

ANTIBACTERIAL EFFECT OF HANDWASHES AGAINST DIFFERENT BACTERIAL STRAINS



Project work by
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OBSERVATION

HAND WASHES	Zone of inhibition (diameter in cm)						
	Gram- negative bacteria			Gram- positive bacteria			
	<i>Esherischia coli</i>	<i>Klebsiella</i>	<i>Vibrio parahaemolyticus</i>	<i>Enterococcus</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus iniae</i>	<i>Mycobacterium</i>
Godrej	1.9	2	1.9	1.6	2.1	1.4	1.7
Wiz	2.1	1.8	1.5	1.8	1.9	2.1	1.8
Lifebuoy	1.5	1.5	1.1	1.6	1.5	1.5	1.3
Savlon	1.4	1.7	2	1.7	1.9	2.4	1.4

RESULT

The antibacterial effect of 4 different handwashes were studied against common infectious bacteria. The handwashes used were Savlon, Lifebuoy, Wiz, Godrej mr.magic . The bacteria taken were 3 gram negative bacteria (Escherichia coli, Klebsiella, Vibrio parahaemolyticus) and 4 gram positive bacteria (Enterococcus, Staphylococcus aureus, Streptococcus iniae, Mycobacterium)

All the handwashes used for the study (savlon, lifebuoy, wiz, Godrej) showed considerable effect against all the bacteria chosen for the study.

In the case of Escherichia coli, the handwash wiz showed the highest zone of inhibition of 2.1cm. The least effective handwash was savlon which showed an inhibitory action of 1.4cm against the bacteria, for Klebsiella, the handwash which showed the most inhibitory effect was Godrej mr.magic which had a zone of inhibition of 2cm. The least effective was lifebuoy with a zone of inhibition of 1.5cm.

In the case of Vibrio parahaemolyticus, savlon showed an inhibitory effect with a zone of inhibition of 2cm. In contrast lifebuoy was least effective with very small zone of inhibition of 1.1cm. While in Enterococcus the wiz handwash exhibited the highest zone of inhibition of 2.5cm. Both Godrej and lifebuoy exhibited only a zone of inhibition of 1cm.

S. aureus, godrej showed the most inhibitory effect with a zone of inhibition of 2.1cm. Lifebuoy was found to be least effective against the bacteria with a zone of inhibition of 1.5cm.

In the case of S. iniae, savlon was found to have a very good inhibitory effect with a zone of inhibition of 2.4cm, while least effective was godrej with a zone of inhibition of 1.4cm.

In the case of mycobacterium wiz handwash showed a good inhibitory effect with a zone of inhibition of 1.8cm and the least effective was lifebuoy with a zone of inhibition of 1.3cm.

From the observations, it is evident that the most sensitive bacteria was Streptococcus iniae against savlon. The least sensitive bacteria was Vibrio parahaemolyticus against lifebuoy. Wiz was the most effective handwash as it is active against the 4 types of bacteria selected in the study.

INTRODUCTION

Liquid handwashes generally contain chemicals, such as alcohol or chlorine, that can kill bacteria. Washing your hands with plain soap and water removes germs, including antibiotic-resistant germs. Keeping your hands clean can prevent the spread of germs, reducing the risk for antibiotic-resistant infections. The major ingredients in antibacterial hand washes are water, cocamidopropyl betaine, lauramido propylamine oxide, lauramine oxide, myristamido propylamine oxide, glycerin, fragrance, citric acid, tetrasodium EDTA. Washing hands can keep you healthy and prevent the spread of respiratory and diarrheal infections. Germs can spread from person to person or from surfaces to people when you touch your eyes, nose, and mouth with unwashed hands. Prepare or eat food and drinks with unwashed hands.

Wiz hand wash have a perfect balance of pH level which is meant to remove germs at the same time it is gentle on your delicate skin. They are responsibly sourced, ethically created and never tested on animals. It is 100% tested for safety (benzophenone-4, sodium benzoate etc.) purpose. It has mild formula which are hard on germs, dirt, bacteria. It is certified and approved by FDA. Wiz care products are derived from premium natural ingredients to keep you protected in every environment. It is the most effective skin cleanser which contains conditioners and moisturizing agents that softens the skin while removing the germs located at every corner.

