine as a disinfectant for semi-critical respiratory equipment handled in nursing care. The study bridges standardized laboratory disinfection testing with nurse-led infection prevention and control (IPC) workflows, positioning the evaluation as foundational evidence before clinical implementation.

As a pre-clinical laboratory-based evaluation, this study has inherent limitations, including the absence of real-time clinical device handling, patient-related variables, and long-term material compatibility assessment. However, this design was intentionally selected to establish foundational safety and efficacy evidence prior to clinical implementation in nursing practice. Reporting of laboratory procedures was guided by modified principles of standardized disinfectant testing and experimental reporting (SNI 1842:2019; AOAC methods), adapted to address applied nursing IPC relevance rather than clinical effectiveness outcomes.

Participants

Biological test subjects were used instead of human participants to model contamination control and waste safety. The bacterial strains used were Staphylococcus aureus (ATCC 6538) and Pseudomonas aeruginosa (ATCC 15442), which served as representative pathogens associated with respiratory device-related healthcare-associated infections (HAIs). Both organisms were selected because they are key indicators in equipment disinfection standards and are highly relevant to airway nursing care. In addition, Danio rerio (zebrafish) larvae were utilized as a toxicity model, as they are widely accepted as a proxy for assessing the environmental safety of disinfectant waste discharge. Since the study did not involve human participants, sample size calculation and recruitment procedures were not applicable. Each laboratory test was performed in triplicate following standard disinfectant testing protocols to ensure reproducibility and consistency of the results. The expert validators involved in the Content Validity Index (CVI) assessment included two senior nursing infection prevention and control (IPC) specialists with more than five years of clinical and academic experience, one clinical pharmacologist, and one biostatistician with expertise in the evaluation of experimental studies.

Instruments

All laboratory instruments were calibrated according to the manufacturer's specifications before data collection. The validity of the measurement methods was

ensured through the use of internationally recognized standard testing protocols, namely AOAC and SNI, which are widely accepted for disinfectant efficacy and physicochemical evaluation. The phenol coefficient assay was conducted based on the reference methods from AOAC 955.11 and SNI 1842:2019, with outcomes recorded as the dilution ratio that produced complete bacterial inactivation within 10 minutes. The pH of the solution was measured using a digital pH meter (Mettler Toledo) calibrated with buffer solutions at pH 4.00 and 7.00. Specific gravity was determined using the pycnometer method with a benchmark clinical range of 1.0–1.2 g/mL. At the same time, viscosity was measured using a rotational viscometer to evaluate flow resistance and solution handling consistency. In addition, toxicity was assessed using an observation sheet based on LC50 endpoint analysis to monitor zebrafish larvae survival over 48 hours. Because no nursing instruments, such as checklists or clinical tools, were tested at this stage, instrument contextualization focused on alignment with nurses' infection prevention and control (IPC) decision-making needs rather than immediate clinical adoption.

Intervention

Disinfection performance was tested under standardized laboratory conditions to simulate reusable airway device reprocessing commonly led by nurses in clinical IPC practice.

Physical Characterization of Chlorhexidine Disinfectant

pH, specific gravity, and viscosity of chlorhexidine solutions at concentrations of 2.5%, 5%, and 7.5% were assessed, as these key factors significantly influence the consistency, efficacy, and germicidal activity of disinfectants (Artasensi et al., 2021).

Antimicrobial efficacy

The phenol coefficient assay was used to evaluate disinfectant effectiveness, with phenol dilution applied as the reference control under identical testing conditions, following national and international standards (SNI 1842:2019; AOAC 955.11:2021). Chlorhexidine concentrations of 2.5%, 5%, and 7.5% were serially diluted and exposed to bacterial suspensions of Staphylococcus aureus and Pseudomonas aeruginosa. The phenol coefficient test remains a standard method for assessing disinfectant efficacy against specific microorganisms (Bakht et al., 2022; M. Singh et al., 2012). Samples were subsequently incubated and cultured on agar to determine the highest dilution capable of achieving complete bacterial inactivation within 10 minutes.

