ANTIBACTERIAL ACTIVITY OF FOUR TOILET DISINFECTANTS AGAINST DIFFERENT BACTERIAL STRAINS

Project work by

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Submitted to

Mahatma Gandhi University, Kottayam

In partial fulfilment of requirement for the Degree of Bachelor of Science in Zoology

2022-23

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CERTIFICATE

This is to certify that the project report entitled "**ANTIBACTERIAL ACTIVITY OF VARIOUS TOILET DISINFECTANTS AGAINST DIFFERENT BACTERIAL STRAINS**" submitted by Ms. Jinan Abdul Lathief, Reg No: AB20ZOO005 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 is her original effort.

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EXAMINERS

1)

2)

DECLARATION

I, hereby declare that this project work entitled "ANTIBACTERIAL ACTIVITY OF FOUR TOILET DISINFECTANTS 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.

JINAN ABDUL LATHIEF SIGNATURE:

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ACKNOWLEDGEMENT

I would like to express my gratitude and appreciation to all those who have contributed to this project. First and foremost, I would like to thank my project guide Dr.Reema Kuriakose for the invaluable guidance, support, and encouragement. Thank you for guiding us along to build a good project work, by providing all the necessary information and the best available resources.

I thank all the teachers of the department of Zoology, St. Teresa's college, Ernakulam who were always willing to encourage and help us in all our efforts.I also thank our non- teaching staffs for their whole- hearted support and help.

My project work would not have been a success without the constant encouragement from my parents and my friends. Their support has kept me motivated and focused on completing this project successfully.

Above all I would like to thank God Almighty for showering his blessings upon me abundantly and for giving me the strength, knowledge, ability, and opportunity to undertake and complete this project work.

TABLE OF CONTENTS

ABSTRACT

Toilets and toilet-acquired infections are a serious problem worldwide often leading to increased deaths, costs and other problems. Toilet hygiene has been promoted as the most important way to prevent these infections. The use of toilet disinfectants is one of the ways to address the barriers to effective hygiene of toilets. In this study, we evaluated the antibacterial efficacy of some popular toilet disinfectants using Kirby Bauer disc diffusion method. Four toilet disinfectants (Dettol, Harpic, Lizol, Zoptik) were tested against different bacterial strains of gram-negative bacteria (Klebsiella, Vibrio parahaemolyticus, Escherichia coli) and gram-positive bacteria (Enterococcus, Staphylococcus iniae, Streptococcus aureus, Mycobacterium). Toilet disinfectants exhibited inhibition zones ranging from 0.8 cm to 3.5cm. The highest zone of inhibition was exhibited by Dettol (3.5cm) against St. iniae. The lowest zone of inhibition was exhibited by Zoptik against St. aureus (0.8cm). Of the toilet disinfectants used for the study, it is evident that the most sensitive bacteria was found to be Mycobacterium.

INTRODUCTION

The control of infections in public toilets specially during mass gathering is a matter of great concern and a major challenge. Public toilets can be potential sources of pathogenic microorganisms because faecal material contains large numbers of microorganisms that can be introduced to surfaces upon excretion. One of the main means of transmission of many classic diseases and many urinary, vaginal and anal infections is from human faeces (Hawker et al. 2001) therefore it is extremely important to provide adequate, hygienic public toilets with a set of guidelines of cleaning and disinfection.

Disinfection is the process of eliminating pathogenic organisms such as bacteria and viruses. Toilet disinfectants or toilet cleaners are designed to clean surfaces in the bathroom, including the walls, floors, countertops, and fixtures. Bathroom cleaners also contain disinfectants or degreasers that can help rid the area of harmful bacteria and debris. It easily removes the tough stains and greasy substances from entire surfaces and adds shine. It can also help keep your toilet clean and free from bacteria, which can reduce the risk of getting sick. Bathroom and toilet cleaners don't require extra toil and excessive rubbing for making bathrooms and toilets clean and safe. Disinfectants are used extensively in toilets, households, hospitals, health care centres to control the growth of microbes on both living tissues and inanimate objects. They are essential parts of infection control practices and aid in the prevention of nosocomial infections (Larson et al. 1991). But a common problem is the selection of disinfectants because different pathogens vary in their response to different disinfectant agents (Russell et al. 1996). Inappropriate choices and inadequate protocols for the disinfection of inanimate surfaces have been a constant and major source of outbreaks of community infections. Bacteria can only evolve from existing bacterial cells and not from inanimate matter (Louis Pasteur, 1862).

