Effectiveness of Commonly Used Disinfectants in Hospitals Against Common Nosocomial Bacteria

By

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing the degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Approval

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On summer, 2023 has been accepted as partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology on 17 August, 2023.

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Ethics Statement

The entire research work has no conflict of interest. No human or animal models were used in this experiment.

This thesis is dedicated to my Family and Friends. For their endless love, support, and encouragement throughout this journey.

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List of Acronyms

HAI	Hospital Acquired Infection
NI	Nosocomial Infection
ICDDR, B	International Centre for Diarrhoeal Disease Research
OT	Operation Theatre
MRSA	Methicillin-Resistant *S. aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
S. aureus	*Staphylococcus aureus*
E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
SSI	Surgical Site Infection
UTI	Urinary Tract Infection
BSI	Blood Stream Infection
ESBL	Extended-Spectrum Beta-Lactamase
CRE	Carbapenem-Resistant *E. coli*
CDC	Centers for Disease Control and Prevention
ICU	Intensive care Unit
CHG	Chlorhexidine gluconate
ARO	Antibiotic-Resistant Organism
WHO	World Health Organization
BHI	Brain Heart Infusion
NA	Nutrient Agar
CFU	Colony Forming Unit
MSA	Mannitol Salt Agar
KPC	*Klebsiella pneumoniae* Carbapenemase
TSA	Tryptic Soy Agar
ml	Mili liter
***µ*l**	Micro liter
mm	Millimetre
TNTC	Too Numeric To Count
R	Resistant
LPS	Lipopolysaccharide
BAC	Benzalkonium Chloride

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Abstract

Nosocomial infections, commonly acquired during hospital stays, pose a significant threat to patient safety and increase healthcare costs. The effective use of disinfectants is crucial for decreasing the transmission of nosocomial bacteria in healthcare settings. This thesis paper aimed to evaluate the effectiveness of various commonly used disinfectants in hospitals against 4 most common nosocomial bacteria (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Acinetobacter* sp.), providing valuable insights for infection control practices. In this study, swab samples were collected before and after the application of disinfectants from two government hospitals and two private hospitals. The surfaces selected for sampling included the operating table in the operation theatre and the floors of the wards, as these areas are where patients typically spend most of their time. The disinfectants that were used during this study were Savlon, Hexisol, Clotex, Shinex, and Perfume Phenyle. Two types of samples were collected from each area: one before and one after the application of disinfectants for surface cleaning. *S. aureus, E. coli, K. pneumoniae,* and *Acinetobacter* sp. were isolated from the collected samples using selective media. Samples were then subjected to serial dilution to find the effectiveness of the disinfectants. The decrease in the number of microbes before and after surface disinfection was quantified using log reduction. The highest log reduction was Perfume Phenyle in the case of the ward as it was able to achieve a 100% reduction percentage. Clotex on the other side was able to only kill 50% of the microbes. In the case of operation theatre, Hexisol was the most effective one with a 100% reduction whereas Clotex was not effective at all as it had a 0% percentage reduction. After that, the isolates were tested for non-susceptible testing, where different concentrations of the disinfectants were applied to determine the zone of inhibition. The inhibition zones were measured to compare the resistance rate of the disinfectants. The results indicated that Shinex and Savlon exhibited strong inhibitory effects on the growth of the isolates, even at reduced concentrations (60% of their concentrations). Conversely, Perfume Phenyle demonstrated more resistance, with minimal or no inhibition zones observed even at the recommended concentration. Hexisol showed comparatively higher resistance even when its high concentrations were used and no concentration was observed to be resistant less than 50% in any concentrations. Whereas, Clotex works up to 90% of its concentration. Among the tested microbes, *S. aureus* displayed less resistance than the others, even at lower concentrations of disinfectants. In conclusion, this study highlights the varying efficacy of different disinfectants against nosocomial bacteria. Shinex and Savlon demonstrated notable effectiveness, while Perfume Phenyle exhibited no efficacy. These findings provide valuable insights into the selection and utilization of disinfectants for effective infection control in healthcare settings.

Chapter 1

Introduction

1.1 Background:

Hospital-acquired infections (HAI) or Nosocomial infections (NI) are the infections that people get when they are admitted to the hospital receiving health care for other issues. This infection is in the healthcare system for a very long period. It has the potential to occur within various healthcare settings, including hospitals, surgical centres, end-stage renal disease facilities, and long-term care facilities. Common pathogens such as bacteria, fungi, viruses, and less common pathogens both can cause this infection. According to a study every year more than 100 million patients are affected by nosocomial infections around the world (Taye et al., 2023). This issue is growing in every country. In high-income countries, the prevalence of HAIs is estimated to be around 7.5%. Conversely, in low- and middle-income countries, the prevalence rate of HAIs can range from 5.7% to 19.2% (Szabo et al., 2022). In Europe, the most common HAI-causing bacteria are from 10 species (Figure 1)