The main ingredients in lifebuoy handwash are Sodium Tallowate, Sodium Palmitate, Sodium Palmate, Aqua, Sodium Palm Kernelate, Glycerin, Parfum, Sodium Lauryl Sulphate, Fatty Acid, Titanium dioxide, Mel (Honey), Curcuma Aromatica (Turmeric) Root Oil, Citrus Aurantium Dulcis (Orange) Juice, Citrus Limon (Lemon) Juice, Fragaria Ananassa (Strawberry) Fruit Juice etc.

Opposed to other antiseptics, Savlon's unique formula offers protection against a wide variety of bacteria including gram positive germs while being gentle on skin. It protects against the H1N1 virus, Rotavirus, Stomach Flu & Flu virus. It is dermatologically tested and is suitable for all skin types.

The major ingredients in savlon hand wash are Aqua, Sodium Laureth Sulfate, Cocamidopropyl Betaine, Cocamide MEA, Glycol Distearate, Acrylates Copolymer, Sodium Chloride, Fragrance, DMDM Hydantoin, Tea Tree Oil, Glycerin, Silver, Tetrasodium EDTA, BHT, Citric Acid, Sodium Hydroxide, Sodium Xylene Sulfonate, Linalool, Benzyl Salicylate, Coumarin, Citral etc.

Godrej Mr.magic hand wash has triclosan and triclocarban which are the most common compounds used as antibacterials. However, other common antibacterial ingredients include benzalkonium chloride, benzethonium chloride, and chloroxylenol.

METHODOLOGY

MATERIALS REQUIRED

Nutrient agar-agar, nutrient broth, distilled water, filter paper, petri plates, conical flasks, test tubes, forceps, cotton plug, sterile swab, autoclave, weighing machine, measuring cylinder, nichrome loop, alcohol, newspaper, ruler, paper, pen etc.

BACTERIAL STRAIN (*Staphylococcus aureus*, *Escherchia coli*, *Klebsiella pneumoniae*, *Streptococcus inium*, *Enerococcus*, *Vibrio parahaemolyticus*)

NUTRIENT BROTH CULTURE

1.3 g of nutrient broth was weighed. It was added to 100ml distilled water and mix well. The broth was prepared in 100 ml conical flask and it was sterilized by autoclaving for 15 minutes and cooled to room temperature. The broth was then poured into sterilized test tubes (each test tube containing 5 ml broth) and closed using a cotton plug.

INOCULATING THE BROTH:

The nutrient broth was inoculated. The cotton plug of both the stock culture to be inoculated was loosened, and then the inoculating loop was flamed to red hot and cooled by waving for few seconds. The cotton plug from the stock culture tube was removed and the mouth was flamed. The cooled sterilized loop was inserted into the culture tube carefully without touching the sides to prevent contamination. A visible amount of the culture was scrapped and removed using the loop and mouth of the tube was plugged back carefully after flaming. By the same procedure, the cap was introduced into the broth using the loop . tube mouth was flamed and recapped after the loop has been withdrawn. The inoculating loop was then reesterilized and the broth culture was gently rotated for the proper mixing of its content. The contents in each test tube were labelled with names of respective microbes and the date was noted. For sufficient bacterial growth, the inoculums were kept for 2-6 hours of incubation.

PREPARATION OF NUTRIENT AGAR (CULTURE MEDIA)

The medium was prepared using 1.3g of nutrient broth and 2 g of nutrient agar. At first, both nutrient broth and agar was weighed out and was made up to 300 ml using distilled water. It was poured into a conical flask and sterilized for 15 minutes in an autoclave at 15 psi. The medium was allowed to cool to an ear bearing heat -15 degree celcius. Cooled agar was poured into petri dishes and waited till it got set. It was then kept upside down. These petri dishes were used for the study.

PREPARATION OF FILTER PAPER DISC:

Filter paper disc was prepared using a punching machine and sterilized using autoclave. The disc was then soaked in the extracts for specific time and was used for anti microbial sensitivity tests.

METHOD

The method used for antibacterial sensitivity was Kirby bauer disc diffusion method. A lawn culture of each bacterium was prepared using sterilized cotton swabs. A sterilized swab was dipped into the bacterial suspension, and moved side to side from top to bottom leaving no space uncovered. The plate is rotated to 90 degree and the same procedure was repeated so that entire plate was coated with bacteria. This procedure was followed for plating all the six different strains of bacteria. Once the lawn had been prepared, the sterilized filter paper impregnated with the medicines to be tested was placed on the plate. This plate was incubated at 37 degree C for 48 hrs. The name of the bacteria was labelled on each plate and was examined for sensitivity (zone of inhibition). The radius of each zone was measured using a standard ruler in centimeters. If the compound is effective against bacteria at certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition which is a measure of the compound effectiveness the larger the clear area around the filter paper, the more effective the compound

KILLING OR DISPOSING:

After the experiment, the bacteria are destroyed by autoclaving the plate for 20 minutes. All the glasswares used for the experiment were also autoclaved to remove any bacteria if present.