The purpose of this study was to evaluate the in vitro antibacterial activity of four toilet disinfectants Dettol, Harpic, Lizol and Zoptik against seven different bacterial strains Escherichia coli, Klebsiella, Vibrio parahaemolyticus, Enterococcus, Mycobacterium

tuberculosis, Staphylococcus aureus and Streptococcus iniae. All the selected disinfectants are conventionally used for the elimination of various types of pathogenic microorganisms, bacterial spores, on inert surfaces of toilets. Many disinfectants are used alone or in combinations (e.g. hydrogen peroxide and peracetic acid) in the health-care setting. Disinfectants include the composition of alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, ortho-phthalaldehyde, hydrogen peroxide, iodophors, peracetic acid, phenolics, and quaternary ammonium compounds.

REVIEW OF LITERATURE

Antibacterials, antiseptics 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 alcohols, phenols, iodine and chlorine. Most of these active agents demonstrate a broad spectrum of antibacterial activity (Waksman, 1947); However, little is known about the 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."

Toilet surface is only clean by appearance whereas a hygienic toilet is both clean and germ-free (Maeda et al., 2008). Toilet seats, especially those in public places, are not cleaned regularly. They harbor germs that could lead to diarrhoea and gastroenteritis. One has greater chances of getting harmful diseases, considering the amount of exposure at public places. Serenity toilet disinfectants remove germs within seconds, making the toilet surface safe and fit to use.

Germs like Salmonella paratyphi, Pseudomonas aeruginosa, Klebsiella pneumoniae and enterococcus foecalis may be present on the surface of the toilet. Such harmful bacteria can cause inflammation, sepsis and various skin infections. Infections include ringworm, scabies, pubic lice, herpes and staph.

Sanitation is the hygienic means of promoting health through prevention of human contact with the hazards of wastes as well as the treatment and proper disposal of sewage wastewater. Hazards can be physical, microbiological, biological or chemical agents of disease (Kampf and Kramer, 2004). Wastes that can cause health problems include human and animal feces, solid wastes, domestic wastewater (sewage, silwage,

greywater), industrial wastes and agricultural wastes. Hygienic means of prevention can be by using engineering solutions (e.g. Sewage and wastewater treatment), simple technologies (e.g. Latrines, septic tanks), or even by personal hygienic practices (e.g. Simple hand washing with soap).

Many toilet disinfectants contain acids and other chemicals that are harmful. Most non-acid toilet disinfectants are relatively safe to use, and have a small impact on the environment.

Disinfectants require a bit more care, but are still fairly safe. Acid cleaners are the most dangerous of all.

There is a need for research on floor surface materials that clean well, do not provide a reservoir for microbes, and do not promote slip, trips, and falls. Additionally, there is a need for improved guidance related to the cleaning and disinfection of floors in hospitals and other health care settings. Reducing chemical and particle exposures from toilet cleaning could significantly improve environmental exposures for workers, patients, and other building occupants because floors are cleaned frequently and have a high surface area, therefore requiring application of substantial amounts of cleaning and disinfecting products throughout health care facilities (Carling et al., 2002).

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.

TOILET DISINFECTANTS :

Harpic, Dettol, Lizol, Zoptik

BACTERIAL STRAINS:

Staphylococcus aureus, *Escherichia coli, Klebsiella pneumoniae, Streptococcus iniae, Enterococcus, Vibrio parahaemolyticus, Mycobacterium tuberculosis*

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 a 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 a 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 the mouth of the tube was plugged back carefully after flaming. By the same procedure, the cap was introduced into the broth using the loop. The tube mouth was flamed and recapped after the loop had been withdrawn. The inoculating loop was then resterilized 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 inoculum was 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 upto 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 celsius. 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 a specific time and was used for antimicrobial 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 degrees and the same procedure was repeated so that the 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 a 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