Toxicity and waste safety

Acute toxicity was assessed using Danio rerio (zebrafish) larvae placed in 6-well plates. Larval survival was observed for 48 hours, and LC50 values were calculated to determine the minimum dilution required before disinfectant waste discharge, supporting environmentally safe infection prevention and control (IPC) waste-handling guidance for nurses (Seo et al., 2022).

Data Collection

Indonesia between August and November 2024. Antimicrobial efficacy testing using phenol coefficient assays against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, along with physicochemical assessments including pH, specific gravity, and viscosity, was performed at PT. Saraswanti Indo Genetech Laboratory. Acute toxicity profiling using *Danio rerio* (zebrafish) larvae was conducted at the Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Malang. All

disinfectant preparation, microbial exposure, survival monitoring, and primary experimental recordings were carried out by the principal investigator, Rustiana Tasya Ariningpraja. No clinical enumerators were involved, as the study remained within the applied experimental evaluation stage. Laboratory personnel acted as local study coordinators, ensuring standardized bacterial culture preparation, zebrafish larvae maintenance, and adherence to predefined contact time and exposure protocols. Additionally, two Infection Prevention and Control (IPC) nursing experts contributed as research assistants to validate the clinical relevance and nurse applicability of chlorhexidine disinfection parameters using the Content Validity Index (CVI) method.

Data Analysis

The experimental outcomes were interpreted as endpoint measurements generated from controlled laboratory testing of chlorhexidine at concentrations of 2.5%, 5%, and 7.5%. To ensure the clinical relevance, practice alignment, and nursing applicability of antimicrobial performance, physicochemical safety, and dilution-based waste management within nurse-led Infection Prevention and Control (IPC) workflows, the study employed expert validation using the Content Validity Index (CVI). Six experts, representing nursing IPC, clinical pharmacology, and biostatistics, evaluated the relevance and practical integration of laboratory parameters using a 4-point item-relevance scale. Item-level CVI (I-CVI) and scale-level CVI (S-CVI) were calculated, supporting the translation of experimental findings into standardized, nurse-driven respiratory device disinfection and waste-handling recommendations.

Ethical Considerations

This study addressed key ethical considerations related to biosafety in handling pathogenic bacteria (Staphylococcus *aureus* and Pseudomonas aeruginosa) and zebrafish larvae as toxicity test models for disinfectant waste safety evaluation. Ethical approval was granted by the Ethics Committee of Universitas Brawijaya, Faculty of Health Sciences (Approval No. 290/EC/KEPK/08/2024). No additional institutional or clinical approvals were required, as the study was conducted in a controlled laboratory environment and remained at the pre-implementation stage, without involving human subjects.

Results

Physical Characterization of Chlorhexidine Disinfectant (pH, Viscosity, Specific Gravity)

The physicochemical properties of chlorhexidine disinfectant were analyzed at three different concentrations: 2.5%, 5%, and 7.5%. The pH values of the samples showed a slight decline with increasing concentration, ranging from 7.97 at 2.5% to 7.80 at 7.5%. Similarly, the specific gravity demonstrated a consistent upward trend, starting at 0.9055 for the 2.5% solution and increasing to 0.9511 for the 7.5% solution, indicating higher density with greater chlorhexidine content. Viscosity also varied across the concentrations, with the 2.5% sample exhibiting the lowest value at 1.482, while the 5% solution had the highest viscosity at 1.790, slightly decreasing to 1.769 for the 7.5% sample (**Table 1**).

Table 1. pH measurement, specific gravity, and viscosity values of chlorhexidine-based disinfectant

Sample	pH	Specific Gravity	Viscosity
Chlorhexidine Disinfectant 2.5%	7.97	0.9055	1.482
Chlorhexidine Disinfectant 5%	7.89	0.9343	1.79
Chlorhexidine Disinfectant 7.5%	7.8	0.9511	1.769

