EFFICACY OF DISINFECTANTS AGAINST MULTI-DRUG-RESISTANT BACTERIA ISOLATED FROM CLINICAL SAMPLES AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA

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By

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A Dissertation Submitted in (Partial) Fulfilment of the Requirements for the Degree of Master of Science in (Microbiology and Immunology) of

the Muhimbili University of Health and Allied Sciences
November, 2024

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled "**Efficacy of disinfectants against multi-drug resistant bacteria isolated from clinical samples at Muhimbili National Hospital, Dar es salaam, Tanzania",** in Partial fulfilment of the requirements for the degree of Master of Science in (Microbiology and Immunology) of the Muhimbili University of Health and Allied Sciences.

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(Main Supervisor)

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Date

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Dr. Salim S Masoud

(Co-Supervisor)

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Date

DECLARATION AND COPYRIGHT

I, **Daniel Paul Mwandu,** declare that this dissertation is my original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature…… **Date**…….

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DEDICATION

This dissertation is dedicated to my lovely wife Neema and our loving son, Ephraim, for their patience during my studies.

ABSTRACT

Background: **Increased multi-drug resistant (MDR) bacterial infections have been associated with increased hospital care costs and mortality due to Health Care Associated Infections (HCAIs). This due fact that, bacteria resistant to disinfectants have also been reported and are on the rise**. Disinfectants are prerequisites for infection control and prevention in healthcare settings. Increased bacteria resistant to antimicrobial agents, including disinfectants, warrants ongoing monitoring efficacy of routinely used disinfectants in hospitals settings. **However, there is a paucity of information on the efficacy of routinely used disinfectants, especially in developing countries, which reflects the need for conducting this study.**

Objective: To evaluate the efficacy of disinfectants against multidrug-resistant bacteria isolated from clinical samples **at Muhimbili National Hospital.**

Materials and Methods: A laboratory-based experiment was conducted to assess the efficacy of disinfectants by using dilution test methods and the quantitative suspension test methods. The Quaternary ammonium compound (QACs), povidone iodine, and sodium hypochlorite (NaOCl) at undiluted and ready to use concentration were collected and tested against four clinical isolates from **blood sample** and two quality control strains: **Methicillin**-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E.coli),* carbapenem-resistant *Acinetobacter baumannii (CR-A. baumannii),* and carbapenemase producer *Klebsiella pneumoniae (*CPKP*),* and reference (quality control) strains (*Escherichia coli* ATCC 35918 and *Staphylococcus aureus* ATCC 25923*).*

Results: All tested bacteria were significantly killed with >99.999% reduction rate at contact exposure of 10 minutes by all selected disinfectants. The 1% QACs killed all tested bacteria at five minutes of exposure, however only gram-positive bacteria were rapid killed after one minute. The 0.5% NaOCl was not efficacious at one minute to all tested bacteria, furthermore at 5 minutes, was significantly only for E. *coli* ATCC 35918 and CRKP. Exposure of 10% povidone iodine for 1 and 5 minutes did not significantly reduced MRSA and carbapenem-resistant *Acinetobacter baumannii*. For QACs, the MICs of both ATCC control strains and MDR bacteria were ranged from 3,125

to 6,250 mg/L and produced the same MBCs of 6250 mg/L. The MICs of MRSA and ESBL-*Escherichia coli* for povidone iodine were twice higher than their reference, and its MBCs was fourfold higher. The MICs of MRSA for NaOCl was four times higher than ATCC-*S. aureus* 25923 while its MBCs was twice higher. **Generally, MDR bacteria shown higher disinfectant tolerance than their corresponding ATCC bacteria strains. By comparison, QACs was highly efficacy than other disinfectants.**

Conclusions: This study found that, **for all tested disinfectants the minimum contact exposure time** to bacteria for 10 minutes is required for complete elimination of bacteria. However, **the QAC was efficacy at 5 minutes contact time**. Therefore, adherence to contact time is critical when disinfection is performed in infection prevention and control.

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LIST OF ABBREVIATIONS

CDC	Centre for Disease Control
CPL	Central Pathology Laboratory
CR	Carbapenem-resistant
CPKP	Carbapenemase producer *Klebsiella pneumoniae*
ESBL	**Extended spectrum β lactamase producers**
FDA	Food and Drug Administration
HCAI	**Health Care Associated Infections**
IPC	Infection prevention and control
LRs	logarithmic reductions
MBC	Minimum bactericidal concentration
MDR	Multi-Drug Resistant
MIC	Minimum inhibition concentration
MNH	Muhimbili National Hospital
MRSA	Methicillin-resistant *Staphylococcus aureus*
MUHAS	Muhimbili University of Health and Allied Sciences
QAC's	Quaternary ammonium compounds
WHO	World Health Organization

DEFINITION OF TERMS:

Antiseptics: a substance (such as isopropyl alcohol or povidone-iodine) that inhibits the growth of microorganisms (such as bacteria) especially in or on living tissue usually used during medical procedures or used to maintain sanitation in place with risk of microorganism contamination.

Disinfectants: Any substance (such as chlorine, hydrogen peroxide, or sodium hypochlorite (NaOCl) that is used primarily on non-living objects to kill living microorganisms (such as bacteria, viruses, and fungi), except bacterial spores

Clinical Samples: are biological materials (such as blood, urine, pus and others) that are collected from humans, generally for examination, diagnostic confirmation, patient monitoring, study, or analysis

Efficacy is the ability of the disinfectant to produce a desired amount of effect, or success in achieving a given goal such as killing of all microbes at recommended exposure contact time.

Multi-Drug Resistant Bacteria: are bacteria isolates that lack susceptibility to at least one agent in three or more antibiotic classes.

Logarithmic reductions: is a mathematical term that is used to express the relative number of living microbes that are eliminated by disinfection. Log reduction = log10 (N_0 /N) Where: N_0 = colony forming units of the microorganisms before exposure to test products(disinfectant)

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Multidrug-resistant (MDR) bacteria have become a major global burden, contributing to increased healthcare-associated infections **(HCAI)** that have been linked to over 50% higher mortality rates (1–3). The prevalence of MDR in patients with bloodstream infection (BSI) is about 70.5%, of which extended-spectrum β-lactamase (ESBL) producers, carbapenem-resistant *Pseudomonas aeruginosa*, and **Methicilli**n-resistant *Staphylococcus aureus* (MRSA) account for the majority **of cases** (4,5).

Contaminated surfaces are the major contributor to the spreading of multidrug-resistant (MDR) strains in hospital settings and have been implicated in causing most nosocomial infections (6). **According to World Health Organization (WHO) report of 2024, it was shown that, *Acinetobacter baumannii* and Enterobacterales order pathogens, are listed as of critical priority because of their ability to transfer resistance genes, while *Staphylococcus aureus* is among the High-priority pathogens** (7). It was revealed that *Acinetobacter* spp. isolated from hospitalized patients and medical equipment were 75% and 74%, respectively (8). *Acinetobacter baumannii* was associated with HAI, with an overall MDR prevalence of 91.0% in long-hospitalized patients (9). Therefore, adhere to proper sterilization and disinfection of inanimate matter could significantly reduce the risk of health care associated infections (10).

The use of antiseptics and disinfectants as one of the most effective strategies for infection prevention and control measures in hospital settings can prevent the spread and colonization of MDR bacteria (11). However, it was shown that MDR bacteria were resistant even to commonly used disinfectants and antiseptics. It is thought they acquired resistance by mutation and carriage of antimicrobial-resistance genes**,** which can cause resistance to disinfectants (12–14).

The rate of disinfectant resistance increased due to the overuse of disinfectants during the COVID-19 pandemic, shown increase in minimum inhibitory concentrations (MICs) for

quaternary ammonium compounds (QACs) disinfectants **from 200 to 16,000 µg/ml** for most isolated Gram-negative bacteria (15). Moreover, it was shown that the MICs and minimum bactericidal concentration (MBCs) of sodium hypochlorite (NaOCl) were 0.2% and 0.8% against MDR Gram-negative respectively**,** which were higher than reference strains (16). Also, in another study, it was shown that the 40.7%, 22.2%, and 24.1% MDR bacterial isolates had higher MICs values for QACs, povidone-iodine, and NaOCl than their corresponding ATCC strains, respectively (17). This is alarming for continuous monitoring of the efficacy of the routinely used antiseptics and disinfectants in hospitals.

1.2 Problem Statement

MDR bacteria are widely found in hospital environments and are major causes of **(HCAI)** (18). Antibiotic-resistant bacteria like MRSA, **carbapenemase** producer, and ESBL producers are the most dangerous and can cause serious infections that are difficult to treat and result in increased morbidity and mortality rates worldwide (19–21). Apart from being multidrug-resistant, these pathogens have shown resistant to commonly used disinfectants. It has been found that certain types of bacteria, particularly MDR gram-negative bacteria like *Acinetobacter baumannii,* have become resistant to commonly used disinfectants with doubled killing time (20, 28, 29).

MDR bacteria in Tanzania cause both community associated infections (CAI) and HCAI, with a mortality rate of 3.7% (24). Hospital environments have been reported often to be contaminated with resistant bacteria, posing a risk of spreading to patients. Recent studies done at Muhimbili National Hospital and Bugando Medical Centre found high rates of contamination with **carbapenem-resistant genes** on hospital beds (6,25).

Muhimbili National Hospital (MNH) uses various disinfectants and antiseptics like NaOCl, povidone iodine, QACs and alcohol-based products to clean the environment, wounds, and during blood withdrawal. However, their efficacy against MDR bacteria has not been evaluated, making it difficult to recommend their usage. Therefore, this study was conducted to investigate the efficacy of commonly used disinfectants against multidrug-resistant bacteria at MNH.

1.3 Rationale of the Study

This research evaluated the efficacy of commonly used disinfectants against multidrug-resistant (MDR) bacteria at Muhimbili National Hospital (MNH). The study established the minimum exposure time required to kill 99.999% of all bacteria, along with the MICs and MBCs for each disinfectant.

These findings are crucial, because **will helps healthcare workers to make informed decisions on proper disinfectants usage with regards to exposure contact time and disinfectants concentration**. This will minimize spreading of MDR bacteria, leading to lower morbidity and mortality rate caused with **HCAI**. **Additionally, these information's will guide selection of the best disinfectants, resulting into reduction of hospitals costs**. Also, this study had sheds light on the burden of antiseptic and disinfectant resistance/tolerance. This information will help health stakeholders to develop specific approaches for combating antimicrobial resistance (AMR), **including establishment of disinfectants stewardship.**

Moreover, this study's insights provide practical guidance for infection prevention and control (IPC) practices that aligning with the National Action Plan on Antimicrobial Resistance (NAP on AMR). Also, the research findings will help to improve MNH policies and processes for appropriate disinfection and decontamination

Therefore, the findings from this study will empower healthcare professionals, policymakers, and the Ministry of Health (MoH) to make evidence-based decisions that enhance patient safety, reduce costs, and combat antimicrobial resistance.

1.4 Conceptual Framework

The study's quantitative and qualitative variables are shown in the figure 1 below. It illustrates how they interact with each other. The figures show that when bacteria from clinical samples such as MRSA, extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL *E. coli*), CR-*A. baumannii* and carbapenemase producer *Klebsiella pneumoniae* (CPKP), are exposed to disinfectants at a recommended concentration and at different exposure time, result in their killing. This can be measured by logarithmic reduction. The MICs and MBCs can also be used to assess the efficacy of disinfectants.