REVIEW OF LITERATURE

Antibacterials, antiseptic, and disinfectants are extensively used in hospitals and other health care settings for a variety of topical and hard surface applications. A wide variety of active chemical agents are found in these products, many of which have been used for 100 years, including alcohol, phenols etc. Most of these active agents demonstrate broad spectrum of antibacterial activity (Waksman,1947). However, little is known about mode of action of these agents in comparison to antibiotics. Widespread use of these products has promoted some speculation on the development of bacterial resistance, in particular whether antibiotic resistance is induced by antiseptics or disinfectants.

Liquid handwashes generally contain chemicals, such as alcohol or chlorine, that can kill bacteria. Washing your hands with plain soap and water removes germs, including antibiotic-resistant germs (Maeda,2008). Keeping your hands clean can prevent the spread of germs, reducing the risk for antibiotic-resistant infections. The major ingredients in antibacterial hand washes are water, cocamido propyl betaine, lauramido propylamine oxide, lauramine oxide, myristamido propylamine oxide, glycerin, fragrance, citric acid, tetrasodium EDTA. Washing hands can keep you healthy and prevent the spread of respiratory and diarrheal infections.

Many handwashes contain chemicals such as alcohol or chlorine that can kill bacteria. Much has been written recently about the potential hazards versus benefits of antibacterial (biocide)-containing handwash. The purpose of this systematic literature review was to assess the studies that have examined the efficacy of products containing triclosan, compared with that of plain soap, in the community setting as well as to evaluate findings that address potential hazards of this use namely the emergence of antibiotic-resistant bacteria. (Larson ,1988)

Inadequate handwashing after defecation and anal cleaning practices in the Indian subcontinent is an important source of faecal-oral transmission of enteric diseases. To better understand the process as traditionally practised, 90 women in semi-rural Bangladesh were observed washing hands after defecation. Several components of handwashing practices were identified: the cleaning agent, using left or both hands; frequency of rubbing hands, type and amount of water used to wash, and the drying of hands on the wearer's clothes. A subsequent experiment was

conducted to assess the effect of currently practised handwashing and drying according to standardised procedure on faecal coliform count of hands. (Hoque,1991)

As a rubbing agent, soil was commonly used (40%); soap was used by 19% and was reported unaffordable by about 81% of the non-users. Good handwashing behaviour was positively associated with better social and economic indicators including education of the women observed. Both hands were unacceptably contaminated after traditional handwashing (the geometric mean count of left was 1,995 and right hand was 1,318 faecal coliform units/hand). After standardising the observed components of handwashing procedures the use of any rubbing agent, i.e. soil, ash or soap, produced similar acceptable cleaning. Use of a rubbing agent (e.g. soil, ash or soap), more rubbing (i.e. six times), rinsing with safer water (e.g. 2 litres of tubewell water) and drying with a clean cloth or in the air produced acceptable bacteriological results.(Hoekstra,2005)

Components of traditional handwashing practices were defined through careful observation, and experiments on handwashing with standardised components showed that efficient and affordable options for handwashing can be developed; this knowledge should be helpful in disease control programmes.

ABSTRACT

Handwashing is thought to be effective for the prevention of transmission of pathogens. However it is not conclusive that handwashing with soap is more effective at reducing contamination with bacteria. The present study was focused to find the effect of 4 most widely used handwashes (wiz handwash, Godrej, lifebuoy, salvon) on different bacterial strains (Escherichia coli, Klebsiella, Vibrio parahaemolyticus, Enterococcus, Staphylococcus aureus, Streptococcus iniae, Mycobacterium).

All the seven bacterial strains are highly sensitive to the 3 handwash used i.e. viz, Godrej and savlon, lifebuoy had less zone of inhibition against those bacteria. Out of the 7 bacterial strains, it is evident that the most sensitive bacteria was Streptococcus iniae against savlon (zone of inhibition of 2.4cm). The least sensitive bacteria was Vibrio parahaemolyticus against lifebuoy (zone of inhibition of 1.1cm).

