# Comparative study on the antibacterial activities of four commercially available antiseptics (Dettol, Hexisol, Oralon and Betadine) against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus, and Pseudomonas aeruginosa.*


Inspiring excellence

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

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May, 2017

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## DECLARATION

There is to declare that the thesis project titled "Comparative study on the antibacterial activities of four commercially available antiseptics- Dettol, Hexisol, Oralon and Betadine against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus,* and *Pseudomonas aeruginosa*" submitted by me has been carried out under the supervision of Dr. M. Mahboob Hossain, professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka. It is further declared that the research work presented here is based on actual and original work carried out by me. Any reference to work done by any other person or institution or any material obtained from other sources has been duly cited and referenced.

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# Acknowledgment

The work I accomplished in pursuance of my B.Sc. project happens to be the first undertaking of this nature I have ever been exposed to. I needed help and encouragement not to be frustrated in the event of repeated failures in my experiments. Fortunately, there were people around me who provided the needed support.

I am grateful to Professor A.A. Ziauddin Ahmad, chairperson of the Department of Mathematics and Natural Science, BRAC University for allowing me and encouraging me to complete my undergraduate thesis.

My regards, gratitude, and appreciation go to my respected Dr. M. Mahboob Hossain, Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University for his constant supervision, constructive criticism, expert guidance, enthusiastic encouragement to pursue new ideas and a good sense of humor and never-ending inspiration throughout the entire period of my research work. It would have been impossible to submit my report without his cordial help.

My deepest gratitude to all the lab employees who provided me with a good working environment with their advice, encouragement, and constant help to make me feel at home in my hard times. I am also thankful to the laboratory assistants and Department Coordination Officers of the MNS department for their help and cooperation in doing my research work.

Finally, I would like to extend my gratitude to the members of my family and friends for their sincere concern and support.

Sakia Binte Azam

# Abstract

The present study is about some commercially available and common antiseptics that are used highly in Bangladesh and other countries as well. Dettol, hexisol, oralon, and betadine those antiseptics and disinfectants that are extensively used in hospitals or home and health care settings for so many purposes. Mainly the antiseptics and disinfect are used to kill the microorganisms or the surface and living tissues. The effort was given to study the effectiveness of these four antiseptics against four well-known bacteria *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and for effectiveness testing purposes the MIC, MBC, and the disk diffusion method was done. Both of the two tests showed that Dettol, Hexisol, Oralon and Betadine had the best antibacterial activity or effectiveness against *Staphylococcus aureus comparing to Klebsiella pneumonia, Pseudomonas aeruginosa,* and *Bacillus cereus*. Against *Staphylococcus aureus,* Dettol was the most effective comparing the two other antiseptics (considering the result of disk diffusion) but considering the dilution method it was found that comparatively Dettol and Betadine were the most effective. Against *Bacillus cereus,* comparatively Dettol was more effective but Hexisol had an almost similar type of effectiveness as like Dettol, and both Oralon, Betadine had more or less the same type of effectiveness and their effectiveness was less than Hexisol and Dettol for *Bacillus cereus*. Against *Pseudomonas aeruginosa,* dittol was very effective compared to Hexisol, Oralon, and betadine. Also, against *Klebsiella pneumoniae*, Dettol was very effective but good effectiveness was also found for hexisol although Betadine and Oralon were less effective in that case. Overall, through this study, it was found that the four antiseptics (Dettol, Hexisol, Oralon and Betadine) have almost very good effectiveness (99.999%) against *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumonia.*

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# List of Abbreviations

| | |
|---|---|
| et.a*l* | And others |
| Etc | Etcetera |
| gm/l | Gram per liter |
| Cfu | Colony forming unit |
| µg | Micro gram |
| µl | Micro liter |
| NA | Nutrient agar |
| NB | Nutrient broth |
| MR | Methyl-red test |
| VP | Voges -Proskauer test |
| TSI | Triple sugar iron test |
| MIC | Minimum inhibitory concentration |
| MBC | Minimum bactericidal concentration |
| V | Volume |
| W | Weight |

# Introduction

## 1.1 Background:

Antiseptics are anti-infective substances that, after topical administration, destroy or inhibit the growth of microorganisms in or on living tissue (skin, mucous membrane, and wound). Antiseptics are applied externally and, to prevent the development of biocide resistance, they are used at concentrations considerably higher than minimal bactericidal concentrations (MBCs). Ideally, antiseptics should have a broad microbicidal spectrum and potent germicidal activity with rapid onset and long-lasting effects. Antiseptic preparations should not be toxic to host tissues/cells and in line with the concept of biocompatibility of medical products, as far as possible, they should not impair the healing process (Müller and Kramer, 2008).

The concept of disinfection includes techniques of microorganisms control by chemical means and of their mechanical removal. Most, but not all, bacteria die during these activities. Chemical disinfectants affect vegetative forms of bacteria and of other microorganisms, while spores are most often resistant to them. It was not until the 19th century that antimicrobial procedures started to appear. Many antibacterial agents were created as a result of the gradual development of knowledge. A significant amount of them are disinfecting agents, that are used to remove microorganisms beyond the human body or antiseptic agents used only on the surface of the body, living tissues. A suitable disinfectant should provide an effective, short-time disinfection, affect the greatest possible number of microorganism species i.e. should have a broad action spectrum, and be well tolerated by the skin. Finally, it should not have an unpleasant smell but should undergo inactivation by blood, pus, and foreign matter (Ziembinska and Szpindor, 2013).

Both antiseptics and disinfectants eliminate disease-causing organisms, notes the Mount Sinai Department of Microbiology. The difference is in how each substance is used. Antiseptics are applied to living skin or tissue to prevent infection, whereas disinfectants are applied to surfaces, equipment, or other inanimate objects. Disinfectants are stronger and more toxic than antiseptics because they are applied to surfaces, not living tissue (Jones, 2014).

Antiseptics and disinfectants are used extensively in hospitals and other healthcare settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections. A wide variety of active chemical agents (or "biocides") are found in these products, many of which have been used for hundreds of years for antisepsis, disinfection, and preservation. In general, biocides have a broader spectrum of activity than antibiotics, and, while antibiotics tend to have specific intracellular targets, biocides may have multiple targets ( McDonnell and Russell, 1999 **).**

The basic principle now widely accepted is that the antimicrobial efficiency of a disinfectant or an antiseptic is examined at three stages of testing (Pelczar et al., 1993). The first stage concerns laboratory tests in which it is verified whether a chemical compound or a preparation possesses antimicrobial activity. For these preliminary screening tests, suspension tests are considered. In the second stage of tests, disinfection procedures and not disinfectants are examined. The last stage takes place in the field and comprises in-situ tests that examine whether, after a normal period of use, germs are still killed by the disinfectant solution (Wijesinghe1 and Weerasinghe2, 2010).

**1.2 Categories of antiseptics or disinfectants based on chemical nature:**

Depending on the chemical nature of disinfectants and antiseptics they can be categorized into several groups. They are alcohols, phenolics, halogens, Quaternary Ammonium Compounds (QACs), and aldehydes. The mode of action of disinfectants and antiseptics differ greatly according to the chemical substance present (Pelczar et al., 1993).

Alcohols are among the most widely used disinfectants and antiseptics. They are colorless hydrocarbons with one or more hydroxyl functional groups. Alcohols are bactericidal and fungicidal but not sporicidal. Some lipid-containing viruses are also destroyed by alcohol (Prescott et al., 2005). The two most popular alcohol germicides are ethanol and isopropanol usually used in about 70-80% concentration (Russel, 1981). The mode of action of alcohol depends upon its concentration. Alcohol with a concentration of 50% and higher dissolves

membrane lipids disrupts cell surface tension and compromises membrane integrity. Ethyl alcohol is used to disinfect surgical instruments, face masks, thermometers etc. Alcohol effectively kills the Staphylococcus aureus (70% Ethyl alcohol concentration in 10min), the Escherichia coli (70% Ethyl alcohol concentration in 2 min), and the Polio virus (70% Ethyl alcohol concentration in 10min) (Prescott et al., 2005).

Phenol was the first widely used antiseptic and disinfectant. Phenolics consist of one or more aromatic carbon rings with added functional groups. The three important substances are alkylated phenols (cresols), chlorinated phenols (chlorophene), and bisphenols(hexachlorophene) (Talaro & Talaro,1996). Phenolics are strongly microbicidal and will destroy vegetative bacteria, fungi, and most viruses (not hepatitis B). However, they are not reliably sporicidal (Talaro & Talaro, 1996). They may be either bacteriostatic or bactericidal, depending on the concentrations used (Pelczar et al., 1993).

Halogens (iodine and chlorine) are important antimicrobial agents (Prescott et al., 2005). Most halogens exert their antimicrobial effect primarily in the non-ionic state. They are highly effective components of disinfectants and antiseptics. Halogens are strong oxidizing agents. They are sporicidal with longer exposure. The major forms used in microbial control among chlorine compounds are liquid and gaseous chlorine, hypochlorites (OCl), and chloramines (NH2-Cl) ((Talaro & Talaro, 1996). They destroy vegetative bacteria and fungi, but not their spores (Reybrouck, 1998).

Chloramine is used presently in drinking water treatment instead of chlorine because it produces fewer disinfection by-products. Hypochlorites (Sodium hypochlorite), often in the form of common household bleach, are used in the home to disinfect drains and toilets. Sodium hypochlorite is the principal ingredient in Clorox which is a household disinfectant and a bleaching agent (Talaro &Talaro,1996). Quaternary Ammonium Compounds (QAC) have positively charged quaternary nitrogen and a long-chain hydrophobic aliphatic chain (Prescott et al., 2005).

The present study was conducted for the detection of the effectiveness of four commercially available antiseptics against four very well-known pathogenic organisms (S.aureous, *Bacillus*

*cereus, Pseudomonas aurogenosa, Klebshiella pneumonia)*. The four selected antiseptics were Dettol, Hexisol, Oralon, and Betadine.

### 1.3 About the Four Antiseptics of the Study:

**Dettol:** An antiseptic cleaning product made by Reckitt Benckiser, was developed based on modifications of phenol, an antiseptic that kills germs, discovered in 1860, by Joseph Lister. The active ingredient in Dettol is chloroxylenol B.P.4.8%w/v. The other ingredients include isopropyl alcohol, pine oil, castor oil soap, caramel, and water. Chloroxylenol; 4-chloro-3,5-dimethylphenol or para-chloro-meta-xylenol is the main compound of Dettol. This antiseptic/disinfectant has the controversy of being slightly toxic by inhalation or ingestion, and can irritate some skin; so, use it wisely. Of course, that's the main active chemical; the remaining are excipients; because Dettol is not a homogeneous mixture or solution; nor a pure substance (Pocetti, 2006).

It is safe and gentle enough to use on the skin and yet powerful enough to also be used as a disinfectant. This is because of its broad spectrum of antimicrobial action. It is effective against gram-positive/negative bacteria, fungi, yeast, mildew, and even the frightening "super-bug" MRSA. It can kill 98% of microbes in just 15 seconds as shown in agar patch studies. The antimicrobial properties of chloroxylenol, the main chemical constituent of Dettol and other chlorinated phenols have been extensively studied (Hugo and Bloomfield 1971a). The antimicrobial properties of the disinfectant against some pathogenic bacteria have earlier been reported (Mellefont et al., 2003).

The Marketing Director of RB (Reckitt Benckiser makers of Nigeria's No 1 antiseptic product Dettol) West Africa, Oguzhan Silivrili said that "a startling fact is that 80 % of hygiene-related illness-causing germs are acquired at home from different surfaces like floors, kitchen tops and washbasins. The new Dettol Multi Surface Cleaner is the first of its kind in the surface-cleaning category providing 10 times better cleaning and germ kill vs detergents and bleach while providing all-day freshness. Dettol can be used on all hard surfaces including tiles, marble, granite, wood, or cement floors to maintain a healthy and hygienic environment within the home (Samguine, 2016).

**Hexisol**: Hand rub is used for Skin Cleansing, Dental Plaque and Bacteria, Gingivitis, Keratitis, Infection Before Any Surgical Procedure, Minor Scalds, and other conditions. Hexisol Hand rub contains Chlorhexidine Gluconate, and Isopropanol as active ingredients.

Hexisol Hand rub works by killing as well as preventing bacterial growth; denaturing cell proteins and deoxyribonucleic acid; interfering with cellular metabolism; and dissolving cell lipoprotein membranes Hexisol hand rub is composed of the following active ingredients (salts):

Chlorhexidine Gluconate (0.5% W/W) and Isopropanol (70%) (Gothner et al., 2007).

Hand-washing was highlighted as the most important measure to prevent nosocomial infections. Caregivers and staff of hospitals were instructed to wash their hands at the sink with hexisol (2.5% v/v chlorhexidine gluconate solution in 70% w/w isopropyl alcohol) before handling the babies (Darmstadt et al., 2005).

Dr. Ishtiaq Mannan, Save the Children's director for Health Nutrition and the HIV/AIDS sector, termed the introduction of this new solution a "game changer" to bring down neonatal deaths in Bangladesh and this game changer antiseptic is chlorhexidine. Chlorhexidine has been seen as a "cheaper and cost-effective" method as it costs only Tk 30 a bottle needed for one child (Hasib, 2015).

**Oralon:** Oralon is a chemical antiseptic, prescribed for gingivitis, cleansing skin and wound areas. Chlorhexidine gluconate is a germicidal mouthwash that reduces bacteria in the mouth; It decreases the number of bacteria in the mouth. Chlorhexidine gluconate oral rinse is used to treat gingivitis (swelling, redness, and bleeding gums). Chlorhexidine gluconate is usually prescribed by a dentist. It comes as a liquid to rinse the affected area as directed by the physician. It is for external use only. Avoid contact with eyes, ears, and mouth; if the medication is accidentally swallowed nausea and stomach upset may occur (Multum, 2012).

**Betadine:** According to Australia's leading well-being pharmacy, Chemmart Pharmacy, Betadine antiseptics are among the most effective antiseptics available, inactivating infecting organisms including bacteria and fungi. Povidone-iodine solutions are a golden-brown color

because of the iodine content. This color shows the area that has been treated and also denotes the activity of the product. As the iodine is depleted from the solution, the color fades. When the color fades to a light yellow, Betadine should be reapplied. In most cases, Betadine antiseptics are non-irritating and non-stinging to the skin. They do not permanently stain the skin or natural fabrics and the treated areas may be bandaged, taped, or otherwise covered. It is mainly used for Cuts wounds and abrasions suggested to apply Betadine antiseptic Liquid undiluted liberally to the injured area using a cotton-tipped applicator or cotton wool as desired. Wipe away from the cut and cover the surrounding area well. Alternatively, Betadine Antiseptic Ointment may be used. It is also suggested for Minor Burns to cool immediately in cold water for 10-20 minutes. If red or blistered, cover with a non-stick sterile dressing and see a doctor. Also suggested for the treatment of Blisters, Tinea (athlete's Foot), Paronychia (Infected skin around the nails), and Ringworm (another name for tinea) (Fogorv, 1999).

### 1.4 Effectiveness of antiseptics to prevent nosocomial infections

Antiseptics and disinfectants are essential parts of infection control and aid in the prevention of nosocomial infections (Larson et al., 1991). Dettol is widely used in homes and healthcare settings for various purposes including disinfection of skin, objects, equipment, as well as environmental surfaces. With prior cleaning before application, the number of microorganisms colonizing the skin and surfaces is greatly reduced (Rutala 1996).

Some of the preoperative measures performed in or near the operating room are helping to keep the postoperative hospital stay short and uneventful, as related to nosocomial infections. Studies indicate that shaving with a razor can injure the skin and increase the risk of infection. If shaving is necessary, it should be performed immediately before the operation and followed with the use of a preoperative antiseptic. Secondly, the skin at the operative site is thoroughly cleaned to remove superficial flora, soil, and debris before the operation to reduce the risk of contaminating the wound with a patient's skin flora. Finally, immediately before the surgery, a preoperative antimicrobial skin preparation is applied to the patient's skin to kill or inhibit more adherent, deep, resident flora. The list of antimicrobial agents commercially available as principal active

ingredients for use in patient preoperative skin-preparation products is relatively short and currently includes alcohols, chlorhexidine gluconate (CHG), and iodophors such as povidone-iodine (PVPI). Only two of these antimicrobial classes, 1) alcohols (specifically ethyl alcohol and isopropyl alcohol) and 2) iodine and iodophors, are currently classified Category 1 (safe and effective) and allowed for patient preoperative skin preparation under the FDA's Tentative Final Monograph for Healthcare Antiseptics (Gentry, 2001).

Approximately 20%–40% of nosocomial infections are caused by cross-transmission via the hands of medical workers, which affects the quality of health care and patients' safety. The World Health Organization, along with the Centers for Disease Control and Prevention (Atlanta, GA, USA), suggested antiseptic hand rubbing as an appropriate method for hand hygiene. (Li et al., 1994). More than 90% of all intravascular device-related septicemias are due to central venous or arterial catheters. The use of 2% chlorhexidine, rather than 10% povidone-iodine or 70% alcohol, for cutaneous disinfection before insertion of an intravascular device and for post-insertion site care can substantially reduce the incidence of device-related infection (Maki et al., 1991).

### 1.5 Bacterial “resistant” to antiseptics or disinfectants

Bacterial resistance to antibiotics is a long-established, widely-studied problem. Increasingly, attention is being directed to the responses of various types of microbes to biocides (antiseptics, disinfectants, and preservatives). Different groups of bacteria vary in their susceptibility to biocides, with bacterial spores being the most resistant, followed by mycobacteria, then Gram-negative organisms, with cocci generally being the most sensitive. There are wide divergencies within this general classification. Thus, (i) spores of Bacillus subtilis are less susceptible to biocides than those of Clostridium difficile: (ii) Mycobacterium chelonae strains may show high resistance to glutaraldehyde and M. avium intracellular is generally less sensitive than M. tuberculosis; (iii) Gram-negative bacteria such as *Pseudomonas aeruginosa,* Providencia spp, and Proteus spp may be difficult to inactivate; (iv) enterococci are less sensitive than staphylococci to biocides and antibiotic-resistant strains of *Staphylococcus aureus* might show low-level biocide resistance. The mechanisms involved in biocide resistance to biocides are becoming better understood. Intrinsic resistance (intrinsic insusceptibility) is found with bacterial

spores, mycobacteria, and Gram-negative bacteria. A special situation is found with bacteria present in biofilms, which can be considered as being an intrinsic resistance mechanism resulting from the physiological (phenotypic) adaptation of cells. Acquired resistance to biocides may arise by cellular mutation or by the acquisition of genetic elements. Plasmid-mediated resistance to some other biocides in Gram-negative bacteria and *s.aures* has been described, but its significance remains uncertain (Russell, 1999). Microorganisms have adapted to biocide exposure by acquiring plasmids and transposons that confer biocide resistance, the same survival strategies to disseminate acquired mechanisms of resistance to biocides as they have for resistance to antibiotics (Sheldon, 2005).