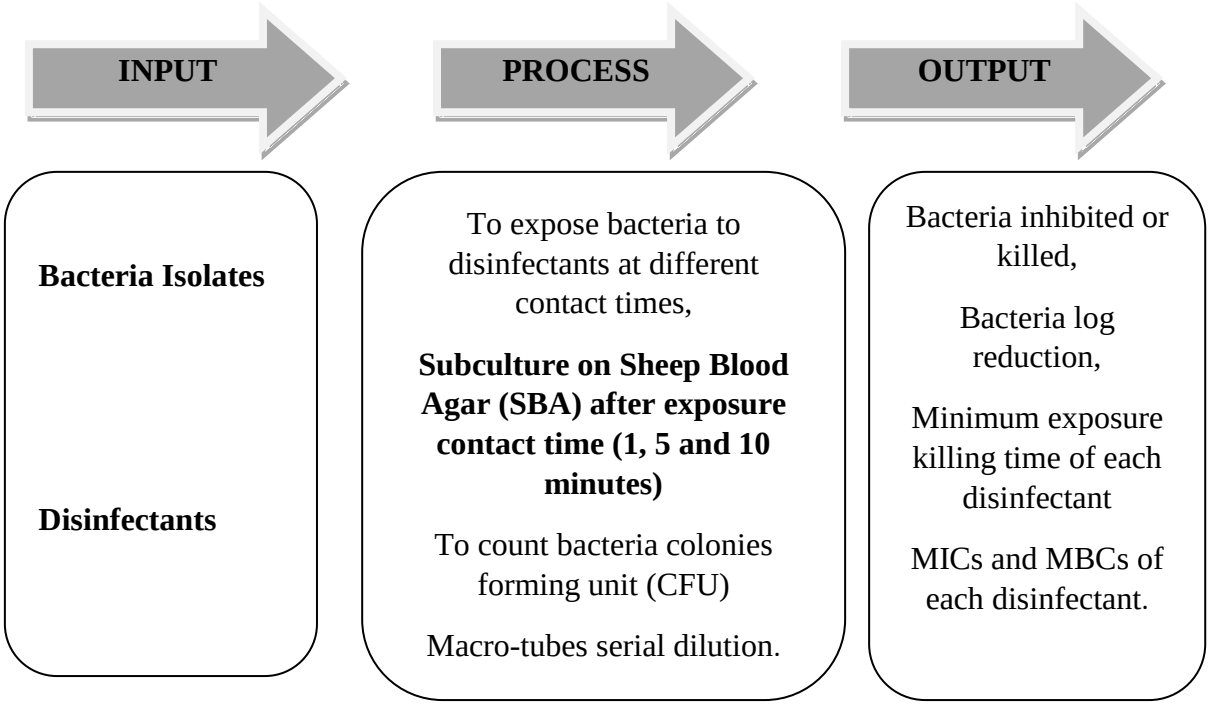


Figure 1: **Conceptual framework narrating the impact of disinfectants on MDR Bacteria**

1.5 Research Questions

What is the efficacy of routinely used disinfectants (QACs, povidone iodine, and NaOCl) against multidrug-resistant bacteria isolated from clinical samples at Muhimbili National Hospital (MNH)?

1.6 Objectives

1.6.1. Broad objective

To evaluate the efficacy of disinfectants **(QACs, povidone iodine, and NaOCl)** against multidrug-resistant bacteria isolated from clinical samples **at MNH.**

1.6.2. Specific objectives

i. To investigate the minimum exposure time of QACs, povidone iodine, and NaOCl required to kill multidrug-resistant bacteria isolated from clinical samples at MNH by 99.999%.
ii. To determine the susceptibility of QACs, povidone iodine, and NaOCl against MDR bacteria isolated from clinical samples at MNH.
iii. To compare the efficacy of routinely used disinfectants in killing MDR bacteria.

1.7 Literature Review

1.7.1 The burden of MDR bacteria in relation to disinfectants

Bacterial antimicrobial resistance (AMR) has been one of the biggest threats to public health in the 21st century. AMR was predicted to kill 10 million individuals annually by 2050 (1). It is of concern that most MDR Bacterial infections are hospital-acquired due to high rates of hospital contamination on both environments and devices. A study done in developed countries revealed that MDR *Acinetobacter* spp. was common in hospitalized patients and critical hospital equipment's by 75% and 74%, respectively (8). Furthermore, a study carried out in Kenya revealed that, **hospital frequently touch surfaces were contaminated with MDR bacteria** by 12.6%. Also, in patient areas were frequently contaminated with meropenem resistant bacteria, which is in a class of last-resort antibiotics (26).

In Tanzania, studies done at Muhimbili National Hospital (MNH) found a high rate of hospital environmental contamination, with a prevalence of MDR gram-negative bacteria up to 61.4% (6). Noteworthy, it was reported from the study done at and Bugando Medical Centre (BMC) that 82.6% of *Acinetobacter* spp. harbored genes encoding carbapenems resistance were contaminants of hospital surfaces (25). This puts forward the need for strengthening infection prevention and control (IPC) measures through the appropriate use of disinfectants and antiseptics to improve patient care.

Despite adopting several strategies for IPC, most gram-negative bacteria have become resistant to antibiotics used in treatment as well as antiseptics and disinfectants. The frequent use of disinfectants and antiseptics has imposed selective pressure, resulting in the emergence of disinfectant-resistant and antiseptic-tolerant bacteria (12,27). This is alarming for continuously assessing the effectiveness of disinfectants and antiseptics in our setting by evaluating factors for biocide agent activity such as the tolerant capacity of MDR bacteria, microbial load, pH, exposure time required to kill bacteria, contact surface, biofilm formations, the susceptible concentration of agents (MICs and MBCs), and mutant selection pressure (28).

1.7.2 Minimum exposure time required for disinfectants to kill MDR bacteria.

The responses of different types of microorganisms to antiseptics and disinfectants vary and depend on the microbiostatic or microbicidal effect. Disinfectant's concentration and contact time are basic on killing bacteria through protein denaturation and cell wall destruction (29). The Food and Drug Administration (FDA) has established specific standards for exposure contact time that determine the efficacy of disinfectants and antiseptics against bacteria. Usually needs the germicide to eradicate 99.9% of bacteria in five minutes on a hard, non-porous surface (30,31). It was revealed that ethanol concentrations, ranging from 30% to 100%, kill most gram-negative bacteria in 10 seconds (32). A study has shown that gram-negative bacteria are more resistant to disinfectants than gram-positive bacteria, particularly *P. aeruginosa*, which required a contact time of more than 19 minutes with QACs while Gram-positive bacterial strains MRSA required only contact times of 15 minutes to be eradicated (23). The findings from the previous study done in Punjab, India by Gaurav Sharma et al, in 2022, had found ≥5 LRs (99.999% bacteria were killed)for 1% QACs at contact time of 5 ,10 and 15 minutes against *S. aureus, pseudomonas aeruginosa, E.coli and Enterococcus hirae* (33). Another study done in Singapore by Eng Lee Tan et al, in 2021, was shown that 10% povidone iodine had ≥5 LRs at 30 seconds and 60 seconds against *Enterococcus faecium, E.coli, Streptococcus pyogenes* and MRSA (34). The finding from the study done by Prisca Damiano et al, in 2023 at Bugando Medical Center, Mwanza, Tanzania had shown LRs of ≥5 at 1 minutes against MDR bacteria for fresh prepared chlorine compound(Sodium dichloroisocyanurate(NaDDC)), but for three-day aged disinfectants LRs range from 0.59(74.1% bacteria were killed) to 1.53(97% of bacteria were killed) (35). **In our setting there is scarcity of information on evolution of minimum exposure contact time required to kill all bacteria. Therefore, evaluating the minimum exposure time for routinely used disinfectants is crucial and might be attribute change in IPC practice so as kill all MDR bacteria, subsequently reduction in mortality case caused by HCAI.**

1.7.3 Disinfectants resistance to multidrug-resistant bacteria

Bacteria reduce susceptibility to antiseptic and disinfectant resistance through various mechanisms. In a study done by Gupta P et al, it was shown that the 40.7% MDR bacteria showed an increase in MIC values for 3.9% w/v QACs compared to ATCC control strains. Also, 18.5%, 22.2%, and 24.1% had increased MICs values for absolute ethanol, 5% w/v povidone-iodine, and NaOCl, respectively, than their corresponding control strains (17). Also, in another study, it was shown that the MICs and MBCs of NaOCl were 0.2% and 0.8% against MDR gram-negative which was higher than reference strains (16). The emergence of bacteria resistant to antibiotics and disinfectants has been associated with the wide use of QACs. After exposure to these compounds, it was reported that *Pseudomonas aeruginosa* became more resistant when slowly exposed to higher amounts of QACs, which led to an increase in MIC from 0.0025% to 0.058% (a 20-fold increase) and was associated with slightly increased resistance to different antibiotics, including carbapenems (36). Also, the highest MIC values of more than 0.5% were reported for benzalkonium chloride against gram-negative bacteria including *P. aeruginosa* which was largely higher than the proposed epidemiological cutoff (below 0.0064% for most species) (31). Chlorine and iodophor compounds also are the most used biocide, it was found that NaOCl kills most bacteria with the highest MIC values among isolates such as *E. faecalis* (3.2%), *Escherichia coli* (1.2%), *P. aeruginosa* (0.8%), and *S. aureus* (1.6%), and yeasts (1.6%)(37,38). **Povidone-iodine is a broad-spectrum antimicrobial agent used as an antiseptic and disinfectant. It denatures proteins and disrupts cell membranes, effectively killing various pathogens. Its low toxicity and versatility make it ideal for skin cleansing, minor wound care, and disinfecting surfaces and equipment in healthcare settings** (10). In another study, it was shown that *S. aureus* and *E. faecalis* were more resistant to alcohol-based products than the other microorganisms. Regarding alcohol-based products, *S. aureus* displayed MICs and MBCs values of 75% and 50%, respectively (39). Betchen M *et al* conducted a study, where MICs and MBCs of the *A. baumannii* strains were determined for commonly used disinfectants and antiseptics, including bleach, ethanol, and isopropanol and it was found that the MICs, MBCs, and MBCs/MICs for bleach was 0.037 µg/ml, 0.098 µg/ml and 2.65, respectively and for ethanol was 5.95 µg/ml, 8.125 µg/ml and 1.37, respectively (14). **Therefore, due to**

limited disinfectants resistance data in our setting, it is essential to evaluate the extent of disinfectant resistance in order to develop effective strategies for eliminating MDR bacteria. This assessment will also support the establishment of disinfectant stewardship programs.

1.7.4 Laboratory methods used to assess the Efficacy of Disinfectants against bacteria.

There are several methods of testing the efficacy of disinfectants, but all disinfectant test methods have the same end goal of determining the disinfectant's ability to kill or inactivate microorganisms. the following are descriptions of the most common and acceptable methods

A. Suspension test method

In this test, a suspended sample of the bacteria from a culture plate is mixed in the disinfectant solution, after exposure to certain contact time, it is subcultured to observe whether it’s killed or not. The test can be done in qualitatively, whereby a bacterial suspension is mixed with disinfectant and cultured to observe whether there is growth or not. In quantitative methods, the number of surviving organisms should be counted and compared to the subculture that was not exposed to disinfectant. The microbicidal effect (ME) is then calculated by subtracting the log reduction from the initial count. A LR of 1 kill 90% of bacteria, while an LR of 2 kills 99%. An LR of 5 or more is needed to kill at least 99.999% of germs, which is the generally accepted standard (40,41). Based on the VAH method 9 which is equivalent to EN 13727, it is important to examine the exposure time at 5, 10, 15, 30, and 60 minutes. After the exposure, the number of bacteria survivors should be used to determine the logarithmic reductions (LRs) (37,38).

