Assessment of Disinfectants efficacy on Nosocomial Bacteria and Determining their Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the degree of B.Sc in Biotechnology

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing 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 which 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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Abstract

Background: Nosocomial infection are increasing daily. It is important to investigate the efficiency of popular Disinfectants and determine their Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against nosocomial bacteria.

Objective: This study aimed to investigate the efficiency of recommended amount of popular disinfectants Savlon, Dettol, Lizol and Savlon spray on nosocomial bacteria *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and to determine the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of Savlon, Dettol, Lizol and Savlon spray on nosocomial bacteria.

Methods: The effects of the disinfectants of Savlon, Dettol, Lizol and Savlon spray against *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* were investigated by dilution and cultured by spread plate method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the disinfectants against total 12 isolates of *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* bacteria were determined by dilution and cultured by spread plate method.

Results: According to dilution and cultured by spread plate method and as per the recommendation of the company of disinfectants, each isolates had colonies or too numerous to count growth. In case of Dettol, the lowest MBC values were found from 750 µL to 1750 µL and lowest MIC values were found from 500 µL to 1500 µL for *S. aureus* and for *E. coli* the lowest MBC were found from 1000 µL to 1750 µL and lowest MIC were found from 750 µL to 1500 µL. Also, from 1500 µL to 2750 µL and from 1200 µL to 2500 µL were found to be the lowest MBC and MIC values for *K. pneumonia*. For *P. aeruginosa* isolate 1, the lowest MBC and MIC value is 5000 µL and 4750 µL, in case of Dettol. Regarding the results of Savlon, the lowest MBC and MIC values were found from 500 µL to 2750 µL and from 250 µL to 2500 µL for *S. aureus*. For *E. coli* the lowest MBC values were from 1250 µL to 3000 µL and lowest MIC values were from 1000 µL to 2750 µL. *K. pneumoniae* isolates lowest MBC values were from 500 µL to 4000 µL and lowest MIC values were from 250 µL to 3750 µL. In case of Lizol, the lowest MBC and MIC values were found from 765 µL to 1800 µL and from 750 µL to 1785 µL for *S. aureus*. For *E. coli* the lowest MBC values were found from 3015 µL to 3150 µL and the lowest MIC values were from 3000 to 3135 µL. In the matter of *K. pneumoniae*, the lowest MBC and MIC value was 915 µL and 900 µL. Unexpectedly, many isolates of these bacteria were found no MIC or MBC against these disinfectants.

Conclusion: According to the findings, the recommendations amount of disinfectants do not work against nosocomial bacteria and the results of MIC and MBC are ten to twenty times higher than recommendations. Consequently, it can lead to health hazard as it may increase nosocomial infections. So, the companies should change their disinfectants efficiency in order to stop nosocomial infections.

Keywords: Nosocomial infections, Effects of disinfectants, Minimum inhibitory concentration (MIC), Minimum Bactericidal concentration (MBC).

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LIST OF ACRONYMS

E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
K. pneumoniae	*Klebsiella pneumoniae*
P. aeruginosa	*Pseudomonas aeruginosa*
NA	Nutrient Agar LB/lb
broth	Luria- Bertani broth µL
Microliter	
ml	Milliliter
L	Liter
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
CFU	Colony-Forming Unit

Chapter 1

Introduction

Nosocomial infection is one of the most concerning problems in the modern world that creates dangerous health issues. Nosocomial infections are acquired while receiving the health care which was absent before admission. Each year, approximately 1.7 million people suffer from nosocomial diseases and almost 99000 deaths occur (Healthcare-Acquired Infections, 2022, para. 3). Both undeveloped and developing countries are facing serious conditions to prevent nosocomial infections. The number of infections in developed countries are not same but they are also not safe. Not only serious patients, old patients and kids but also nurses, doctors and other stuffs can be affected too. Hence, it is better to prevent nosocomial infection by killing the bacteria. The most effective and common element to kill bacteria are disinfectants. Besides, most of the companies claim that their disinfectants kill 99% bacteria. Disinfectants are harsh chemical substances to kill bacteria, viruses and other microorganisms that causes infection (Safeopedia, 2021, para 1.). Disinfectants are used in houses, offices and hospitals in order to not only clean the dust but also to kill the pathogenic organisms.