## 1.6 Organisms that are used in the study

***Staphylococcus aureus***: It is a major human pathogen that causes a wide range of clinical infections. It is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections (Tong, 2015). *S. aureus* strains can express a wide array of potential virulence factors including surface proteins that promote adherence to damaged tissue, bind proteins in blood to help evade antibody-mediated immune responses, and promote iron uptake. The organism also expresses several membrane-damaging toxins and superantigen toxins that can cause tissue damage and the symptoms of septic shock, respectively (Foster, 2004).

***Bacillus cereus***: *Bacillus cereus* is a Gram-positive aerobic or facultatively anaerobic, motile, spore-forming, rod-shaped bacterium that is widely distributed environmentally. While *B. cereus* is associated mainly with food poisoning, it is being increasingly reported to be a cause of serious and potentially fatal non-gastrointestinal tract infections. The pathogenicity of *B. cereus*, whether intestinal or nonintestinal, is intimately associated with the production of tissue-destructive exoenzymes. Among these secreted toxins are four hemolysins, three distinct phospholipases, an emesis-inducing toxin, and proteases. The major hurdle in evaluating *B. cereus* when isolated from a clinical specimen is overcoming its stigma as an insignificant contaminant. *B. cereus* produces a potent β-lactamase conferring marked resistance to β-lactam antibiotics (Bottone, 2010).

***Pseudomonas aeruginosa*:** It is one of the leading nosocomial pathogens worldwide. Nosocomial infections caused by this organism are often hard to treat because of both the intrinsic resistance of the species (it has constitutive expression of AmpC β-lactamase and efflux pumps, combined with a low permeability of the outer membrane), and its remarkable ability to acquire further resistance mechanisms to multiple groups of antimicrobial agents, including β-lactams, aminoglycosides and fluoroquinolones. *P. aeruginosa* represents a phenomenon of bacterial resistance since practically all known mechanisms of antimicrobial resistance can be seen in it: derepression of chromosomal AmpC cephalosporinase; production of plasmid or integron-mediated β-lactamases from different molecular classes (Strateva and Yordanov, 2009 ). It is also mentioned that *Pseudomonas aeruginosa* is a gram-negative, opportunistic pathogen and a significant cause of acute and chronic infections in patients with compromised host defenses. Evidence suggests that within infections *P. aeruginosa* encounters oxygen limitation and exists in microbial aggregates known as biofilms (Filiatrault et al., 2006).

***Klebsiella pneumoniae***: Bacteria belonging to the genus Klebsiella frequently cause human nosocomial infections. In particular, the medically most important Klebsiella species, *Klebsiella pneumoniae*, accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections. Nosocomial *Klebsiella* infections are caused mainly by *Klebsiella pneumoniae*, the medically most important species of the genus. The principal pathogenic reservoirs for the transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel.

Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks (Podschun and Ullmann, 1998).

### 1.7 Aims and objectives:

Dettol, Hexisol, Oralon, and Betadine are very available and common types of antiseptics or disinfectants for the people of Bangladesh. So, in this work, an attempt was made to study the effectiveness of these antiseptics against four well-known bacteria *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae*. Mainly effort was given to:

- To detect the MIC and MBC of the selected antiseptics and disk diffusion method was also done so that the effectiveness of those four commercially available antiseptics can be observed through the result of the study.
- To observe the comparative antibacterial activities of the common antiseptics through the study.

# Literature Review

**Donnell and Russell, 1999** studies on the modes of action of antiseptics and disinfectants against fungi, viruses, and protozoa have been rather sparse. Furthermore, little is known about the means whereby these agents inactivate prionsA battery of techniques are available for studying the mechanisms of action of antiseptics and disinfectants on microorganisms, especially bacteria. These include examination of uptake, lysis, and leakage of intracellular constituents, perturbation of cell homeostasis effects on model membranes, inhibition of enzymes, electron transport, and oxidative phosphorylation interaction with macromolecules, effects on macromolecular biosynthetic processes, and microscopic examination of biocide-exposed cells. Additional and useful information can be obtained by calculating concentration exponent's values and relating these to membrane activity. Many of these procedures are valuable for detecting and evaluating antiseptics or disinfectants used in combination. Similar techniques have been used to study the activity of antiseptics and disinfectants against fungi, in particular yeasts. Additionally, studies on cell wall porosity may provide useful information about intracellular entry of disinfectants and antiseptics. It was concluded that apart from certain specific examples such as silver, other metals, and organomercurials, plasmids were not normally responsible for the elevated levels of antiseptic or disinfectant resistance associated with certain species or strains. An increase in an antibiotic MIC can have significant consequences, often indicating that the target organism is unaffected by its antimicrobial action. Increased biocide MICs due to acquired mechanisms have also been reported and in some cases misinterpreted as indicating resistance. Gram-negative bacteria are generally more resistant to antiseptics and disinfectants than are non-sporulating, non-mycobacterial gram-positive bacteria. Based on these data, there is a marked difference in the sensitivity of *S. aureus* and *E. coli* to QACs (benzalkonium, benzethonium, and cetrimide), hexachlorophene, diamidines, and triclosan but little difference in chlorhexidine susceptibility. *P. aeruginosa* is considerably more resistant to most of these agents, including chlorhexidine, and (not shown) *Proteus* spp. possesses an above-average resistance to cationic agents such as chlorhexidine and QACs.

**Wijesinghe1 and Weerasinghe2, 2010** studied three disinfectants and antiseptics (Dettol, Lysol, and Chlorox), and in vitro activity of these disinfectants and antiseptics was studied against two challenge strains *Staphylococcus aureus* and *Pseudomonas aeruginosa* were analyzed. All these disinfectants and antiseptics were tested at four different concentrations and three different contact times. Results showed that using double concentration than the recommended use dilution of Lysol had a significant impact on killing both *S. aureus and P. aeruginosa*. Dettol achieved a 99.999% killing of *S. aureus* within 5 minutes at the recommended use dilution and 15-minute contact time showed to be the best in achieving an effective killing of *P. aeruginosa* at the recommended use dilution. The results of Chlorox indicated that a 15-minute contact time was far more effective against S. aureus than the 5-minute contact time specified by the manufacturer. Chlorox achieved a 99.999% killing of *P.aeruginosa* within a contact time of 15 minutes at all concentrations tested. It revealed that the prolonged contact times increased the activity of Chlorox irrespective of the concentration used. At 1 minute contact time, none of the products achieved a 5 log reduction against both *S. aureus* and *P. aeruginosa.* The overall results suggest that Chlorox was the best disinfectant against both *S. aureus* and *P. aeruginosa* at the recommended use dilution.

**Saha et al., 2009** studied the antimicrobial effects of six antiseptics and disinfectants against five pathogenic bacteria. Different pathogens responded differently to different antiseptics and disinfectants. The antibacterial effects of the antiseptics and disinfectants were also concentration dependent. Six types of antiseptics and disinfectants, namely Dettol (Chloroxylenol), Savlon (Chlorhexidine Gluconate and Cetrimide), Iodine, Phenyl, Formalin, and Hydrogen peroxide ($H_2O_2$); and five pathogenic bacteria, *Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Klebsiella species and Escherichia coli* were used in this experiment. For each test, 100ml Luri Bartini broth was inoculated with a few cells of a pathogenic bacterium and incubated at 37°C for 24 hours in a rotary sacker rotated at 120 rpm. After incubation, 1ml of broth culture was spread uniformly on a nutrient agar plate with a sterile glass spreader. The plate was air-dried for a few minutes. Sterile filter paper discs were soaked with 100%, 50%, 25%, 10%, and 5% concentration of commercial form of different antiseptics and disinfectants. Then these discs were placed on inoculated nutrient agar plates which were incubated at 37°C for 24 hours. After incubation, clear zones indicated inhibition of the growth of the microorganisms.

The zones around the discs were measured and recorded. Dettol and Savlon showed moderate antibacterial effects. Antibacterial effect of Dettol was better against *S. aureus*, *S. typhi* and E.coli than against *S. dysenteriae* and K. sp. Similarly, Savlon was more effective against *S. aureus,S. typhi* and *S.dysenteriae* than against *E. coli*. Iodine showed better antibacterial efficacy against *S. aureus* and *E. coli* than against the remaining pathogens. Of these three pathogens, only *S.dysenteriae* was sensitive to a saturated solution of iodine.

# Materials and Methods

## 2.1 Collection of materials

**Collection of antiseptics**: The required antiseptics were collected from a pharmaceutical shop beside the BRAC University, Mohakhali.

**Collection of organisms**: All organisms were collected from the microbiology laboratory's stock at BRAC University. In the lab, all organisms are cultured and stocked in a refrigerator.

## 2.2 Place of experiment

The experiment took place on the 18th floor under lab supervisor Ms.Shamima Akhtar, Microbiology Laboratory of BRAC University, building number – ub02.

# Materials

## 2.3.1 Nutrient Broth Medium:

Nutrient Broth was used for the cultivation of a wide variety of microorganisms.
In the early 1900s, the American Public Health Association (APHA) suggested the formula of Nutrient Agar as a standard culture medium used in water testing.
Nutrient Broth is the same formulation as Nutrient Agar, only Agar has been omitted.
Nutrient Broth Medium is a general-purpose medium used for the examination of water and dairy products according to Standard Methods for the Examination of Water and Wastewater (1) and Dairy Products (2) in accordance with IP. It can also be used for cultivating several less

fastidious microorganisms. Beef extract and peptone provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients to the non-fastidious organisms. Sodium chloride maintains the osmotic equilibrium of the medium.

### Preparation of nutrient broth solution:

In 1000 ml purified/distilled water 25 grams was Suspend. Heat if necessary to dissolve the medium completely. It was sterilized by autoclaving at 10 lbs pressure (115°C) for 30 minutes or alternatively at 15 lbs pressure (121°C) for 15 minutes or as per the validated cycle.

Appearance: Cream to a yellow homogeneous free-flowing powder

## 2.3.2 Nutrient Agar:

Usually, it is used as a general-purpose medium for the growth of a wide variety of non-fastidious microorganisms. It consists of peptone, beef extract, and agar. This relatively simple formulation provides the nutrients necessary for the replication of a large number of non-fastidious microorganisms. Nutrient Agar/broth is used for the cultivation and maintenance of non-fastidious organisms as well as the enumeration of organisms in water, sewage, dairy products, feces, and other materials.

**Preparation of Nutrient Agar**

Nutrient agar and broth are available commercially in powdered (free-flowing, homogeneous) form.

1. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e., 23 gm dehydrated nutrient agar (see the manufacturer instruction) in 1000 ml distilled water.
2. It was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder
3. Then the medium was sterilized by autoclaving (121°C for 15 min)

4. The medium was dispensed into tubes or plates and left the agar medium to solidify and store.
5. The pH of the medium was determined (pH 6.8 +/- 0.2) with a pH meter and adjusted if necessary.

**Uses of Nutrient Agar/broth**

1. For the enumeration of organisms in water, sewage, dairy products, feces, and other materials.
2. For the cultivation and maintenance of non-fastidious species.

**Quality Control**

- The color of prepared Nutrient Agar will be light amber, very slightly to slightly opalescent
- The pH of the prepared media should be 6.8 ± 0.2 (Acharya, 2016).

## 2.3.3 Physiological saline

Saline is useful as a diluent to maintain cell integrity and viability because it lacks properties that may interfere with biochemical reactions and/or antibiotic susceptibility tests. The concentration of sodium chloride in 0.85% (normal) Saline provides osmotic protection for microbial cells. Normal saline is used for preparing microbial suspensions when it is necessary to deliver a set number of microbes to an identification test battery, antimicrobial agents, or growth media used for disk susceptibility testing. It is also used to prepare stock solutions and serial dilutions of antimicrobial agents. The Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS) recommends the use of 0.85% Saline to adjust the turbidity of bacterial suspensions to help maintain cell integrity and viability.

# Methods

## 2.4 Selective Isolation of bacteria from stock culture

From each stock culture plate, single colonies were taken and were streaked on the selective agar plate using four four-way streaking techniques. All the sample plates were incubated at $37^0$c for 24 hours and then preserved at $4^0$C.

Morphological characteristics of isolates:

The colony morphology of various isolates was examined from the plates (according to 'Microbiological Laboratory Manual' by Cappuccinos and Sherman, 1999) and recorded on the basis of colony appearance characters as like size, form, pigmentation, margin, elevation, and opacity (Table 1),( Fig: 1.1).

## 2.5 Biochemical Identification:

Different biochemical tests were performed according to the methods described in the Microbiology Laboratory Manual (Cappuccino et al., 2005). The biochemical tests carried out were:

1) Motility test or Indole test
2) Methyl-red test
3) Voges-Proskauer test
4) Triple sugar iron test
5) Catalase test
6) Citrate utilization test
7) Oxidase test

(Table 2, Fig 1.2)

## 2.5.1Motility test (Indole activity test)

This test detects indole, a by-product of metabolic degradation of the amino acid tryptophan. Bacteria that are positive for indole production possess tryptophanase, the enzyme involved in hydrolyzing and deaminating tryptophan to indole. Some bacteria have the ability to propel themselves through liquids by means of flagella. In semi-solid agar media, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye. Non-motile bacteria generally give growths that are confined to the stab-line, have sharply defined margins and leave the surrounding medium clearly transparent. Motile bacteria typically give diffuse; hazy growths that spread throughout the medium rendering it slightly opaque.

1) The test was carried out in motility indol eurea semisolid media

2) One suspected isolated colony was touched with a straight wire and was stabbed carefully into down the tubes without touching the bottom.
3) Following incubation, the tubes were observed for the presence of motile organisms which will disperse through the medium leaving the stab line spread and making the tube turbid.
4) Production of cherry red reagent layer after addition of Kovac's reagent in MIU medium demonstrates that the substrate tryptophan has been was hydrolyzed which indicates indole positive reaction (Fig1.2D).

## 2.5.2 Methyl red (MR) test

The Methyl Red (MR) test is a colorimetric pH indicator test that detects mixed acid producers and is based upon the final hydrogen ion concentration reached by a culture in glucose broth after prolonged incubation (48 to 72 hours) at 35$^{o}$C. So, this test determines whether the microbe performs mixed acids fermentation when supplied with glucose. Types and proportions of fermentation products produced by anaerobic fermentation of glucose are one of the key taxonomic characteristics that help to differentiate various genera of enteric bacteria. The large

amounts of produced acid after inoculation show a significant decrease in the pH of the media The pH at which methyl red detects the acidity of the medium is lower than that for other indicators; it ranges from pH 6.0 (yellow) to pH 4.4 (red).

1)The bacterium to be tested was inoculated into potassium phosphate broth (MR-VPbroth), which contained dextrose, peptone, and potassium phosphate, and was incubated at 37°C for 24h.

2) Over the 24 hours the mixed-acid-producing organism was expected to produce sufficient acid to overcome the phosphate buffer and remain acidic.

3) The pH of the medium was tested by the addition of five drops of MR reagent. The development of the red color was taken as positive. MR-negative organisms produced a yellow color (Fig 1.2B).

### 2.5.3 Voges -Proskauer test

Several members of *Enterobacteriaceae* produce acetylmethylcarbinol (acetoin) as a major end product of glucose fermentation and smaller quantities of mixed acids. Acetoin is a neutral compound produced from pyruvate (the pivotal compound of glucose fermentation) via the butylene glycol pathway and this compound can be detected by the Voges-Proskauer (VP) test. So, the active product in the medium formed by bacterial metabolism is acetyl methyl carbinol, a product of the butylenes glycol pathway. Pyruvic acid, the pivotal compound in the fermentative degradation of glucose, is further metabolized through various metabolic pathways, depending on the enzyme systems possessed by different bacteria.

1) The bacterium to be tested was inoculated into potassium phosphate broth (MR-VP broth) and incubated for 24 hours.

2) Barritt's reagent A was added to the test broth and shaken.

3) Barrit's reagent B was added and the tube was allowed to stand for 15 min.

4) The appearance of red color was taken as a positive test, a negative tube might be held for an hour after the addition of reagents (Fig1.2C).

## 2.5.4 Triple Sugar Iron (TSI) test

TSI agar slant is a screening medium used to identify the ability of gram-negative bacilli to ferment carbohydrates (glucose, sucrose, and/or lactose) and/or to produce hydrogen sulfide.

This test was performed to assess the mode of sugar utilization. This test is done by stabbing the butt of the media and streaking the bacteria over the slant of Triple Sugar Iron (TSI) agar media.

1) To inoculate, an isolated colony from the respective agar plate was picked with a cool, sterile needle, and stabbed into the TSI, (Himedia, India) containing dextrose, lactose, and sucrose butt.
2) Incubated with caps loosened at 37°C for overnight and examined after 24 hours for carbohydrate fermentation, $CO_2$, and $H_2S$ production.
3) A yellow (acidic) color in the butt indicated that the organism being tested was capable of fermenting all three sugars, whereas a red (alkaline) color in the slant and butt indicated that the organism being tested is a non-fermenting.
4) Detection of $H_2S$ production identified by black precipitation in the butt of the tube.
5) $CO_2$ gas production was indicated by splitting and cracking of the medium (Fig 1.2A).

## 2.5.5 Catalase Test

Catalase is an enzyme that splits $H_20_2$ into water and $O_2$. This test is performed to differentiate between groups of microorganisms based on catalase production.

1) A small amount of bacterial colony was transferred from the

respective agar plate to the surface of a clean, dry glass slide using a clean toothpick.

2) A drop of the catalase reagent (Hydrogen Peroxide) was placed onto the slide and mixed.

3) A positive result gave a rapid evolution of oxygen within 5-10 seconds and was proven by a bubbling reaction.

4) A negative result showed no bubbles or only a few scattered bubbles

5) Following incubation, the tubes showed one of the following

(Fig: 1.2C).

## 2.5.6 Citrate Utilization Test

Simmons citrate agar shows the ability of organisms to utilize citrate as a carbon source. Some members of *Enterobacteriaceae* can utilize citrate as the sole source of carbon for growth. Simmons citrate agar contains sodium citrate as the sole source of carbon, ammonium dihydrogen phosphate as the sole source of nitrogen, other nutrients, and the pH indicator bromthymol blue.

1)Colourless bacterial colonies were picked from the respective agar plate by a straight wire and inoculated into the slope of Simmon's citrate agar (Oxoid ltd, England) and incubated overnight at $37^0$C.