B. Diffusion test method

The diffusion test has been used for a long time, as a starting point to assess a given microorganism susceptibility to different chemical agents. In this test a test organism is inoculated on Mueller-Hinton agar plate by using confluent lawn method. Followed by placing of filter paper disks impregnated with known concentrations of disinfectants or by punching wells then a known concentration of disinfectants should be filled into respective

well. The disinfectants or chemical diffuses from the circular disk into the agar and interacts with the growing bacteria. disinfectant activity is observed by formation of a clear circular zone of inhibition around the disinfectants which must be compared to standard for interpretation (42).

C. Disinfectant dilution test methods

Dilution tests are useful on determination of disinfectant's MICs, and MBCs. The disinfectants/antiseptics should be diluted in series into tube contain broth, then test bacterial suspension is added to each tube. The MIC is determined by looking through the tubes for the lowest concentration that inhibits visible growth (turbidity/cloudiness). To determine the MBCs, tubes with no visible growth are inoculated onto agar media (43).

D. Capacity test (Kelsey-Sykes test)

This method used to assess the disinfectant's capacity, which is the ability to continue acting even when the amount of contamination increases. In a capacity test, the disinfectant is put to the test over and over again by adding bacterial suspension one after another until its killing power is depleted. Tests of capacity replicate real-world scenarios such as cleaning and instrument decontamination (40).

E. Stimulated use test method

In this method, the volunteers are required to wash their hands with a five-milliliter of soap for a minute, afterwards rinse them under running water and dry them with paper towels. Then the hand will be immersed into media containing the test organism followed by washing by using test product(disinfectants). The effectiveness of disinfectants was evaluated by comparing the bacteria survivors reduction factor before and after applying disinfectants (44,45)

F. Carrier test method

These methods used to test the efficacy of disinfectants on medical devices and environmental surfaces, also known as VAH 15 method corresponding to EN 14561 (bactericidal). In this process, medical instruments are contaminated by being submerged in a liquid that contains cultures of test organisms, drying them afterwards, and exposing

them to a disinfectant solution for a specific period of time. Followed by a subculture of the disinfected medical instrument. The efficacy of disinfectants/antiseptics is determined by log reduction(44,45) Additionally, the test can be conducted in both dirty and clean environments and makes use of smooth glass surfaces and disinfectant concentrations higher than those likely to be encountered in the field (46).

G. In-use test

It is useful in identifying contaminated disinfectants. 1 ml of disinfectant is added into 9 ml of an inactivator-containing diluent. The diluted sample are subcultured on nutrient agar plates. Followed by incubating one at 37 °C for 24 to 72 hours. Any plate with growth indicates contamination (47,48).

H. Practical test (surface disinfectant test)

It used to measure the time-concentration relationship of the disinfectant; practical tests are performed under real-life conditions. The goal is to see if the proposed use dilution is still adequate in the conditions that it would be used in. The surface disinfection tests are the most well-known practical tests. The test surface (a small tile, a stainless-steel disc, etc.) is contaminated with a standardized inoculum of the test bacteria and dried: then a definite volume of the disinfectant solution is distributed over the carrier; after the given exposure time, the number of survivors is determined; the reduction is determined quantitatively by comparing the survivors in this control series with the survivors in the test series (47).

J. Suspension time-kill test (time-kill curve)

It is the most appropriate method for determining the bactericidal or fungicidal effect. It is an effective in determination of the antimicrobial agent's dynamic interaction with the microbial strain. The time-kill test reveals an antimicrobial effect that is time-dependent or concentration-dependent. It involves directly inoculating a liquid test substance with a high concentration of test microorganisms and then calculating the percentage of test microorganisms killed over time. The percentage of dead cells relative to the growth control is then calculated by counting the number of living cells (CFU/mL) in each tube using the agar plate count method. In general, a bactericidal effect is obtained with a lethality percentage of 90% for 6 hours, which is equivalent to 99.9% for 24 hours (49).

I. Surface time kill test

In this test, a microbial culture is inoculated onto several sterile test surfaces, the test product is applied to each surface for specific time, and the surviving microorganisms are cultured and counted to determine the efficacy of disinfectant (50).

Table 1 : **Advantages and disadvantages of each laboratory method used to assess the efficacy disinfectant against bacteria** (51)**.**

S/No	METHOD	ADVANTAGES	DISADVANTAGES
1	Suspension test	• It makes better contact between the disinfectant and microbes. • It is simpler to conduct tests. • It enables microorganism counting throughout the procedure • well standardized	• It does not confer the real situation/environment, because microbes are not attached to a specific surface. • Minor dilution errors made during disinfectant solution preparation can have a significant impact on test results. • High viscosity disinfectants may not be distributed evenly in the test suspension
2	Diffusion test	• It is simple and less cost, • It allows testing a large number of microorganisms and antimicrobial agents, • Easy to interpret result produced	• It provides limited information, just about susceptibility and resistance to the chemical agents tested. • It is incapable of distinguishing between bacteriostatic and bactericidal activities, and • The differences in zone sizes cannot be used to compare chemical agent efficacies.
3	Dilution tests	This method is frequently used to determine the MICs and MBCs It gives quantification on susceptibility and resistance to	• It's complicated method that involves a series of dilutions to obtain MICs and MBCs • Difficult to interpret the results provided.

S/No	METHOD	ADVANTAGES	DISADVANTAGES
		the chemical agents tested.	
4	Capacity test	Good for dilution concentration to be used	• Difficulty in comparing several procedures
5	Stimulated use test	Reliable for assessing the activity of chemical agents in a real situation	• Difficulty in comparing several procedures.
6	Carrier test	• It is more accurately depicts real conditions. Because microbes adhere to a carrier mimics their natural environment.	• It is difficult to regulate the quantity of microorganisms recovered due to the death or loss of microorganisms during the procedure. • It too complex.
7	In-use test	Reliable test to assess microbial contamination in used solution of disinfectants	• Difficulty in comparing several procedures
8	Practical test (surface disinfectant test)	It assesses the activity of chemical agents in real working condition	• It is complicated and relies on numerous variables; the smoothness or roughness of the surface may have an impact on the final result.
9	Suspension time-kill test	• It is fast and economical in term of cost. • Their final results are reproducible. • Contact time can be easily study • It is standardized Procedure	• It can be difficult to relate to the elimination of microorganisms from a surface. • Due to fact that different broths or suspensions may interact with or inactivate disinfectant, the procedure in which the microbial inoculum is prepared can have an affect the test

S/No	METHOD	ADVANTAGES	DISADVANTAGES
			result.
10	Surface Time-Kill Test	• The dried film of microorganisms used in this test provides realistic to the experimental model, • It is less cost	• It is conducted under ideal laboratory conditions, which may not reflect real-world conditions. • The test does not consider the presence of organic matter, which can interfere with the effectiveness of disinfectants and antiseptics. • The test does not consider the presence of biofilms, which are communities of microorganisms that can form on surfaces and increase their resistance to disinfection. • The test does not account for the presence of air pockets or other factors that can interfere with the disinfection process.

CHAPTER TWO

2.0 METHODOLOGY

2.1 Study Design and Setting

A laboratory-based experiment was conducted to evaluate the efficacy of disinfectants against multidrug-resistant bacteria isolates from clinical specimens at Muhimbili National Hospital (MNH). The study was involved collection of routinely used disinfectants from MNH, which is the main tertiary hospital in Tanzania and a teaching hospital for the Muhimbili University of Health and Allied Science.

Muhimbili National Hospital is located in Dar es Salaam, which is the largest city in Tanzania, with a geographical area of 139.3 square kilometers in total. Dar es Salaam is the commercial capital of Tanzania, as well as one of the fastest-growing cities in Africa. According to the latest national census, the city population is 7,404,689, with a 5.08 percent inter-censual growth rate. Muhimbili National Hospital (MNH) as a tertiary hospital serving the majority of people living in Dar es Salaam. In addition to that, the hospital also receives patients from regional referral hospitals. One of the key features of MNH is its Central Pathology Laboratory (CPL), which is a well-equipped laboratory for diagnose various diseases. Moreover, its microbiology unit is capable of identifying MDR bacteria.

2.2 Study Duration

The study was conducted between February and June 2024.

2.3 Study Unit and Sample Size

Testing bacterial isolates

The study unit were bacteria characterized as MDR isolates from clinical samples at MNH and two ATCC control strains. **Four MDR bacteria obtained from blood samples collected between August and November, 2023, which were well** characterized as MRSA, ESBL-producing *Escherichia coli*, CPKP and CR- *A. baumannii* were included. **Additionally, the CPKP included in this study was used in the previous where was well characterized by using Whole-genome sequencing (WGS) and detected to harbor antibiotic resistance genes and QACs resistance genes**. All these isolates were retrieved from the archived samples at the central pathology laboratory (CPL) **which were stored at**

– 40ºC for research activities and epidemiological purpose. Susceptible strains *Escherichia coli* (ATCC 35918), and *Staphylococcus aureus (*ATCC 25923) were obtained from the National Public Health Laboratory (NPHL) and were included as control.

Table 2: **Antibiotic susceptibility pattern of bacteria isolated from clinical samples**

ISOLATES	**PRM**	**CN**	**AK**	**AMP**	**FEP**	**CAZ**	**AUG**	**CRO**	**MEM**	**SXT**
Klebsiella pneumoniae	CPKP	R	R	R	R	R	R	R	R	R
Acinetobacter baumannii	CR-AB	S	R	R	R	R	R	R	R	
Escherichia coli	ESBL	S	S	R	R	R	R	R	S	R
ISOLATES	**PRM**		**C**	**CIP**	**E**	**CD**	**CN**	**TE**	**FOX**	
Staphylococcus aureus	MRSA		S	R	R	R	R	R	R	

Key: *PRM=Phenotypic resistance Mechanism, R=Resistance, S=sensitive, CIP=Ciprofloxacin, CN=Gentamycin, AK=Amikacin, AMP=ampicillin, FEP = Cefepime, CAZ=Ceftazidime, AUG=Augmentin, CRO = Ceftriaxone, MEM = Meropenem, SXT= Trimethoprim/sulfamethoxazole, TE=Tetracycline, FOX=cefoxitin, C= Chloramphenicol, E-Erythromycin and CD=Clindamycin.

Disinfectants

Thee three disinfectants commonly used at the Muhimbili National Hospital were included in this study. These included QACs, povidone iodine and NaOCl at ready to use solution and undiluted.

***Table 3*: Details of routinely used disinfectants at MNH**

Types of Disinfectants	**Brand name**	**Manufacturer**	**Active ingredients**	**Application**
Quaternary ammonium compound (QACs),	Sani Higene	SIRMAXO Chemicals Private Limited, Mumbai, India	It contains Alkyl benzyl ammonium chloride, Didecyl dimethyl ammonium chloride and polymeric biguanide Hydrochloride	Applied immediately to clean and disinfect surface areas such as the floor, mattresses, and other items.
Sodium hypochlorite (NaOCl)	ZAP Bleach	Manufactured for Muhimbili Health National Medical Hospital (MHNMH) by TARMAL INDUSTRIES LTD P.O.BOX 283 DSM	It contains free chlorine	Applied immediately to clean and disinfect surface area, also for equipment decontamination at 10 minutes exposure
Iodophor compounds	povidone iodine	Brookes Pharma (Private) Limited, 58 & 59, Sector 15, Korangi Industrial Area, Karachi, Pakistan.	povidone iodine	Used for wounds dressing, in 24 to 72 hours and applied immediately during sample collection as an antiseptic

Collection of disinfectants available from wards

For the ready-to-use concentration, three set of 200 mls of each disinfectant were collected into four sterile Falcon conical tubes (Servicebio, made in China), each containing 50 mls. **The disinfectants were collected from ward 35, 36, 37 and Paediatric burn unit ward.** The collection was taken place once a day, in the morning between 0830 and 0930 h for three days. For each undiluted disinfectant 100mls was collected once from the MNH stores into sterile flat conical flask. The sampled disinfectants were transported from MNH to MUHAS in a clean, cool box to prevent any contamination. As a safety measure, all disinfectants collected were checked for sterility. This was done by using In-use test methods to detect any contamination. Where by 1 milliliter of disinfectant was added with 9 milliliters of sterile normal saline (Alfa Pharmaceuticals Limited, plot No14, Mbagala industries area, Dar es salaam, Tanzania). Which is then was subcultured on 5% Sheep blood Agar (Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK made in the United Kingdom). Then was incubated for 24-hours at 37^{o} C, if there are any bacterial growth colonies on the plate, it indicates that the disinfectant was contaminated.