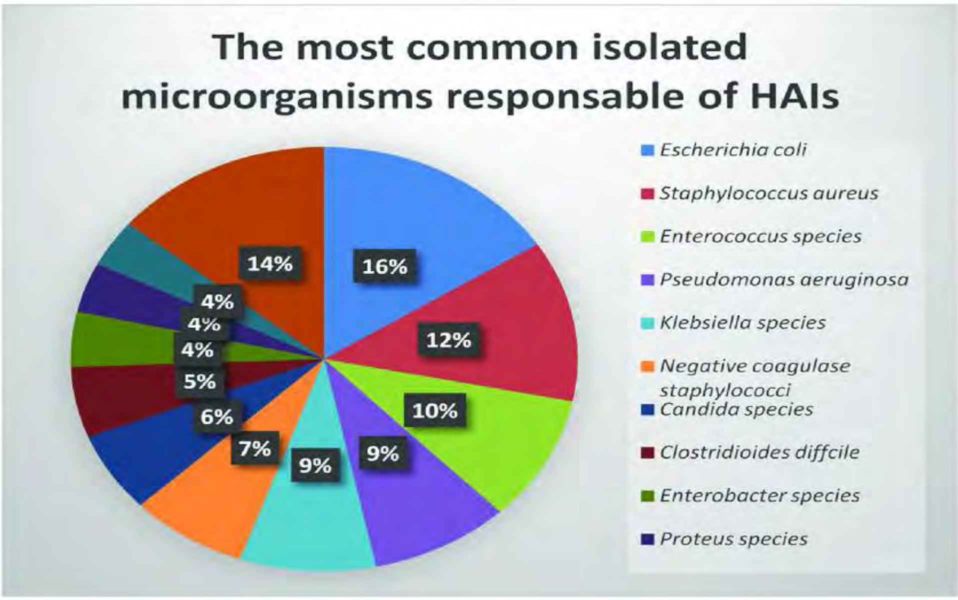

Figure 1.1 Most common microbes found in HAIs (Szabo et al., 2022)

In recent years, this issue is also growing in Bangladesh as well. Studies have shown rates of NIs in Bangladesh may exceed 30% in some hospitals which is more than the percentage in developing countries (Sm et al., 2016). This infection can not only affect the immune-compromised sick patients but also the health care workers and staff. According to a study conducted by ICDDR, B, on 226 healthcare workers those who worked on the surveillance wards, 27% of them experienced a respiratory illness during the study period. Also, in the same study it is observed that 30 of the 40 healthcare workers on the adult medicine ward experienced respiratory illness compared with 6%-24% of staff members on other wards (Icddr, b, 2011). This rate is quite alarming as the hospitals in Bangladesh are overly crowded and most of the staff have poor or average knowledge of NI (Rahman et al., 2017).

A study conducted on Tertiary hospitals in Bangladesh found *E. coli* was reported to be the most common microorganism responsible for nosocomial infections, accounting for 55.9% of cases. Other identified organisms included *Pseudomonas* sp. (33.3%), *Proteus* sp. (12.7%), *S. aureus* (5.9%), *Klebsiella* sp. (4.9%), and *Acinetobacter* sp. (3.9%) (Mohiuddin et al., 2012). These microbes tend to cause many kinds of infectious diseases which are quite deadly for the patients. Sometimes they can cause sepsis and even death. That is why, it is crucial to eliminate these microbes from the Hospital environment to lessen the effect. That is why, CDC has published guidelines on what type and kind of disinfectants need to be used to prevent these microbes from spreading from surface to patients (Rutala & Weber, 2008). However, the number of resistant microbes against disinfectants is increasing very vastly. In 2015, it was estimated that within the European Union (EU), reports were around 671,689 instances of infections stemming from bacteria that are resistant to antibiotics. Out of these cases, approximately 63.5% were classified as NI, indicating that they were acquired within healthcare facilities (Carlie et al., 2020). In studies, it has been observed that isolates found in patients are more resistant against antimicrobials such as 2% Steranios, Deconex HLDPA, and Microzed Quatenol (Tapouk et al., 2020).

However, there is not much information or research on the situation of HAI or NI in Bangladesh. Being one of the overpopulated countries, the rate of NI is quite higher but there is no sufficient research on it. Even though there are guidelines published by the government to prevent NI but there are no regulations over it. That is why, the objective was to find out the situation that the hospitals are in. During the study, the disinfectants that are being used were Savlon, Hexisol, Perfume Phenyl, Shinex, and Clotex.

1.2 Characteristics of Chosen Microbes

In this study, previous research has been followed to find out which microbes specifically bacteria are found in the hospital environment. It was found that microbes that are now a major concern are *Pseudomonas aeruginosa, S. aureus, Enterococci* sp. , *K. pneumoniae,* and *Enterobacter* sp. and also these are resistant pathogens specifically methicillin-resistant *S. aureus* (MRSA). MRSA is recognized for inducing significant morbidity and mortality among patients who are hospitalized (Tolera et al., 2018). The microbes that are chosen for this specific study are opportunistic bacteria. They are commonly found in the skin, gut, or environment. However, they do not cause any harm until they enter the bloodstream.

E. coli can cause various types of infections in healthcare settings, including urinary tract infections (UTIs), surgical site infections (SSIs), bloodstream infections (BSIs), pneumonia, meningitis, and gastrointestinal infections (Ramos et al., 2020). Some strains of *E. coli* have developed resistance to commonly used antibiotics, making infections more challenging to treat. For example, Extended-Spectrum Beta-Lactamase (ESBL) producing *E. coli* and Carbapenem-Resistant *E. coli* (CRE) are of concern in healthcare settings (Legese et al., 2017)

S. aureus is Gram-positive bacteria that are cocci-shaped and has the tendency to arrange in clusters that look "grape-like." *S. aureus* has also raised concern especially MRSA which is more virulent than other strands as it is resistant to antibiotics. *S. aureus* is commonly found in the skin of a healthy individual. It causes many common bacterial infections in humans such as skin tissue and soft tissue infections, UTIs which are associated with catheters, SSIs, and pulmonary infections (e.g., pneumonia and empyema) (MRSA | CDC, n.d.). According to CDC, infections due to *S. aureus* are most concerning as it is difficult to treat. Because *S. aureus* has strands that are resistant to antibiotics (Ramos et al., 2020).