2) If the organism had the ability to utilize citrate, the medium would change from green to Prussian blue color; a negative slant would have no growth of bacteria and would remain green

(Fig: 1.2D).

### Oxidase Test

An oxidase test was performed to differentiate between enteric and non-
Enteric bacteria.

1) A loop full of bacteria from the nutrient agar plate was streaked onto a piece of filter paper (Whatman, 1MM).
2) A few drops of oxidase reagent (N, N, N′, N′-tetramethyl-p-phenylenediamine) were added to the streaked bacteria on the filter paper.
3) Positive reactions turned the bacteria from violet to purple within 1 to 30 seconds. Delayed reactions were ignored.

## 2.6 MIC and MBC Test

The MIC is the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism in tubes. Dilution methods are the most appropriate ones for the determination of MIC values, since they offer the possibility to estimate the concentration of the tested antimicrobial agent in the agar (agar dilution) or broth medium (macrodilution or microdilution). Either broth or agar dilution method may be used to quantitatively measure the *in vitro* antimicrobial activity against bacteria and fungi. MIC value recorded is defined as the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the microorganism tested, and it is usually expressed in µg/ml or mg/l. There are many approved guidelines for dilution antimicrobial susceptibility testing of fastidious or non-fastidious bacteria, yeast, and filamentous fungi. The most recognized standards are provided by the CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). As advised, these guidelines provide a uniform procedure for testing that is practical to perform in most clinical microbiology laboratories (Balouiri et al., 2016).

The MBC is the lowest concentration of antibiotic required to kill a particular bacterium. At the end of 24 h of incubation, the tubes were read for the MIC and then the MBC was determined by sampling all the macroscopically clear tubes (1 dilution below the MIC was used for the levels to be assessed in the MBC assay). The suspension was inoculated onto plates of blood agar or nutrient agar (here in the study the nutrient agar is used). The plates were incubated for 24 h at 37 °C (Yilmaz, 2012). Working bacteria culture was adjusted to be equal to 0.5 McFarland standards ($1 \times 10^8$ cfu/ml) (Zainol et al., 2013). Bactericidal antibiotics usually have an MBC equal to or very similar to the MIC, whereas bacteriostatic antibiotics usually have an MBC significantly higher than the MIC (Street, 2014).

**Methods that were followed during the MIC and MBC study**:

1. Dilution was done in this way: 5 fold, 10 fold, 20 fold, 40 fold, 80 fold, 160 fold, 320 fold and so on.
2. The required amount of nutrient broth, and psychological saline was prepared the day before the dilution of every experiment.
3. Subculture of four selected bacteria was also done one day before the dilution so that fresh cultured bacterial growth could be obtained.
4. For the dilution at first bacterial suspension was prepared.
5. Then the dilution process was done for all tubes, the dilution process or measurement for dilution is given in 2.1A
6. Then the dilution tubes were incubated at $37^0$C for 24 hours.
7. After the incubation of the dilution tubes, 100 µl solutions from each dilution tube were transferred to the large Petri dish through a pipette and then it was spread with the help of a spreader.
8. After the spreading, the plates were incubated at $37^0$C for 24 hours
9. After the incubation period, the bacterial growths were observed for each plate and the MIC and MBC were also identified through the observation of the growth of bacteria.

MIC was the plate that had very little or minimum growth and 1 dilution below the MIC was used for the levels to be assessed in the MBC assay (Table 3, Result 2.6.1, Fig 1.3).

**The measurement of dilution:**

The total volume was 5000µl for every dilution tube

For the first tube, 5 fold dilution: antiseptic amount was 1000µl, bacterial suspension was 100µl and nutrient broth was 3900µl.

For the second tube, 10fold dilution: antiseptic amount was 500µl, bacterial suspension was 100µl and nutrient broth amount was 4400µl.

For the third tube, 20 fold dilution: antiseptic amount was 250µl, bacterial suspension was 100µl and nutrient broth amount was 4650µl.

For the fourth tube, 40 fold dilution: antiseptic amount was 125µl, bacterial suspension was 100µl and nutrient broth amount was 4775µl.

For the fifth tube, 80 fold dilution: antiseptic amount was 62.5µl, bacterial suspension was 100µl and nutrient broth amount was 4837.5µl

For the six no. tube, 160 fold dilution: antiseptic amount was 31.25µl, bacterial suspension was 100µl and nutrient broth amount was 4868.75µl

For the $7^{th}$ tube, 320 fold dilution: antiseptic amount was 15.625µl, bacterial suspension was 100µl and nutrient broth amount was 4884.38µl

For $8^{th}$ tube, 640 fold dilution: antiseptic amount was 7.82µl, bacterial suspension was 100µl and nutrient broth amount was 4892.19µl

Also in some cases, 420 fold dilution is considered,

For 420 fold dilution, the antiseptic amount was 11.9µl, bacterial suspension was 100µl and the nutrient broth amount was 4888.1µl

## 2.7 Agar disk-diffusion method

Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeast testing. Although not all fastidious bacteria can be tested accurately by this method, the standardization has been made to test certain fastidious bacterial pathogens like streptococci, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Neisseria gonorrhoeae,* and *Neisseria meningitidis*, using specific culture media, various incubation conditions and interpretive criteria for inhibition zones.

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, an antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism, and then the diameters of inhibition growth zones are measured. Antibiogram provides qualitative results by categorizing bacteria as susceptible, intermediate, or resistant. Therefore, it is a typing tool based on the resistance phenotype of the microbial strain tested, its outcomes also guide clinicians in the appropriate selection of initial empiric treatments, and antibiotics used for individual patients in particular situations. However, since bacterial growth inhibition does not mean bacterial death, this method cannot distinguish bactericidal and bacteriostatic effects.

Moreover, the agar disk-diffusion method is not appropriate to determine the minimum inhibitory concentration (MIC), as it is impossible to quantify the amount of the antimicrobial agent diffused into the agar medium. Nevertheless, an approximate MIC can be calculated for some microorganisms and antibiotics by comparing the inhibition zones with stored algorithms.

Nevertheless, disk-diffusion assay offers many advantages over other methods: simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease of interpreting results provided (Balouiri et al., 2016).

**Procedure:**

1. Nutrient agar plates were obtained that contained solid nutrient agar.
2. The backside of the plate was divided into 4 equal sections using the marker.
3. Any two antiseptics and a bacteria were chosen. Also, a positive control and a negative control were chosen. For the Positive control (that can inhibit bacterial growth) the ciprofloxacin was chosen and as a negative control normal Physiological saline was chosen, which cannot inhibit bacterial growth.
4. The 4 sections and the initials of the bacteria were labeled on the backside of the plate for the antiseptic or disinfectant.
5. Then the bacterial suspension was prepared and vortex properly for well-mixing
6. Then a sterilized cotton swab was used to dip inside the tube containing bacterial suspension and then the surface of the nutrient agar plate was spread properly through that cotton swab which contained the bacterial suspension.
7. Then, each of the two filter paper disc was soaked with a different antiseptic (10 µl of a specific amount of antiseptic) and one filter paper disc was soaked with saline solution, and an antibiotic disc (ciprofloxacin) was taken.
8. The forceps were flamed and each filter paper disc was transferred to the nutrient agar taking care to place it in the center of the appropriate marked section. Also, the antibiotic disc was placed in its proper position.

9. The discs were tapped lightly with the forceps to make sure it adheres to the agar when the plate was inverted.

10. After that, the plates were incubated at $37^0$C for 24 hours and then the zones were observed and measured with the measuring scale. (Table 4)

**2.8. Data analysis:** The data were analyzed using the software Microsoft excel version 10.

# Result

In the present study, the effectiveness of 4 antiseptics (Dettol, Hexisol, Oralon, Betadine) was observed using the organisms (*S. aureus, Klebsiella pneumonia, B. cereus, Pseudomonas aeruginosa)* as test organism. Before MIC and MBC tests, the biochemical test was done to confirm the identity of the bacteria.

Table1: Biochemical test to confirm the identity of the organism

| Name of the Organism | Selective Media | Characteristics of the Appearance Of colony |
|---|---|---|
| *1.S. aureus* | 1. Mannitol salt agar (MSA) | 1. Ferments Mannitol, acid is produced and changes the pH of the medium to acidic. Yellow colonies; may have yellow halo around colonies. Basic shape of colony: Circular, Elevation: Convex, Margin: entire. |
| 2. *Klebsiella pneumoniae* | 2.MacConkey agar | 2. Mucoid, convex, lactose positive colonies. Pink to brick red colonies with or without a zone of precipitated bile. |
| 3. *B. cereus* | 3.Bacillus Cereus Selective Agar | 3. Have distinctive turquoise green to peacock blue color colony surrounded by a zone of precipitate of the same color. Microscopic examination for the presence of lipid globules in the vegetative cells.Overall blue colonies with opaque halo. |
| 4. *Pseudomonas aeruginosa* | 4.Cetrimide Agar | 4. Mucoid colonial phenotype, production**of** water-soluble pigmented colonies. Have yellow-green to blue color colonies which indicate the production of pyocyanin (blue-green), and pyoverdine (yellow-green, fluorescent). Colonies are smooth, flat, mucoid, grape like odor. |

Table 2: Result of Biochemical test

| **Organisms** | Biochemical tests | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TSI Fermentation | | | | | |
| | Indole production test | Methyl red reaction test | Voges Proskauer reaction test | Citrate utilization test | Slant | Butt | $CO_2$ | $H_2S$ | Catalase activity test | Oxidase activity test |
| *Staphylococcus aureus* | - | + | + | + | | | - | - | + | - |
| *Pseudomonas aeruginosa* | - | - | - | + | **K** | **K** | - | - | + | + |
| *Klebsiella pneumoniae* | - | - | + | + | **A** | **A** | + | - | + | - |
| *Bacillus cereus* | - | - | - | - | **A** | **A** | - | - | + | + |
| | | | | | | | | | | |

## 2.6.1 MIC and MBC Test Result

### 2.6.1.1 Against *Bacillus.cereus* the observed result is given below:

After dilution and the incubation for 24 hours, 100µl of the diluted solution from each test tube was spread in nutrient agar plate for another 24 hours and the result for each antiseptic are -

**For Dettol**: In a bottle of 100ml, 4.8% w/v or 48000µg/ml chloroxylenol was present.

Chlorhexanol at a concentration of 9600µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 4800µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 2400 µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 1200 µg/ml (40 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of 0.4x101/100 µl($4x10^1$/ml) in the nutrient agar plate.

Chlorhexanol at a concentration of 600 µg/ml (80 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $1.0\times10^1$ /100 µl ($1.00\times10^2$ /ml) in the nutrient agar plate.

Chlorhexanol at a concentration of 300 µg/ml (160 fold dilution) effectively killed almost 100% (99.999963%) of the organism present in the test tube and showed a CFU of $3.7\times10^1$ /100 µl ($3.70\times10^2$ /ml) in the nutrient agar plate.

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC of Dettol against *Bacillus .cereus* was 1200 µg/ml (1200µg chloroxylenol per 1ml ) and MBC of Dettol was 2400 µg/ml ( Fig 1.3A ).

**For Hexisol:**

In a bottle of 100ml, 0.5% w/v or 5000µg/ml chlorhexidine gluconate was present. Chlorhexidine gluconate at a concentration of 1000 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 500 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 250 µg/ml (20 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.3\times10^{1}$/100 µl ($3\times10^{1}$/ml) in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 125µg/ml (40 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $1.0\times10^{1}$ /100 µl ($1.00\times10^{2}$ /ml) in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 62.5µg/ml (80 fold dilution) effectively killed almost 100% (99.99972%) of the organism present in the test tube and showed a CFU of $2.8\times10^{1}$ /100 µl ($2.80\times10^{2}$ /ml) in nutrient agar plate

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC of Hexisol against *Bacillus.cereus* was 250 µg/ml (250µg chlorhexidine gluconate per 1ml), and MBC was 500 µg/ml (Fig 1.3B).

**For Oralon:**

In a bottle of 100ml, 0.2% w/v or 2000µg/ml chlorhexidine gluconate was present.

Chlorhexidine gluconate at a concentration of 400 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 200 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 100 µg/ml (20 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.4\times10^{1}$/100 µl ($4\times10^{1}$/ml) in the nutrient agar plate.

Chlorhexanol at a concentration of 50 µg/ml (40 fold dilution) effectively killed almost 100% (99.9998%) of the organism present in the test tube and showed a CFU of $1.2\times10^{1}$/100 µl ($1.20\times10^{2}$/ml) in the nutrient agar plate.

Chlorhexanol at a concentration of 25 µg/ml (80 fold dilution) effectively killed almost 100% (99.9997%) of the organism present in the test tube and showed a CFU of $3.0\times10^{1}$/100 µl ($3.0\times10^{2}$/ml) in the nutrient agar plate.

Chlorhexanol at a concentration of 12.5 µg/ml (160 fold dilution) effectively killed almost 100% (99.9993%) of the organism present in the test tube and showed a CFU of $6.1\times10^{1}$/100 µl ($6.1\times10^{2}$/ml) in the nutrient agar plate.

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC of Oralon against *Bacillus.cereus* was 100µg/ml (100µg chlorohexidine gluconate per 1ml), and MBC was 200 µg/ml (Fig 1.3C).

**For Betadine:**

In a bottle of 100ml, 5% w/v or 50000µg/ml Povidone-iodine was present.

Povidone-iodine at a concentration of 10000 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 5000 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 2500 µg/ml (20 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.1\times10^{1}$/100 µl ($1\times10^{1}$/ml) in nutrient agar plate

Povidone-iodine at a concentration of 1250 µg/ml (40 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.4\times10^{1}$/100 µl ($4\times10^{1}$/ml) in the nutrient agar plate

Povidone-iodine at a concentration of 625 µg/ml (80 fold dilution) effectively killed almost 100% (99.99987%) of the organism present in the test tube and showed a CFU of $1.3\times10^{1}$/100 µl ($1.3\times10^{1}$/ml) in the nutrient agar plate

Povidone-iodine at a concentration of 312.5 µg/ml (160 fold dilution) effectively killed almost 100% (99.9997%) of the organism present in the test tube and showed a CFU of $3.0\times10^{1}$/100 µl ($3.0\times10^{2}$/ml) in the nutrient agar plate

So, after observing the turbidity and also observing the colonies of plate, it was detected that MIC of Oralon against *Bacillus.cereus* was 2500µg/ml (2500µg povidone iodine per 1ml), and MBC was 5000 µg/ml (Fig: 1.3D).

**2.6.1.2Against *Pseudomonas aeruginosa,* the observed result is given below**:

After dilution and the incubation of 24 hours, 100µl of the diluted solution from each test tube was spreaded in a nutrient agar plate for another 24 hours and the results for each antiseptic are -

**For Dettol**: In a bottle of 100ml, 4.8% w/v or 48000µg/ml chloroxylenol was present.

Chlorhexanol at a concentration of 9600µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 4800µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 2400µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 1200µg/ml (40 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 600µg/ml (80 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 300 µg/ml (160 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $2.85\times10^2$ /100 µl($2.850\times10^3$ /ml) in nutrient agar plate

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC of Dettol against *Pseudomonas aeruginosa*was 600µg/ml (600µg chloroxylenol per 1ml) and MBC was 600 µg/ml (Fig1.3E).

**For Hexisol:**

In a bottle of 100ml, 0.5% w/v or 5000µg/ml chlorhexidine gluconate was present.

Chlorhexidine gluconate at a concentration of 1000 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 500 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 250 µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 125µg/ml (40 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.1\times10^1$ /100 µl ($1\times10^1$ /ml) in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 62.5 µg/ml (80 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.3\times10^1$ /100 µl ($3\times10^1$ /ml) in the nutrient agar plate

Chlorhexidine gluconate at a concentration of 31.25 µg/ml (160 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.7\times10^1$ /100 µl ($7\times10^1$ /ml) in nutrient agar plate

Chlorhexidine gluconate at a concentration of 15.62 µg/ml (320 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $1.60\times10^{2}$ /100 µl ($1.600\times10^{3}$ /ml) in nutrient agar plate

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC of Hexisol against *Pseudomonas aeruginosa* was 125µg/ml (125µg chlorhexidine gluconate per 1ml), and MBC was 250 µg/ml (Fig 1.3F).

**For Oralon:**

In a bottle of 100ml, 0.2% w/v or 2000µg/ml chlorhexidine gluconate was present.

Chlorhexidine gluconate at a concentration of 400 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 200 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 100 µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 50 µg/ml (40 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.2\times10^{1}$/100 µl ($2\times10^{1}$/ml) in nutrient agar plate

Chlorhexanol at a concentration of 25 µg/ml (80 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.3\times10^{1}$/100 µl ($3\times10^{1}$/ml) in nutrient agar plate

Chlorhexanol at a concentration of 12.5 µg/ml (160 fold dilution) effectively killed almost 100% (99.9982%) of the organism present in the test tube and showed a CFU of $1.80\times10^2$/100 µl ($1.8\times10^3$/ml) in nutrient agar plate

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC of Oralon against *Pseudomonas aeruginosa* was 50µg/ml (50µg chlorohexidine gluconate per 1ml), and MBC was 100 µg/ml (Fig 1.3G).

**For Betadine:**

In a bottle of 100ml, 5% w/v or 50000µg/ml Povidone-iodine was present.

Povidone-iodine at a concentration of 10000 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 5000 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 2500 µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 1250 µg/ml (40 fold dilution) could not effectively kill the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed TNTC colonies in the nutrient agar plate.

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC and MBC of betadine against *Pseudomonas aeruginosa* was 2500µg/ml (2500µg povidone iodine per 1ml) (Fig 1.3H).

**2.6.1.3Against *Klebsiella pneumoniae,* the observed result is given below:**

After dilution and the incubation of 24 hours, 100µl of the diluted solution from each test tube was spreaded in a nutrient agar plate for another 24 hours and the results for each antiseptic are-

**For Dettol:** In a bottle of 100ml, 4.8% w/v or 48000µg/ml chloroxylenol was present.

Chlorhexanol at a concentration of 9600µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 4800µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 2400µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 1200µg/ml (40 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 600µg/ml (80 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 300 µg/ml (160 fold dilution) effectively killed almost 100% (99.998%) of the organism present in the test tube and showed a CFU of $1.26\times10^2$/100 µl ($1.260\times10^3$ /ml) in nutrient agar plate

Chlorhexanol at a concentration of 150 µg/ml (320 fold dilution) effectively killed almost 100% (99.998%) of the organism present in the test tube and showed a CFU of $1.80\times10^2$/100 µl ($1.800\times10^3$ /ml) in the nutrient agar plate.