The most common nosocomial bacteria are *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *E. coli* causes urinary tract infection, surgical cite infection, bloodstream infection and pneumonia etc. (Nagarjuna et al.2015). In hospital patients. *K. pneumoniae* causes urinary tract infection, pneumonia, septicemias and soft tissue infection

(Podschun and Ullmann, 1998.). *Staphylococcus aureus* is the most common cause of nosocomial wound infection and diseases (Jensen et al.1999). *Pseudomonas aeruginosa*, a gram-negative bacterium, is one of the most resistant agents of nosocomial infection (Labovska, 2021). *P. aeruginosa* is the reason of most common nosocomial respiratory tract infection. Such as: ventilator associated pneumonia (VAP), sinusitis, otitis media and tracheitis (Labovska, 2021).

These bacteria not only spread common diseases but also nosocomial infections too. The most common risk factors are poor hygiene, contaminated objects and surfaces of the medical products and hospital rooms. Family members, other patients and hospital staff also can transmit the bacteria if there is insufficient isolation of patients and lack of hygienic. Most of the bacteria are now antibiotic resistant. This is an alarming factor for world. So, it would be better if these bacteria could be killed before spreading. Consequently, most of the individuals uses disinfectants in hospital and houses to kill the microorganisms.

Many disinfectants which mostly contain sole chemicals have been investigated against different bacteria. In an article, Glutaraldehyde (GLU) (Doganay, Turkey) (2.0%), quaternary ammonium compounds (QAC) (Dr. Schumacher, Germany) (0.05%), polyhexanide (PHMB) (Mertsel, Turkey) (0.2%) and chlorhexidine gluconate (CHX) (Merkez Ilac, Turkey) (2.0%) were disinfectants used against *P. aeruginosa*. (Tekintas, Y.2020). CHX (7.81–31.25 -g/mL) had the lowest MIC values, whereas GLU (1250−5000 -g/mL), QAC and PHMB MIC values were found