2.4 Laboratory Procedures

The laboratory experiments were carried out at the microbiology laboratory of MUHAS

A: Determination of the minimum exposure time of disinfectants that required to kill multidrug-resistant bacteria

The quantitative suspension method was used in this study to determine the efficacy of disinfectants against MDR bacteria at a specific contact time. It was involved suspending the bacteria in a liquid medium and exposing them to the disinfectant for a specified time. Afterward, the bacteria from the medium were subcultured on agar plates to observe bacteria survival, including the enumeration of bacteria survivors, and the log reduction determination. The following are step-by-step instructions on how this method was carried out in this study. These are:

i. Bacterial colonies preparation

The study begins by obtaining fresh cultures from archived bacterial strains including MRSA, ESBL-producing *E. coli*, CPKP, and CR-*A. baumannii*. This was done through inoculation on 5% Sheep blood Agar (Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK made in the United Kingdom) followed by incubation at 37 °C for 24 hours. After growth bacteria re-identification was done starting from gram stain, then Analytical

Profile Index (API) 20 E for gram negative while gram positive was subjected to catalase and coagulase tested, also the phenotypic antibiotic resistance mechanism was done using Double Disc Synergy Test (DDST) for ESBL confirmation, Modified Carbapenemase Inactivation Method (MCIM) for confirmation of carbapenemases producers and MRSA was tested by using Cefoxitin disc. The re-identified and confirmed bacteria were used in this study whereby, from culture plate using a sterile cotton swab, three to four colonies of each bacterial strain were taken and transferred to 5 ml of sterile normal saline (Alfa Pharmaceuticals Limited, plot No14, Mbagala industries area, Dar es salaam, Tanzania). The mixture was thoroughly mixed, and turbidity was compared with the 0.5 McFarland standard, approximately 1.5×10^8 colony-forming units per mL (CFU/mL). A subculture was used within 72 hours**.**

ii. Disinfectant solutions preparation

For this objective the disinfectant at recommended ready-to-use concentrations (rtu) of 1% QACs, 10% povidone iodine, and 0.5% NaOCl were mixed well and exposed to testing organisms. Then their efficacies were assessed.

iii. Microbial Analysis

This test was conducted under clean conditions, with 0.3% Fetal Bovine Serum (FBS) (SAFC Industriestrasse 25, CH-9471, Buchs, Switzerland). To perform the quantitative suspension test, disinfectant solutions containing 0.3% Fetal Bovine Serum (FBS) (SAFC Industriestrasse 25, CH-9471, Buchs, Switzerland) was prepared just before the test begins. 8 ml of the disinfectant was mixed thoroughly with 1 ml of a 0.3% albumin solution, also 8 ml of the sterile normal saline (Alfa Pharmaceuticals Limited, plot No14, Mbagala industries area, Dar es salaam, Tanzania) was mixed thoroughly with 1 ml of a 0.3% albumin solution to make a standard control which was used on comparison. Then both solutions were thoroughly mixed with 1 ml of the test bacteria suspensions. The killing contact time of disinfectant was evaluated by using VAH method 9, recommended by the Germany Association for Applied Hygiene (44,45) and equivalent to British Standard Institute (BSI) for Chemical disinfectants and antiseptics BS EN 13727:2012+A2:2015 (52). To assess the bactericidal activity of the disinfectant, the evaluation was included different contact time interval (1 minute, 5 minutes, 10 minutes) at 20^{o}C temperature. After contact time, 0.5 ml of suspension was mixed with 4.5 mls of Neutralizer (Phosphate buffer solution (PBS), JOURILABS, Addis Ababa Ethiopia) for 5 Minutes, then 1µL of a

mixture was taken and inoculated on 5% Sheep blood Agar (Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK made in the United Kingdom). After 24 hours the plates were observed for bacteria growth, enumeration was done to plates to quantify the number of bacteria survivors. This procedure was repeated in triplicate in three days for each disinfectant, making total run of nine for each isolate. This was done to ensure the reliability of the data obtained. However, the mean values were used during data analysis.

iv. Result Interpretation

Evaluation was done by calculating log reduction values by the following formula: log reduction (LRs) = log (number of organisms per milliliter before exposure) - log (number of organisms per milliliter after exposure). An LRs of 1 is equal to a killing of 90% of the bacteria, and an LRs of 2 means 99% killed. The accepted requirement, the LRs should be equals or greater than 5: at least 99.999% of the germs are killed (40,41).

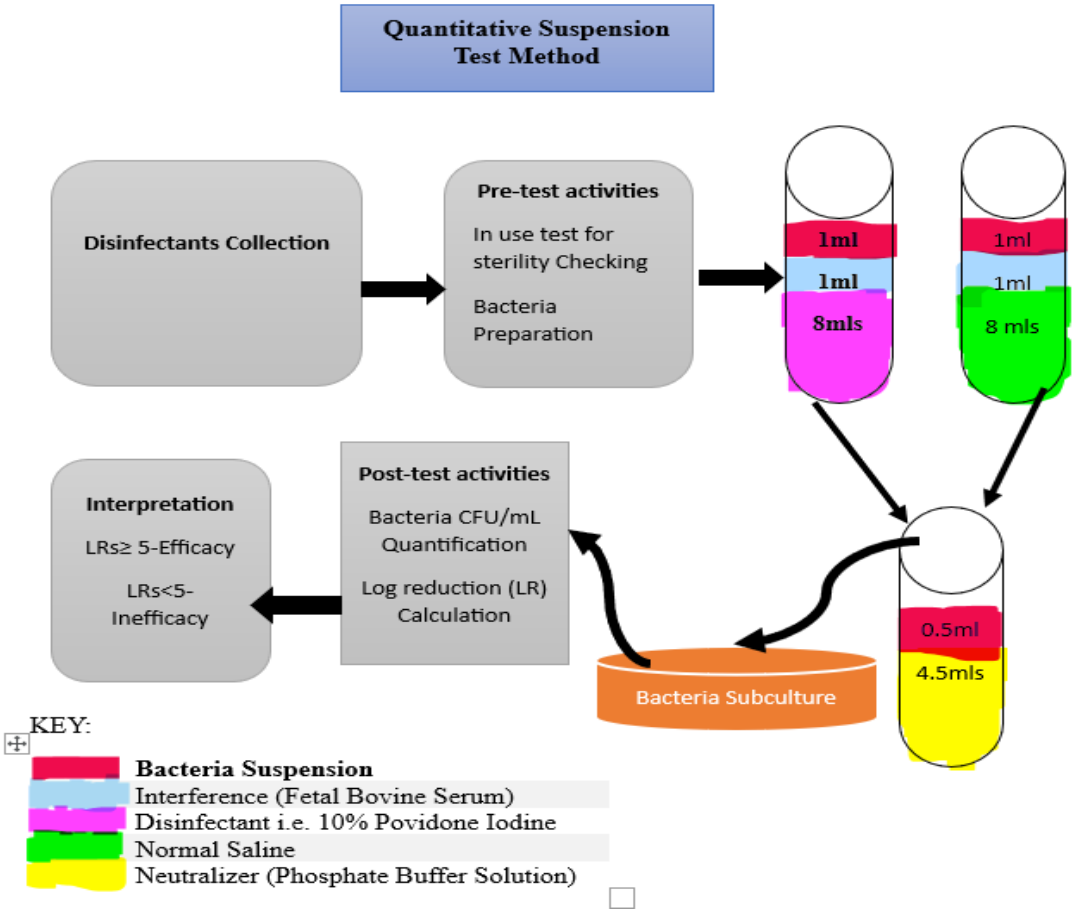


Figure 1:Illustrative scheme showing procedures for quantitative suspension test method (created by Author)

B: Determination of the MICs and MBCs of disinfectants against multidrug-resistant bacteria.

Disinfectant dilution test method was used in this study to evaluate the efficacy of disinfectants by determining disinfectant's MICs and MBCs. This was done by making, a serial dilution into microtube containing Mueller Hinton Broth (Deben Diagnostic Ltd, UK) (43). The following are step-by-step instructions on how disinfectant dilution test methods was carried out in this study.

i. Bacterial colonies preparation

To perform the test, the suspension described on A(i) above, which contains about 1.5×10^8 CFU/mL, was diluted into Mueller Hinton Broth (Deben Diagnostic Ltd, UK) by 1:10 prior involved into the test.

ii. Disinfectant solutions Preparation

Undiluted solutions were used, such as 10% QACs, 10% povidone iodine, and 3.5% NaOCl then their efficacies were assessed. Each solution was shaken well before use or tested.

iii. Microbial Analysis

Twelve screw tubes were prepared and distributed with 1 mL of Mueller Hinton Broth (Deben Diagnostic Ltd, UK). The first tube was added with 1 mL of tested disinfectant, and the process of stirring and transferring 1 mL to the next tube was continue until tube number 11. 100µL of test bacteria suspension was added to all tubes except for flask number 11, serving as the negative control. The tubes were incubated at 37^{o}C for 24 hours (48-50). To determine the MBCs, tubes were sub-cultured on 5% Sheep Blood Agar. Positive and negative controls are labeled as Tubes 12 and 11, respectively (51). This procedure was repeated twice for each disinfectant to ensure the reliability of the data. The percentages concentration was converted into mg/L as it well known that 1 % concentration is equivalent to 1g in 100 ml (10,000mg/L). Therefore, 10% povidone iodine is equivalent to 100,000 mg/L.

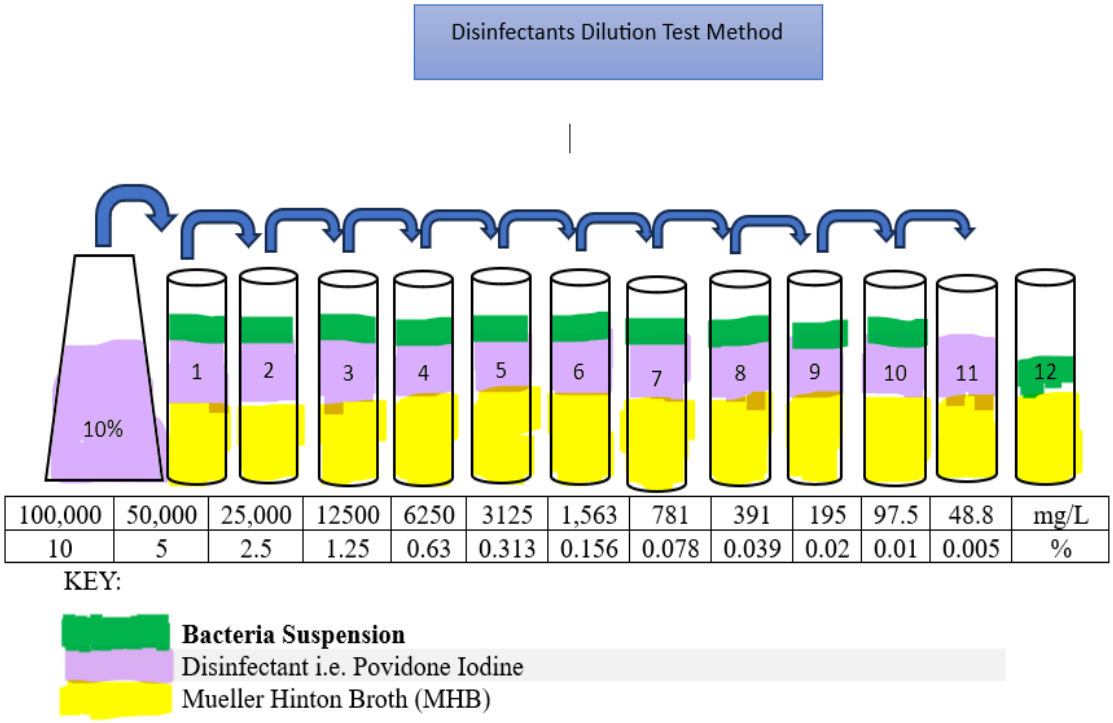


Figure 3: **Illustrative scheme showing successive dilution method (created by Author).**

iv. Result Interpretation

After incubation, the highest dilution tube that with no turbidity was considered as the MICs. The MBC was determined as the highest dilution tube that shows no bacterial growth after subculture. Also, the MICs obtained was compared with previous studies. The MICs of all bacteria ranged from 390 to 780 mg/L for NaOCl while for povidone iodine, ranged from 64 to 512 mg/L and for QACs, ranged from 0.2 to 16,000 mg/L (16,53–55).