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC of Dettol against *Klebsiella pneumoniae* was 300µg/ml (300µg chloroxylenol per 1ml) and MBC was 600 µg/ml (Fig 1.3I ).

**For Hexisol:**

In a bottle of 100ml, 0.5% w/v or 5000µg/ml chlorhexidine gluconate was present.

Chlorhexidine gluconate at a concentration of 1000 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 500 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 250 µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 125 µg/ml (40 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 62.5 µg/ml (80 fold dilution) effectively killed almost 100% (99.998%) of the organism present in the test tube and showed a CFU of $1.62\times10^2$ /100 µl ($1.620\times10^3$ /ml) in nutrient agar plate

Chlorhexidine gluconate at a concentration of 31.25 µg/ml (160 fold dilution) could not effectively kill the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed TNTC colonies in the nutrient agar plate.

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC for Hexisol against *Klebsiella pneumoniae*was 62.5µg/ml (62.5µg chlorhexidine gluconate per 1ml), and MBC was 125 µg/ml (Fig 1.3J).

**For Oralon:**

In a bottle of 100ml, 0.2% w/v or 2000µg/ml chlorhexidine gluconate was present.

Chlorhexidine gluconate at a concentration of 400 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 200 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 100 µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 50 µg/ml (40 fold dilution) effectively killed almost 100% (99.9988%) of the organism present in the test tube and showed a CFU of $1.20\times10^2$/100 µl ($1.200\times10^3$/ml) in nutrient agar plate

Chlorhexidine gluconate at a concentration of 25 µg/ml (80 fold dilution) effectively killed almost 100% (99.9984%) of the organism present in the test tube and showed a CFU of $1.58\times10^2$/100 µl ($1.580\times10^3$/ml) in nutrient agar plate

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC for Oralon against *Klebsiella pneumoniae*was 50µg/ml (50µg chlorohexidine gluconate per 1ml), and MBC was 100 µg/ml (Fig 1.3K ).

**For Betadine:**

In a bottle of 100ml, 5% w/v or 50000µg/ml Povidone-iodine was present.

Povidone-iodine at a concentration of 10000 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 5000 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 2500 µg/ml (20 fold dilution) effectively killed almost 100% (99.998%) of the organism present in the test tube and showed a CFU of $1.42\times10^2$ /100 µl ($1.420\times10^3$/ml) in nutrient agar plate

Povidone-iodine at a concentration of 1250 µg/ml (40 fold dilution) effectively killed almost 100% (99.997%) of the organism present in the test tube and showed a CFU of $2.94\times10^2$/100 µl ($2.940\times10^3$/ml) in nutrient agar plate

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC for Betadine against *Klebsiella pneumoniae* was 2500µg/ml (2500µg povidone iodine per 1ml), and MBC was 5000 µg/ml (Fig 1.3L ).

**2.6.1.4 Against *Staphylococcus aureus,* the observed result is given below**:

After dilution and the incubation of 24 hours, 100µl of the diluted solution from each test tube was spreaded in nutrient agar plate for another 24 hours and the result for each antiseptic are-

**For Dettol:** In a bottle of 100ml, 4.8% w/v or 48000µg/ml chloroxylenol was present.

Chlorhexanol at a concentration of 9600µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 4800µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexanol at a concentration of 2400µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 1200µg/ml (40 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 600µg/ml (80 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 300µg/ml (160 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate

Chlorhexanol at a concentration of 150 µg/ml (320 fold dilution) effectively killed almost 100% (99.998%) of the organism present in the test tube and showed a CFU of $2.5\times10^{1}$/100 µl ($2.50\times10^{2}$ /ml) in the nutrient agar plate.

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC for Dettol against *Staphylococcus aureus* was 150µg/ml (150 µg chloroxylenol per 1ml) and MBC was 300 µg/ml (Fig 1.3M).

**For Hexisol:**

In a bottle of 100ml, 0.5% w/v or 5000µg/ml chlorhexidine gluconate was present.

Chlorhexidine gluconate at a concentration of 1000 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 500 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 250 µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 125 µg/ml (40 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 62.5 µg/ml (80 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 31.25 µg/ml (160 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $2.40\times10^2$/100 µl ($2.400\times10^3$ /ml) in nutrient agar plate

Chlorhexidine gluconate at a concentration of 15.62 µg/ml (320 fold dilution) could not effectively kill the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed TNTC colonies in the nutrient agar plate.

So, after observing the turbidity of the tube and also observing the colonies of the plate, it was detected that MIC for Hexisol against *Staphylococcus aureus*was 31.25µg/ml (31.25µg chlorhexidine gluconate per 1ml), and MBC was 62.5 µg/ml (Fig 1.3).

**For Oralon:**

In a bottle of 100ml, 0.2% w/v or 2000µg/ml chlorhexidine gluconate was present.

Chlorhexidine gluconate at a concentration of 400 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 200 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 100 µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 50 µg/ml (40 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Chlorhexidine gluconate at a concentration of 25 µg/ml (80 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $0.1\times10^1$ /100 µl ($1.0\times10^1$/ml) in nutrient agar plate

Chlorhexidine gluconate at a concentration of 12.5 µg/ml (160 fold dilution) effectively killed almost 100% (99.999%) of the organism present in the test tube and showed a CFU of $5.8\times10^1$ /100 µl ($5.80\times10^2$/ml) in nutrient agar plate

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC for Oralon against*Staphylococcus aureus*was 25µg/ml (25µg chlorohexidine gluconate per 1ml), and MBC was 50 µg/ml. (Fig 1.30)

**For Betadine:**

In a bottle of 100ml, 5% w/v or 50000µg/ml Povidone-iodine was present.

Povidone-iodine at a concentration of 10000 µg/ml (5 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 5000 µg/ml (10 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 2500 µg/ml (20 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 1250 µg/ml (40 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 625 µg/ml (80 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 32.5 µg/ml (160 fold dilution) effectively killed 100% of the organism present in the test tube (concentration of the bacteria was mentioned in the 2.6 section of the maerials and methods chapter) and showed no growth in the nutrient agar plate.

Povidone-iodine at a concentration of 59.5 µg/ml (420 fold dilution) effectively killed almost 100% (99.9999%) of the organism present in the test tube and showed a CFU of $1.0\times10^{1}$/100 µl ($1.00\times10^{2}$/ml) in nutrient agar plate

Povidone-iodine at a concentration of 50.5 µg/ml (500 fold dilution) effectively killed almost 100% (99.9998%) of the organism present in the test tube and showed a CFU of $2.0\times10^{1}$/100 µl ($2.00\times10^{2}$/ml) in nutrient agar plate

So, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC for Betadine against *Staphylococcus aureus* was 59.5 µg/ml (59.5µg povidone iodine per ml), and MBC was 312.5 µg/ml (Fig 1.3P).

## Table 3: Result of the MIC and MBC of antiseptics against the selected bacteria

| Name of organism | Dettol | | Hexisol | | Oralon | | Betadine | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| *Bacillus.cereus* | 1200 µg/ml | 2400 µg/ml | 250 µg/ml | 500 µg/ml | 100 µg/ml | 200 µg/ml | 2500 µg/ml | 5000 µg/ml |
| *Staph.aureus* | 150 µg/ml | 300 µg/ml | 31.25 µg/ml | 62.5 µg/ml | 25 µg/ml | 50 µg/ml | 59.5 µg/ml | 50.0 µg/ml |
| *K.Pneumoniae* | 300 µg/ml | 600 µg/ml | 62.5 µg/ml | 125 µg/ml | 50 µg/ml | 100 µg/ml | 2500 µg/ml | 5000 µg/ml |
| *Pseudomonas. aurogenus* | 600 µg/ml | 600 µg/ml | 125 µg/ml | 250 µg/ml | 50 µg/ml | 100 µg/ml | 2500 µg/ml | 2500 µg/ml |

## Table 4: Result of disk diffusion method

| Name of organism | Antibiotic ciprofloxicine | Saline | Dettol | Hexisol | Oralon | Betadine |
|---|---|---|---|---|---|---|
| *S.aureus* | 30 mm | NO | 28 mm | 18 mm | 17 mm | 16 mm |
| *K.Pneumoniae* | 29 mm | NO | 15 mm | 16 mm | 9 mm | 11 mm |
| *Bacillus.cereus* | 25 mm | NO | 18 mm | 15 mm | 12 mm | 12 mm |
| *Pseudomonas. aurogenus* | 35 mm | NO | 12 mm | 16 mm | 13 mm | 11 mm |