9.77–78.13 -g/mL and 0.12–1.95 -g/m (Tekintas, Y.2020). In another article, High level disinfectants (Steranios 2%, Deconex HLDPA, and Microzed Quatenol) were tested for their antibacterial effects by determining their minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) against *Enterococcus faecalis* ATCC 29212 and *Burkholderia cepacia* ATCC 10673 (Tapouk, A.F.2020). The result showed that, *E. faecalis*, as gram-positive bacterium, was more susceptible to high level disinfectants compared to gram-negative *B. cepacia* and the MIC = MBC values of 2% Steranios, Deconex HLDPA and Microzed Quatenol against *E. faecalis* and *B. cepacia* were 0.31, 9.77, 2.2 mg/L and 9.8, 78.13, 70.31 mg/L, respectively (Tapouk, A.F.2020). A report regarding Dettol showed that, the efficacy of the liquid disinfectant Dettol against nosocomial *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* was investigated. The result showed that dilutions of the disinfectant were not immediately lethal to the microorganisms and showed with the survival curves (EL Mahmood, A. M.2008). Research showed that the antimicrobial inhibitory effects of five common antiseptics [Chlorhexidine (CHX), Hydrogen peroxide (H_2O_2), Iodine, Ethanol and Dettol] were investigated using agar well diffusion method against organisms' methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa* (Al-Talib.H.2019). Also, the study revealed that bacteria were resistant to ethanol by all concentrations (AlTalib.H.2019). The result showed that H_2O_2 was the most effective antiseptics than the others followed by CHX. The study bacteria were found to be crucially susceptible to the routinely used antiseptics tested (Al-Talib.H.2019). The result also includes that the undiluted concentrations of the antiseptics showed variable zones of inhibition against the tested organisms, on MRSA it ranged from 25 mm (CHX) to 30 mm in other antiseptics, on *A. baumannii* 20 mm (CHX) to 34mm Dettol, on E. coli 20 mm Dettol to 38 mm (H_2O_2), on *Klebsiella spp*. 20 mm Dettol to 24 mm (CHX), whereas on *P. aeruginosa* it ranged from 13 mm Iodine to 30 mm (H_2O_2). The minimal inhibitory concentration (MIC) of chlorhexidine concentration against MRSA and *P. aeruginosa* was 10%, while *A. baumannii* was 20% (AlTalib.H.2019). Another research exhibited that once the instruments were exposed to bacterium, they were immersed in four commercially available disinfectants: Durr Dental solution, Bacillol, Dettol, and Savlon and then Culture streaks were taken at 5, 10, and 15 min of contact time and growth of organisms was observed on culture media (Venkatachalam.N.2020). The result showed that as per the immersion time factor, Durr system and Bacillol were more efficient than Dettol and Savlon (Venkatachalam.N.2020). Another article overviewed the comparison about disinfects effectiveness of 2% glutaraldehyde and 4.8% chloroxylenol on tooth extraction instruments at the Department of Oral Surgery, Faculty of Dentistry, University of North Sumatera and the result showed that the comparison between glutaraldehyde and chloroxylenol showed a significant difference to the total bacteria count on instrument after disinfection ($p=0.014 < 0.05$) (Riza.A.2018). Also, article showed that the mixture of Savlon with seven chemical disinfectants (Hibitane, Formalin, Septicin, Savlon, Dettol, Iodine and Biotic) against Gram negative *bacilli*, Gram positive *cocci* and Gram-positive *bacilli* and result appeared that Hibitane had the highest effect at (88.23, 88.88 and 100%) concentrations on Gram negative *bacilli*, Gram positive *cocci* and Gram-positive *bacilli* (Shareef.Y.A.2005). The effect of disinfectant on bacterial resistance of antibiotic, so bacteria treated with Sub-Minimum Inhibitory Concentrations of Savlon mixed with other disinfectants which lead to an increase of resistance of some and the sensitivity of others (Shareef.Y.A.2005). Each articles showed that each method and procedure was different and was applied on different bacteria. These researches proves that some works were done with Dettol. But in the case of Savlon, Lizol and Savlon spray, hardly any work was performed to investigate their efficiency.

Savlon, Dettol, Lizol and Savlon spray are most common disinfectants used in Bangladesh and South Asia. Almost every hospital uses these disinfectants daily to clean floors, medical tools, washing cloths etc. These disinfectants are also demandable in common households for daily cleaning. Most of the time, people use these disinfectants as per their instruction which includes dilution. But it is unknown that if their recommendation is worth to kill mentioned bacteria. It is also mandatory to use right amount of disinfectant to kill bacteria. Thus, these harsh chemicals cannot harm the nature and human health.

This study aimed to investigate the recommended efficiency of popular disinfectants Savlon, Dettol, Lizol and Savlon spray on nosocomial bacteria *E. coli, S. aureus, K. pneumoniae, P. aeruginosa* and to determine the minimum inhibitory concentrations and the minimum bactericidal concentration of Savlon, Dettol, Lizol and Savlon spray on nosocomial bacteria.

Chapter 2

Material and Method

2.1. Bacterial Isolates

Escherichia coli, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomoas aeruginosa* isolates were collected from Department of Microbiology, of Dhaka Community Medical. The antimicrobial characteristics are also reported in Table 1. Bacterial Isolates were stored in T1N1 media at room temperature.