2.5 Variables

2.5.1. Dependent variable

Outcome used to assess disinfectants efficacy such as the minimum Exposure times, Bacteria logarithmic reductions (LRs), MICs and MBCs.

2.5.2. Independent variable

Study units which were experiments input to obtain an outcome, these are tested bacteria strains and disinfectants

2.6 Data Entry and Statistics Analysis:

Data Entry and Analysis was done by using Excel Microsoft spreadsheets software. Interpretation was done as per German Association for Applied Hygiene guideline (VAH methods 07 and 09) this was done by observation of change on log reduction using the equation, a viable number of bacteria before the addition of disinfectant (A) minus a viable number of bacteria after the addition of disinfectant(B). The equation was simplified as, Log Reduction = $\log_{10}$ (A/B). The disinfectant with LRs $\geq$ 5 was regarded as efficacy: at least 99.999% of the germs are killed. Also, the MICs and MBCs findings were compared with reference strain. Data was presented in tables and figures with a minimum exposure time, bacterial logarithmic reduction, MICs, and MBCs to describe the findings.

2.7 Ethical Considerations

The Muhimbili University of Health and Allied Sciences Institutional Review Board (MUHAS IRB) through Department of Microbiology and Immunology under School of diagnostic medicine was contacted for ethical approval for this study. Ethical Approval (**MUHAS-REC-12-2023-2001**) was given.

Waiver of ethical consent and permission to access the testing organisms and disinfectant products, was obtained from the Directorate of Research, Training, and Consultancy at MNH and permission was issued through letter with **Ref. No.: MNH/CRTCU/Perm/2024/041**.

To ensure the safety of both humans and the environment, any materials contaminated with test organisms during procedures, were autoclaved to eliminate any potential hazard prior disposal. Once sterilized, these materials were sent to a restricted area (incinerator), where they are burned into ashes. This process was done to prevent any potential risks of environmental contamination**.**

2.8 Dissemination Plan

The results of this study were presented to the Department of Microbiology and Immunology. The final report will be available at the MUHAS library. The study's findings will also be presented at scientific conferences and published in a peer-reviewed medical journal.

CHAPTER THREE

3.0 RESULTS

3.1 Bacteria Colony Forming Unit per Milliliter (CFU/mL) before and after disinfectants exposure

For all three disinfectants, there was no bacterial growth after 10 minutes of contact time. However, at 5 minutes contact time 1% QACs inhibited growth for all bacteria. Bacteria exposure to 10% povidone iodine and 0.5% NaOCl at 5 minutes produced varying bacterial growth **characteristics**: from no growth to growth of 4×10^3 on MRSA. ATCC-*E. coli* and Carbapenemase producer *Klebsiella pneumoniae* did not grow at 5 minutes exposure for both 10% povidone iodine and NaOCl. Exposure of NaOCl at one minute did not significant inhibit, the growth of any tested bacteria (**Table 4 and Figure 4**).

Table 4: Bacteria Colony Forming Unit per Milliliter (CFU/mL) on disinfectants exposure

Disinfectant's Name	**Contact time**	**Mean CFU/mL of Tested Bacteria**					
		ATCC-*E. coli*	ESBL-*E. coli*	CPKP	CR- *A. baumannii*	ATCC-*S. aureus*	MRSA
0.5% NaOCl	Initial	2.58×10^5	3.39×10^5	3.54×10^5	5.61×10^5	5.29×10^5	5.99×10^5
	1 min	1.10×10^4	1.40×10^4	1.90×10^4	1.80×10^4	1.70×10^4	2.30×10^4
	5 min	0.00×10^0	3.00×10^3	0.00×10^0	2.00×10^3	2.00×10^3	4.00×10^3
	10 min	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0
10% Povidone iodine	Initial	2.42×10^5	3.17×10^5	4.31×10^5	5.24×10^5	5.73×10^5	6.16×10^5
	1 min	0.00×10^0	0.00×10^0	0.00×10^0	1.00×10^3	2.00×10^3	5.00×10^3
	5 min	0.00×10^0	0.00×10^0	0.00×10^0	1.00×10^3	1.00×10^3	2.00×10^3
	10 min	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0
1% QACs	Initial	3.08×10^5	3.49×10^5	4.39×10^5	5.77×10^5	6.55×10^5	5.95×10^5
	1 min	0.00×10^0	1.00×10^3	1.00×10^3	1.00×10^3	0.00×10^0	0.00×10^0
	5 min	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0
	10 min	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0	0.00×10^0

KEY: MRSA=Methicillin-resistant *Staphylococcus aureus*, ESBL-*E. coli*= Extended spectrum Beta-lactamase producing *Escherichia coli*, CPKP=Carbapenemase producer *Klebsiella pneumoniae*, CR- *A. baumannii* =Carbapenem-resistant *Acinetobacter* ***baumanii,***

ATCC-*E coli=Escherichia coli*, American Type Culture Collection 35918, and ATCC-*S. aureus*= *Staphylococcus aureus*, American Type Culture Collection 25923

3.2 The Minimum Exposure Time for Disinfectants to kill MDR Bacteria

At 10 minutes of exposure time all disinfectants **exhibited** a significant log reduction (LRs) of ≥ 5(99.999% of all tested bacteria were killed), showing that were efficacious. For 1% QACs, significant LRs of ≥5 was observed to all examined bacteria at 5 minutes of exposure. Moreover, MRSA showed LRs ≥5 even at 1 min contact time. Nevertheless, QACs could only exhibit LR ≥5 for gram-positive bacteria (ATCC- *S. aureus* and MRSA) and ATCC- *E. coli* at a 1-minute exposure time. ESBL *Escherichia coli,* CPKP and CR- *A. baumannii* did not significantly decrease during a contact period of one minute, resulting to log reduction of two.

The 10% povidone iodine was efficacious in killing ATCC- *E. coli,* ESBL- *Escherichia coli*, and CRKP at exposure times of 1, 5, and 10 minutes. At 1- and 5-minute exposure time, it could not kill the three bacteria (CRAB, ATCC- *S. aureus*, and MRSA).

The 10% NaOCl **was** not efficacious at 1-minute exposure time to all examined bacteria. At 5 minutes of exposure time, it was able to kill only ATCC-*E. coli* and CRKP with ≥5 LRs but no other bacteria.

Table 5: **Logarithmic reduction of tested bacteria for each disinfectant**

Disinfectant's Name	**Tested organism**	**Initial log value, for suspension without disinfectant**	**CONTACTCTIME AND LOG REDUCTION**		
			1 min, LR (%)	5 min, LR (%)	10 min, LR (%)
0.5% NaOCl	**ATCC-*E coli***	5	1(90.000)	>5 (99.999)	>5 (99.999)
	ESBL-*E. coli*	6	1(90.000)	2(99.000)	>5 (99.999)
	CRKP	6	1(90.000)	>5 (99.999)	>5 (99.999)
	CR-*A. baumannii*	6	1(90.000)	2(99.000)	>5 (99.999)
	ATCC-*S. aureus*	6	1(90.000)	2(99.000)	>5 (99.999)
	MRSA	6	1(90.000)	2(99.000)	>5 (99.999)
10% Povidone Iodine	**ATCC-*E coli***	5	>5 (99.999)	>5 (99.999)	>5 (99.999)
	ESBL-*E. coli*	6	>5 (99.999)	>5 (99.999)	>5 (99.999)
	CRKP	6	>5 (99.999)	>5 (99.999)	>5 (99.999)
	CR- *A. baumannii*	6	2(99.000)	2(99.000)	>5 (99.999)
	ATCC-*S. aureus*	6	2(99.000)	2(99.000)	>5 (99.999)
	MRSA	6	2(99.000)	2(99.000)	>5 (99.999)

Table 1: Continued

Disinfectant's Name	Tested organism	Initial log value, for suspension without disinfectant	CONTACTCTIME AND LOG REDUCTION		
1% QACs	**ATCC-*E coli***	5	>5 (99.999)	>5 (99.999)	>5 (99.999)
	ESBL-*E. coli*	6	2(99.000)	>5 (99.999)	>5 (99.999)
	CRKP	6	2(99.000)	>5 (99.999)	>5 (99.999)
	CR- *A. baumannii*	6	2(99.000)	>5 (99.999)	>5 (99.999)
	ATCC-*S. aureus*	6	>5 (99.999)	>5 (99.999)	>5 (99.999)
	MRSA	6	>5 (99.999)	>5 (99.999)	>5 (99.999)

3.3 The disinfectants susceptibility pattern against multidrug-resistant bacteria

The MICs of NaOCl required to inhibit bacterial growth differ for each bacterial strain**,** with higher MICs (1094 mg/L) observed among CPKP and MRSA. The same for MBCs was higher in CPKP and MRSA (Table 6). The MICs of MRSA for NaOCl was fourth time higher than ATCC- *S. aureus*. The ESBL-*Escherichia coli* and MRSA had two times increased in MBCs for NaOCl than ATCC-*E. coli* and ATCC- *S. aureus*, respectively. The MICs and MBCs of 10% povidone were higher against all bacteria tested, with ESBL-*Escherichia coli*, CR- *A. baumannii* and MRSA displaying highest MIC. The MBC of 10% povidone iodine for both ESBL- *Escherichia coli* and MRSA was two time higher than their MICs. The MICs of MRSA and ESBL-*Escherichia coli* for povidone iodine were twofold higher than their reference ATCC strains. The MICs and MBC for QACs were higher against all MDR bacteria and reference strains. The MBC of QAC were twofold higher than the MIC for ATCC- *Escherichia coli*, CPKP, ATCC-*S. aureus* and MRSA**. (Table 5).**