## Figure 1.1 Bacterial Growths on Selective Media

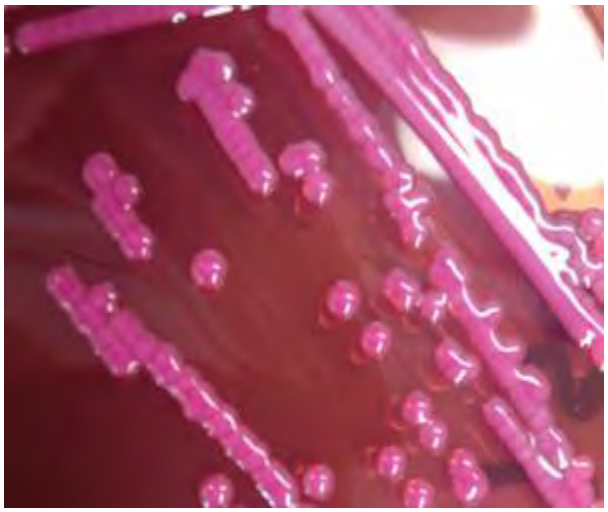


Fig: *Klebsiella pneumoniae*on macconkey agar

Fig: *staphylococcus aureus* on MSA agar

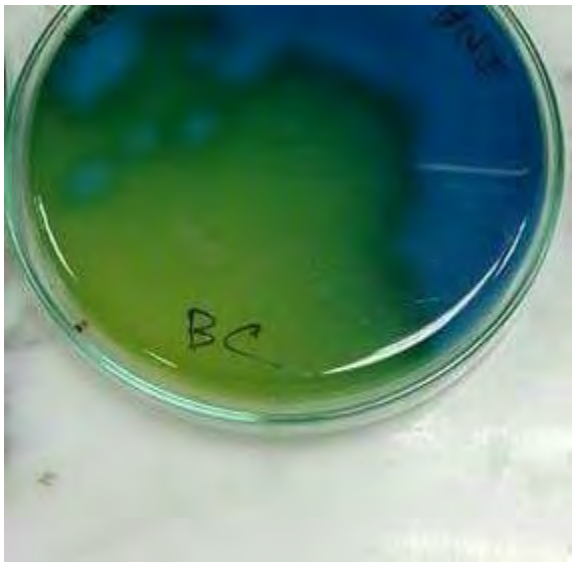



Fig: *Bacillus cereus* on BC agar

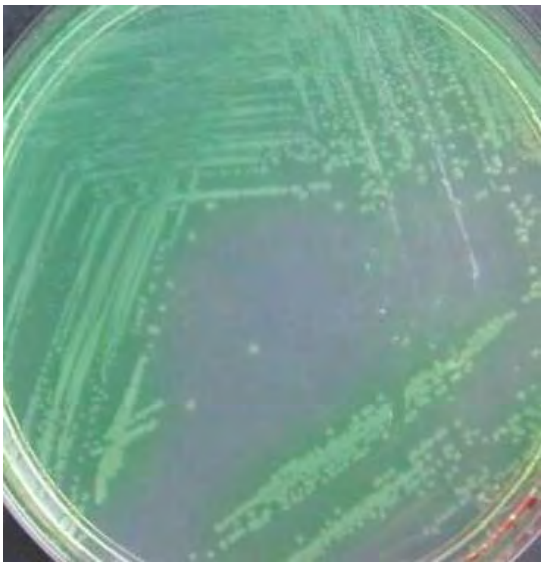


Fig: *Pseudomonas aeruginosa* on cetrimide agar

## Figure 1.2 Biochemical test


Fig 1.2A: TSI test

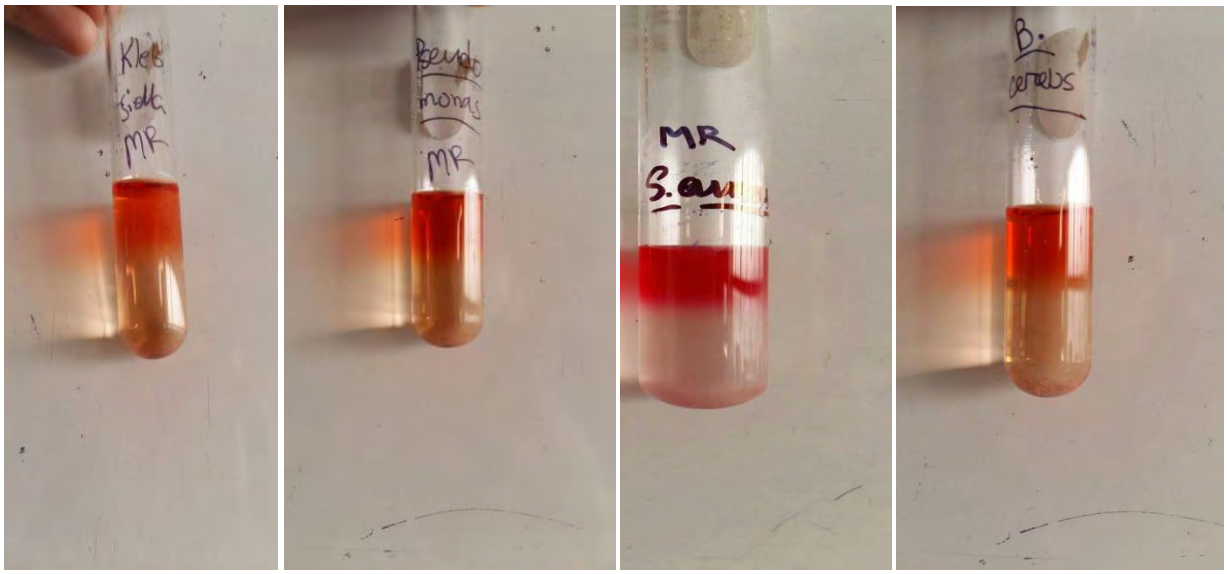



Fig1.2B: MR test

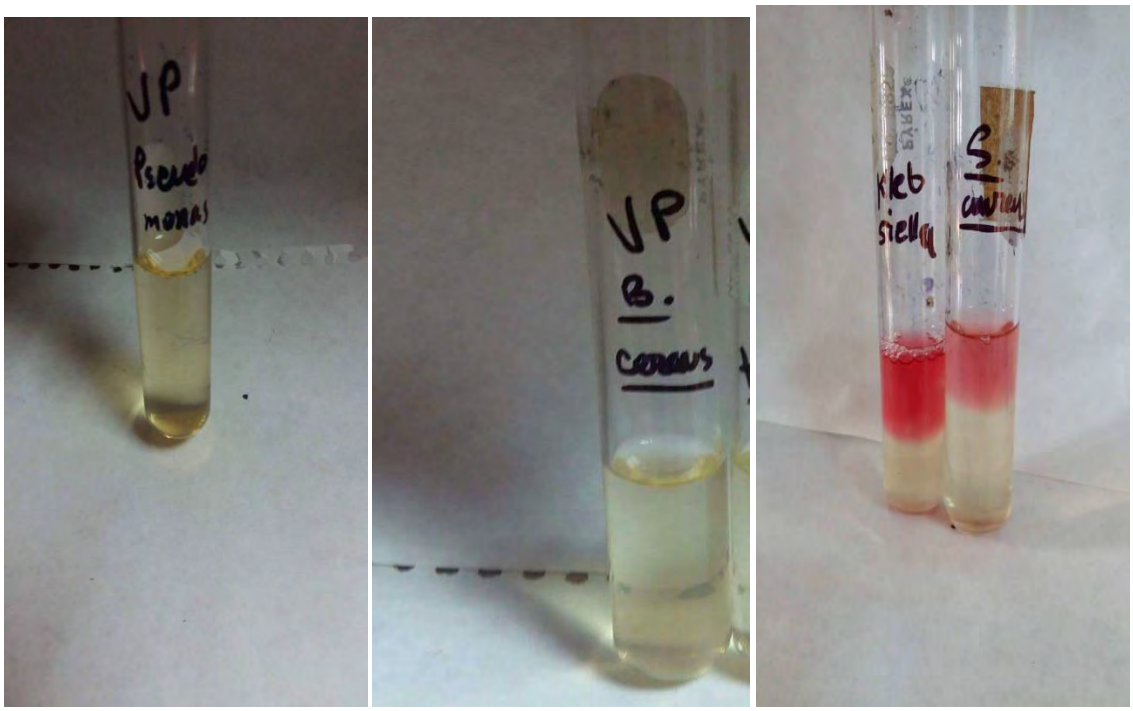



Fig1.2C: VP test

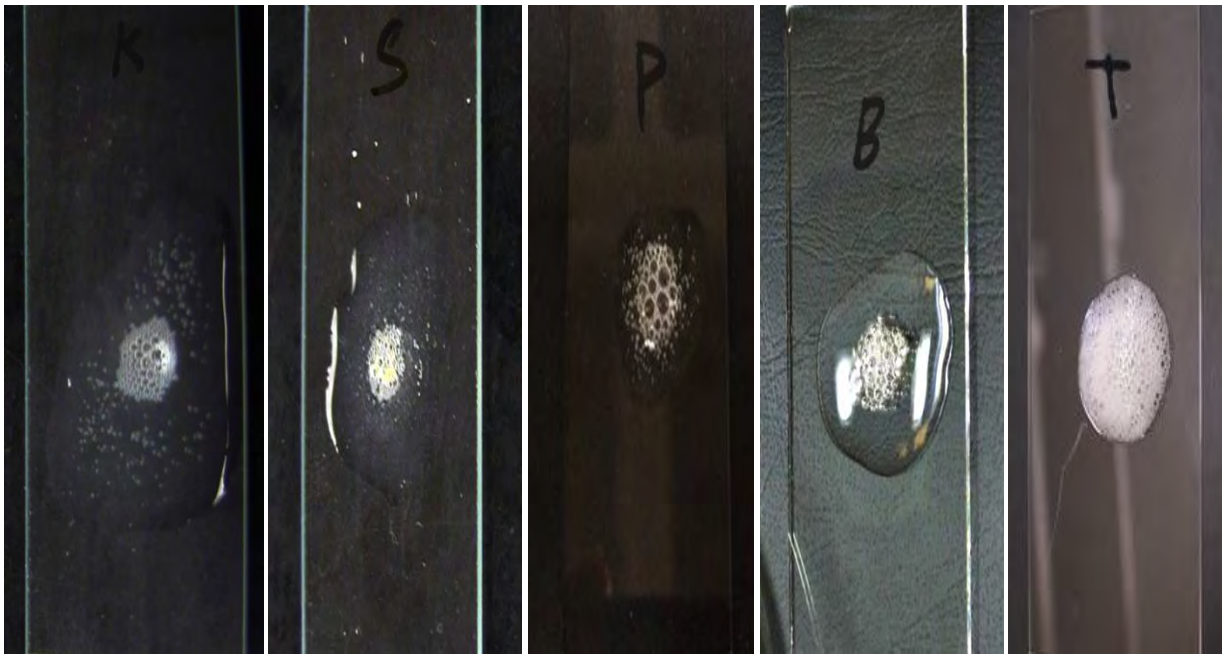



Fig 1.2C: Catalase test

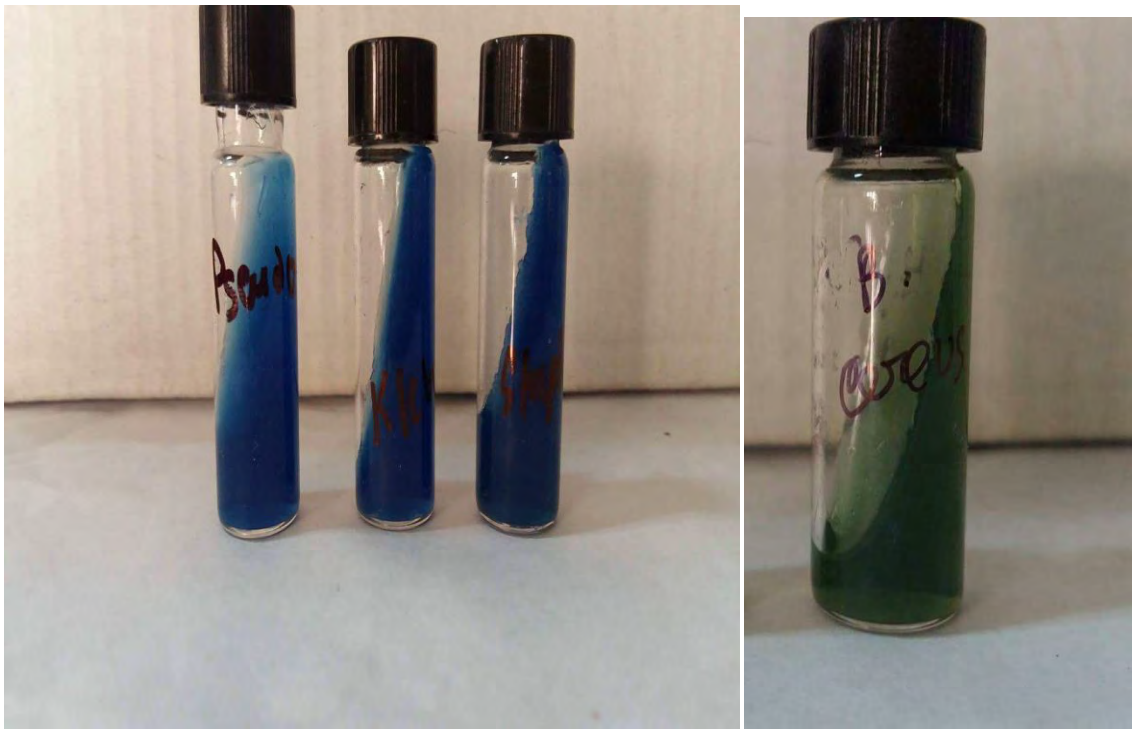
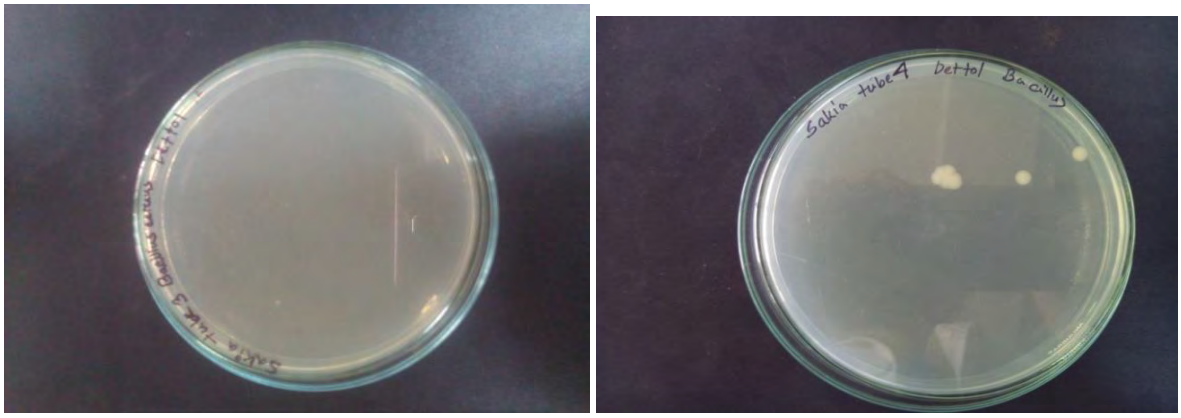


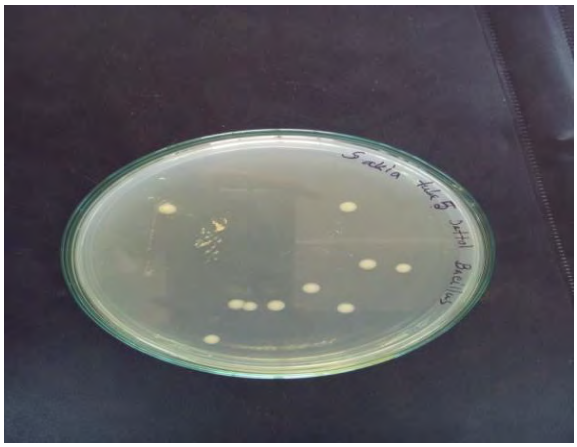
Fig1.2D: Citrate test

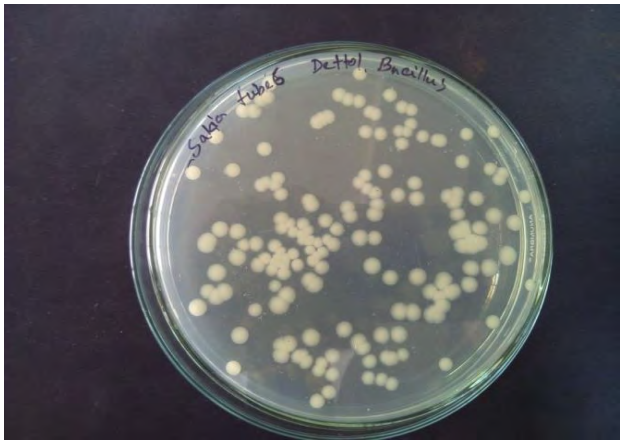
## Figure1.3 MIC and MBC test




2400 µg/mlchloroxylenol, no colony

1200µg/mlchloroxylenol,CFU $0.4\times10^{1}$/100µl

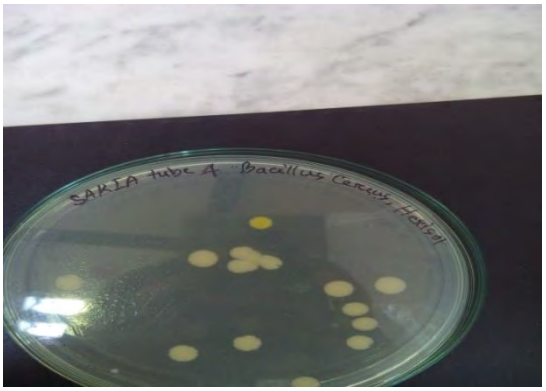




600µg/ml chloroxylenol, 300µg/mlchloroxylenol,

CFU $1.0\times10^{1}$/100µl CFU $3.7\times10^{1}$/100 µl

**Fig 1.3A**: MIC and MBC test of Dettol against *B. cereus*

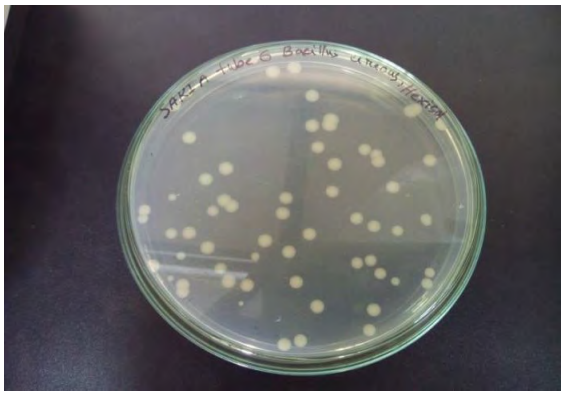


250µg/mlchlorhexidine gluconate, 250µg/ml chlorhexidine gluconate,

CFU $0.3\times10^{1}$/100µl CFU $0.3\times10^{1}$/ 100µl


62.5µg/ml chlorhexidine gluconate,

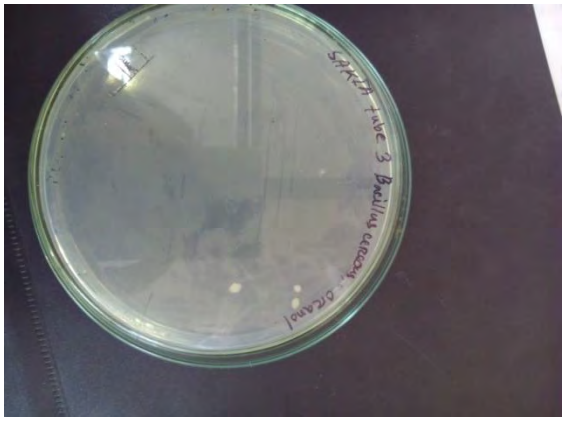
CFU $2.8\times10^{1}$ /100µl



31.25µg/ml chlorhexidine gluconate,

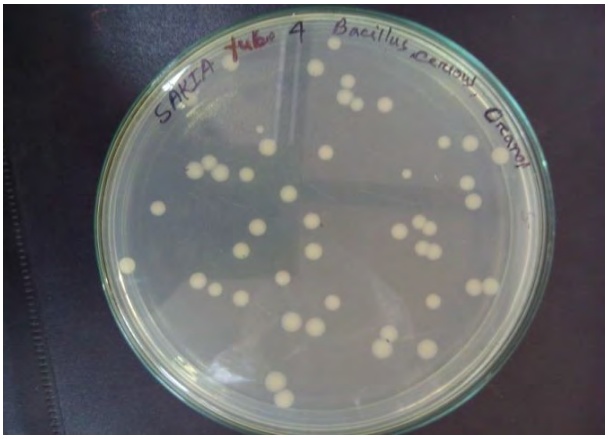
CFU $5.5\times10^{1}$ /100µl

**Fig 1.3B**: MIC and MBC test of Hexisol against *B. cereus*



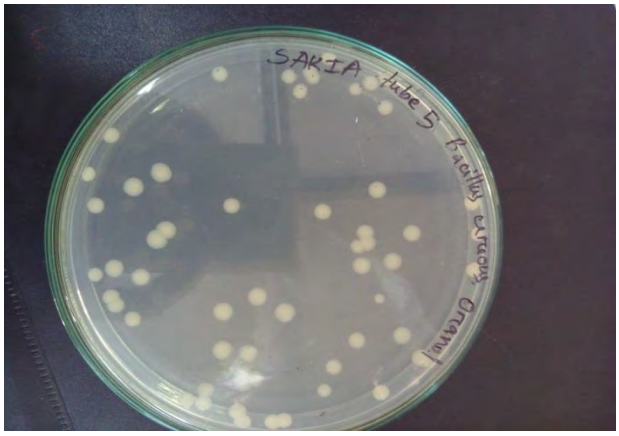
100µg/ml chlorhexidine gluconate,

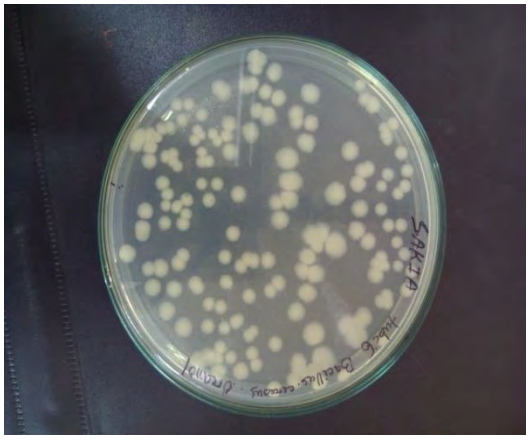
CFU $0.4\times10^{1}$/100 µl




50µg/ml chlorhexidinegluconate,

CFU $1.2\times10^{1}$/100 µl

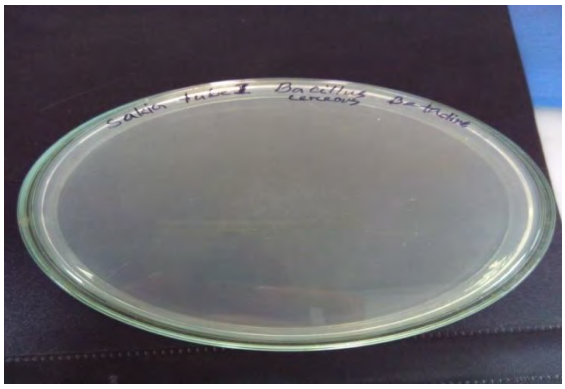


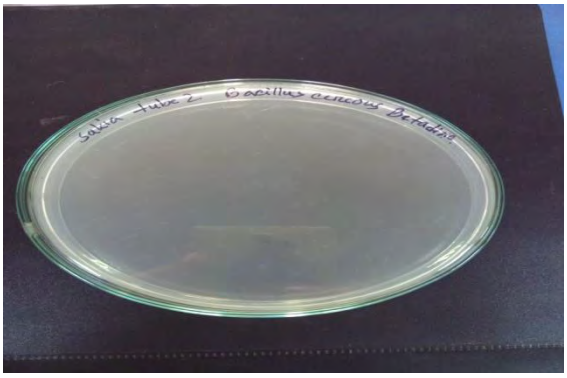



25µg/ml chlorhexidine gluconate, 12.5µg/ml chlorhexidine gluconate

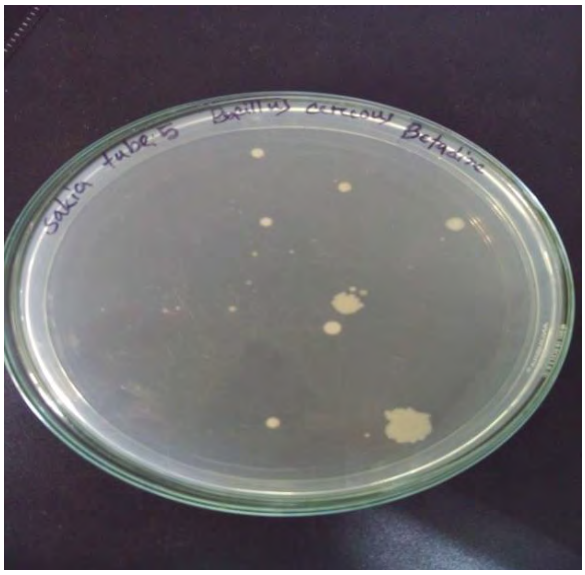
CFU $3.0\times10^{1}$/100µl CFU $6.1\times10^{1}$/100 µl

**Fig1.3C**: MIC and MBC test of Oralon against *B. cereus*





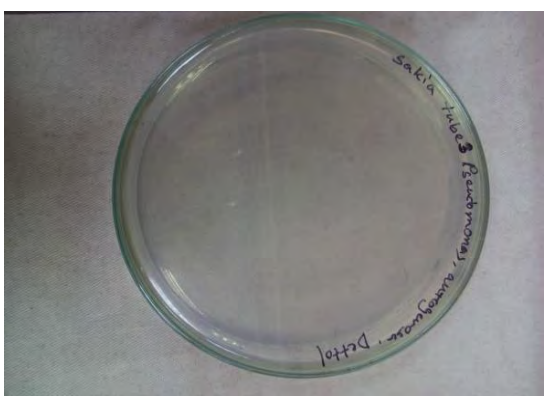
10000µg/ml Povidone-iodine, no colony 5000µg/ml Povidone-iodine, no colony



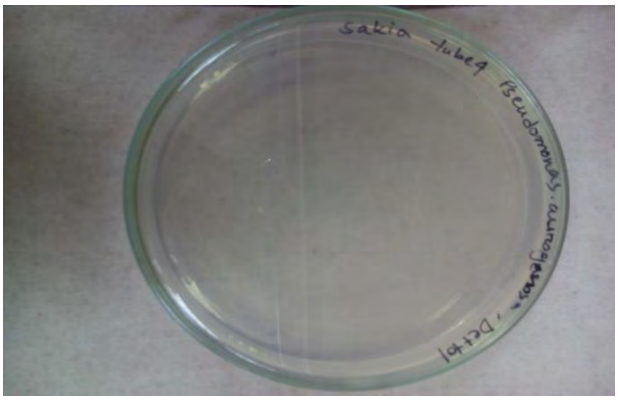
2500µg/ml Povidone-iodine, 625µg/mlPovidone-iodine,

CFU $0.1\times10^{1}$ /100µl CFU $1.3\times10^{1}$/100µl

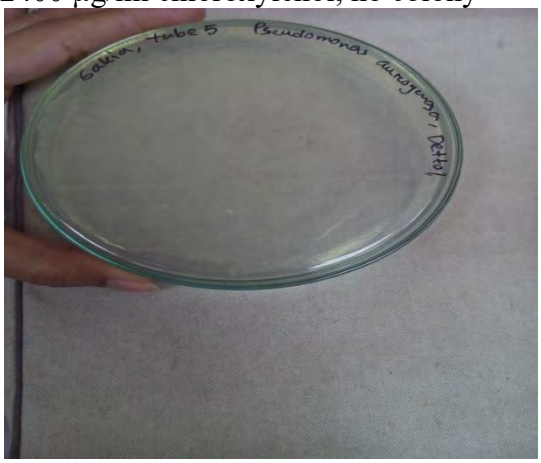
**Fig1.3D**: MIC and MBC test of Betadine against *B. cereus*