Table 1: Antibiotic Susceptibility of the nosocomial bacteria that were used in this study

Antibiotic	**Bacteria**											
	Gram Positive Bacteria			**Gram Negative Bacteria**								
	***S. aureus* Isolates**			***E. coli* Isolates**			***K. pneumoniae* Isolates**			***P. aeruginosa* Isolates**		
	1	2	3	1	2	3	1	2	3	1	2	3
MRP 10	-	-	-	S	S	S	S	-	R	S	S	S

IPM 10	-	-	-	S	S	S	S	S	I	R	R	S
CT 10	-	-	-	R	R	R	R	-	R	I	I	R
PIT 100/10	-	-	-	S	S	S	S	S	R	S	S	R
FEP 30	-	-	-	I	R	R	R	-	R	S	S	R
CIP	-	R	-	-	-	-	-	R	-	-	-	-
SXT	-	S	-	-	-	-	-	S	-	-	-	-
ATM 30	R	-	R	-	-	-	-	-	-	-	-	-
VA 30	R	S	R	-	-	-	-	-	-	-	-	-
LNZ 30	R	S	R	-	-	-	-	-	-	-	-	-
TGC15	S	-	S	-	-	-	-	-	-	-	-	-

MRP10: Meropenem. IPM10: Imipenem. CT10: Colistin10. PIT100/10: Pipercilin. FEP30: Cefepime. CIP: Ciprofloxacin. SXT: Sulfamethoxazole. ATM30: Aztreonam. VA30: Vancomycin. LNZ30: Linezolid. TGC15: Tigecycline.

2.2. Disinfectants that were used

Dettol (chloroxylenol (comprises 4.8% of Dettol's total admixture), terpineol and isopropyl alcohol), Savlon (Cetrimide 3.0% W/V and Chlorhexidine Gluconate 0.3% W/V. Also Contains: Isopropyl Alcohol, Terpineol, Liquid Deodoriser, Benzyl Benzoate, D-Gluconolactone, Sodium Hydroxide and Purified Water), Lizol (benzalkonium chloride solution and etc.), Savlon Spray (Alcohol Ethoxylate) were disinfectants used in this study. In this study total disinfect was focused, (not on the sole chemicals). Disinfectants were diluted in Luria-Bertani broth.

2.3. Testing the Effects of Disinfectants on Bacteria

The Effects of disinfectants on Bacteria isolates was determined by dilution and cultured by spread plate method followed by (Tapouk et al., 2020). The method includes that isolates were grown on a Nutrient Agar and incubated at 37°C for 24 h. Bacterial Suspension was prepared by using LuriaBertani broth and adjusted to 0.08-0.13 OD at 625 nm wavelength by Spectrophotometer. Bacteria suspensions were constant and not diluted. According to each disinfectant’s instruction, the disinfectants were diluted in Luria-Bertani broth and the total amount was constant for 5ml which is presented in Table2. After preparing the dilution, 50 µL of bacteria suspension were inoculated to each diluted tube. After waiting for recommended time (For Dettol 5 minutes, Savlon and Lizol 3 minutes and Savlon spray 10 seconds), 40μL of each tube was cultured by spread plate method on the surface of Na at 37°C for 20h-24h. After the incubation, the effects of disinfectants were determined by observing the colonies and the colonies were counted by Colony-Forming Unit method.

Table 2: Recommended amount of disinfectants and used amount in the procedure

Disinfectants	**Uses**	**Amount as per the instruction**	**Amount In 5ml**
Lizol	Cleaning floor of house and Bathroom	1 cup in 4L	15 μL
Dettol	Shower	1 Table S. in 10L	3 μL
	Washing cloths and instruments, cleaning floor both in house and hospital. Washing instruments of childbirth	1:40	125 μL
	Treatment of cut and bite of insects Shaving	1:20	250 μL
Savlon	Shower	3 table S. in 10L	9 μL
			22.5 μL
	Cleaning towel, napkin etc. Cleaning cloths and utensils	2Table S. in 1L	60 μL
			150 μL
	Cleaning floor, kitchen and bathroom	2 Table S. in 2L	30 μL
			75 μL
			150 μL
	Treatment of cut and bite of insects Shaving Cleaning outside of body	5 Tea S. in ½ L	150 μL
			250 μL