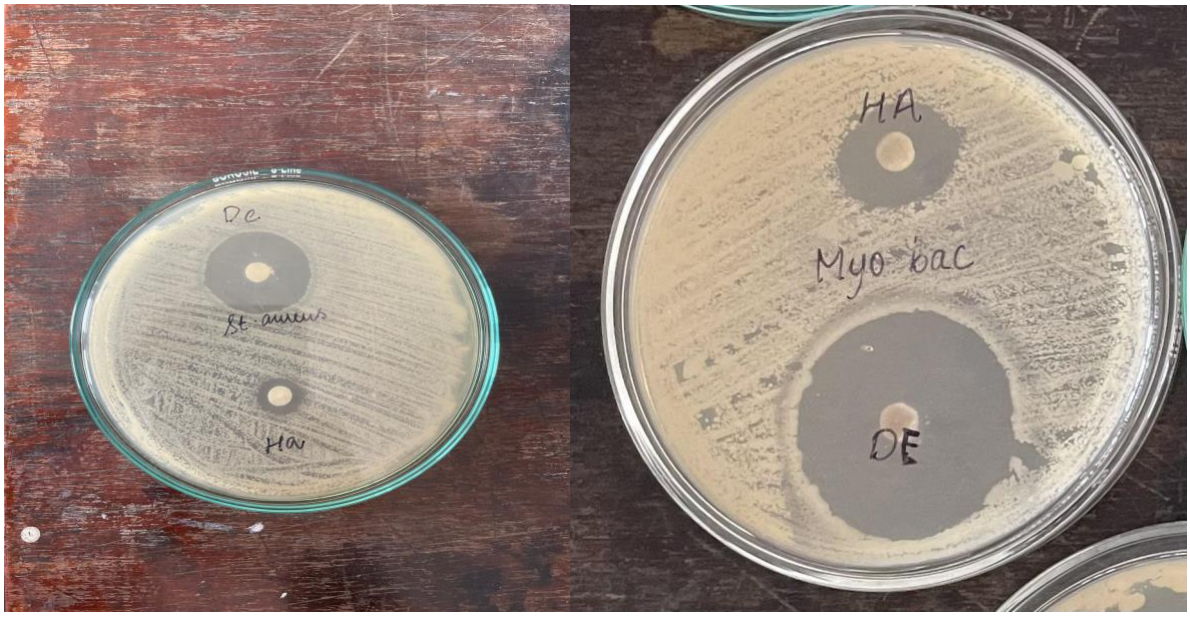
KILLING OR DISPOSING:

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.