Acinetobacter sp. are Gram-negative Coccobacilli commonly found in the soil and environment which can reach the hospital environment and enter into the bloodstream of patients. They rank on the fourth position (after *P. aeruginosa*, *S. aureus,* and *K. pneumoniae*) among the most frequent HAI causing microbes(Rebic et al., 2018). *Acinetobacter* can cause common infections such as UTIs, BSIs, SSIs, and pneumonia just like the other microbes. It has been established as 'alert' because it has emerged as a cause of ICU infection. (Rebic et al., 2018)

K. pneumonia is a type of gram-negative bacteria. This bacterium is mostly spread from person to person but less commonly through the environment according to CDC. This can cause pneumonia and other kind of infections just like the other microbes. This is also prone to be resistant to antibiotics which is why it is also concerning.

1.3 Disinfectants and their active ingredients

The disinfectants that were used during the collection of the samples were Savlon, Hexisol, Clotex, Perfume Phenyle, and Shinex (Fig 1.2). Except Hexisol all the disinfectants are used both in the ward and OT. n hospital settings, the primary objective of employing these disinfectants is to lower the likelihood of patients acquiring nosocomial infections. Insufficient disinfectant usage is a contributing factor to numerous nosocomial infections. Various commercial disinfectants are employed to forestall and manage such infections, each with its own set of strengths and weaknesses. Employing efficient and safe disinfection methods that cause minimal harm to equipment and staff stands as a fundamental disinfection principle (Pirsaheb et al., 2016). However, it is also essential to make sure the environment is also kept clean as much as possible. To make sure that different antimicrobials with different active ingredients are used.

Figure 1.2: Disinfectants used

These disinfectants have active ingredients that specifically target microbes so that the microbes get destroyed within a very short period. According to the ingredients written in its bottle of the Savlon, the active ingredients found are Cetrimide 3.0% w/v and Chlorhexidine Gluconate (CHG) 0.3% w/v (SAVLON ANTISEPTIC LIQUID, n.d.).

Cetrimide is a quaternary ammonium compound that is commonly used as an antiseptic and disinfectant. Cetrimide is a substance which is toxic. It inhibits the growth of many bacteria by releasing the nitrogen and phosphorous from their cells. Due to the release of nitrogen and phosphorous the bacteria end up having slow metabolism or killing the bacteria eventually. On the other hand, CHG has a cationic (positively charged) structure. It interacts with the negatively charged components of microbial cell membranes, such as phospholipids, disrupting their structure and integrity (Figure 1.3). This destabilization leads to the leakage of cellular contents and eventual cell death. ("Chlorhexidine Facts," n.d.)

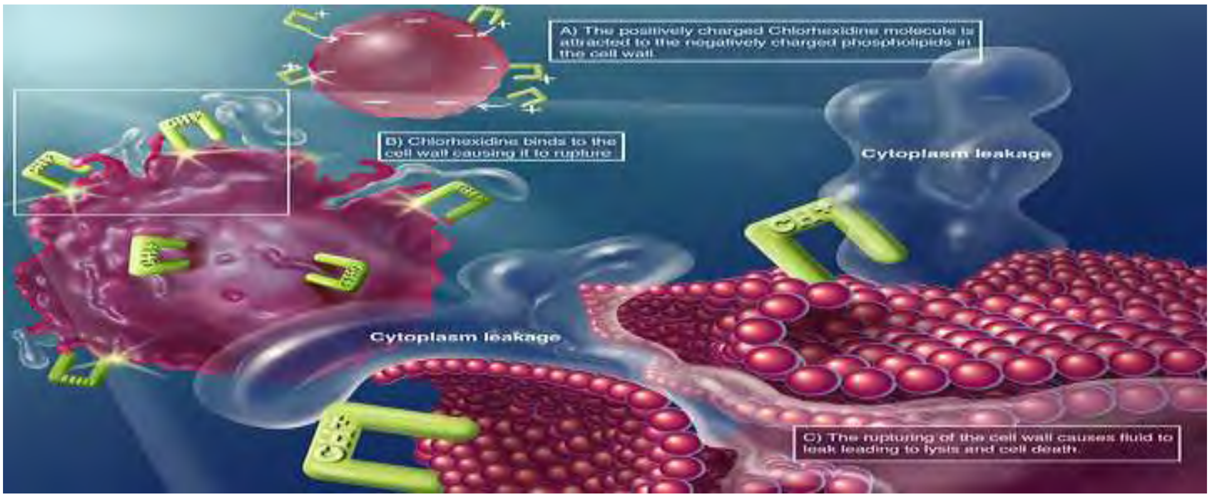

Figure 1.3: Mechanism of action of chlorhexidine gluconate (*Chlorhexidine Facts: Mechanism of Action*, n.d.)

Hexisol is an alcohol-based hand rub contains 2% CHG combining with 70% Isopropyl alcohol. Both of these ingredients together are effective for both rapid and persistent reduction of bacteria. The combination is effective as Isopropyl alcohol immediate kills the transient and resident microorganisms and 2% CHG binds to the superficial cell layers of the microbes and provides a residual, or persistent, antimicrobial property that does not allow restoration of microorganisms (Hexisol, n.d.).