S.=Spoon, 1 cup=12ml, 1Tea spoon=3ml, 1table Spoon=6ml

As per the Disinfectants instruction: 1Tea spoon=5ml, 1table Spoon=15ml

2.4. Determination of Minimum bactericidal concentration and Minimum inhibitory concentrations of disinfectants

Minimum inhibitory concentration and Minimum bactericidal concentration of disinfectants were determined to study the specific effect of disinfectants on different Bacteria. For this purpose, same process was followed for bacterial suspensions. The disinfectants were diluted in Luria-Bertani broth and the total amount was constant for 5ml which is presented in Table3 and Table 4. Table 3: Dilution Factor of Savlon and Dettol and their converted amount in 5ml

Disinfectants	**Dilution Factor**	**Amount In 5ml**
Savlon and Dettol	1:20 (Recommended)	250 µL
	2:20	500 µL
	3:20	750 µL
	4:20	1000 µL
	5:20	1250 µL
	6:20	1500 µL
	7:20	1750 µL
	8:20	2000 µL
	9:20	2250 µL
	10:20	2500 µL
	11:20	2750 µL
	12:20	3000 µL
	13:20	3250 µL
	14:20	3500 µL
	15:20	3750 µL
	16:20	4000 µL
	17:20	4250 µL
	18:20	4500 µL
	19:20	4750 µL
	20:20	5000µL

µL: Microliter. ml:Mililiter. L: Liter

After preparing the dilution, 50 μL of bacteria suspension were inoculated to each diluted tube. After waiting for recommended time (For Dettol 5 minutes, Savlon and Lizol 3 minutes and Savlon Spray 10 seconds), 40μL of each tube was cultured by spread plate method on the surface of Na at 37°C for 20h-24h. After the incubation, the lowest concentration that inhibited the growth of bacteria was determined as the minimum inhibitory concentration (MIC) value and the minimum concentration of Disinfectants that results in bacterial death was determined as minimum bactericidal concentration (MBC).

Table 4: Disinfectant Lizol's amount in Cups in 4L and their converted amount in 5ml

Disinfectants	**Cups in 4L**	**Amount in 5ml**
Lizol	1 (Recommended)	15 μL
	10	150 μL
	20	300 μL
	30	450 μL
	40	600 μL
	50	750 μL
	60	900 μL
	70	1050 μL
	80	1200 μL
	90	1350 μL
	100	1500 μL
	110	1650 μL
	120	1800 μL
	130	1950 μL
	140	2100 μL
	150	2250 μL
	160	2400 μL
	170	2550 μL
	180	2700 μL

	190	2850 µL
	200	3000 µL
	210	3150 µL
	220	3300 µL
	230	3450 µL
	240	3600 µL
	250	3750 µL
	260	3900 µL
	270	4050 µL
	280	4200 µL
	290	4350 µL
	300	4500 µL
	310	4650 µL
	320	4800 µL

µL: Microliter. ml: Milliliter. L: Liter. 1Cup: 12ml

Chapter 3

Results

3.1. Effects of Disinfectants on Nosocomial Bacteria

The effects of disinfectants on different Bacteria are exhibited in Table 5. In the instance of Lizol direct, three isolates of *S. aureus* were discovered to have no growth, while only one isolate of *E. coli* was detected with 175 CFU/ml, according to the results of the dilution techniques and spread plate method. *K. pneumoniae* isolates were found to be no growth except the isolate 1 which had 1225 CFU/ml; besides isolate 1 contained 9600 CFU/ml, *P. aeruginosa* isolates were found to be uncountable growth. In the matter of 15µL Lizol, 3µL Dettol and 125µL Dettol, all of the bacteria isolates contained uncountable growth of bacteria. Again, for 250µL of Dettol, one isolates of *S. aureus* and two isolates of *K. pnuemoniae* had colonies and other isolates were found to be too numerous to count. Unexpectedly, all the bacteria isolates were found to be uncountable in the case of 9µL Savlon, 22.5µL Savlon, 30µL Savlon and 60µL Savlon. One isolate of *E. coli* acquired colonies, and other isolates were discovered to be uncountable for both the 75µL and 150 µL of Savlon. Finally, in the case of 250 µL of Dettol, one isolate of *S. aureus*, one isolate of *E. coli*, and two isolates of *K. pneumoniae* had colonies, while the remaining isolates were discovered to be uncountable. The results of Savlon spray for every isolate of *S. aureus, E. coli*, *K. pneumoniae* and