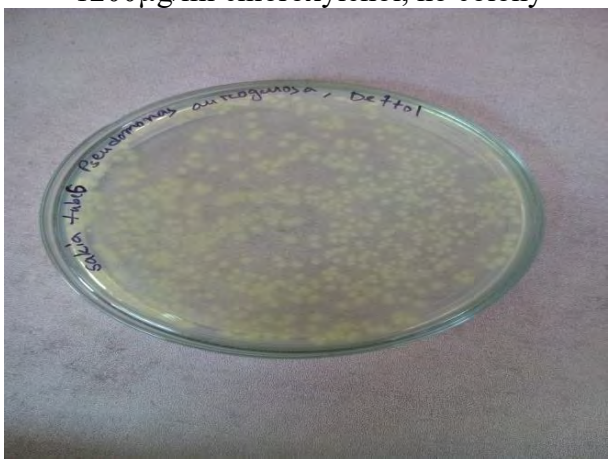
2400 µg/ml chloroxylenol, no colony



1200µg/ml chloroxylenol, no colony

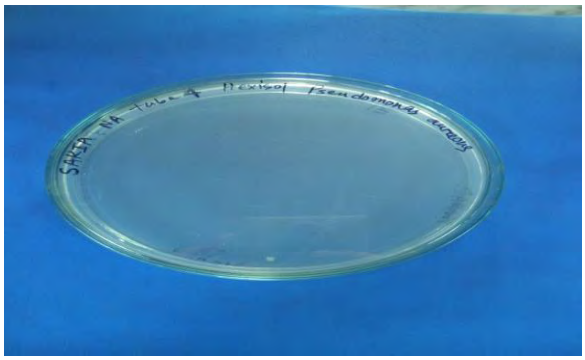


600µg/ml chloroxylenol, no colony



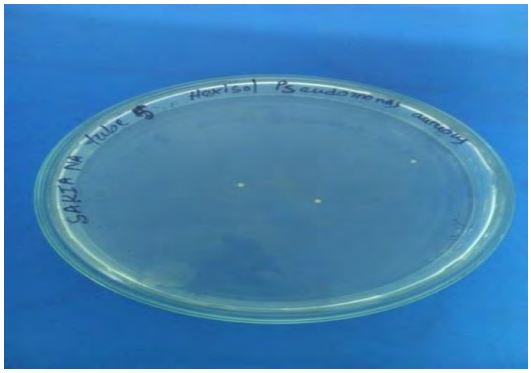
300µg/ml chloroxylenol, CFU $2.85\times10^{2}$/100µl

**Fig1.3E**: Dettol Against *Pseudomonas aeruginosa*



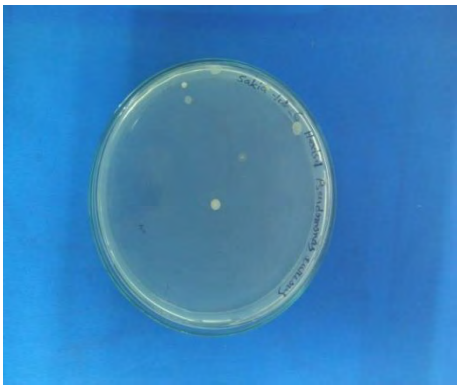
125µg/ml chlorhexidine gluconate,

CFU $0.1\times10^{1}$ /100µl



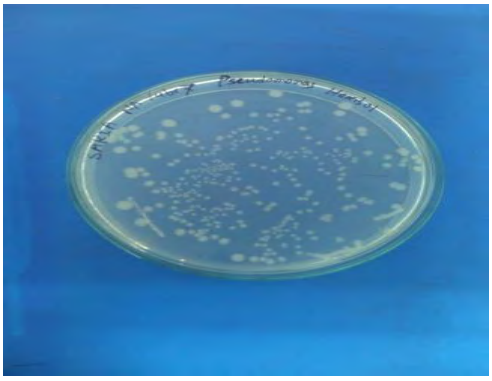
62.5µg/ml chlorhexidine gluconate

CFU $0.3\times10^{1}$/100 µl



31.25µg/ml chlorhexidine gluconate

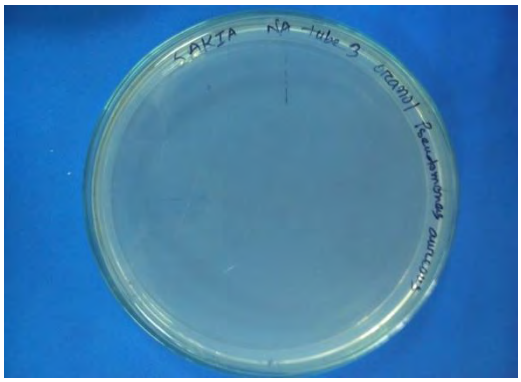
CFU $0.7\times10^{1}$/100 µl



15.62µg/ml chlorhexidine gluconate

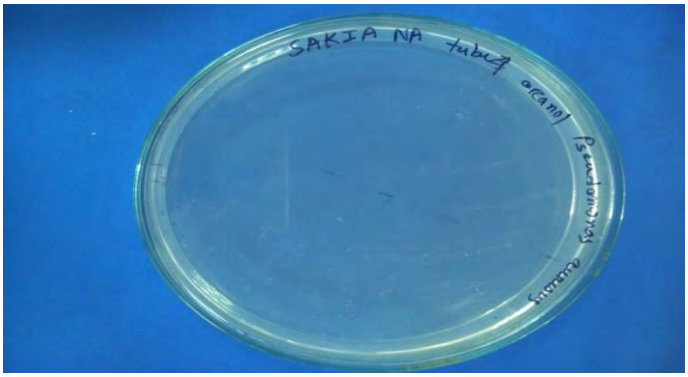
CFU $1.60\times10^{2}$/100 µl

**Fig1.3F:** Hexisol Against *Pseudomonas aeruginosa*



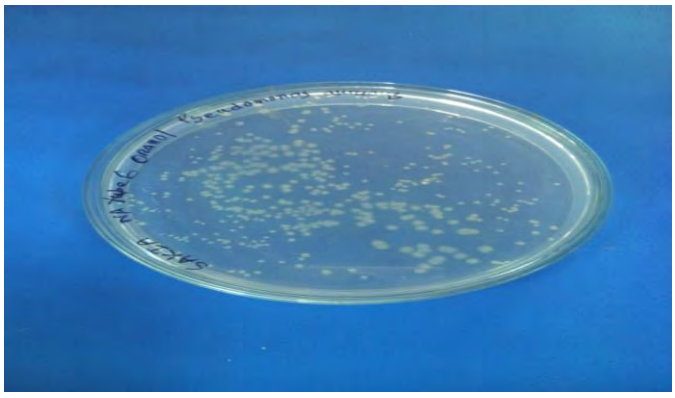
100µg/ml chlorhexidine gluconate,

no colony



50µg/ml chlorhexidine gluconate,

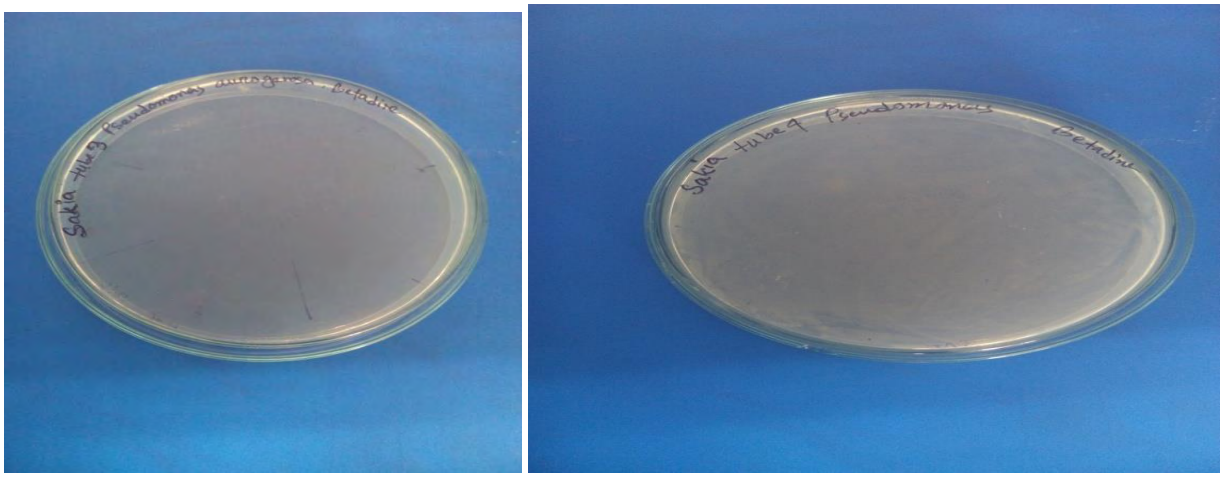
CFU $0.2\times10^{1}$/100µl



12.5µg/ml chlorhexidine gluconate

CFU $1.80\times10^{2}$/100µl

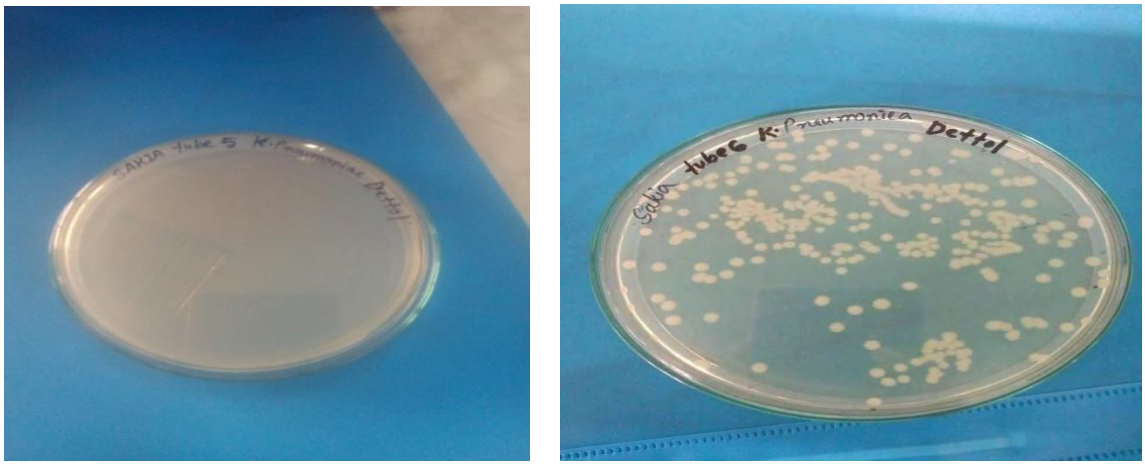
**Fig1.3G**: Oralon Against *Pseudomonas aeruginosa*



2500µg/ml Povidone-iodine, no colony

1250µg/ml Povidone-iodine, TNTC colonies

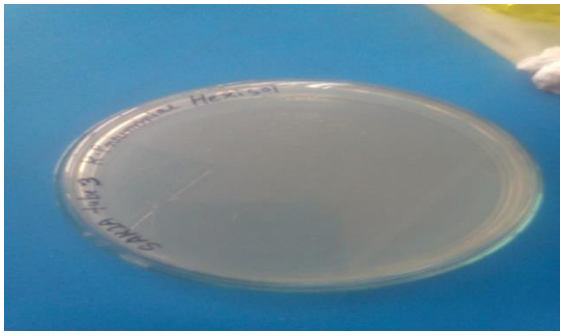
**Fig1.3H**: Betadine Against *Pseudomonas aeruginosa*



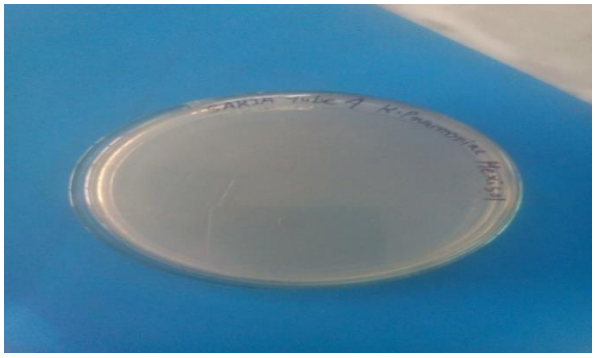

600µg/ml chloroxylenol no colony

300µg/ml chloroxylenol, CFU $1.26\times10^{2}$/ 100 µl1

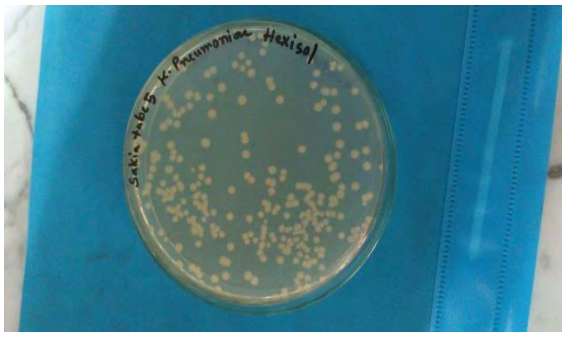
**Fig1.3I**: Dettol against *Klebsiella pneumonia*



250µg/ml chlorhexidine gluconate, no colony

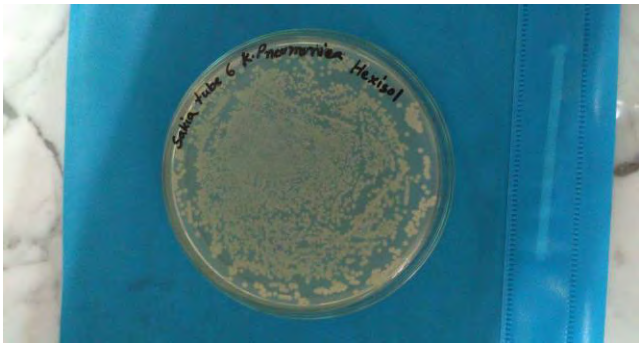


125µg/ml chlorhexidine gluconate, no colony



62.5µg/ml chlorhexidine gluconate,

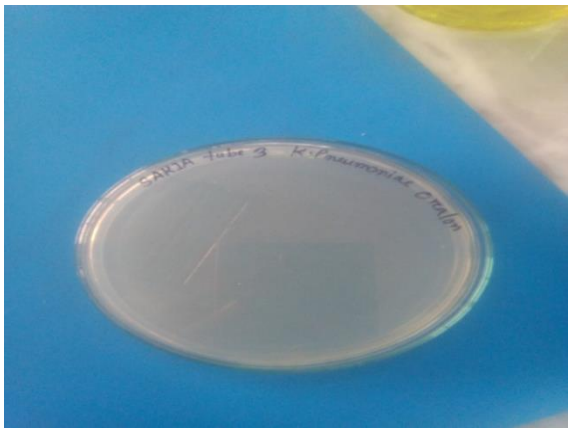
CFU $1.62\times10^2$/100 µl



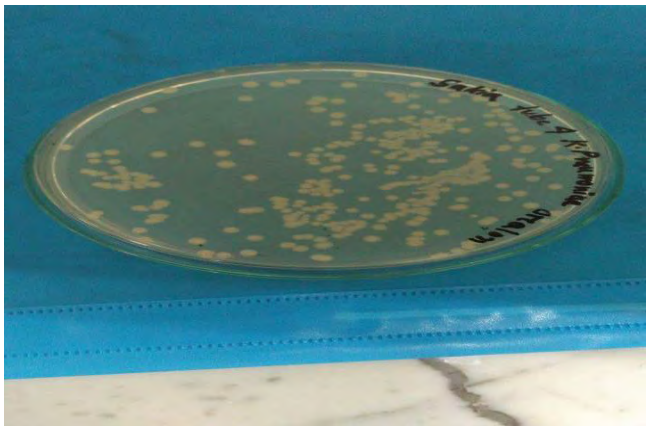
31.25µg/ml chlorhexidine gluconate

TNTC colonies

**Fig1.3J:** Hexisol against *Klebsiella pneumoniae*

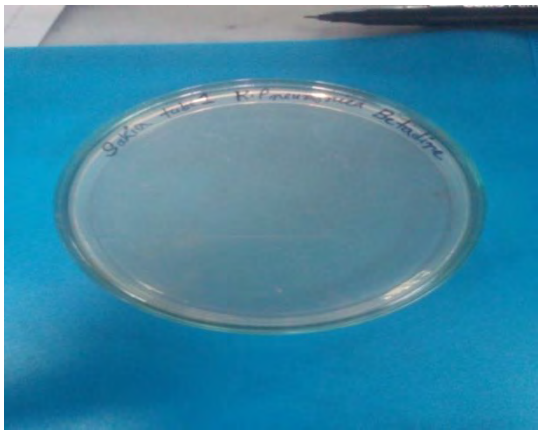


100µg/ml chlorhexidine gluconate, no colonies

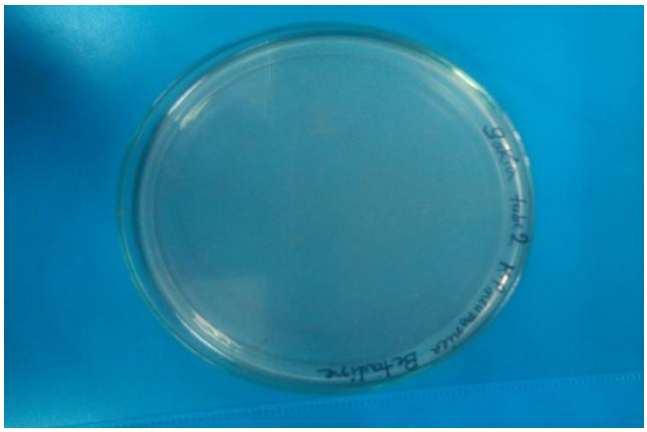


50µg/ml chlorhexidine gluconate, CFU $1.20\times10^2$/µl

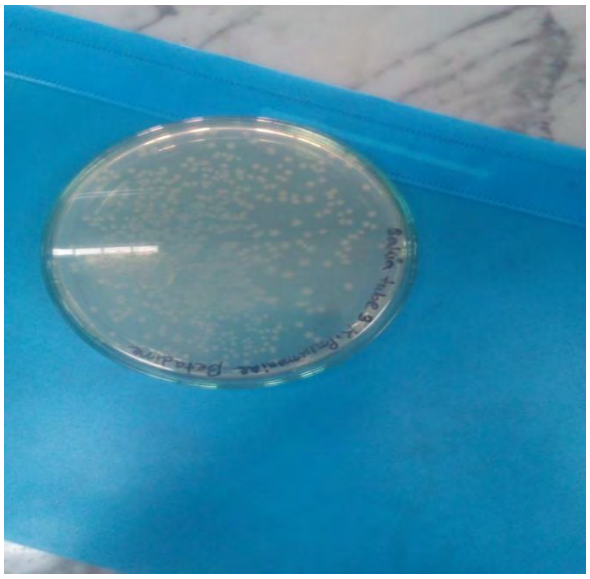
**Fig1.3K**: Oralon against *Klebsiella pneumoniae*