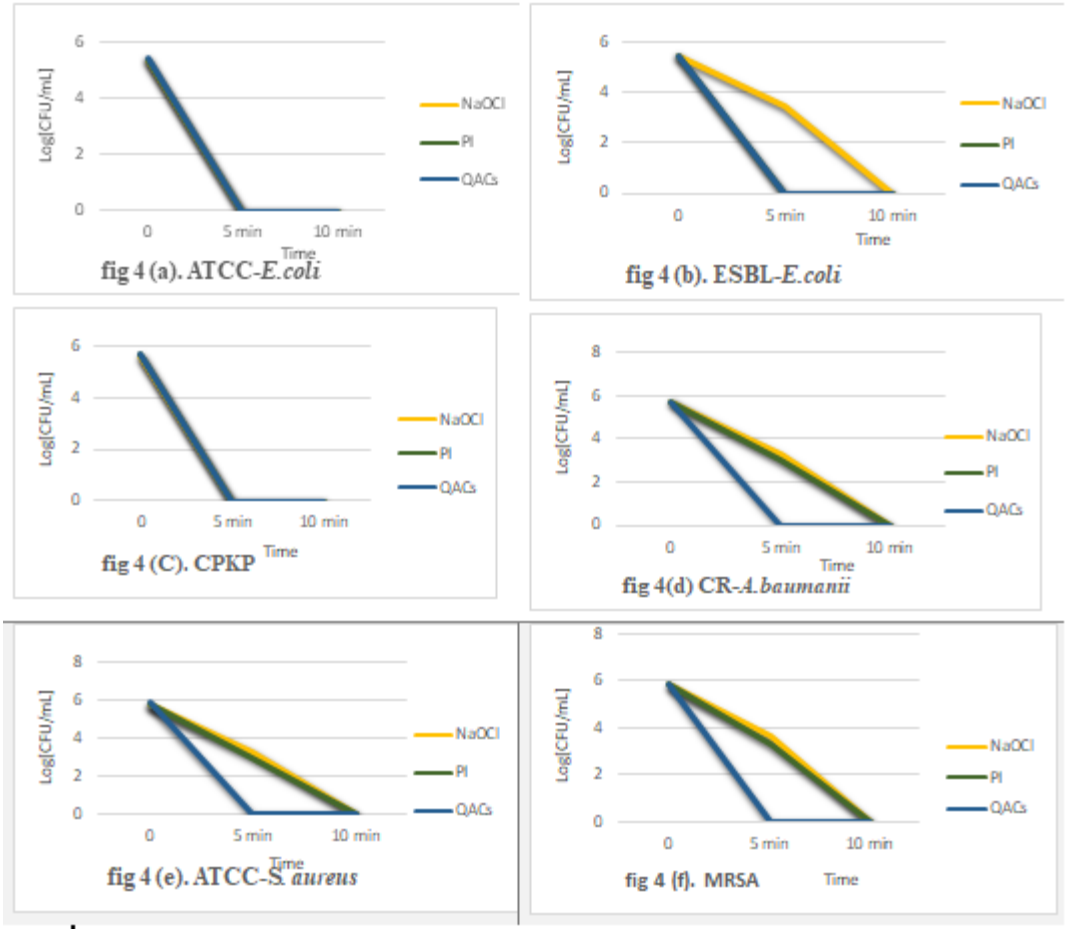
Table 6: **Minimum inhibitory concentration (MICs) and Minimum bactericidal concentration (MBCs) of routinely used disinfectants for tested bacteria**

Disinfectant's MICs	**Tested Bacteria**											
	ATCC-*E. coli*		ESBL-*E. coli*		CRKP		CR- *A. baumannii*		ATCC- *S. aureus*		**MRSA**	
	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L
NaOCl-3.5%	0.06	547	0.06	547	0.11	1094	0.06	547	0.03	273	0.11	1094
PI- 10%	0.31	3125	O.63	6250	0.31	3125	O.63	6250	0.31	3125	O.63	6250
QACs-10%	0.31	3125	O.63	6250	0.31	3125	O.63	6250	0.31	3125	0.31	3125
MBCs	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L
NaOCl-3.5%	0.06	547	0.11	1094	0.11	1094	0.06	547	0.06	547	0.11	1094
PI- 10%	0.31	3125	1.25	12500	0.63	6250	O.63	6250	0.31	3125	1.25	12500
QACs-10%	O.63	6250	O.63	6250	O.63	6250	O.63	6250	O.63	6250	O.63	6250

Legend: QACs- quaternary ammonium compounds; PI - povidone iodine; sodium hypochlorite – NaOCl; MRSA-Methicillin-**Resistant** *Staphylococcus aureus*; ESBL-*E. coli*-Extended spectrum Beta-lactamase producing *Escherichia coli*; CPKP-Carbapenemase producer *Klebsiella pneumoniae,* CR- *A. baumannii* -Carbapenem-resistant *Acinetobacter* ***baumannii,*** ATCC-*E coli-Escherichia coli,* American Type Culture Collection 35918, and ATCC-*S. aureus- Staphylococcus aureus,* American Type Culture Collection 25923

3.4 Comparison of efficacy for routinely used disinfectants in killing MDR bacteria

Figures 5 (A to F) compares the efficacy of three disinfectants against each bacterium tested. 1% QACs were able to kill all tested bacteria at 5 and 10 minutes with significant LR≥5, as shown in figures 4(a), 4(b), 4(c), 4(d), 4(e), and 4(f). However, povidone iodine was able to kill three bacteria (ATCC- *E. coli*, CPKP, and ESBL-*Escherichia coli*) at 5 and 10 minutes with LR≥5. While NaOCl could kill only two bacteria strains (CPKP and ATCC- *E. coli*) with LR ≥5 at 5 and 10 minutes. Therefore, it was shown that quaternary ammonium compounds (QACs) are the most efficacious disinfectants, followed by povidone iodine, and NaOCl was the least efficacy. (**See Figure 4(a-f**).



KEY: QACs- quaternary ammonium compounds; PI - Povidone iodine; Sodium hypochlorite– NaOCl

***Figure 4*: (a- f) Bacteria Survival after 5- and 10-minutes of disinfectants exposure**

CHAPTER FOUR

4.0 DISCUSSION, STRENGTH AND LIMITATION OF THE STUDY

4.1 Discussion

Because there is an incredible burden of antibiotic resistance worldwide, bacteria are even more resistant to routinely used disinfectants, which are essential for infection prevention and control. This underscores the need for continuous monitoring of the efficacy of the commonly used disinfectants in our setting. This study found that disinfectant exposure time is essential for killing bacteria. All disinfectants demonstrated efficacy at 10 minutes of exposure, effectively killing 99.999% of all tested bacteria. We also found QACs to be efficacious at 5 minutes, but not povidone iodine or NaOCl. Furthermore, we observed that different concentrations (MIC, MBC) inhibited or killed each bacterium, highlighting the need for high disinfectant concentrations that enable the killing of all microbes. The study also found that, MDR bacteria were more tolerant to disinfectants compared to susceptible bacteria strains.

This study found that 1% QACs was efficacious at both 5 and 10 minutes for all tested bacteria but not at one-minute, where only **Gram**-positive bacteria were eliminated. The recommended exposure contact time for surface disinfection is one minute (56). A previous study conducted in 2022, found that exposure times of five minutes and above, were required for completely kill all bacteria (33). During the data collection, we observed that, exposure time of one minute was applied for surface disinfection at the study site. Therefore, exposure at one minute could have only killed **Gram**-positive bacteria, while **Gram** negative would have survived, posing risk for infection prevention and control. Hence, long exposure is required to kill **Gram** negative bacteria, as complex structure of cytoplasmic membrane in these bacteria makes difficult to be killed (15,22,57,58). Therefore, it is important for the cleaner to adhere to the recommended time depending on the types of disinfection intended to be performed.

Our study observed that, 10% povidone iodine was effective against all **Gram**-negative bacteria when exposed for 1, 5, or 10 minutes. The only type of bacteria that did not respond well was carbapenem-resistant *Acinetobacter baumannii*, which showed less

reduction, similar to MRSA. Previous studies conducted about ten years ago demonstrated the greater efficacy of 10% povidone iodine against a wide range of microorganisms, including MRSA, with a minimum killing exposure time of 30 seconds to 60 seconds (34,38,59–61). During sample collection and scrubbing, it is recommended that povidone iodine should be applied and left on the skin for two minutes, while for wound dressing should be administered and allowed to contact the wound for 72 hours(56). Despite the observation that, the practice only adheres to the contact time for wound dressing, there is no habit of waiting for two minutes during sample collection. This exposure time may not kill MRSA and carbapenem-resistant *Acinetobacter baumannii,* which were tolerant of povidone iodine. Therefore, the contact time during scrubbing and swabbing prior to surgery and sample collection should be more than five minutes for better efficacy of povidone iodine.

Although, one minute contact exposure to 0.5% NaOCl is recommended for surface disinfection and ten minutes for soaking instruments and equipment during decontamination (56). Our study observed that 0.5% NaOCl completely killed all tested bacteria in 10 minutes, not in one minute. These results were similar to those of a systematic review study done using practical methods that mimicked real conditions, which showed that for NaOCl to eradicate all bacteria, it required a contact time of 10 to 30 minutes (62). However, our results were different from those of Damiano et al.'s in 2023, which showed a significant decrease in bacteria after one minute for freshly prepared sodium dichloroisocyanurate (NaDDC) (35). The difference seen between NaOCl from this study and sodium dichloroisocyanurate (NaDDC) from a previous study could be because: first, the disinfectant that was collected might not have been made within a day; second, it may not have been diluted properly; and third, the two types of disinfectant are made of different substances. The 0.5% NaOCl was in solution form, while 0.5 % sodium dichloroisocyanurate (NaDDC) was in tablet form; their strength might be different because of their physical properties rather than their chemical nature (22). Hence, extension to contact time is required for completely eradicate all germs during surface disinfection.

We observed that the MICs of MDR bacteria for NaOCl ranged from 0.06% to 0.11% (547 to 1,094 mg/L). These findings correspond to previous studies in 2018 , which showed that the MICs of frequently isolated bacteria ranged from 0.039% to 0.078% (390 to 780 mg/L) (16,37,63). This indicates that the MICs of MDR bacteria for NaOCl are still within acceptable ranges and were lower than the ready to use concentration of 0.5% (5,000 mg/L). Therefore, because of their biocidal nature, they can still be useful as long as proper preparation is adhered to and organic loads are cleaned prior to disinfection for greater efficacy (64).

A recent study done by Boyce JM et al, in 2023, had shown that, the MICs of bacteria for QACs were ranged from 200 to 16,000 mg/L (15). Our study findings were within that ranges, which showed, the MICs of bacteria were ranged from 3125 to 6250 mg/L for QACs. Thus, our study and that recent study, had significantly finding exceeded those of previous studies which were done more than three years ago, which had shown MICs for QACs were ranged from 2.4 to 156 mg/L (55,65,66). This shows that, under suboptimal usage of QACs, MDR bacteria my acquire resistance genes likes qacE and qacΔE1 through horizontal gene transfer or plasmid gene transfer (37,67). Furthermore, an increase in QACs consumption especially at substantial concentration has directly linked to antibiotic resistance, due to its comparable chemical structure (15,36). Therefore, it is crucial to practice disinfectant stewardship in order to minimize an unnecessary increase in bacterial resistant to both antibiotics and disinfectants.

The MICs of bacteria ranged from 0.31 to 0.63% (3,125 to 6,250 mg/L) for povidone iodine. These findings showed an increase in MICs compared with the previous study done 5 years ago by Antonelli A. et al., which showed that the MICs of various bacteria were ranged from 64 to 512 mg/L (53). Although in vitro and in vivo studies have demonstrated that 10% povidone iodine is effective, with quick and superior bactericidal action against a wide range of microorganisms (61). The increase in MICs, particularly among MDR bacteria, is of concern.

However, there was insufficient data to compare the increase in MBCs of disinfectants over time between susceptible and MDR bacteria in the most recent studies (68). Subsequently, this study found surprising increases in MBCs of povidone iodine against

MDR bacteria. MRSA and ESBL *Escherichia coli* exhibited MBCs four times greater than those of their reference bacteria strain. These results can simply indicate that povidone-iodine resistance is growing. Moreover, carbapenemase producer *Klebsiella pneumoniae* showed a high susceptibility to all disinfectants, indicating that antibiotic resistance for some bacteria strains might be caused by alternative resistance mechanisms that are independent of the microbicide resistance gene (20,65).