In the present study conducted on antibacterial action of different types of handwashes, it was evident that antibacterial agents are extensively used to inhibit or kill the bacterial growth.

CONCLUSION

In the present project title “antibacterial effect of different types of handwashes”, on various types of bacteria. It can be concluded that wiz handwash is most effective antibacterial handwash than savlon and lifebuoy. The results demonstrate that handwashing with non-antibacterial soap is much more effective in removing bacteria from hands than handwashing with water only.

All the seven bacterial strains (Escherichia coli, Klebsiella, Vibrio parahaemolyticus, Enterococcus, Staphylococcus aureus, Streptococcus iniae, Mycobacterium) are highly sensitive to the 3 handwash used (i.e. wiz, Godrej and savlon) while lifebuoy had less zone of inhibition against those bacteria. Out of the 7 bacterial strains, it is evident that the most sensitive bacteria was Streptococcus iniae against savlon. The least sensitive bacteria was Vibrio parahaemolyticus against lifebuoy.

From the study it can be concluded that wiz handwash is most effective than lifebuoy, Godrej and savlon on different bacterial strains.

DISCUSSION

In the present study entitled bacterial effect of handwashes [Godrej, wiz, savlon, lifebuoy] on different strain of bacteria (Escherchia coli, Klebsiella, Vibrio parahaemolyticus, Enterococcus, Staphylococcus aureus, Streptococcus iniae, Micobacterium). It is evident that the handwashes used were effective against all the bacteria used for the study.

Liquid handwashes generally contain chemicals, such as alcohol or chlorine, that can kill bacteria. Washing your hands with plain soap and water removes germs, including antibiotic-resistant germs. Keeping your hands clean can prevent the spread of germs, reducing the risk for antibiotic-resistant infections.

Wiz handwash exhibit more inhibition against the bacteria such as E.coli, Enterococcus, S.iniae & Micobacterium. Wiz hand wash have a perfect balance of pH level which is meant to remove germs at the same time it is gentle on your delicate skin. They are responsibly sourced, ethically created and never tested on animals..

The major ingredients in antibacterial hand washes are water, cocamido propyl betaine, lauramido propylamine oxide, lauramine oxide, myristamido propylamine oxide, glycerin, fragrance, citric acid, tetrasodium EDTA,

The main ingredients in the wiz hand wash are aloe vera, rose water and almond. Aloe vera has potent antibacterial, antifungal, and antiviral properties. The antimicrobial effects of Aloe vera have been attributed to the plant's natural anthraquinones which have demonstrated in vitro inhibition of Mycobacterium tuberculosis and Bacillus subtilis. It kill bacteria, viruses and fungi owing to its naturally-occurring antiseptic qualities.

The almond extracts exhibited significant inhibitory effect on bacterial growth. Rose water has antiseptic and antibacterial properties that can help wounds heal faster. These properties can help to clean and fight off infection of cuts and burns. They can also help cuts, burns, and even scars heal faster.

Savlon hand wash shows significant inhibition against bacteria such as *Vibrio parahaemolyticus* and *S. pneumoniae*. Savlon is highly effective against common household germs. Opposed to other antiseptics, Savlon's unique formula offers protection against a wide variety of bacteria including gram positive germs while being gentle on skin. It is even protective against the H1N1 virus, Rotavirus, Stomach Flu & Flu virus. It is dermatologically tested and is suitable for all skin types.

The antibacterial effect of Savlon handwash is due to two antiseptics, cetrimide and chlorhexidine gluconate also contain 66.5% alcohol .

Cetrimide (CTR) is a cationic surfactant, a quaternary ammonium derivative, which has demonstrated its effectiveness against gram-positive and gram-negative bacteria, and it exhibit antifungal activity. Generally applied as a topic antiseptic, it is not toxic.

Chlorhexidine gluconate (CHG) is one of the most commonly used antiseptic, and is active against Gram-negative, Gram-positive bacteria, yeast and fungi. However, over use may lead to reduced susceptibility of different bacteria to CHG. (Luby,2007)

Godrej Mr.magic hand wash shows the zone of inhibition against *Klebsiella* & *S.aureus*. It has triclosan and triclocarban which are the most common compounds used as antibacterials. However, other common antibacterial ingredients include benzalkonium chloride, benzethonium chloride, and chloroxylenol.