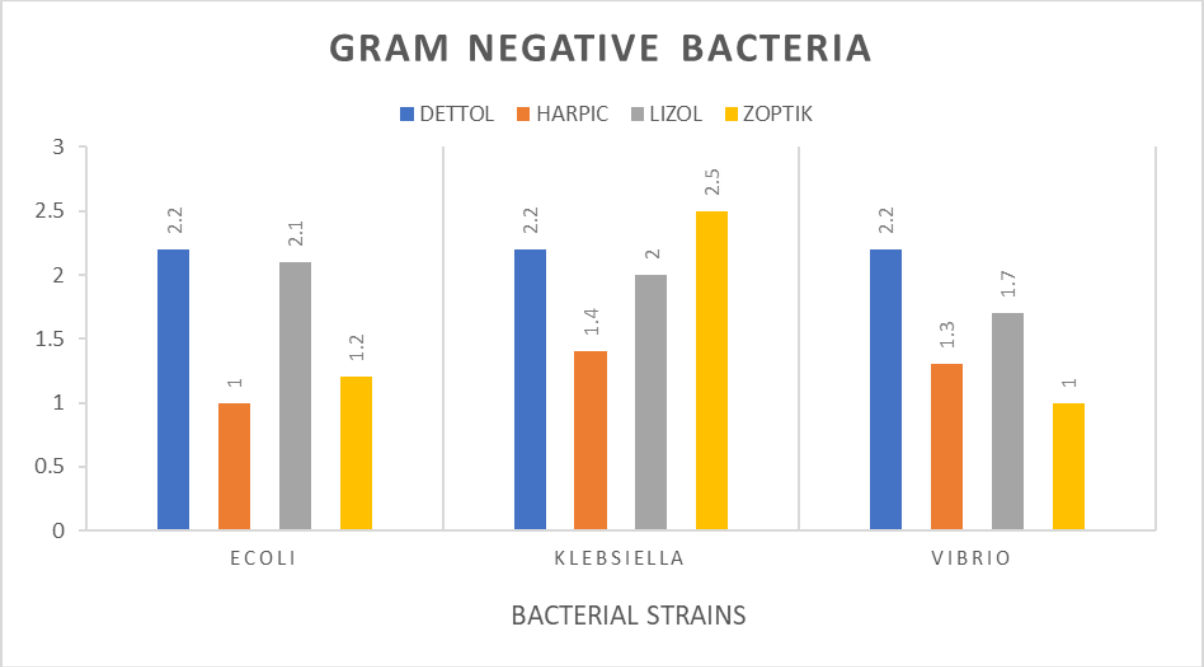
OBSERVATIONS

Toliet Disinfectants	Zone of Inhibition (Diameter in cm)						
	Gram negative bacteria			Gram positive bacteria			
	Escherichia coli	Klebsiella	Vibrio parahaemolyticus	Enterococcus	Staphylococcus aureus	Streptococcus iniae	Mycobacterium
Harpic	1	1.4	1.3	1.1	1	1.4	3.1
Dettol	2.2	2.2	2.2	2.5	2	3.5	3.2
Lizol	2.1	2	1.7	2.5	2.2	1.6	2.1
Zoptik	1.2	2.5	1	1	1.5	0.8	1

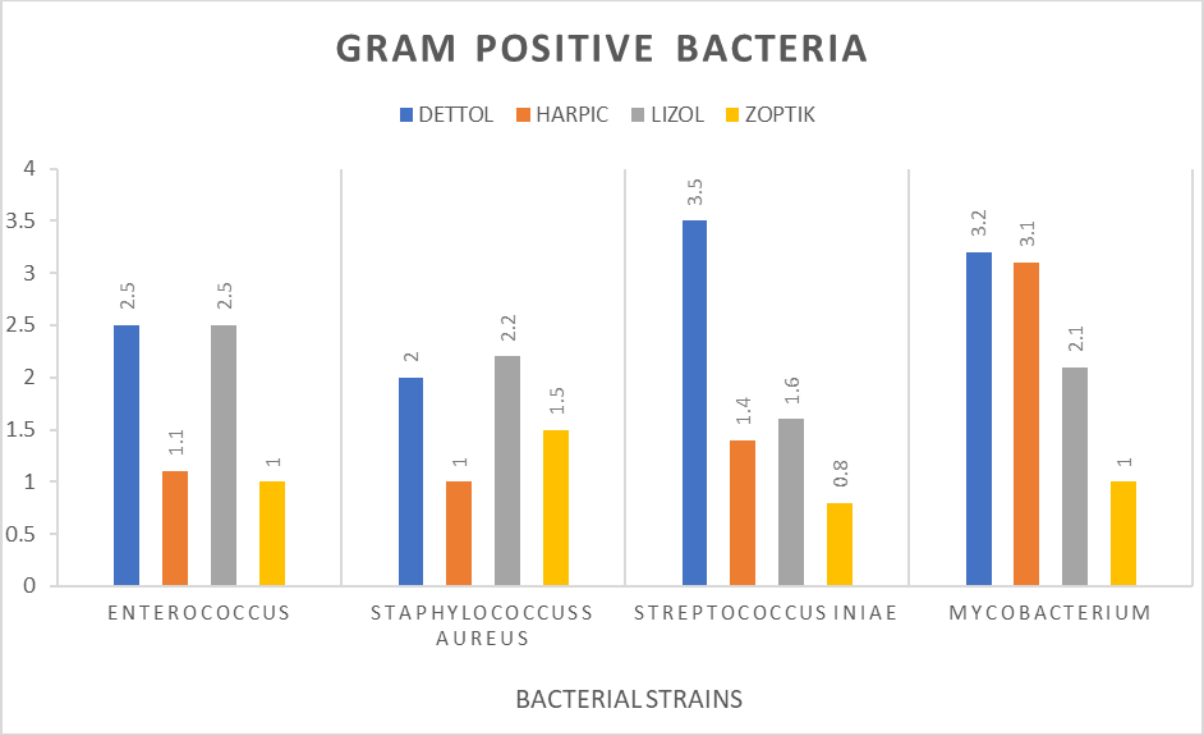
Table showing Antibacterial activity of various toilet disinfectants against different bacteria