Phenol Coefficient Test Results

The results of the phenol coefficient testing for chlorhexidine-based disinfectants against P. aeruginosa and S. aureus reveal a clear trend in antimicrobial efficacy. The data show that as the concentration of the disinfectant increases, its phenol coefficient also rises, indicating enhanced bactericidal activity. For P. aeruginosa, the phenol coefficient values are 2.50, 4.38, and 6.25 for the 2.5%, 5%, and 7.5% concentrations, respectively. Similarly, for S. aureus, the phenol coefficients are 3.12, 5.00, and 7.50 at the corresponding concentrations. These findings underscore the direct correlation between disinfectant concentration and its effectiveness in inhibiting bacterial growth. Additionally, S. aureus demonstrates a generally higher phenol coefficient, suggesting a higher sensitivity to chlorhexidine at similar concentrations (**Tables 2 & 3**).

Table 2. Phenol coefficient values of chlorhexidine-based disinfectant against *Pseudomonas aeruginosa*

Disinfectant	Concentration	Dilution	5 min	10 min	15 min	Phenol Coefficient
Phenol	5%	1: 60	-/-	-/-	-/-	80
		1:70	-/-	-/-	-/-	
		1:80	+/+	-/-	-/-	
		1:90	+/+	+/+	+/+	
		1:100	+/+	+/+	+/+	
Chlorhexidine	2.50%	1:150	-/-	-/-	-/-	Phenol Coefficient (a/b) = 200 ÷ 80 = 2.50
		1:200	+/+	-/-	-/-	
		1:250	+/+	+/+	+/+	
		1:300	+/+	+/+	+/+	
		1:350	+/+	+/+	+/+	
Chlorhexidine	5%	1:250	-/-	-/-	-/-	Phenol Coefficient (a/b) = 350/80 = 4.38
		1:300	-/-	-/-	-/-	
		1:350	+/+	-/-	-/-	
		1:400	+/+	+/+	+/+	
		1:450	+/+	+/+	+/+	
Chlorhexidine	7.50%	1:400	-/-	-/-	-/-	Phenol Coefficient (a/b) = 500/80 = 6.25
		1:450	-/-	-/-	-/-	
		1:500	+/+	-/-	-/-	

Disinfectant	Concentration	Dilution	5 min	10 min	15 min	Phenol Coefficient
		1:550	+/+	+/+	+/+	
		1:600	+/+	+/+	+/+	

Table 3. Phenol coefficient values of chlorhexidine-based disinfectant against *Staphylococcus aureus*

Disinfectant	Concentration	Dilution	5 min	10 min	15 min	Phenol Coefficient
Phenol	5%	1: 60	-/-	-/-	-/-	80
		1:70	-/-	-/-	-/-	
		1:80	+/+	-/-	-/-	
		1:90	+/+	+/+	+/+	
		1:100	+/+	+/+	+/+	
Chlorhexidine	2.50%	1:150	-/-	-/-	-/-	Phenol Coefficient (a/b) = 250/80 = 3.12
		1:200	-/-	-/-	-/-	
		1:250	+/+	-/-	-/-	
		1:300	+/+	+/+	+/+	
		1:350	+/+	+/+	+/+	
Chlorhexidine	5%	1:250	-/-	-/-	-/-	Phenol Coefficient (a/b) = 400/80 = 5.00
		1:300	-/-	-/-	-/-	
		1:350	-/-	-/-	-/-	
		1:400	+/+	-/-	-/-	
		1:450	+/+	+/+	+/+	
Chlorhexidine	7.5%	1:500	-/-	-/-	-/-	Phenol Coefficient (a/b) = 600/80 = 7.50
		1:550	-/-	-/-	-/-	
		1:600	+/+	-/-	-/-	
		1:650	+/+	+/+	+/+	
		1:700	+/+	+/+	+/+	

Acute Toxicity Test Results

The exposure to chlorhexidine disinfectants resulted in 100% mortality among zebrafish larvae across all concentrations (2.5%, 5%, and 7.5%) after 48 hours. In each of these groups, no larvae survived, and all exhibited reduced movement (**Table 4**). The results indicate a clear dose-dependent effect, with the disinfectants proving lethal to the larvae at all tested concentrations. In contrast, the water control group showed no signs of mortality or reduced movement, highlighting the harmful impact of chlorhexidine exposure on zebrafish larvae.