P. aeruginosa were found to be too numerous to count except isolate 1 of *K. pneumoniae* that had 3674 CFU/ml.

Table 5: Effects of Disinfectants on Nosocomial Bacteria (constant total value of 5ml)

Disinfectants		**Bacteria**											
		***S. aureus* Isolates (CFU/ml)**			***E. Coli* Isolates (CFU/ml)**			***K. pneumoniae* Isolates (CFU/ml)**			***P. aeruginosa* Isolates (CFU/ml)**		
		1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Lizol	Direct	NG	NG	NG	175	NG	NG	1225	NG	NG	9600	TNTC	TNTC
	15 µL	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
Dettol	3 µL	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	125 µL	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	250 µL	240000	TNTC	TNTC	TNTC	TNTC	TNTC	65500	TNTC	2000	TNTC	TNTC	TNTC
Savlon	9 µL	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	22.5 µL	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	30 µL	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	60 µL	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	75 µL	TNTC	TNTC	TNTC	TNTC	123333.3	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	150 µL	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	250 µL	26000	TNTC	TNTC	TNTC	12500	TNTC	TNTC	33000	71400	TNTC	TNTC	TNTC
Savlon Spray	Direct	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	3674	TNTC	TNTC	TNTC	TNTC	TNTC

NG: No Growth. TNTC: Too Numerous To Count

3.2. Minimum Bactericidal Concentration (MBC) of disinfectants

Minimum Bactericidal Concentration of disinfectants is exhibited on Table 6. In the case of Dettol, the lowest values for *S. aureus* isolates, were determined to be 1500 µL, 750 µL, and 1750 µL. In the case of *E. coli* isolates, the lowest values were 1500 µL,1750 µL,1000 µL and 1750 µL,1500 µL,2750 µL were found to be the lowest values for *K. pneumoniae*, For *P. aeruginosa* isolate 1, the lowest value is 5000 µL and no MBC were found for other two isolates. Regarding Savlon, for the isolates of *S. aureus*, *E. coli*, and *K. pneumoniae*, the lowest values were determined to be 2500 µL, 500 µL, 2750 µL, 3000 µL, 2250 µL, 1250 µL, 3750 µL, 500 µL, and 4000 µL, respectively. Unexpectedly, no MBC was reported for isolates of *P. aeruginosa*. The lowest values for Lizol were identified to be 1800 µL, 765 µL, and 1665 µL for *S. aureus* isolates, no MBC value was discovered for *E. coli* isolate 1 but 3150 µL and 3015 µL were observed for isolates 2 and 3, respectively. Surprisingly, only isolate 2 of *K. pneumonia*e had the lowest value of 915 µL, while the other two isolates had no MBC values. For *P. aeruginosa* isolates in the case of Lizol as well, no MBC was observed.

Table 6: Minimum Bactericidal Concentration of disinfectants on bacteria (Constant value of 5ml)

Disinfectants	**Bacteria**											
	S. aureus **Isolates**			***E. coli*** **Isolates**			***K. pneumoniae*** **Isolates**			***P. aeruginosa*** **Isolates**		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Dettol (µL)	1500	750	1750	1500	1750	1000	1750	1500	2750	5000	N	N
Savlon (µL)	2500	500	2750	3000	2250	1250	3750	500	4000	N	N	N
Lizol (µL)	1800	765	1665	N	3150	3015	N	915	N	N	N	N

N: No MBC was found.