Perfume Phenyle disinfectant claims to kill 99% of germs and is made up of Turpentine, Caustic Soda also known as Sodium Hydroxide, Carbolic, etc. Turpentine is used in various antimicrobials in oil form. Turpentine oil has been shown to have chemical components that have the potential to be the greatest inhibitory against *S. aureus* and *E. coli*. (Wijayati et al., 2019). Sodium Hydroxide or popularly known as Caustic Soda is used to clean surfaces as it can kill microbes (Elekhnawy et al., 2020).

The active ingredients of Shinex are Benzalkonium Chloride solution, EDTA, preservatives, etc. According to Pereira and Tagkopoulos (2019), Benzalkonium Chloride (BACs) is widely used as an active ingredient in disinfectants for various settings like homes, industries, farms, and healthcare facilities and it is also approved to use on surfaces both indoors and outdoors. The reason behind the antimicrobial properties of BAC is believed to be its ability to disrupt the interactions between molecules. This disruption can lead to the separation of lipid bilayers in the cell membranes of bacteria, compromising their ability to control the permeability of the cell. As a result, important cellular contents can escape from the cell, which contributes to the antimicrobial effect of BAC (Benzalkonium: Uses, Interactions, Mechanism of Action | DrugBank Online, n.d.). The second active element is Ethylenediaminetetraacetic Acid also known as EDTA is used for clinical purposes for gram-positive and gram-negative microorganisms, fungi, and yeast. EDTA has been proven to eliminate Mg^{2+} and Ca^{2+} ions from the external cell wall of Gram-negative bacteria. This action releases approximately 50% of the Lipopolysaccharide (LPS) molecules and exposes the phospholipids in the inner membrane. This, in turn, enhances the effectiveness of other antimicrobial substances (Finnegan & Percival, 2015).

1.4 Why is it a concern

The effect of Hospital-acquired infections (HAIs) is more effective both for the patients and the economy of the country. HAIs caused by antibiotic-resistant organisms (AROs) represent a significant impact on patient morbidity and mortality and also is a burden on the economy of the healthcare system (Alfa et al., 2015). Hand hygiene has been underscored by the World Health Organization as a critical approach for diminishing NIs (Sm et al., 2016). But only cleaning hands cannot reduce the effect of these infections. The risk of HAI has been estimated to be 2% to 25% more in developing countries or low income countries than that in resource-rich countries or high income countries with exceeding 25% infected patients (Sm et al., 2016).

Nosocomial infection not only affects the health of the patient but also affects the health of healthcare workers such as nurses or staff. Because healthcare workers have to come into close proximity to the infected patients while working inside the hospital. According to a study conducted by Journal BiNet, the frequency of nosocomial infections ranges from 3.0% to 20.7% in terms of prevalence rate, while the occurrence rate falls within the range of 5% to 10% as the incidence rate. Also, according to the report of WHO, approximately 8.7% of nosocomial infections were observed among patients receiving hospital care (BiNET, n.d.). As time passes by these nosocomial infection-causing microbes are getting more resistant to antibiotics. On the other hand, the patients have to extend the duration of hospital admission due to complications caused by this infection. The rate of mortality also increasing day by day. If the infection rate is not increased, the microbes will not work against any antibiotics and soon there will be no treatment for these infections. That is why, it is necessary to reduce the microbes found in the environment of the hospital.

1.5 Aims and Objectives

1. Study the effectiveness of disinfectants in the hospital
2. Isolate bacteria from respective hospitals
3. Check the efficacy of disinfectants in vitro

Chapter 2

Method and Materials

2.1 Study Design and Sample Collection

In this study, the effectiveness was evaluated of the disinfectants In-situ (in the hospital) and also In-vitro Assay in Brac University Laboratory. In case of hospitals, the samples were collected before and after the use of disinfectants from various surfaces of the hospitals following the method by Nourbakhsh (2016) and Boteju et al. (2020).

The swab samples were collected from the Ward and OT before and after the use of disinfectants. The Ward and OT where the samples were collected are shown in Table 1. The swab samples were collected using a sterile swab from 1inch^2 area in normal saline and transferred in our lab within 2 hours at 4°C. Then in the lab, the targeted microbes were isolated and further experiments were done. The isolated microbes from the previous one was tested for disinfectant efficacy in the lab (Table 1)

Table 1. Places from where the sampling site is mentioned

Sample	Sample Site
Operation Theatre Before	1inch^2 area of the OT Table where the patient is laid during surgery before using disinfectant
Operation Theatre After	Same 1inch^2 area after using disinfectant and visibly airdrying
Ward Before	1inch^2 Floor surface which gets frequently cleaned before using disinfectants
Ward After	Same 1inch^2 area after using disinfectant and visibly airdrying

2.2 Bacterial Culture

2.2.1 Sample processing

After reaching the lab, each sample swab was inoculated in 5ml of Brain Heart Infusion (BHI) Broth for enrichment. The media was then incubated at 37°C overnight.