Comparing all three disinfectants, QACs were found to be more efficacious than povidone, iodine, and NaOCl. This is because QACs work quickly to cause cell lysis (14,22,57). Although QACs were more efficacious, their effectiveness varies based on concentration and exposure time. Therefore, it is important to consider other factors such as the type of pathogen, the side effects on the user following exposure, and compatibility with different surfaces (69).

4.2 Strength of the Study

The use of quantitative suspension methods allowed reproducibility of the results on evaluation by using log reduction. Also, disinfectant susceptibility results added value to the findings, including the remarkable resistance observed in povidone iodine.

4.3 Limitations of the Study

Only multidrug-resistant strains of MRSA, ESBL *Escherichia coli*, CR-*A. baumannii*, and carbapenemase-producer *Klebsiella pneumoniae* were used rather than including a wide range of different microorganisms. This because, these bacteria are most prevalent in our setting. Viral strains were not included due to the laboratory setup constraint for culturing the viruses, and fungal strains were not involved due to their lesser isolation. Molecular characterization of disinfectant and antibiotic resistance genes was not done due to insufficient funding and limited study time.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Disinfectants have a vital role in avoiding the spread of infectious diseases in hospitals, as well as, in the community. The findings from this study revealed that, the minimum exposure contact time required to eradicate all bacteria is between 5 and 10 minutes. It was found that QACs, are more efficacy than povidone iodine, and NaOCl. Additionally, MDR bacteria revealed high disinfectant tolerance when compared to ATCC reference bacteria, this showed a notable rise in disinfectant resistance. However, carbapenemase producer *Klebsiella pneumoniae* showed a high susceptibility to all disinfectants, indicating that antibiotic resistance for some bacteria might be due to alternative resistance mechanisms that are independent of the microbicide resistance gene.

5.2 Recommendations

- QACs should be used for surface disinfection rather than NaOCl. This due to fact that the procedure of surface disinfection is done immediately without wait longer contact time.
- The 0.5% NaOCl maintained significant efficacy at 10 minutes, then should be continuing used for equipment/instrument decontamination.
- Due to remarkable disinfectant's resistance, it is necessary to evaluate their efficacy continuously, including establish a regular surveillance for disinfectants available in the markets.
- Disinfectants stewardships should be established, so as to combat antimicrobial resistance (AMR), this will help to aligning with the National Action Plan on Antimicrobial Resistance (NAP on AMR).

REFERENCES

1. Shankar Pr. Book review: Tackling drug-resistant infections globally. Arch Pharm Pract. 2016;7(3):110.

2. Lim C, Takahashi E, Hongsuwan M, Wuthiekanun V, Thamlikitkul V, Hinjoy S, et al. Epidemiology and burden of multidrug-resistant bacterial infection in a developing country. Elife. 2016;5(September):1–18.

3. CDC. Antibiotic Resistance Threats in The United States 2019. Cdc. 2019;10.

4. Manyahi J, Kibwana U, Mgimba E, Majigo M. Multi-drug resistant bacteria predict mortality in bloodstream infection in a tertiary setting in Tanzania. PLoS One 2020;15(3):1–11.

5. Seni J, Moremi N, Matee M, van der Meer F, DeVinney R, Mshana SE, et al. Preliminary insights into the occurrence of similar clones of extended-spectrum beta-lactamase-producing bacteria in humans, animals and the environment in Tanzania: A systematic review and meta-analysis between 2005 and 2016. Zoonoses Public Health. 2018;65(1):1–10.

6. Joachim A, Manyahi J, Issa H, Lwoga J, Msafiri F, Majigo M. Predominance of Multidrug-Resistant Gram-Negative Bacteria on Contaminated Surfaces at a Tertiary Hospital in Tanzania: A Call to Strengthening Environmental Infection Prevention and Control Measures. Curr Microbiol. 2023;80(5):148.

7. OMS. WHO bacterial priority pathogens list, 2024 [Internet]. Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. 2024. 72 p.

8. Weinberg SE, Villedieu A, Bagdasarian N, Karah N, Teare L, Elamin WF. Control and management of multidrug resistant Acinetobacter baumannii: A review of the evidence and proposal of novel approaches. Infect Prev Pract. 2020;2(3):100077.

9. Yadav SK, Bhujel R, Hamal P, Mishra SK, Sharma S, Sherchand JB. Burden of multidrug-resistant acinetobacter baumannii infection in hospitalized patients in a tertiary care hospital of Nepal. Infect Drug Resist. 2020;13:725–32.

10. Rutala W., Weber DJ. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008:https://www.cdc.gov/infectioncontrol/guidelines/disinfection/. 2019;(May).

11. Cheon S, Kim MJ, Yun SJ, Moon JY, Kim YS. Controlling endemic multidrug-resistant acinetobacter baumannii in intensive care units using antimicrobial stewardship and infection control. Korean J Intern Med. 2016;31(2):367–74.

12. Liu WJ, Fu L, Huang M, Zhang JP, Wu Y, Zhou YS, et al. Frequency of antiseptic resistance genes and reduced susceptibility to biocides in carbapenem-resistant Acinetobacter baumannii. J Med Microbiol. 2017;66(1):13–7.

13. Babaei MR, Sulong A, Hamat RA, Nordin SA, Neela VK. Extremely high prevalence of antiseptic resistant quaternary ammonium compound E gene among clinical isolates of multiple drug resistant acinetobacter baumannii in Malaysia. Ann Clin Microbiol Antimicrob. 2015;14(1):1–5.

14. Betchen M, Giovinco HM, Curry M, Luu J, Fraimow H, Carabetta VJ, et al. Evaluating the Effectiveness of Hospital Antiseptics on Multidrug-Resistant Acinetobacter baumannii: Understanding the Relationship between Microbicide and Antibiotic Resistance. Antibiotics. 2022;11(5).

15. Boyce JM. Quaternary ammonium disinfectants and antiseptics: tolerance, resistance and potential impact on antibiotic resistance. Antimicrob Resist Infect Control. 2023;12(1):1–14.

16. Köhler AT, Rodloff AC, Labahn M, Reinhardt M, Truyen U, Speck S. Evaluation of disinfectant efficacy against multidrug-resistant bacteria: A comprehensive analysis of different methods. Am J Infect Control. 2019;47(10):1181–7.

17. Gupta P, Bhatia M, Gupta P, Omar BJ. Emerging Biocide Resistance among Multidrug-Resistant Bacteria: Myth or Reality? A Pilot Study. J Pharm Bioallied Sci. 2018;10(2):96–101.

18. Wang M, Wei H, Zhao Y, Shang L, Di L, Lyu C, et al. Analysis of multidrug-resistant bacteria in 3223 patients with hospital-acquired infections (HAI) from a tertiary general hospital in China. Bosn J Basic Med Sci. 2019;19(1):86–93.

19. Morris FC, Dexter C, Kostoulias X, Uddin MI, Peleg AY. The Mechanisms of Disease Caused by Acinetobacter baumannii. Front Microbiol. 2019;10(JULY).

20. Almaghrabi MK, Joseph MRP, Assiry MM, Hamid ME. Multidrug-Resistant Acinetobacter baumannii: An Emerging Health Threat in Aseer Region, Kingdom of Saudi Arabia. Can J Infect Dis Med Microbiol. 2018;2018.

21. Exner M, Bhattacharya S, Christiansen B, Gebel J, Goroncy-Bermes P, Hartemann P, et al. Antibiotic resistance : What is so special about multidrug-resistant Gram-negative bacteria ? Antibiotikaresistenz : Was ist so besonders an den Gram-negativen. GMS Hyg Infect Control. 2017;12:1–24.

22. Russell AD. Bacterial resistance to disinfectants: Present knowledge and future problems. J Hosp Infect. 1999;43(SUPPL. 1):57–68.

23. Jalal NA, Al-Atyyani RA, Al-Said HM, Ashgar SS, Faidah H, Johargy AK, et al. Comparative Assessment of Antimicrobial Efficacy of Seven Surface Disinfectants against Eight Bacterial Strains in Saudi Arabia: An In Vitro Study. Microbiol Res (Pavia). 2023;14(3):819–30.

24. Manyahi J, Matee MI, Majigo M, Moyo S, Mshana SE, Lyamuya EF. Predominance of multi-drug resistant bacterial pathogens causing surgical site infections in Muhimbili national hospital, Tanzania. BMC Res Notes. 2014;7(1):1–7.

25. Silago V, Mruma EC, Msemwa B, Mtemisika CI, Phillip S, Ndagula RA, et al. Predominance of Acinetobacter spp., Harboring the blaIMP Gene, Contaminating the Hospital Environment in a Tertiary Hospital in Mwanza, Tanzania: A Cross-Sectional Laboratory-Based Study. Pathogens. 2022;11(1):1–11.

26. Odoyo E, Matano D, Tiria F, Georges M, Kyanya C, Wahome S, et al. Environmental contamination across multiple hospital departments with multidrug-resistant bacteria pose an elevated risk of healthcare-associated infections in Kenyan hospitals. Antimicrob Resist Infect Control. 2023;12(1):1–9.

27. van Dijk HFG, Verbrugh HA, Abee T, Andriessen JW, van Dijk HFG, ter Kuile BH, et al. Resisting disinfectants. Commun Med. 2022;2(1):1–5.

28. Li J, Xie S, Ahmed S, Wang F, Gu Y, Zhang C, et al. Antimicrobial activity and resistance: Influencing factors. Front Pharmacol. 2017;8(JUN):1–11.

29. Skelley J. Open source tactics: Bargaining Power for strategic litigation. J Intellect Prop. 2016;16(1):1–35.

30. US EPA. Product Performance Test Guidelines: OCSPP 810.2300 Sanitizers for Use on Hard Surfaces--Efficacy Data Recommendations [EPA 712-C-07-091]. 2012;13.

31. Gray HK, Arora-Williams KK, Young C, Bouwer E, Davis MF, Preheim SP. Contribution of Time, Taxonomy, and Selective Antimicrobials to Antibiotic and Multidrug Resistance in Wastewater Bacteria. Environ Sci Technol. 2020;54(24):15946–57.

32. Morton HE. the Relationship of Concentration and Germicidal Efficiency of Ethyl Alcohol. Ann N Y Acad Sci. 1950;53(1):191–6.

33. Gaurav S, Kiranjeet K, Rajesh K. Bactericidal Activity of Different Generations of Quaternary Ammonium Disinfectants as per EN 1276: A Comparative Study. Int J Zool Investig. 2022;08(02):857–66.

34. Tan EL, Johari NH. Comparative in vitro evaluation of the antimicrobial activities of povidone-iodine and other commercially available antiseptics against clinically relevant pathogens. GMS Hyg Infect Control. 2021;16:Doc05.

35. Damiano P, Silago V, Nyawale HA, Mushi MF, Mirambo MM, Kimaro EE, et al. Efficacy of disinfectants on control and clinical bacteria strains at a zonal referral hospital in Mwanza, Tanzania: a cross sectional hospital-based study. Sci Rep [Internet]. 2023;13(1):1–7.

36. Joynson JA, Forbes B, Lambert RJW. Adaptive resistance to benzalkonium chloride, amikacin and tobramycin: The effect on susceptibility to other antimicrobials. J Appl Microbiol. 2002;93(1):96–107.

37. Kampf G. Antiseptic stewardship: Biocide resistance and clinical implications. Antiseptic Stewardship: Biocide Resistance and Clinical Implications. 2018. 1–694 p.

38. Durani P, Leaper D. Povidone-iodine: Use in hand disinfection, skin preparation and antiseptic irrigation. Int Wound J. 2008;5(3):376–87.

39. Alajlan AA, Mukhtar LE, Almussallam AS, Alnuqaydan AM, Albakiri NS, Almutari TF, et al. Assessment of disinfectant efficacy in reducing microbial growth. PLoS One. 2022;17(6 June):1–18.