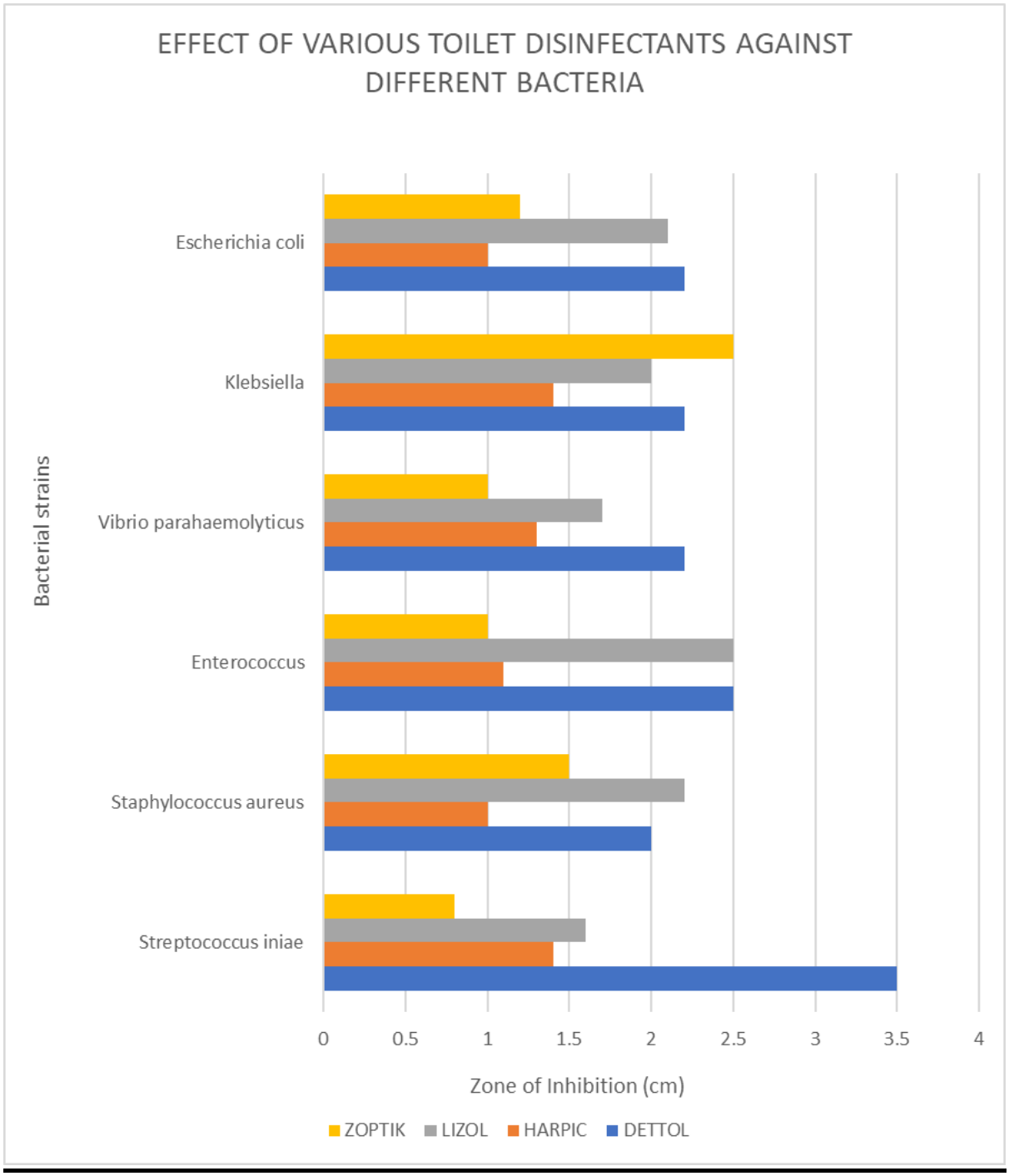
Photos showing zone of inhibition by various toilet disinfectants



Graph - 1 showing the zone of inhibition by different Gram - negative bacteria bacteria against toilet disinfectants.



Graph - 2 showing the zone of inhibition produced by different Gram - positive against toilet disinfectants.