Table 4. Mortality and physiological responses of zebrafish larvae after 48 hours of chlorhexidine exposure

Sample	Initial Number of Larvae	Larvae Dead (24 hrs)	Larvae Dead (48 hrs)	Survival Percentage (%)	Mortality Percentage (%)	Larvae with Reduced Movement	Reduced Movement Response (%)
Chlorhexidine Disinfectant 2.5%	30	30	30	0	100	30	100
Chlorhexidine Disinfectant 5%	30	30	30	0	100	30	100
Chlorhexidine Disinfectant 7.5%	30	30	30	0	100	30	100
Water Control	30	0	0	100	0	0	0

Expert Justification Using Content Validity Index (CVI)

Table 5 illustrates that six experts evaluated nine statements regarding the laboratory findings on chlorhexidine as a disinfectant for semi-critical respiratory equipment. Overall, almost all statements achieved I-CVI ≥ 0.78, indicating validity. Six items reached full validity (I-CVI = 1.00), and two items were valid at 0.83. In contrast, one item regarding the relationship between high concentration and antimicrobial resistance scored 0.67 and did not meet validity criteria. The S-CVI/Ave of 0.92 exceeds the ≥ 0.90 threshold, demonstrating strong agreement among experts. These results suggest that the laboratory interpretation of chlorhexidine effectiveness is scientifically robust and supports its potential use as a disinfectant for nebulizers in HAI prevention (Polit & Beck, 2010).

Table 5. Expert Evaluation of Chlorhexidine Findings Using CVI

No	Statement	Experts Agree (Score 3–4)	I-CVI	Interpretation
1	Increasing phenol coefficient with higher chlorhexidine concentrations demonstrates valid antimicrobial efficacy.	6	1	Valid
2	Phenol coefficient results against Pseudomonas aeruginosa and Staphylococcus aureus reflect reasonable differences in bacterial sensitivity.	6	1	Valid
3	The interpretation that S. aureus is more sensitive than P. aeruginosa to chlorhexidine is supported by experimental results and literature references.	6	1	Valid
4	pH measurements of chlorhexidine solutions (2.5–7.5%) indicate relevant chemical stability.	6	1	Valid
5	Increasing specific gravity with concentration can be interpreted as an indicator of higher active ingredient content.	6	1	Valid
6	Viscosity changes reflect valid physical characteristics affecting solution stability and usability.	5	0.83	Valid
7	Decreased viscosity at 7.5% (compared to 5%) was correctly interpreted as a potential effect of intermolecular interactions.	6	1	Valid
8	The relationship between high chlorhexidine concentration and potential development of antimicrobial resistance is relevant in the context of sub-lethal exposure.	4	0.67	Not Valid

No	Statement	Experts Agree (Score 3–4)	I-CVI	Interpretation
9	Optimal chlorhexidine concentration adjustment (e.g., 5% as the midpoint of viscosity and effectiveness) can be concluded from the results.	5	0.83	Valid

Table 6 illustrates the determination of Optimal Chlorhexidine Concentration Based on Phenol Coefficient, Physicochemical Properties, and CVI. The optimal concentration of chlorhexidine was determined by integrating three main parameters: bactericidal efficacy (phenol coefficient), solution characteristics (pH, viscosity, specific gravity), and content validity through CVI.

Table 6. Optimal Chlorhexidine Concentration for Semi-Critical Respiratory Equipment Disinfection

Disinfectant	Phenol Coefficient (S. aureus)	Phenol Coefficient (P. aeruginosa)	pH	Specific Gravity (g/mL)	Viscosity (cP)	CVI (Validity)	Overall Interpretation
Chlorhexidine 2.5%	3.12	2.5	7.97	0.9055	1.482	Valid	Effective, but low viscosity and low specific gravity may shorten the contact time
Chlorhexidine 5%	5	4.38	7.89	0.9343	1.79	Valid	Effective, stable pH, highest viscosity for prolonged contact, balanced specific gravity → considered optimal
Chlorhexidine 7.5%	7.5	6.25	7.8	0.9511	1.769	Valid	Highest bactericidal effect, but higher density and lower practical usability for routine clinical application