Triclocarban is an antibacterial agent that is particularly effective against Gram-positive bacteria such as *Staphylococcus aureus*. It is a bacteriostatic compound that has been found in antibacterial soaps and other personal care products.

Triclosan is a widely accepted broad spectrum antimicrobial agent proven to be effective against many gram-positive and gram-negative bacteria. Triclosan acts by blocking bacterial fatty acid biosynthesis. The addition of Gantrez copolymer has been shown to enhance the antimicrobial activity of triclosan.

Lifebuoy hand wash was the least effective one when compared to others. The main ingredients in lifebuoy handwash is Lauric acid (LA) which has a broad spectrum of anti-microbiological activities against enveloped viruses and various bacteria, and might be useful to protect against microbial infection and control the balance and distribution of bacteria in human gut microbiota.

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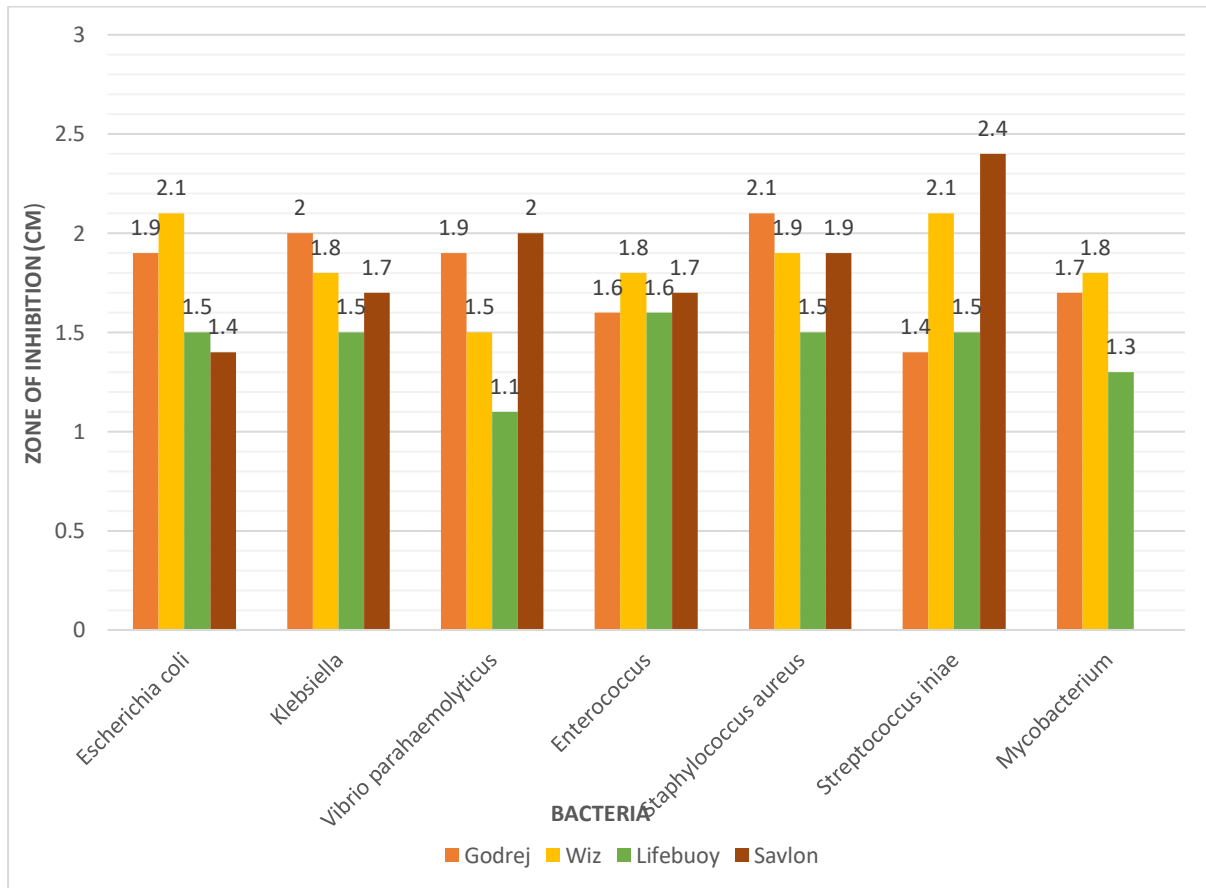
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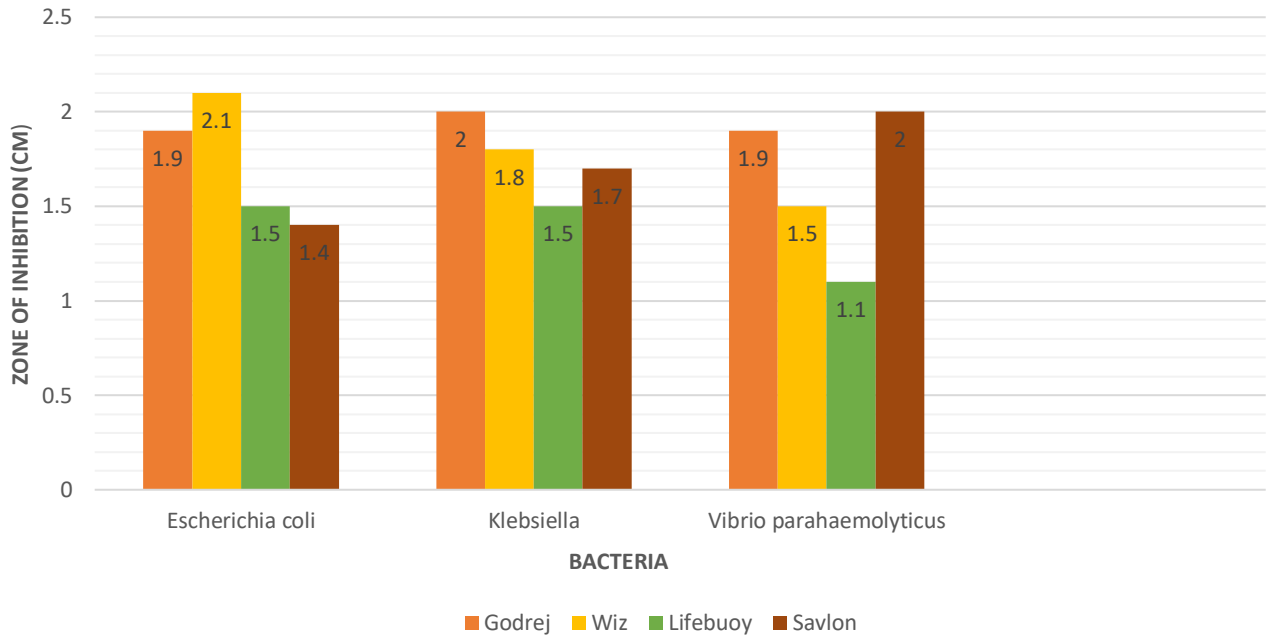
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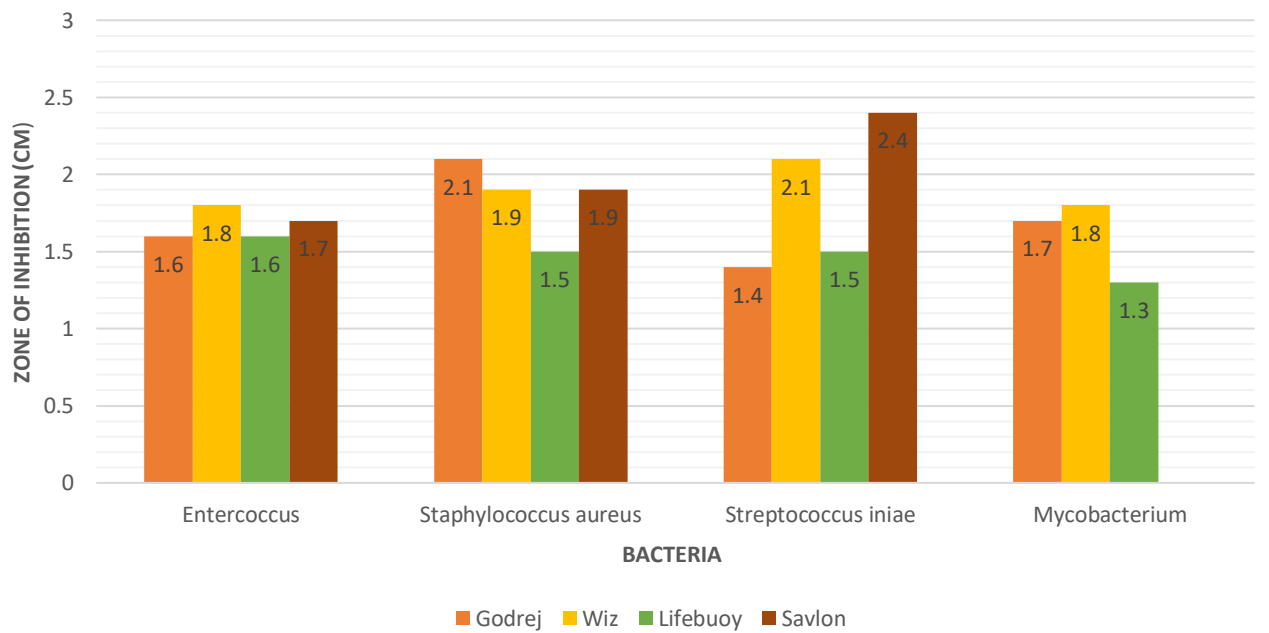
ZONE OF INHIBITION SHOWN BY HANDWASHES AGAINST DIFFERENT BACTERIAL STRAINS