Graph - 3 showing the zone of inhibition produced by different bacteria against various Toilet disinfectants.

RESULT

This study aimed at demonstrating and assessing the antibacterial activity of 4 different toilet disinfectants/cleaners (Harpic, Dettol, Lizol and Zoptik) against 7 different bacterial strains (Escherichia coli, Klebsiella, Vibrio parahaemolyticus, Enterococcus, Staphylococcus aureus, Streptococcus iniae and Mycobacterium tuberculosis). And the toilet disinfectants exhibited inhibition zones against all 7 bacterial strains used for the study and zones of inhibition ranging from 0.8 to 3.5 cm.

The bacterial strain Mycobacterium tuberculosis had the highest antibacterial activity ranging from 1 to 3.2 cm followed by Klebsiella and the least sensitive bacteria was St. iniae (0.8 to 1.4) and Vibrio parahaemolyticus (1 to 2.2). In case of the bacteria Escherichia coli, Dettol showed highest sensitivity with a zone of inhibition of 2.2 cm, similarly the zones of inhibition (2.2) were the same towards Klebsiella and Vibrio parahaemolyticus, while Harpic exhibited least sensitivity towards E.coli with a zone of inhibition of 1cm. Dettol exhibited the highest zone of inhibition of 3.5 cm towards the Gram-positive bacteria St.iniae and in contrast, Zoptik was found to show the least effect towards St.iniae with a zone of inhibition of 0.8 cm in the antibacterial activity.

Considering the Gram-positive bacteria Enterococcus, Lizol and Dettol showed similar zones of inhibition (2.5 cm). While, Harpic and Zoptik showed least sensitivity with a zone of inhibition of 1.1 cm and 1 cm respectively. The bacterial strain St.aureus showed considerable effects, with Lizol being the most sensitive with a zone of inhibition of 2.2 cm and Harpic exhibited least sensitivity with a zone of inhibition of 1 cm.

Of the toilet disinfectants used for the study, it is evident that the most significant sensitive bacteria were Mycobacterium tuberculosis. And the least sensitive bacteria was St.iniae and Vibrio parahaemolyticus. Dettol was the most effective disinfectant against the bacteria used for the study. It showed considerable inhibitory effect against

all the bacteria taken with a highest zone of inhibition of 3.5 cm. But in the case of Zoptik the inhibitory effects were lesser towards all the bacteria chosen.

DISCUSSION

The present project aimed to analyse the antibacterial effect of 4 Toilet disinfectants (Harpic, Dettol, Lizol, Zoptik) against 3 Gram-negative bacteria (Escherichia coli, Klebsiella,Vibrio parahaemolyticus) and 4 Gram-positive bacteria (Enterococcus, Staphylococcus aureus, Streptococcus iniae, Mycobacterium tuberculosis).

Public toilets can result in a buildup of pathogens within surfaces of the toilet and other areas. Surface contamination could occur within a short time of flushing. So, the disinfecting of these surfaces using chemical and physical processes is essential. The efficiency of disinfectants varies greatly depending on various factors, some are specific for each disinfectant while others depend on the type of microorganisms. Disinfectants are chemical agents with an immediate and sustained activity which destroys microorganisms to such a level mandated for hygienic and surgical indications.

All toilet cleaners are not antibacterial, research by other scientists has shown antimicrobial effects against staphylococcus aureus, influenza a flu virus, rhinovirus, escherichia coli, enterobacter aerogenes and salmonella enterica Microorganisms are continuously acquiring resistance to new disinfectants, as a result, no single disinfectant will be appropriate for all pathogens (Tortora et al. 1998). Therefore, it is necessary to evaluate the effectiveness of a disinfectant against a specific pathogen so that an appropriate agent can be easily selected.

In this study, different types of toilet disinfectants had significant antibacterial activities. However, there were few which showed comparatively greater antibacterial activity and some showed lesser antimicrobial activity.

Dettol was the most effective disinfectant among all the toilet cleaners used in the present study against all the bacteria taken. Dettol is widely used in homes and healthcare settings for various purposes including disinfection of skin, objects and equipment, as well as environmental surfaces. With prior cleaning before application, the number of microorganisms colonising the skin and surfaces are greatly reduced (Rutala et al. 1996). The antimicrobial properties of chloroxylenol, the main chemical

constituent of Dettol and other chlorinated phenols have been extensively studied (Hugo and Bloomfield 1971a). A study conducted by Oke et al. revealed that Dettol was effective only against P. aeruginosa whereas it was not effective against S. aureus and E. coli.