2.2.2 Serial Dilution

Serial dilution was done to count the number of bacterial colonies present in the sample. From each BHI tube 1ml of stock sample was transferred to a 9% saline solution tube and mixed evenly using vortex. Up to 16 dilutions were done and plated in Nutrient Agar (Figure 2.2.2) to compare the CFU/inch^2 of the samples. Log reduction was calculated to compare the before and after effectiveness of disinfectants by using the following formula:

Log10 Reduction Factor (RF) = Log10 Prevalue - Log10 Postvalue (Singh et al., 2012)

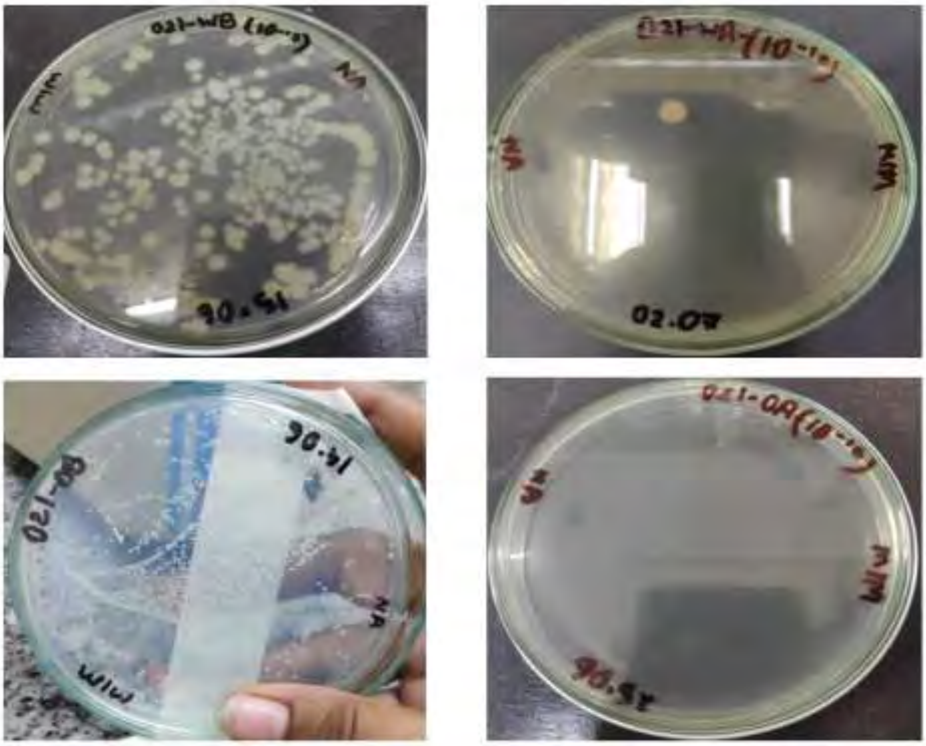

Figure 2.2.2: Dilutions plated on Nutrient Agar (10^{12} and 10^{16})

2.2.3 Bacteria Isolation:

After incubation, selective media were used to identify specific organisms which were *S. aureus, Acinetobacter* sp., *K. pneumoniae*, and *E. coli*. The medias used were Mannitol Salt Agar (MSA) media, Leeds Acinetobacter Agar media, and HiChrome KPC Agar media (Figure 2.2.3).

Each of the swabs were steaked in selective media plates and incubated at 37°C for 24 hours. After incubation, the organisms were identified based on the colour on the media. Single colonies from each identified organisms were grown in Nutrient Agar for 24 hours at 37°C.

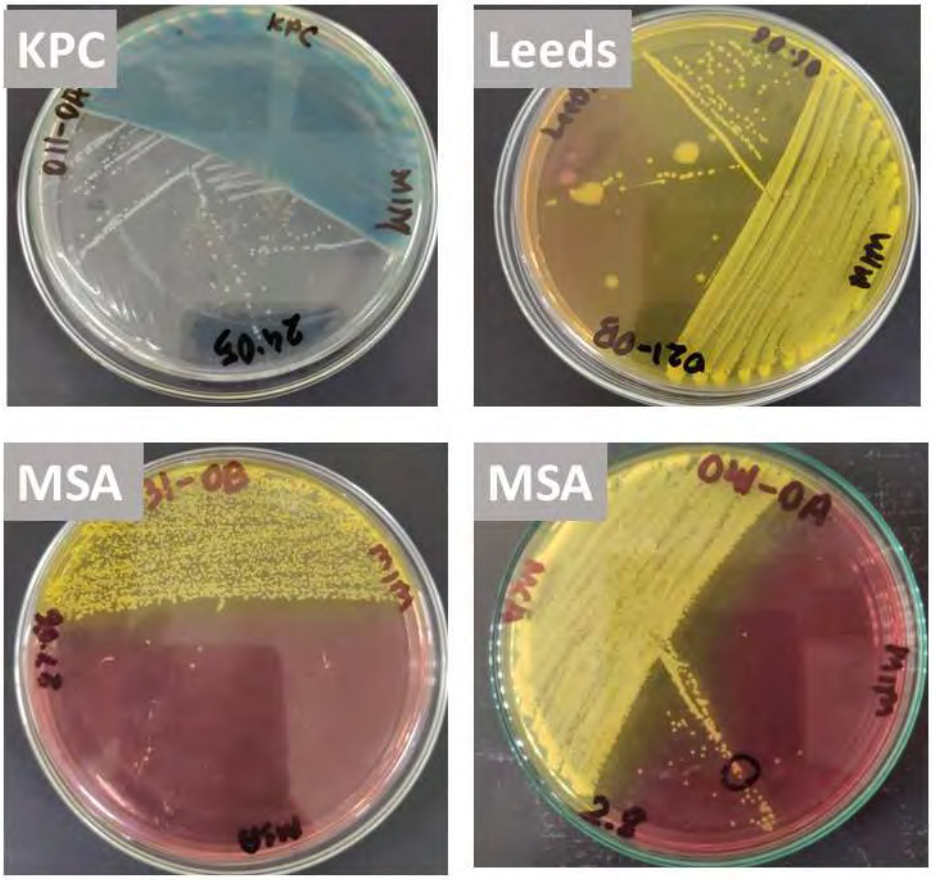

Figure 2.2.3: Bacterial growth on different selective media

2.2.4 Efficacy Test:

The efficacy test of the disinfectant was done by the method described by Montagna et al. (2019). According to this study agar diffusion test was done. The microbes were suspended in a saline solution (sterile) at a concentration of 0.5 McFarland (1.5×10^8 CFU/ml). They were then evenly spread onto Tryptic Soy Agar (TSA) using sterile swabs and a rolling technique.