3.3. Minimum Inhibitory Concentration of disinfectants

Minimum Inhibitory Concentration of disinfectants is exhibited on Table 7. In the case of Dettol, the lowest values for *S. aureus* isolates were determined to be 1250 µL, 500 µL, and 1500 µL. In the case of *E. coli* isolates the lowest values were 1250 µL,1500 µL,750 µL and 1750 µL,1500µL,2750 µL were found to be the lowest values for *k. pneumoniae*, For *P. aeruginosa* isolate 1, the lowest value is 4750 µL and no MIC were found for other two isolates. In case of Savlon, for the isolates of *S. aureus*, *E. coli*, and *K. pneumoniae*, the lowest values were determined to be 2250 µL, 250 µL, 2500 µL, 2750 µL, 2000 µL, 1000 µL, 3500 µL, 250 µL and 3750 µL, respectively. Unexpectedly, no MIC was reported for any isolates of *P. aeruginosa*. The lowest values for Lizol were identified to be 1785 µL, 750 µL, and 1650 µL for *S. aureus* isolates. No MIC value was discovered for *E. coli* isolate 1 but 3135 µL, and 3000 µL MIC values were found for *E. coli* isolates 2 and 3, respectively. Surprisingly, only isolate 2 of *K. pneumoniae* had the lowest value of 900µL, while the other two isolates had no MIC values. In the case of *P. aeruginosa* isolates, for Lizol as well no MIC was observed.

Table 7: Minimum Inhibitory Concentration of disinfectants on bacteria (Constant value of 5ml)

Disinfectants	**Bacteria**											
	S. aureus **Isolates**			***E. coli*** **Isolates**			***K. pneumoniae*** **Isolates**			***P. aeruginosa*** **Isolates**		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Dettol (µL)	1250	500	1500	1250	1500	750	1500	1250	2500	4750	N	N
Savlon (µL)	2250	250	2500	2750	2000	1000	3500	250	3750	N	N	N
Lizol (µL)	1785	750	1650	N	3135	3000	N	900	N	N	N	N

N: No MIC was found.

Chapter 4

Discussion:

Pathogenic microbes are responsible for many human diseases. Disinfectants is a chemical substance which can diminish or eradicate potentially harmful microorganisms and prevent the transmission of these organisms.

The growth of all isolates of *S. aureus, E. coli, K. pneumoniae* and *P. aeruginosa* were found to be uncountable when 1 cup of Lizol in 4liter water was used as per the instruction in case of cleaning floor of house and bathroom.

Surprisingly, all of the isolates of all bacteria showed uncountable growth of colonies when tested in 10-liter water with 1 table spoon of Dettol. While testing in diluted Dettol by 1:40, all of the bacterial isolates were found as uncountable growth in case of washing cloths and instruments, cleaning floor both in house and hospital and washing instruments of childbirth. Also, all of the isolates of all bacteria showed uncountable growth of colonies despite of three isolates of bacteria which were the 1st isolate of *S. aureus* the 1st and 3rd isolates of *K. pneumoniae*. These three isolates formed 240000 CFU/ml, 65500 CFU/ml and 2000 CFU/ml colonies respectively.

When 9 µL Savlon is diluted in 4091 µL LB, 22.5 µL Savlon is diluted in 4977 µL LB, 30 µL Savlon is diluted in 4970 µL LB, 60 µL Savlon is diluted in 4940 µL LB and 150 µL Savlon is diluted in 4850 µL LB all of the isolates of all bacteria were found uncountable growth of colonies. Only the second isolate of *E. coli* had countable growth of 123333.3 colonies when 75 µL of Savlon were diluted in 4925 µL of LB, whereas the other isolates exhibited uncountable growth of colonies. When 250 µL Savlon is diluted in 4750 µL LB, four of the isolates were found to have countable growth which are 1st isolate of *S. aureus*, 2nd isolate of *E. coli*, 2nd and 3rd isolates of *K. pneumoniae* having 26000, 12500, 33000, and 71400 growths of colonies respectively and rest of the isolates having uncountable growths. When direct Savlon spray was mixed with LB, only 1st isolate of *K. pneumoniae* was shown countable colonies which was 3674. The remaining bacterial isolates exhibited an indefinite number of colonies.