Integration of these parameters indicates that while all chlorhexidine concentrations are effective against *S. aureus* and *P. aeruginosa*, the physical characteristics and practical applicability determine the optimal choice. Chlorhexidine 2.5% is effective, but low viscosity and low specific gravity may reduce surface contact stability. Chlorhexidine 7.5% achieves the highest phenol coefficient but is denser, potentially less practical for routine use. Chlorhexidine 5% offers the best balance: high phenol coefficient, stable pH, highest viscosity for prolonged contact, and moderate specific gravity. CVI validation confirms expert agreement that 5% is the optimal laboratory-based concentration for safe and effective nurse-applied disinfection of semi-critical respiratory devices.

Discussion

Physicochemical Properties

The physicochemical characteristics of chlorhexidine disinfectants, namely pH, specific gravity, and viscosity, are critical determinants of formulation stability, handling feasibility, and antimicrobial performance (Rutala & Weber, 2019, 2023). pH influences chemical stability and antimicrobial activity, while viscosity affects sprayability, surface coverage, and retention time. Specific gravity contributes to surface adhesion and droplet behavior during application. Collectively, these

properties influence disinfectant deposition and interaction with target surfaces, particularly in electrostatic or spray-based applications commonly used for equipment reprocessing (Artasensi et al., 2021; Chauhan et al., 2023).

The observed pH values across tested concentrations remained within a near-neutral range, suggesting acceptable chemical stability and a lower potential for mucosal or respiratory irritation compared with more acidic disinfectants, such as chlorine-based agents (Rhee et al., 2023). Despite this, the pH range of 7.97 to 7.80 remains within the neutral spectrum, indicating that the disinfectant is neither significantly acidic nor alkaline. Due to its pH being closer to neutral, the disinfectant is likely to pose a lower risk compared to more acidic formulations, such as chlorine-based disinfectants, which can have a higher potential for irritation or respiratory disturbances (Clausen et al., 2020).

The specific gravity of the solutions showed a consistent upward trend, increasing from 0.9055 at 2.5% to 0.9511 at 7.5%. This change indicates an increase in the density of the solutions, which is closely tied to the concentration of active ingredients. While higher specific gravity can suggest enhanced antimicrobial efficacy, as it may improve the disinfectant's ability to adhere to and interact with surfaces, it is important to note that the primary factor determining effectiveness is the solubility of the active ingredients in water (Maillard & Pascoe, 2024). According to CDC guidelines for disinfectants, solubility is a key requirement for ensuring proper performance (Rutala & Weber, 2019). Therefore, a concentration range of 5% to 7.5% would be recommended for situations requiring stronger antimicrobial action, as these concentrations optimize both the effectiveness and the stability of the formulation.

The viscosity of the disinfectants varied across concentrations, with a notable peak at the 5% formulation (1.790), followed by a slight reduction at 7.5% (1.769). This non-linear behavior might be attributed to changes in intermolecular interactions at higher concentrations. The moderate viscosity of the 5% solution suggests an optimal balance between fluidity and thickness, which could enhance the uniform application and retention of the disinfectant on surfaces. Viscosity plays a crucial role in ensuring effective disinfectant performance, as it allows for more even distribution and better adherence to surfaces, increasing the interaction time with microorganisms (Sivamani Chidambaram et al., 2024). In contrast, the slight decrease in viscosity at 7.5% might improve ease of handling without significantly compromising coverage, making it easier to apply without sacrificing efficiency. Therefore, viscosity directly influences the efficiency, comfort, and overall effectiveness of disinfectant use, ensuring optimal performance across various surfaces.

Antimicrobial Efficacy

The phenol coefficient test results provide compelling evidence of the bactericidal efficiency of chlorhexidine disinfectants, with a clear positive correlation between concentration and antimicrobial effectiveness. Against *P. aeruginosa*, the phenol coefficients progressively increased from 2.50 at a 2.5% concentration to 6.25 at 7.5%. A similar trend was observed for *Staphylococcus aureus*, where the values increased from 3.12 to 7.50 across the same concentration range. These findings emphasize that higher chlorhexidine concentrations are significantly more effective at bacterial inhibition.