For the disinfectant solutions, 10µl of each disinfectant was transferred to sterile tubes. Initially, a 100% concentration of each disinfectant was prepared. The concentration was gradually reduced by adding distilled water. The subsequent concentrations were prepared by adding 9ml of antiseptic to a tube containing 1ml of distilled water, resulting in a 90% concentration. The remaining concentrations were prepared in the same descending manner. The antiseptic concentrations used in this study ranged from 100% to 10% for Savlon and Shinex, 30% for Hexisol, and 60% for Clotex and Perfume Phenyle.

Additionally, sterile paper disks with a diameter of 6mm were soaked in 10µl of each disinfectant solution, dried in the air, and placed on the surface of the inoculated plate. The plates were kept for an hour at room temperature and then incubated at 37°C for 24 hours.

The effectiveness of each disk was determined by measuring the diameter of the zone of inhibition surrounding it. If the zone measured more than 8mm in diameter, the microbes were considered sensitive to the disinfectant (Figure 2.2.4). The paper disks were also removed to check for the presence or absence of growth underneath them. Each test was triplicated to confirm and evaluate the results. Control tests were conducted using standard strains of *K. pneumonia, E. coli, S. aureus, and Acinetobacter* sp. Positive control plates using the standard strains were tested for zones using same incubation conditions, whereas a paper disk soaked with 10µl of sterile saline served as the negative control. All the experiments were carried out using sterile techniques.

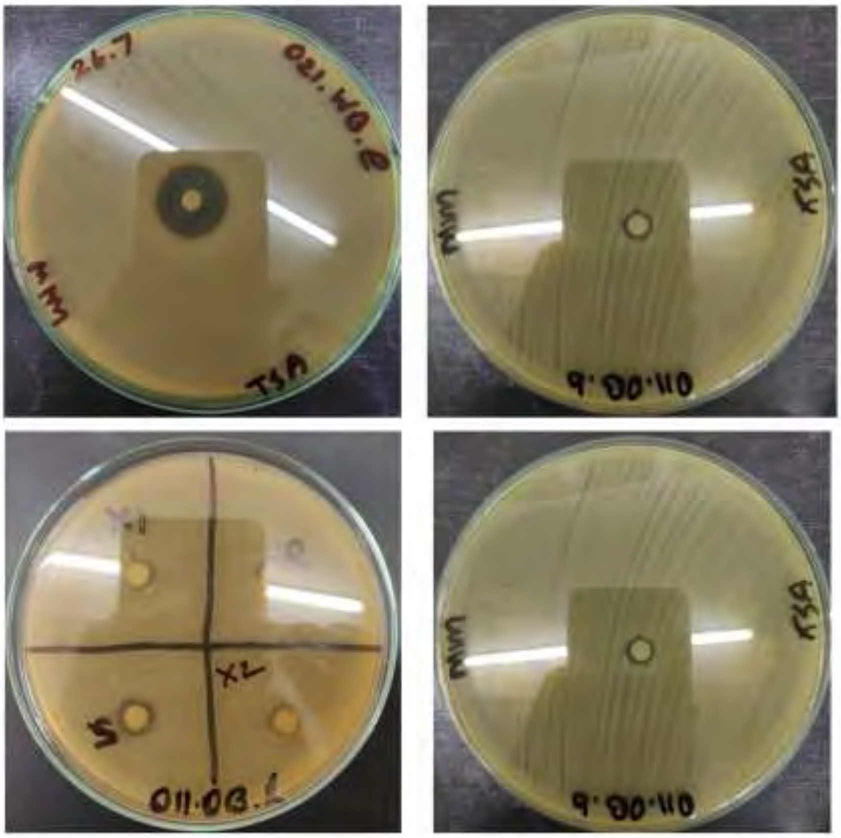

Figure 2.2.4: Zone of inhibition using different concentrations

Chapter 3

Result and Observation

3.1 Effect of Disinfectants in Different Dilution

Samples had been collected from surfaces that are frequently cleaned using disinfectants. A total of 48 isolates had been found which there are 17 *Acinetobacter* sp. , 16 *K. pneumoniae*, 8 *E. coli,* and 7 *S. aureus*. The disinfectants were Hexisol, Savlon, Clotex, Shinex, and Perfume Phenyle. The comparison between both before and after the cleaning procedure has shown in Table 2 and Table 3.

Table 2 shows surface disinfection on ward surface showed log reduction of Savlon which was 4.3 and 0.3 for Clotex. The maximum log reduction was shown by Perfume Phenyle which was 17.3 log reduction. Savlon and Perfume Phenyle showed effective killing percentage whereas Clotex showed very poor result.

Similarly, Table 3 shows in OT the log reduction of Clotex and Perfume Phenyle was 0 and 0.3 respectively. On the other hand, for Hexisol it was more than 21 which was the only disinfectant to achieve a good result.