The minimum concentration of an antibacterial agent needed to kill a bacterium is known as the Minimum Bactericidal Concentration (MBC). In case of this article, antimicrobial agent is disinfectants. When 6, 3 and 7 cups of Dettol were diluted with LB respectively, for each isolate of *Staphylococcus aureus*, the MBC of Dettol was reported. Regarding *E. coli* isolates 6, 7, 3 cups of Dettol were once more coupled with Lizol one at a time, yielding the MBC of Dettol for *E. coli* isolates. Similar to this, MBC of Dettol against *K. pneumoniae* were found by dilution with 7, 6:20, and 11 cups of Dettol. The first isolate of *P. aeruginosa* has MBC in case of Dettol, whereas the other two isolates do not.

In the event of *S. aureus*, *E. coli* and *P. aeruginosa* isolates, MBCs of Savlon were noted by diluting 10,2,11 cups; 12, 9,5 cups and 15,2,16 cups into LB broth. No MBCs were found in case of *P. aeruginosa* Isolates. By diluting 120,51,111 cups of LB, MBCs of Lizol for *S. aureus* were determined. There was no MBC for *E. coli* isolate 1 with Lizol. But for the other two isolates, MBCs of Lizol were discovered by diluting 210 and 200 cups of Lizol into LB broth. In the instance of Lizol, the first and third isolates of *K. pneumoniae* did not have MBCs, but interestingly, the second isolate did, and it was discovered after dilution of 61 cups of Lizol with LB broth.

A minimum inhibitory concentration, or MIC, is the amount of an antimicrobial substance or agent required to inhibit bacteria from growing visibly. For each isolate of Staphylococcus aureus, 5, 2, and 6 cups of Dettol were diluted with LB, and the MIC of Dettol was recorded. In order to achieve the MIC of Dettol for the three isolates of *E. coli*, 5, 6, 2 cups of Dettol were once more combined with LB broth respectively. By diluting with 6, 5 and 11 cups of Dettol, MIC of Dettol against *K. pneumoniae* were discovered in a manner similar to this. In the instance of Dettol, only the first isolate of *P. aeruginosa* possesses MIC; the other two isolates do not. MICs of Savlon were established by dilutions of 9, 1, 10 cups; 11, 8, 4 cups and 15,1,16 cups into LB broth for isolates of *S. aureus*, *E. coli*, and *P. aeruginosa* respectively. Concerning *P. aeruginosa* isolates, no MICs were found. MICs of lizol for *S. aureus* were calculated by diluting 119,50,110 cups of Lizol. Lizol had no MIC for *E. coli* isolate 1. However, for the remaining two isolates, MICs of lizol were discovered. In the case of lizol, the first and third isolates of *K. pneumoniae* did not have MICs, but intriguingly, the second isolate did, and it was found after dilution of 60 cups of Lizol with LB broth.

Chapter 5

Conclusion

The two most prominent bacterial species found in the lungs of cystic fibrosis (CF) patients are *Staphylococcus aureus* and *Pseudomonas aeruginosa*, both of which are linked to worse clinical outcomes (Camus et al.,2021). A wide variety of nosocomial and community infections are caused by the pathogenic enterobacteria *Escherichia coli* and *Klebsiella pneumoniae* in humans. Therefore, selecting the best disinfectants to fight harmful pathogens is essential for daily living. This study intends to help individuals pick the best disinfectants by assisting them in understanding the efficacy of various disinfectants.

Expected result was not discovered for the most of the cases according to their instruction which is very harmful for human and causes severe and persistent infections in humans. Also, it was discovered that Savlon spray has no effects towards the bacteria. That is why it did not have MIC and MBC. It indicates that bacteria will remain alive regardless of the amount of Savlon spray used. Dettol showed the lowest MIC and MBC against 2nd isolates of *S. aureus*. In the matter of Savlon, it had lowest MICs and MBCs against 2nd isolates of *S. aureus* and *K. pneumoniae*. Lizol showed lowest MIC and MBC against 2nd isolates of *S. aureus*.

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