GRAM NEGATIVE BACTERIA



GRAM POSITIVE BACTERIA



CERTIFICATE

This is to certify that the project report entitled “**ANTIBACTERIAL EFFECT OF HANDWASHES AGAINST DIFFERENT BACTERIAL STRAINS**” submitted by Ms. Maria Joshy, Reg No: AB20ZOO007 in partial fulfilment of the requirement of Bachelor of Science degree of Mahatma Gandhi University, Kottayam, is a bonafide work under my guidance and supervision and to my best knowledge, this her original effort.

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EXAMINERS

- 1)
- 2)

DECLARATION

I, hereby declare that this project work entitled “**ANTIBACTERIAL EFFECT OF HANDWASHES AGAINST DIFFERENT BACTERIAL STRAINS**” is submitted to St. Teresa’s College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in partial fulfilment of the requirement of Bachelor of Science degree in Zoology. This work has not been undertaken or submitted elsewhere in connection with any other academic course and the opinions furnished in this report are entirely my own.

NAME: MARIA JOSHY

SIGNATURE:

REGISTER NO.: AB20ZOO007

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ZONE OF INHIBITION OF HANDWASHES AGAINST BACTERIA



Fig:1 Showing zone of inhibition of handwashes against *Streptococcus iniae*



Fig:2 Showing zone of inhibition of handwashes against *Vibrio parahaemolyticus*



Fig:3 Showing zone of inhibition of handwashes against Mycobacterium

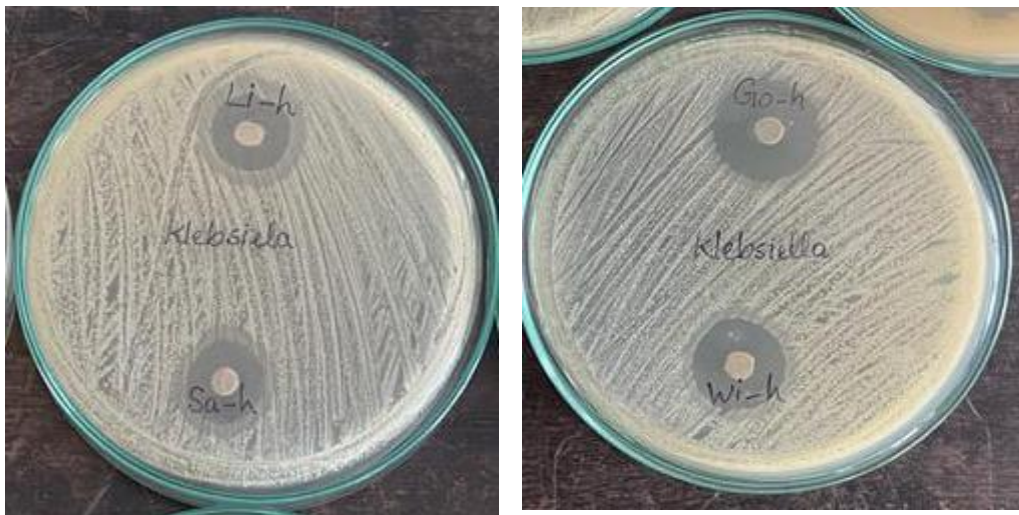


Fig:4 Showing zone of inhibition of handwashes against Klebsiella



Fig:5 Showing zone of inhibition of handwashes against *Staphylococcus aureus*



Fig:6 Showing zone of inhibition of handwashes against *Enterococcus*