40. Rideal S, Ridkat EK, Sciver A. The testing of disinfectants. Br Med J. 1927;2(3470):79–80.

41. Sajed A. Determination of Efficacy of Disinfectants against Pathogenic Microorganisms from the Environment of Tertiary Care Hospital. Ophthalmol Updat. 2021;19(2):61–5.

42. Fitzpatrick SR, Garvey M, Flynn J, O' Brien B, Gleeson D. Use of different methods for the evaluation of teat disinfectant products. J Appl Anim Res. 2022;50(1):31–8.

43. Rodr C, Alonso-calleja C, Garc C, Carballo J, Capita R. Bactericidal Concentration (MBC) for Twelve Antimicrobials. Biology (Basel). 2022;11(Mic):46.

44. Commission VAHD. for VAH certification of chemical disinfection procedures. 2017.

45. VAH Disinfectants Commission. REQUERIMENTS AND METHODS for VAH certification of chemical disinfection procedures. 2015;(April).

46. Springthorpe VS, Sattar SA. Carrier tests to assess microbicidal activities of chemical disinfectants for use on medical devices and environmental surfaces. J AOAC Int. 2005;88(1):182–201.

47. Singh M, Sharma R, Gupta PK, Rana JK, Sharma M, Taneja N. Comparative efficacy evaluation of disinfectants routinely used in hospital practice: India. Indian J Crit Care Med. 2012;16(3):123–9.

48. Khor SY, Jegathesan M. In-use testing of disinfectants in Malaysian government hospitals. Med J Malaysia. 1977;32(1):85–9.

49. Pfaller MA, Sheehan DJ, Rex JH. Determination of Fungicidal Activities against Yeasts and Molds : Lessons Learned from Bactericidal Testing and the Need for Standardization. 2004;17(2):268–80.

50. Uchejeso OM. Time Kill Kinetics Study of Commonly Used Disinfectants against Pseudomonas aeruginosa in Federal Medical Centre, Umuahia-Nigeria. Am J Biomed Sci Res. 2020;7(3):262–8.

51. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity : A review $. J Pharm Anal. 2016;6(2):71–9.

52. British Standards Institution. Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1). Eur Comm Stand. 2009;3(phase 1).

53. Antonelli A, Giovannini L, Baccani I, Giuliani V, Pace R, Rossolini GM. In vitro antimicrobial activity of the decontaminant hybenx® compared to chlorhexidine and sodium hypochlorite against common bacterial and yeast pathogens. Antibiotics. 2019;8(4):4–8.

54. Smock E, Demertzi E, Abdolrasouli A, Azadian B, Williams G. Antiseptic Efficacy of Povidone Iodine and Chlorhexidine Gluconate Skin Preparation Solutions Used in Burns Surgery. J Burn Care Res. 2018 Apr 20;39(3):440–4.

55. Rozman U, Duh D, Cimerman M, Turk SŠ. Hygiene of Medical Devices and Minimum Inhibitory Concentrations for Alcohol-Based and QAC Disinfectants among Isolates from Physical Therapy Departments. Int J Environ Res Public Health. 2022;19(22).

56. Ministry of Health. Infection prevention and control guidalines: The United Republic of Tanzania. 2018. 1–256 p.

57. Sanjeeb Mohapatra LY, Shin Giek Goh CN a, A, You Luhua NHT, Karina Yew-Hoong Gin. Quaternary ammonium compounds of emerging concern: Classification, occurrence, fate, toxicity and antimicrobial resistance. 2022;(November).

58. Gerba CP. Quaternary ammonium biocides: Efficacy in application. Appl Environ

Microbiol. 2015;81(2):464–9.

59. Cheng A, Sun HY, Tsai YT, Wu UI, Chuang YC, Wang JT, et al. In vitro evaluation of povidone-iodine and chlorhexidine against outbreak and nonoutbreak strains of Mycobacterium abscessus using standard quantitative suspension and carrier testing. Antimicrob Agents Chemother. 2018;62(1):1–10.

60. Guilhermetti M, Hernandes SED, Fukushigue Y, Garcia LB, Cardoso CL. Effectiveness of Hand-Cleansing Agents for Removing Methicillin-Resistant Staphylococcus aureus From Contaminated Hands. Infect Control Hosp Epidemiol. 2001;22(02):105–10.

61. Lepelletier D, Maillard JY, Pozzetto B, Simon A. Povidone iodine: Properties, mechanisms of action, and role in infection control and staphylococcus aureus decolonization. Antimicrob Agents Chemother. 2020;64(9):1–13.

62. Gallandat K, Kolus RC, Julian TR, Lantagne DS. A systematic review of chlorine-based surface disinfection efficacy to inform recommendations for low-resource outbreak settings. Am J Infect Control. 2021;49(1):90–103.

63. Köhler AT, Rodloff AC, Labahn M, Reinhardt M, Truyen U, Speck S. Efficacy of sodium hypochlorite against multidrug-resistant Gram-negative bacteria. J Hosp Infect. 2018;100(3):e40–6.

64. Jones IA, Joshi LT. Biocide use in the antimicrobial era: A review. Molecules. 2021;26(8).

65. Vereshchagin AN, Frolov NA, Egorova KS, Seitkalieva MM, Ananikov VP. Quaternary ammonium compounds (QACs) and ionic liquids (ILs) as biocides: From simple antiseptics to tunable antimicrobials. International journal of molecular sciences. 2021 Jun 24;22(13):6793.

66. Mazzola PG, Jozala AF, Novaes LCDL, Moriel P, Penna TCV. Minimal inhibitory concentration (MIC) determination of disinfectant and/or sterilizing agents. Brazilian J Pharm Sci. 2009;45(2):241–8.

67. Chowdhury CS, Khan JA, Khanam J, Nila SS, Ahmed S, Haque N, et al. Detection

of Biocide Resistance Genes (qacE and qacΔE1) in Pseudomonas spp Isolated from Patients with CSOM at Mymensingh Medical College Hospital, Bangladesh. Mymensingh Med J. 2021 Oct;30(4):954–9.

68. Aftab R, Dodhia VH, Jeanes C, Wade RG. Bacterial sensitivity to chlorhexidine and povidone - iodine antiseptics over time : a systematic review and meta - analysis of human - derived data. Sci Rep. 2023;1–9.

69. Artasensi A, Mazzotta S, Fumagalli L. Back to basics: Choosing the appropriate surface disinfectant. Antibiotics. 2021;10(6).

APPENDICES

Appendix I: Data Collection Tool 01

Disinfectant Name…….. …...Collection Date …… Day…

Contact time	Test number	ATCC-*E. coli*	ESBL-*E. coli*	ATCC-*S. aureus*	MRSA
Initial	**T1**				
	T2				
	T3				
1 min	**T1**				
	T2				
	T3				
5 min	**T1**				
	T2				
	T3				
10 min	**T1**				
	T2				
	T3				

Appendix II: Data Collection Tool 02

s/n	Disinfectants	Exposed organism: i.e. MRSA, ESBL *E. coli, Escherichia coli* (ATCC 35918), and *Staphylococcus aureus (*ATCC 25923)			
				1st run	2nd run
1	**Sodium hypochlorite (NaOCl)**	Concentration (% and mg/L)	MIC		
			MBC		
2	**Quaternary ammonium compound (QACs),**	Concentration (% and mg/L)	MIC		
			MBC		
3	**Povidone Iodine**	Concentration (% and mg/L)	MIC		
			MBC		

Appendix III: Ethical Clearances

UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

OFFICE OF THE DIRECTOR – RESEARCH, PUBLICATIONS AND INNOVATION

Ref. No.DA.282/298/01.C/2001 Date: 19/12/2023

MUHAS-REC-12-2023-2001
DANIEL PAUL MWANDU
COLLEGE OF MEDICINE
MUHAS

RE: APPROVAL FOR ETHICAL CLEARANCE FOR A STUDY TITLED: EFFICACY OF DISINFECTANTS AGAINST MULTI-DRUG RESISTANT BACTERIA ISOLATED FROM CLINICAL SAMPLES AT MUHIMBILI NATIONAL HOSPITAL. DAR ES SALAAM, TANZANIA.

Reference is made to the above heading,

I am pleased to inform you that the Chairman has on behalf of the University Senate, approved ethical clearance of the above-mentioned study, on recommendations of the Senate Research and Publications Committee meeting accordance with MUHAS research policy and Tanzania regulations governing human and animal subjects research.

APPROVAL DATE: 19/12/2023
EXPIRATION DATE OF APPROVAL: 19/12/2024

STUDY DESCRIPTION:
Purpose:

The purpose of this laboratory-based experiment study is to determine the efficacy of disinfectants against multidrug-resistant bacteria isolated from clinical samples at Muhimbili National Hospital.

The approved protocol and procedures for this study is attached and stamped with this letter, and can be found in the link provided:
https://irb.muhas.ac.tz/storage/Certificates/Certificate%20-%2014801.pdf and in the MUHAS archives.

The PI is required to:

1. Submit bi-annual progress reports and final report upon completion of the study.
2. Report to the IRB any unanticipated problem involving risks to subjects or others including adverse events where applicable.
3. Apply for renewal of approval of ethical clearance one (1) month prior its expiration if the study is not completed at the end of this ethical approval. You may not continue with any research activity beyond the expiration date without the approval of the IRB. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.
4. Obtain IRB amendment (s) approval for any changes to any aspect of this study before they can be implemented.
5. Data security is ultimately the responsibility of the investigator.
6. Apply for and obtain Data Transfer Agreement (DTA) from NIMR if data will be transferred to a foreign country.
7. Apply for and obtain Material Transfer Agreement (MTA) from NIMR, if research materials (samples) will be shipped to a foreign country.
8. Apply for and obtain permission to publish the results from National Institute for Medical Research. The application should be before submitting to a publisher and not concurrently through the email publications@nimr.or.tz.
9. Any researcher, who contravenes or fail to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III section 10 (2)
10. The PI is required to ensure that the findings of the study are disseminated to relevant stake holders.
11. PI is required to be versed with necessary laws and regulatory policies that govern research in Tanzania. Some guidance is available on our website https://drp.muhas.ac.tz/.

Muhimbili University of Health and Allied Sciences
DIRECTOR
Research, Publications & Innovations
Box 65001

Dr. Nahya Salim Masoud,

Chairperson, MUHAS Research and Ethics Committee

Appendix IV: Introduction Letter

THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

MUHIMBILI NATIONAL HOSPITAL

In reply please quote:

Ref. No.: MNH/CRTCU/Perm/2024/041 Date: 24th January, 2024

Head of Department
Central Pathology Laboratory
Muhimbili National Hospital

RE: PERMISSION TO COLLECT DATA AT MNH

Name of Student	**Daniel Paul Mwandu**
Title	**"Efficacy of Disinfectants Against Mult-Drug Resistant Bacterial Isolated From Clinical Samples at MNH Dar es Salaam Tanzania."**
Institution	Muhimbili University of Health and Allied Sciences
Supervisor	Dr. Joel Manyahi (MUHAS)
Co – supervisor	Dr. Salim Masoud (MNH)
Period	24th January 2024 to 30th May, 2024

Approval has been granted to the above principal investigators to collect data at MNH. Dissemination of study findings at MNH shall be overseen by **Dr. Salim Masoud** (MNH) supervisor.

Kindly ensure the named principal investigator abide to the ethical principles and other conditions of the research approval.

Sincerely, P.O. Box 65000 DAR-ES-SALAAM

Dr. Charles K. Pallangyo
For: Head of Clinical Research, Training and Consultancy Unit

c.c DCSS
c.c Dr. Salim Masoud
c.c Daniel Paul Mwandu

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