10000µg/mlPovidone-iodine, no colony

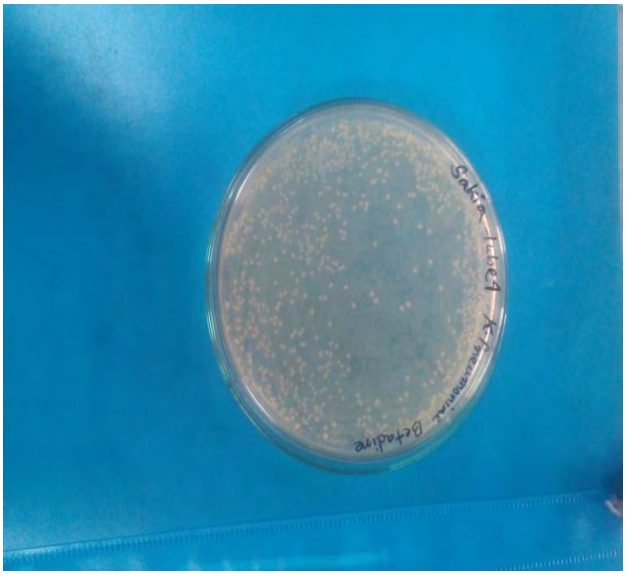


5000µg/mlPovidone-iodine, no colony



2500µg/ml Povidone-iodine,

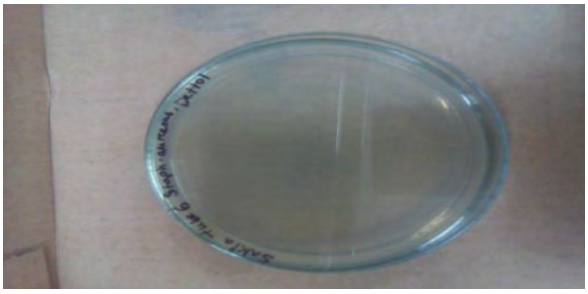
CFU $1.42\times10^2$/100µl



1250µg/mlPovidone-iodine,

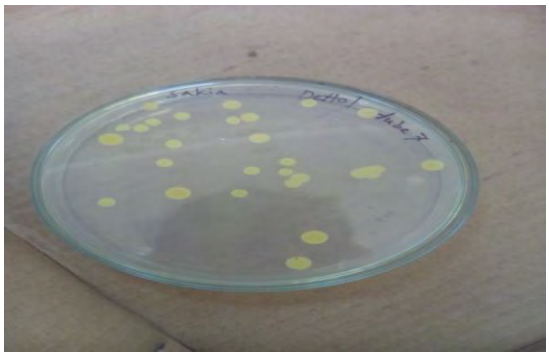
CFU $2.94\times10^2$ / 100µl

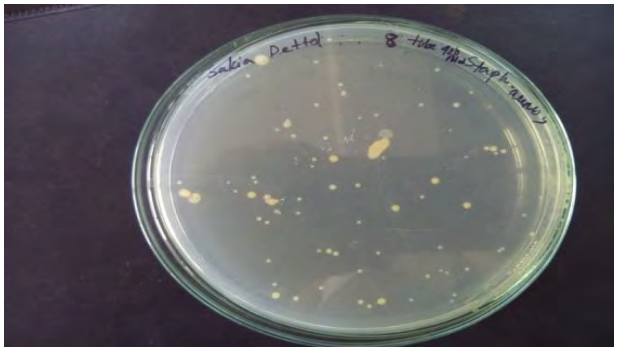
**Fig1.3L**: Betadine against *Klebsiella pneumoniae*



600 µg/ml chloroxylenol, no colony

300 µg/ml chloroxylenol, no colony



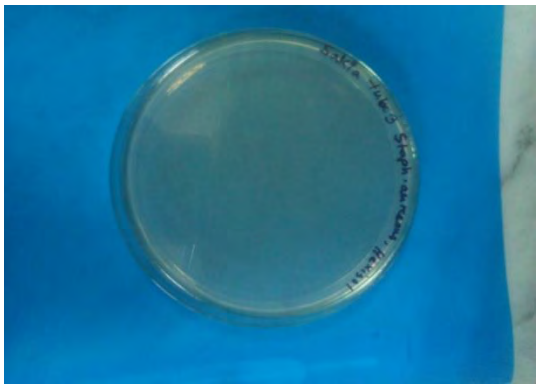


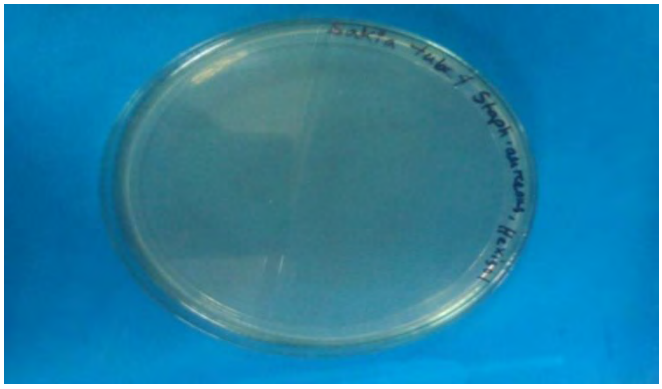
150 µg/ml chloroxylenol,

CFU $2.5\times10^{1}$ /100µl

114.2µg/mlchloroxylenol,$4.0\times10^{1}$colonies

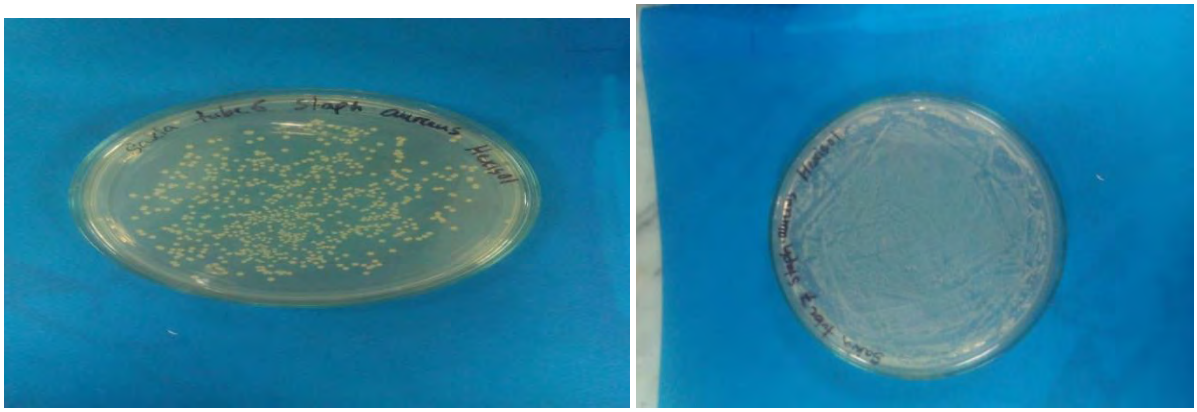
**Fig1.3M**: Dettol against *s.aureus*





250µg/mlchlorhexidine gluconate, no colony

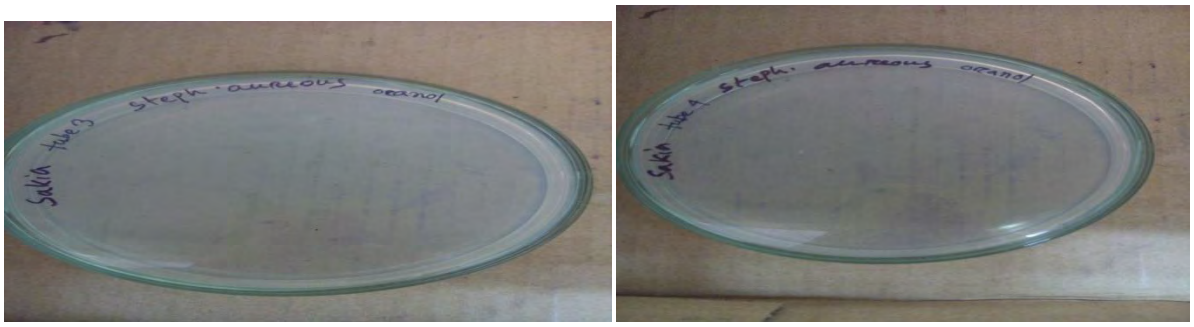
125µg/ml chlorhexidine gluconate, no colony



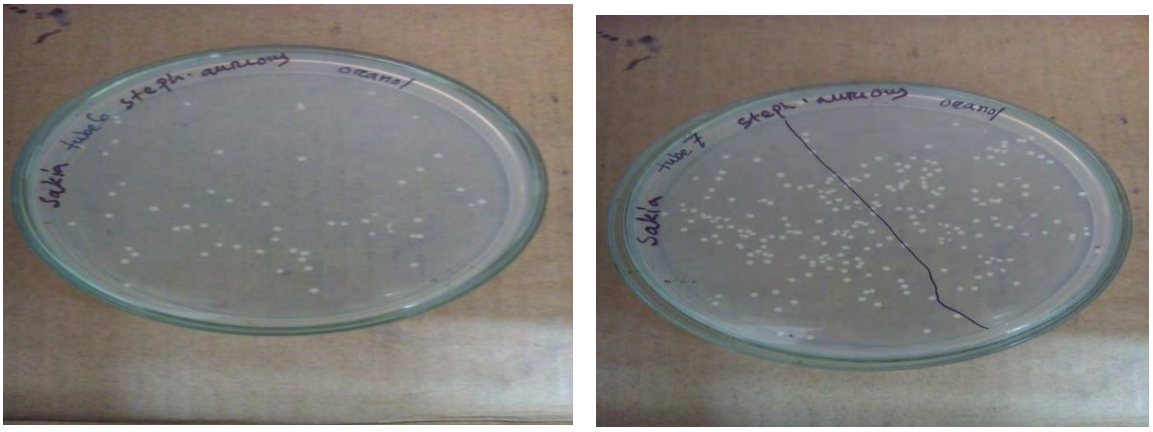
31.25µg/ml chlorhexidine gluconate, 15.62µg/ml chlorhexidinegluconate,TNTC

CFU $2.40\times10^{2}$ /100µl

**Fig1.3N**: Hexisol against *s.aureus*



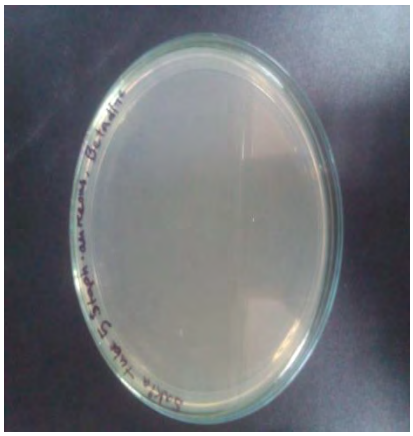
100µg/mlchlorhexidine gluconate, no colony 50µg/ml chlorhexidine gluconate, no colony



12.5µg/ml,chlorhexidinegluconate, 6.25µg/ml,chlorhexidinegluconate,

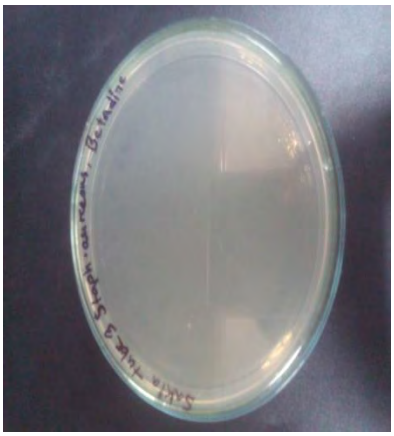
CFU $5.8\times10^{1}$ /100µl CFU $1.80\times10^{2}$ /100µl