Table 2. Results of surface disinfection activity by different disinfectants on Ward

Hospital No	Disinfectant Name	Number of CFU/inch^2 present on the surface before disinfection	Number of CFU/inch^2 present on the surface after disinfection	Log reduction in CFU/inch^2 after disinfection	Percent kill after disinfection
011	Savlon	8×10^{21}	4×10^{17}	4.3	99.99%
031	Clotex	4×10^{17}	2×10^{17}	0.3	50%
041	Perfume Phenyle	8×10^{17}	Nil	17.9	100%

Table 3. Results of surface disinfection activity by different disinfectants on Operation Theatre

Hospital No	Disinfectant Name	Number of CFU/inch^2 present on the surface before disinfection	Number of CFU/inch^2 present on the surface after disinfection	Log reduction in CFU/inch^2 after disinfection	Percent kill after disinfection
011	Hexisol	8×10^{21}	Nil	21.9	100%
021	Shinex	TNTC	Nil		
031	Clotex	2×10^{17}	2×10^{17}	0	0%
041	Perfume Phenyle	2×10^{18}	1×10^{18}	0.3	50%

3.2 Efficacy Test on Isolates

The antimicrobial effect of the disinfectants is shown from Table 4 to Table 8. It indicates the percentage of resistance of the disinfectants using different concentrations. For this result, a previous paper had been followed (Montagna et al, 2019). According to this paper, the microorganisms were considered sensitive when the zones of inhibition were >8mm overall and the other ones are considered Resistant (R). Each isolate was triplicated and then the results were evaluated.

Table 4. Percentage of resistance based on different concentrations of Savlon

Bacteria (n)	Percentage of Non-Susceptible Isolates Concentration of Disinfectants (%)									
	100	90	80	70	60	50	40	30	20	10
Acinetobacter sp. (17)	29.41	58.82	47.1	58.82	64.72	76.47	82.35	76.41	82.35	82.35
E. coli (8)	25	37.5	50	62.5	37.5	75	75	62.5	87.5	87.5
K. pneumoniae (16)	6.25	18.75	62.5	50	50	62.5	81.25	68.75	68.75	75

S. aureus (7)	0	28.57	14.43	14.43	14.43	28.57	28.57	28.57	14.43	14.43
Total	16.67	37.5	47.92	50	47.92	68.75	72.92	64.58	68.75	70.83

Here it indicates that different concentrations of Savlon worked well in inhibiting the growth of the microbes around the discs. After 60% concentration, the isolates showed more than 50% resistance.

Table 5. Percentage of resistance based on different concentrations of Hexisol

Bacteria(n)	**Percentage of Non-Susceptible Isolates**							
	Concentration of Disinfectants (%)							
	100	**90**	**80**	**70**	**60**	**50**	**40**	**30**
Acinetobacter sp. (17)	64.71	76.47	70.6	70.6	70.6	76.47	88.24	76.47
E. coli (8)	37.5	62.5	50	50	87.5	75	75	75
K. pneumoniae (16)	62.5	62.5	68.75	62.5	68.75	68.75	68.75	68.75
S. aureus (7)	14.3	28.57	28.57	28.57	14.3	14.3	14.3	14.3
Total	52.08	62.5	60.42	58.33	64.58	64.58	66.67	64.58

Here this table indicated that Hexisol could not inhibit zones much even when the 100% concentration was used.

Table 6. Percentage of resistance based on different concentrations of Clotex

Bacteria(n)	**Percentage of Non-Susceptible Isolates**				
	Concentration of Disinfectants (%)				
	100	**90**	**80**	**70**	**60**
Acinetobacter sp. (17)	35.3	47.06	70.59	82.35	94.12
E. coli (8)	50	62.5	75	100	87.5
K. pneumoniae (16)	31.25	43.75	68.75	100	93.75
S. aureus (7)	71.43	57.14	28.57	85.71	100
Total	41.67	50	64.58	91.67	93.75

This table indicates how Clotex gave 50% resistance when 90% concentration was used. But lower than that, isolates were resistant to all of them.

Table 7. Percentage of resistance based on different concentrations of Shinex

Bacteria(n)	**Percentage of Non-Susceptible Isolates**									
	Concentration of Disinfectants (%)									
	100	**90**	**80**	**70**	**60**	**50**	**40**	**30**	**20**	**10**
Acinetobacter sp. (17)	52.94	29.41	29.41	52.94	64.71	76.47	64.71	76.47	82.36	82.36
E. coli (8)	25	25	25	50	37.5	62.5	50	87.5	87.5	75
K. pneumoniae (16)	31.25	31.25	62.5	50	56.25	75	75	75	75	68.75
S. aureus (7)	14.29	0	14.29	28.57	14.29	14.29	28.57	0	14.29	14.29
Total	35.42	25	37.5	47.92	50	64.58	60.42	66.67	70.83	66.67

Here Shinex showed excellent resistance in the case of every bacteria. It showed less resistance than the other ones. However, a concentration of up to 60% was useful as it showed less resistance. For concentrations lower than 60% microbes were resistant.