Harpic is another strong disinfectant and cleaning liquid. The popularity of Harpic liquid toilet cleaner owes to the unique efficaciousness and handy applicability of the product, resulting in a clean & odour free toilet system. It is very powerful in action, removing even the most stubborn stains, dirt, etc. imparting a shining surface. By its action, the disease-causing bacteria are killed, and very agreeable and hygienic clean conditions are established. Its thick liquid formula with powerful cleaning agents kills 99.9 percent of germs. Harpic is a commonly used toilet cleaning solution and contains hydrochloric acid (10%) as the active ingredient, along with butyl oleylamine and others in a water solution. Harpic exhibited a strong inhibition zone of 3.1 against mycobacterium. And it shows less effectiveness against E.coli and St.aureus. Harpic causes harmful effects on the skin, skin allergies and immunotoxicity. It can cause severe skin burns and eye damage when it comes in contact, and it can also cause respiratory irritation. Most importantly it is very toxic to aquatic life in the long run. Harpic's ingredients are harmful to aquatic life and have a long-lasting impact on the water bodies into which the wash water is discharged.

Lizol eliminates viruses, bacteria, soap scum, and tough grease. It is an all-Purpose cleaner and can be used throughout your bathroom surfaces and other surfaces such as: Household: Walls. to offer a powerful cleaning action and removes 99.9% germs. Lizol liquid disinfectant ensures 10 times better cleaning and germ protection. It has a refreshing fragrance and ensures that your bathroom surfaces are spotless and shining. The ingredients present in the lizol bathroom cleaner are Sodium laureth sulphate, Citric acid, Hydrochloric acid, Dipropylene glycol and 2-Butoxyethanol. Active Ingredient is Benzalkonium chloride. However, this chemical has been known to have adverse effects on human health. It is known to cause skin irritation, itching, and redness. In

high concentrations, it can be toxic to the eyes and respiratory system. It can also lead to dry and irritated throat, causing coughing and soreness.

In this experiment, Lizol exhibited a strong inhibition of 2.5 cm against Enterococcus. The active ingredient benalkonium chloride in lizol showed superior action or equivalence to most other products with broad microbial action, including spores, and progressive action with longer exposure time and concentration. Even in the present study it has better results as a disinfectant agent. Lizol showed less effectiveness towards St. iniae with a zone of inhibition 1.6 cm. But it has high sensitivity towards the ingredient chloroxylenol in Dettol (Oke et al. 2013)

Zoptik is a disinfectant to decrease water's surface tension and lift dirt and oils off surfaces, so it can be easily rinsed away. The main active ingredient was triclocarban and it showed a moderate sensitivity towards the bacterial strains used in the study, it has better results as a disinfectant agent. The main active ingredient was Triclosan which is now being replaced by triclocarban in many other disinfectants, partly because of problems such as development of bacterial resistance to triclosan. In general, the triclocarban-containing disinfectants and handwashes seemed to be less effective considering other chemicals. In the present study Zoptik was the only disinfectant which is not commonly available.

CONCLUSION

The antibacterial activity of various toilet disinfectants (Dettol, harpic, Lizol, Zoptik) was tested against 3 Gram-negative (Escherichia coli, Klebsiella, Vibrio parahaemolyticus) and 4 Gram- positive (Enterococcus, Staphylococcus aureus, Streptococcus iniae, Mycobacterium) bacteria using the Kirby-Bauer disc diffusion method.

All the bacteria were sensitive to the disinfectants taken. The most sensitive bacteria were Mycobacterium and Klebsiella. Mycobacterium showed zones of inhibition ranging from 1 – 3.2 cm while for Klebsiella the range was from 1.4 – 2.5 cm. Dettol was the most effective disinfectant showing a good inhibitory effect against all the bacteria taken in the study. On the other hand, Zoptik was the least effective medicine showing least sensitivity towards all the bacteria taken.

From the study it can be concluded that Dettol toilet disinfectant is more effective than Harpic, Lizol and Zoptik on different bacterial strains.

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