Interestingly, the phenol coefficients for *S. aureus* were consistently higher than

those for P. aeruginosa at all concentrations, suggesting greater susceptibility of Gram-positive bacteria to chlorhexidine. This disparity is likely due to structural differences between the bacterial cell walls (Torrens & Cava, 2024; Yoo, 2018). Gram-positive bacteria possess a thicker peptidoglycan layer, which chlorhexidine may penetrate more effectively compared to the outer membrane of Gram-negative bacteria, such as *P. aeruginosa* (Cheung et al., 2012; Mai-Prochnow et al., 2016). This observation aligns with existing literature and supports chlorhexidine's potential as a versatile disinfectant, particularly in settings where Gram-positive pathogens pose a higher risk.

Acute Toxicity

Acute toxicity testing using zebrafish larvae demonstrated that chlorhexidine exhibits marked toxicity to aquatic organisms under direct exposure conditions. The absence of mortality in the water control group supports the attribution of observed effects to chlorhexidine exposure rather than experimental conditions. These findings highlight the importance of evaluating disinfectant waste handling and dilution before environmental discharge, particularly in healthcare settings where routine disposal may occur (Sahoo et al., 2024). Although laboratory toxicity findings cannot be directly extrapolated to real-world environmental concentrations, they provide an early warning regarding potential ecological risks associated with improper disposal. Therefore, dilution and neutralization before waste discharge should be considered as precautionary measures within nurse-led infection prevention workflows (Wang et al., 2022).

As well as contributing to environmental pollution, the improper disposal of disinfectant waste can significantly exacerbate the development of antimicrobial resistance. Excessive use of chemicals in disinfectants introduces these substances into urban ecosystems, where they impose unprecedented selective pressure on microorganisms. Research conducted by Hu et al. (2023) on environmental samples from hospital surroundings revealed that the presence of disinfectant-related chemicals elevated the concentration of antibiotic resistance genes by 1.4 to 5.8 times, with a corresponding 20.1-fold increase in the relative abundance of resistance genes under heightened selective pressure (Hu et al., 2023). These chemicals were particularly effective in enhancing microbial resistance mechanisms, promoting cross-resistance in bacteria, and facilitating the horizontal transfer of resistance genes, ultimately shaping the resistance gene profile.

To minimize environmental and health risks, used disinfectants should be neutralized before disposal. This can be achieved through bioassay-based protocols and appropriate dilution, ensuring reduced toxicity and preventing the spread of antimicrobial resistance (Wang et al., 2020). Secure storage and controlled deactivation are essential for safe waste management.

Expert Validation Using Content Validity Index (CVI)

Expert validation using the Content Validity Index (CVI) supported the relevance and applicability of the study findings within nursing infection prevention practice. High agreement among experts confirmed the consistency of antimicrobial trends, physicochemical suitability, and the practical feasibility of chlorhexidine application for respiratory equipment reprocessing. One item related to high-concentration chlorhexidine and antimicrobial resistance yielded lower consensus, reflecting ongoing uncertainty in the literature and reinforcing the need for

cautious interpretation. (Weissheimer et al., 2023).

Overall, expert agreement supported the selection of moderate chlorhexidine concentrations as providing a balance between antimicrobial effectiveness, handling feasibility, and safety considerations. This validation strengthens the translational relevance of the findings for nurse-led disinfection practices (Ge et al., 2023; Polit & Beck, 2010).

Current Application of Chlorhexidine in Semi-Critical Respiratory Device Disinfection and Nursing Practice

Chlorhexidine is increasingly applied as an antiseptic in healthcare settings due to its broad-spectrum antimicrobial activity and effectiveness in preventing hospital-acquired infections. It is commonly used in 2% or 4% concentrations for skin antisepsis, particularly with chlorhexidine-impregnated cloths, which significantly (Clarke et al., 2023). In oral care, concentrations of 0.12% to 0.2% are employed, especially for preventing ventilator-associated pneumonia (Singh et al., 2022). The effectiveness of these antiseptic applications is influenced by factors such as method of application, frequency, and patient-specific conditions.