Table 8. Percentage of resistance based on different concentrations of Perfume Phenyle

Bacteria(n)	**Percentage of Non-Susceptible Isolates**					
	Concentration of Disinfectants (%)					
	100	**90**	**80**	**70**	**60**	**R**
Acinetobacter sp. (17)	100	100	100	94.12	100	100
E. coli (8)	100	100	100	100	75	100
K. pneumoniae (16)	100	100	100	100	94.12	100
S. aureus (7)	94.12	100	100	100	94.12	100
Total	97.92	100	100	97.92	91.67	100

Among all the disinfectants, Perfume Phenyle showed a very dissimilar result. The zones were not clear at all. For most of the isolates, there were no zones at all. As a result, they were resulted as resistant even at 100% concentration. The Recommended concentration also showed the isolates are resistant to Perfume Phenyle.

In the case of all the disinfectants except Perfume Phenyle, *S. aureus* showed proper zones for every concentration. It was less Resistant than other microbes even for the lower concentrations (10%).

Chapter 4

4.1 Discussion

According to studies it has been observed that the most common HAI causing pathogens are Coliforms (40.02%), *Acinetobacter* sp. (25.28%), and *S. aureus* (10.54%). Also, *Acinetobacter* sp. (34%), and *E. coli* (15%) are the predominant bacteria causing HAIs (Boteju et al., 2020). These nosocomial infections are proven to be deadly for the health of the patients because they already have weak immune systems. That is why, it is very important to make sure the surface which comes in contact with the body of the patient is free of microorganisms. The five disinfectants Savlon, Hexisol, Clotex, Perfume Phenyle, and Shinex were all tested in hospital environment and in vitro both.

In the case of Ward, different disinfectants showed variable results. According to the calculation of Singh et al. (2012), Savlon was successful in reducing 99.99% of microbes and Perfume Phenyle was able to achieve 100% killing of the microbes. Both of these results are more than what the disinfectants claimed. However, Clotex could not emit more than 50% microbes (Table 2). On the other hand, the operation theatre is a very crucial place that needs to be cleaned properly every day. In this study, Clotex showed 0% effectiveness against the microbes as the log reduction showed whereas Perfume Phenyle showed only 50%. Only Hexisol showed 100% effectiveness after cleaning the surface (Table 3).

For the efficacy test, the inhibition zone of the disinfectants had been compared. The one with the most inhibitory zone (>8mm) is considered to have the most bactericidal effect. The first disinfectant which is Savlon had shown a very clear and effective zone on most of the nosocomial bacteria. According to the guideline published by Savlon, the active ingredients such as Cetrimide and CHG, are effective against bacteria and fungi as well. It showed less resistance to *S. aureus* even with a low concentration (10%). But it was more resistant with lesser inhibitory zones than the other ones. After using the most concentrated solution of Savlon, it showed almost 16.67% resistance which was the most of them all (Table 4). The zones gradually decreased with the decrease of the Savlon concentration. The concentration which had 60% Savlon showed more than 50% non-susceptible effect on all of the microbes. So, it can be said that Savlon showed moderate efficacy against all the microbes just as was observed in the study conducted by Saha et al. (2011).

Hexisol is one of the most common hand rubs that is used in our daily life. It was a very popular source of antibacterial solution during covid outbreak as it was believed to kill SARS-CoV-2 from both skin and other surfaces. Hexisol contains 2% CHG in 70% Isopropyl alcohol, which is effective for both rapid and continuous decrease of bacterial load across various body regions for a large number of organisms. However, in this study, Hexisol showed very poor zones against all of the microbes. The concentrated solution showed 52.08% resistance which was the least among all the concentrations (Table 5).

The third disinfectant that was used for this study was Clotex. This particular disinfectant is also used in the hospital environment. It showed very poor zones against the microbes. Concentrated Clotex showed almost 41.67% resistance whereas 90% concentration only showed less than 50% effectiveness (Table 6).

The disinfectant that also gave a good result was Shinex. It showed to be very effective against all microbes. The inhibitory zones were larger in diameter than all the other ones. For most of the disinfectants, the zones decreased with concentration, and the same scenario was observed in the case of Shinex (Table 7). The concentrated solution showed some visible zones but with decreasing concentration of the disinfectant, the resistance increased. Finally, it was observed that when 60% concentration was used it showed 50% resistance.

Perfume Phenyle is one of the most used disinfectants from the very early days. It is even used in households to remove bacteria to keep the home clean and fresh. However, the result of this study was quite unusual than the other ones. The full concentration showed no effective zones except for one *S. aureus*. Up to 60% concentration was tested but there were not very effective zones observed (Table 8). To make sure there was no mistake, the recommended concentration was also tested. But still, there were almost no visible zones. So, it can be said that almost all the microbes are resistant to Perfume Phenyle which is similar to the result of the study conducted by Saha et al. (2011) where Phenyle showed very poor effectiveness.

From observing all the results, it can be observed that Shinex and Savlon worked better than inhibiting the microbes around it than the other three even at lower concentrations. In contrast, Perfume Phenyl, and Hexisol did not work much. Also, in comparison to other microbes *S. aureus* was less resistant even in very lower concentrations.

4.2 Conclusion

The outcomes showed that for different microorganisms the results fluctuate depending on the disinfectants that are used. Savlon, Perfume Phenyle, and Hexisol showed effectiveness when used to clean the surface in the hospital. For the efficacy test, Shinex and Savlon showed good results against all 4 microbes that were used in this study. In comparison to other ones, Shinex recorded the most noteworthy zone of inhibition for *S. aureus* (35mm) which was unusual compared to the traditional disinfectants that are used. Almost all the disinfectants were observed to be working for *S. aureus* even in the lowest concentrations. However, no proper zones were observed for Perfume Phenyle at all as it is one of the oldest ones that are still used for other purposes as well.

Chapter 5

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