Despite its wide use for antisepsis, chlorhexidine application as a disinfectant for semi-critical medical devices remains limited. Hutauruk et al. (2021) demonstrated that 2.5% chlorhexidine effectively eradicated bacterial colonies and biofilms on tracheostomy cannulas (Hutauruk et al., 2021). Similarly, Ariningpraja et al. (2024) assessed chlorhexidine concentrations of 2.5%, 4%, and 5% against *Klebsiella pneumoniae*, confirming antimicrobial efficacy even at the lowest concentration (Ariningpraja et al., 2024).

These findings highlight chlorhexidine's potential as a disinfectant for semi-critical respiratory devices. From a nursing-applied perspective, nurses play a pivotal role in integrating chlorhexidine disinfection into standardized, evidence-based workflows. By managing proper concentration, contact time, and safe handling, nurses ensure optimal microbial reduction and prevent cross-contamination during routine respiratory equipment reprocessing. Nurse-led implementation of chlorhexidine disinfection strengthens infection prevention and control (IPC) practices, particularly in high-risk settings such as tracheostomy care and mechanically ventilated patients.

Implications and limitations

This study contributes applied pre-clinical evidence by integrating antimicrobial efficacy, physicochemical characterization, and expert validation to inform nurse-led disinfection practices for reusable semi-critical respiratory equipment. The findings support a structured approach to disinfectant selection that considers both laboratory effectiveness and practical usability within nursing workflows.

Nevertheless, several limitations should be acknowledged. The antimicrobial evaluation was limited to two bacterial species, and toxicity assessment was restricted to zebrafish larvae as an environmental proxy. The controlled laboratory setting does not fully capture clinical variables such as organic load, device material heterogeneity, or real-world handling variability. Additionally, long-term environmental and resistance-related outcomes were not directly evaluated. Future studies should incorporate broader microbial panels, extended environmental assessments, and field-based evaluations to enhance generalizability and clinical relevance.

Relevance to Practice

From a nursing and healthcare perspective, this study provides actionable guidance for the safe and effective use of chlorhexidine in disinfecting semi-critical respiratory devices. Nurses can implement the 5% concentration formulation, ensuring optimal viscosity, pH stability, and bactericidal activity, while minimizing risks associated with overly dilute or overly dense solutions. By adhering to evidence-based disinfection protocols, including proper contact time, dilution, and handling, nurses can reduce microbial contamination, prevent cross-infection, and strengthen hospital infection prevention and control programs. Institutions may also leverage these findings to develop standardized workflows and staff training programs for consistent, safe, and effective device disinfection.

Conclusion

Chlorhexidine demonstrates strong bactericidal activity against Staphylococcus aureus and Pseudomonas aeruginosa, with favorable physicochemical properties observed at a 5% concentration. Expert validation using the Content Validity Index confirms the scientific reliability and nursing applicability of these findings. Integrating laboratory-based efficacy with practical usability supports the use of 5% chlorhexidine as a feasible option for nurse-led disinfection of semi-critical respiratory devices, particularly in resource-limited and reusable-device settings common across international nursing practice. However, as this study was conducted at a pre-clinical laboratory level, further clinical and field-based studies are required to evaluate real-world effectiveness, workflow integration, and long-term safety before broader implementation.

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CrediT Authorship Contributions Statement

Rustiana Tasya Ariningpraja: Conceptualization, Methodology, Project administration, Supervision, Literature review, Formal analysis, Writing – original draft, Writing – review & editing.

Lucky Jayadi: Instrument development, Data curation, Validation, Statistical analysis.

Andini Maslukha: Investigation, Data collection, Field coordination, Participant management.

Nuraeni Effendy: Investigation, Data collection, Field coordination, Data quality control.

Conflicts of Interest

There is no conflict of interest.

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Supplementary Materials (OPTIONAL)

Supplementary File S1: Research Instrument contains the full questionnaire used for data collection.

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