**Fig1.3 O**: Oralon against *s.aureus*



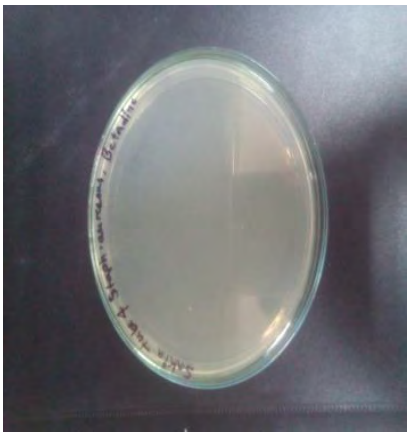
2500µg/ml Povidone-iodine,

no colony



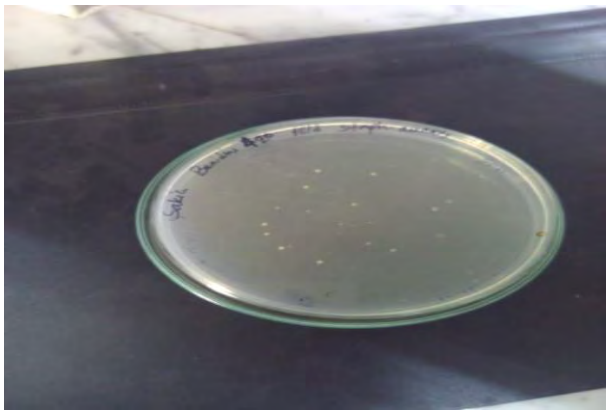
1250µg/mlPovidone-iodine,

no colony



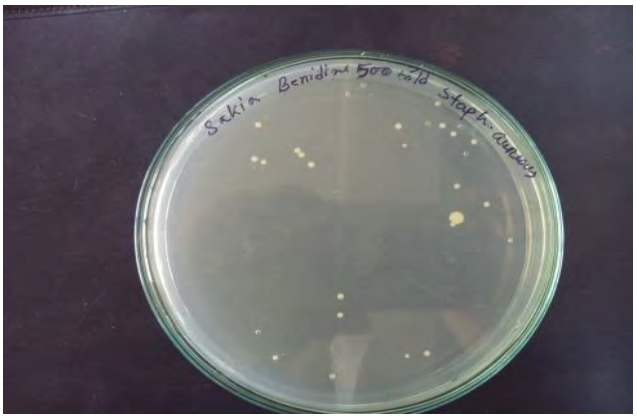
625µg/mlPovidone-iodine,

no colony



59.5 µg/ml Povidone-iodine,

CFU $1.0\times10^{1}$ /100µl




50.5µg/mlPovidone-iodine,

CFU $2.0\times10^{1}$ /100µl

**Fig1.3 P**: Betadine against *s.aureus*

## Figure 1.4 Agar disk diffusion test

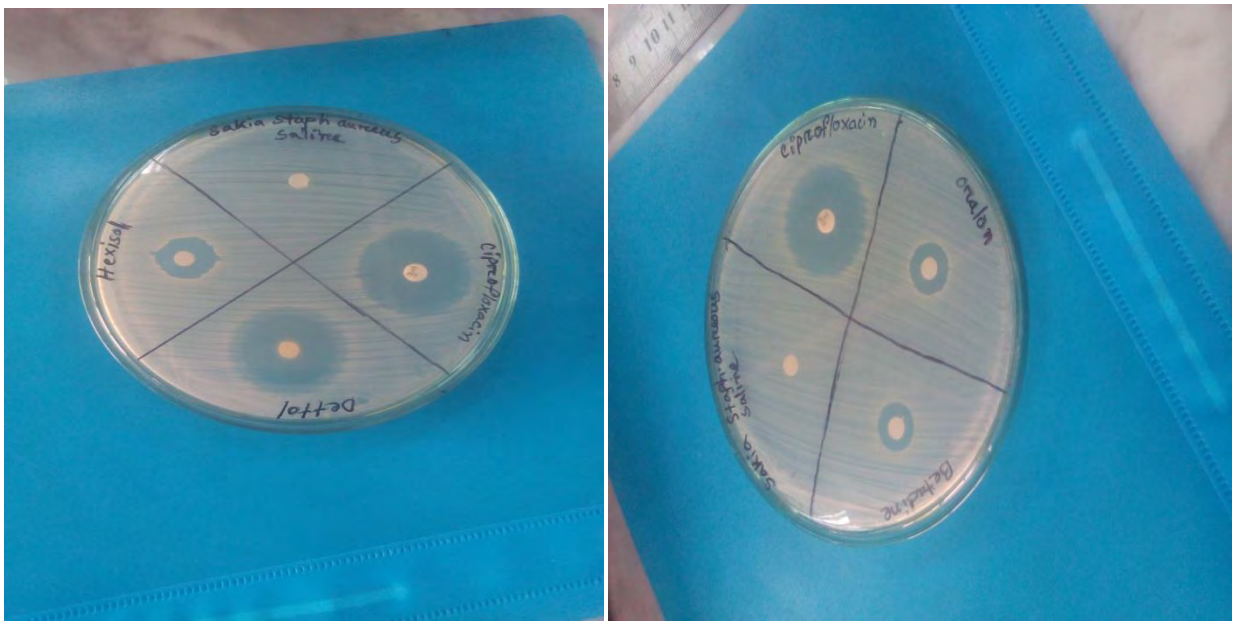



Fig: Disk diffusion test against ***Staphylococcus aureus***

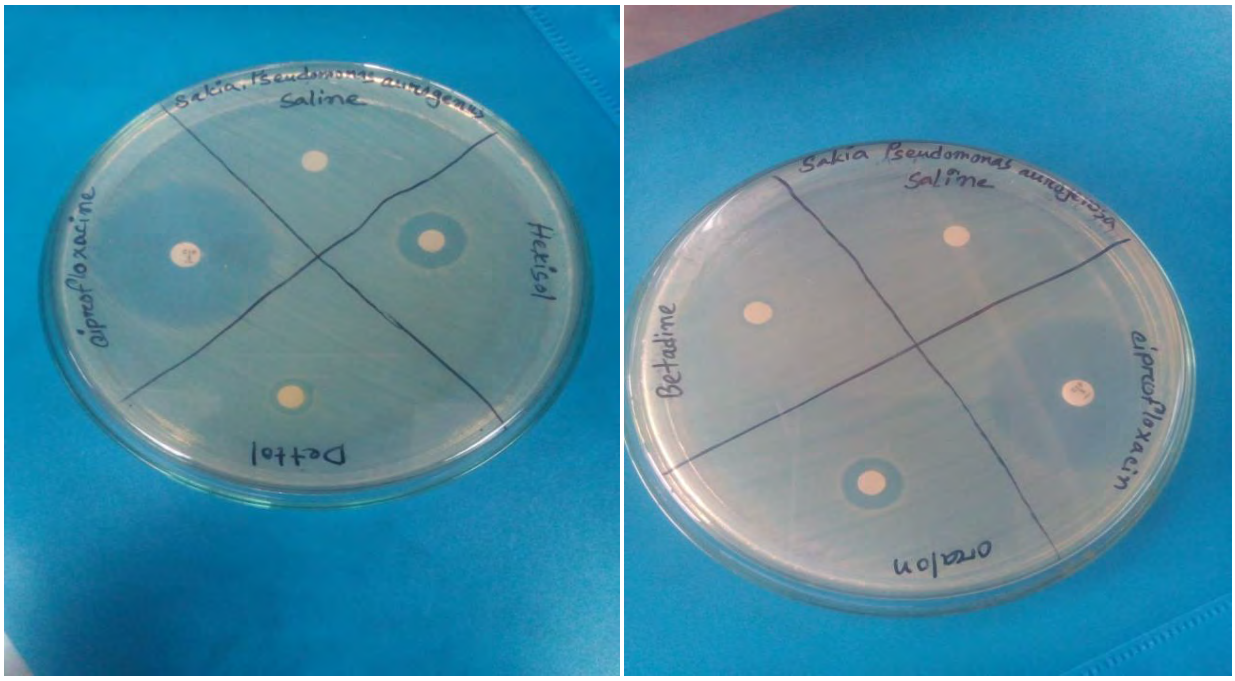



Fig: Disk diffusion test against ***Pseudomonas aeruginosa***

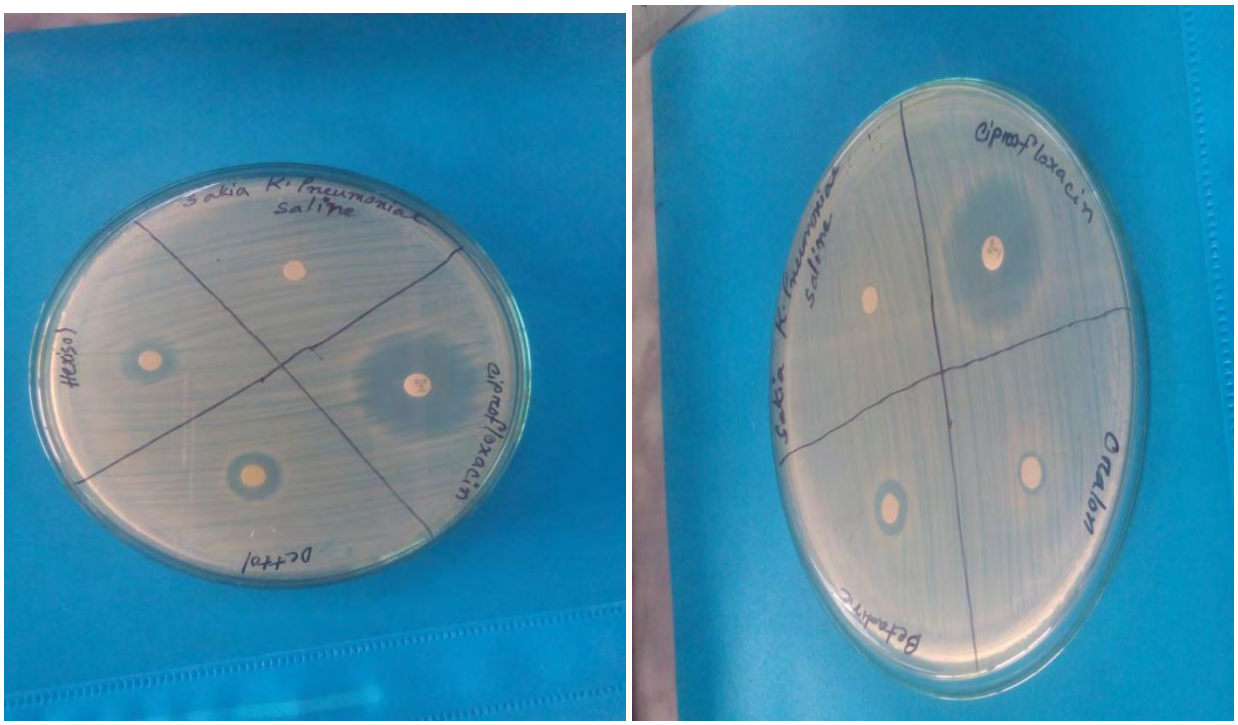



Fig: Disk diffusion test against ***Klebsiella pneumoniae***

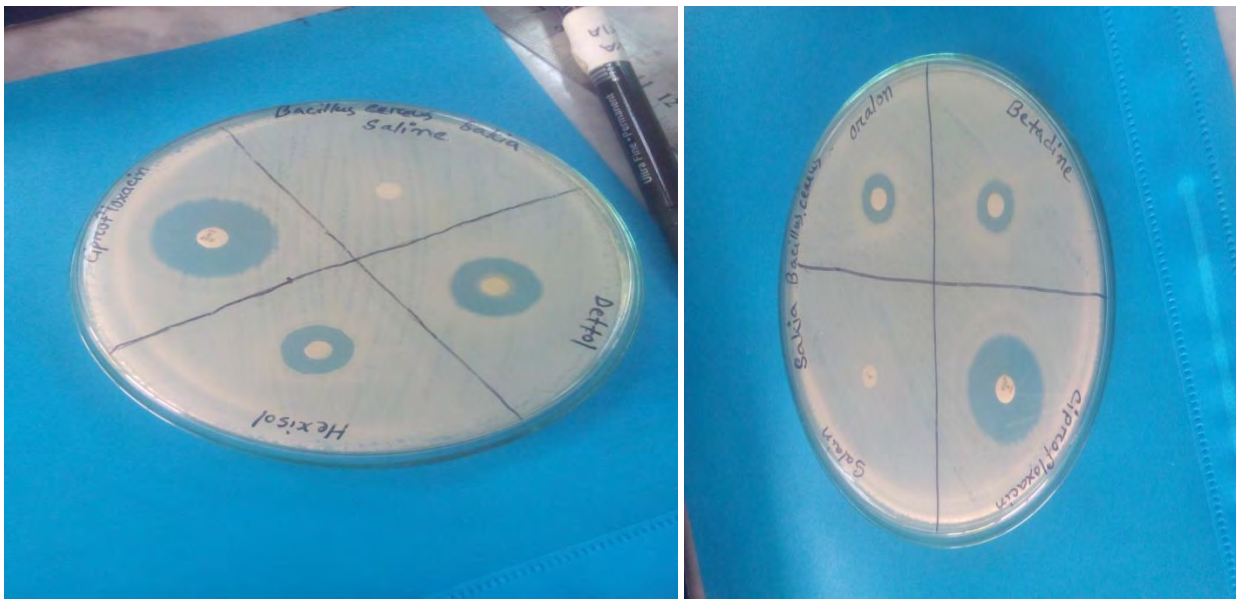



Fig: Disk diffusion test of ***Bacillus cereus***

# Graph

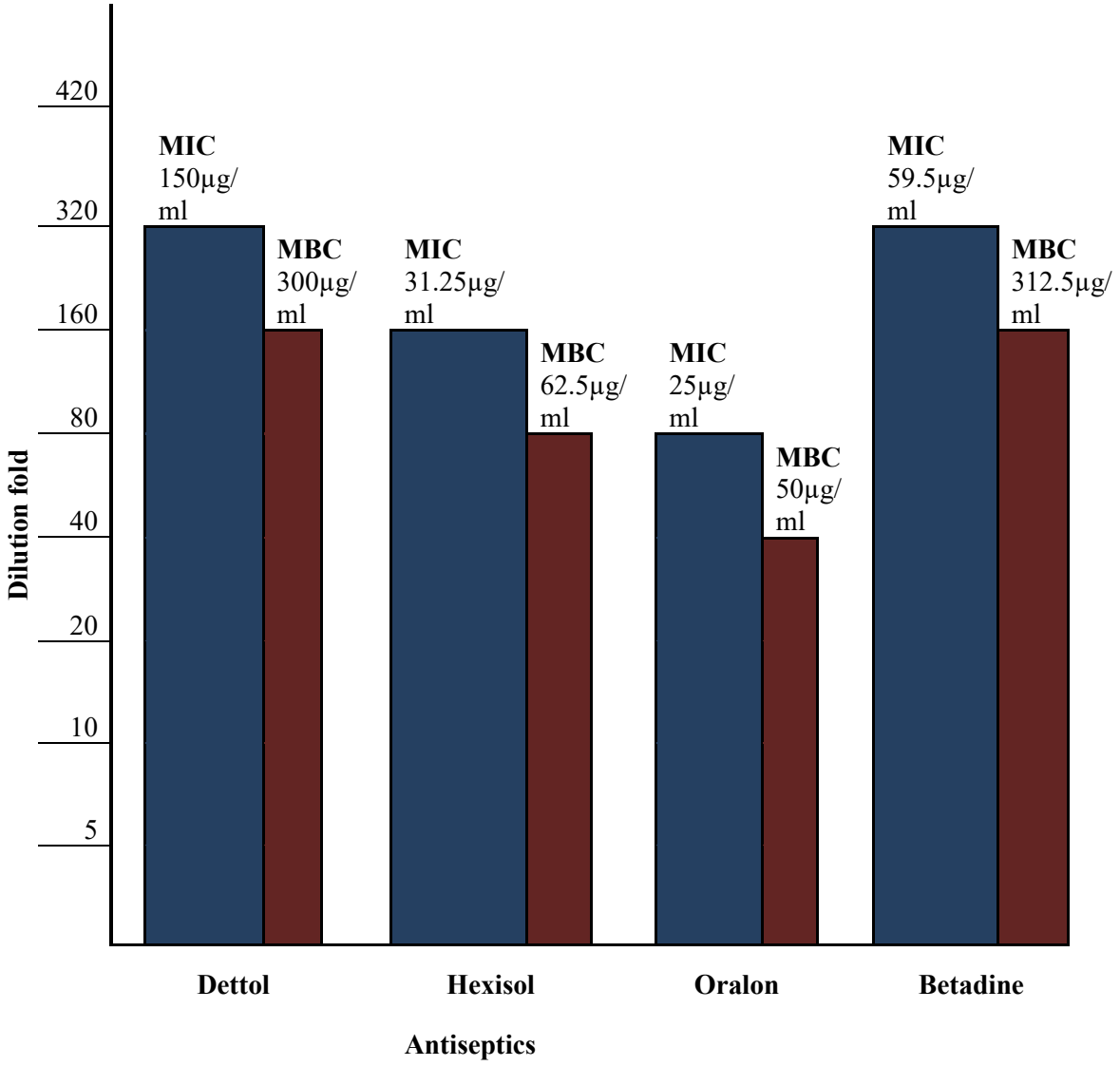



**Fig:** **Comparative Chart of MIC & MBC of the Antiseptics against *Staph. aureus***

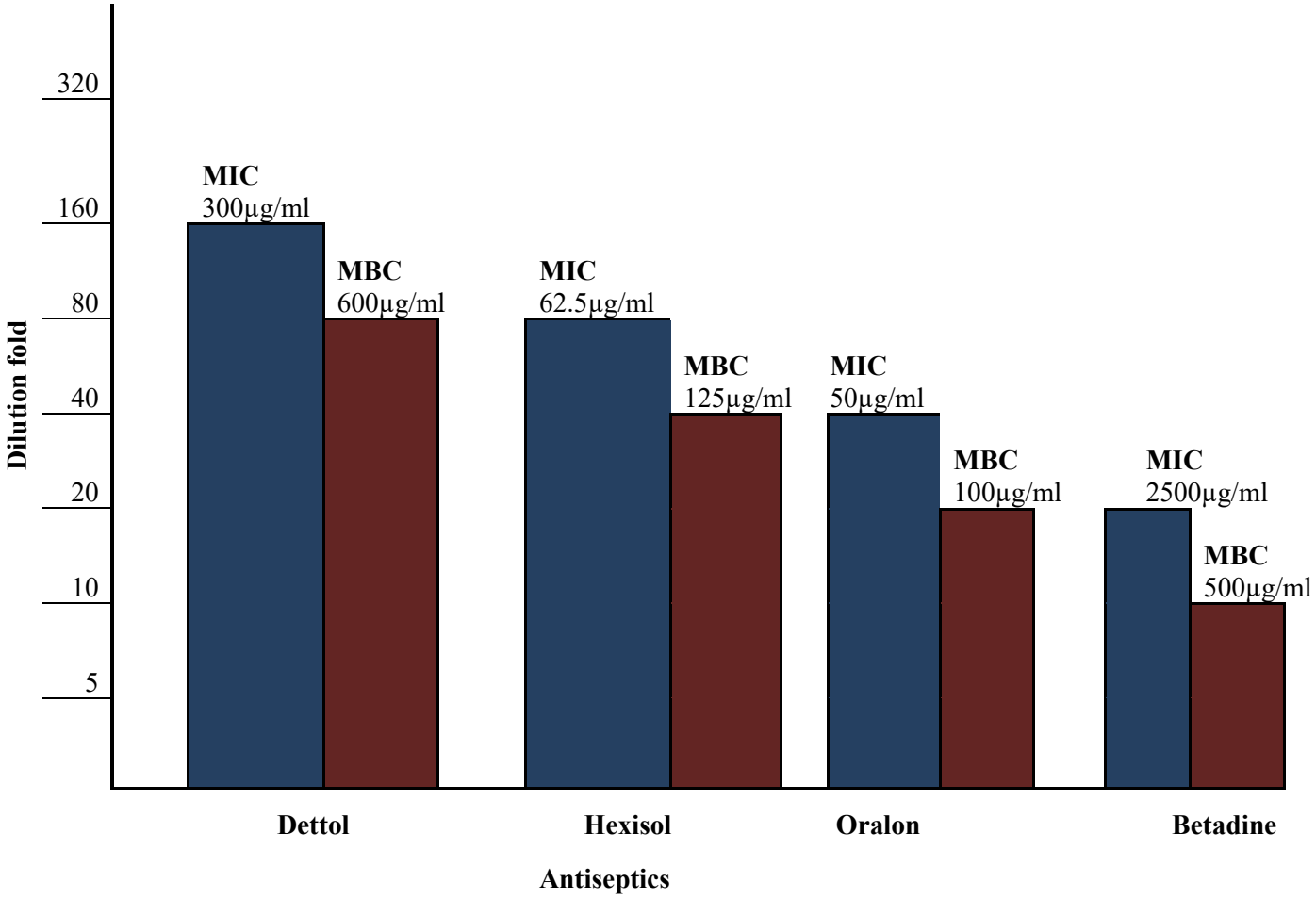



**Fig:** **Comparative Chart of MIC & MBC of the Antiseptics against *Klebsiella pneumoniae***

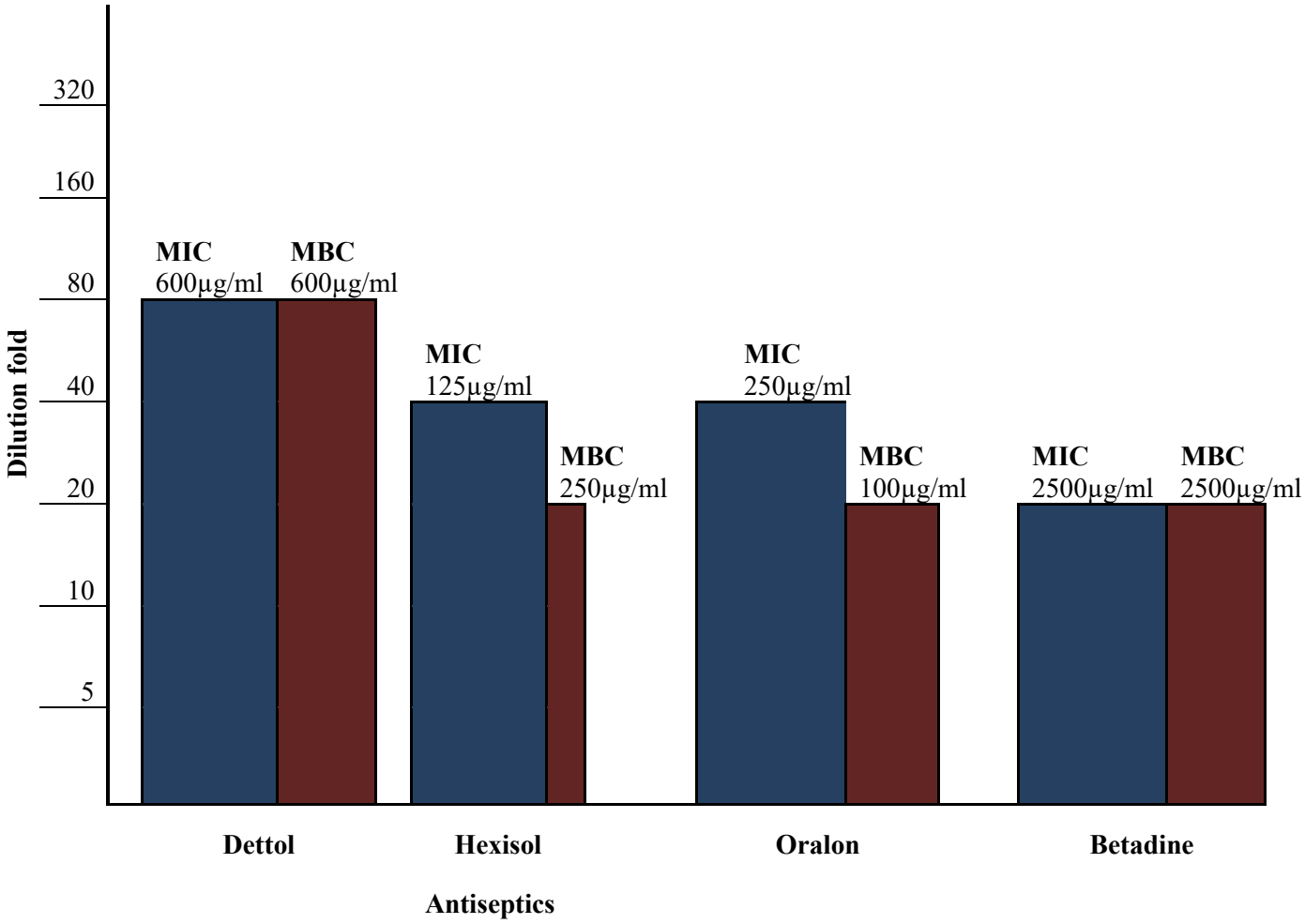



**Fig:** **Comparative Chart of MIC & MBC of the Antiseptics against *Pseudomonas aeruginosa***

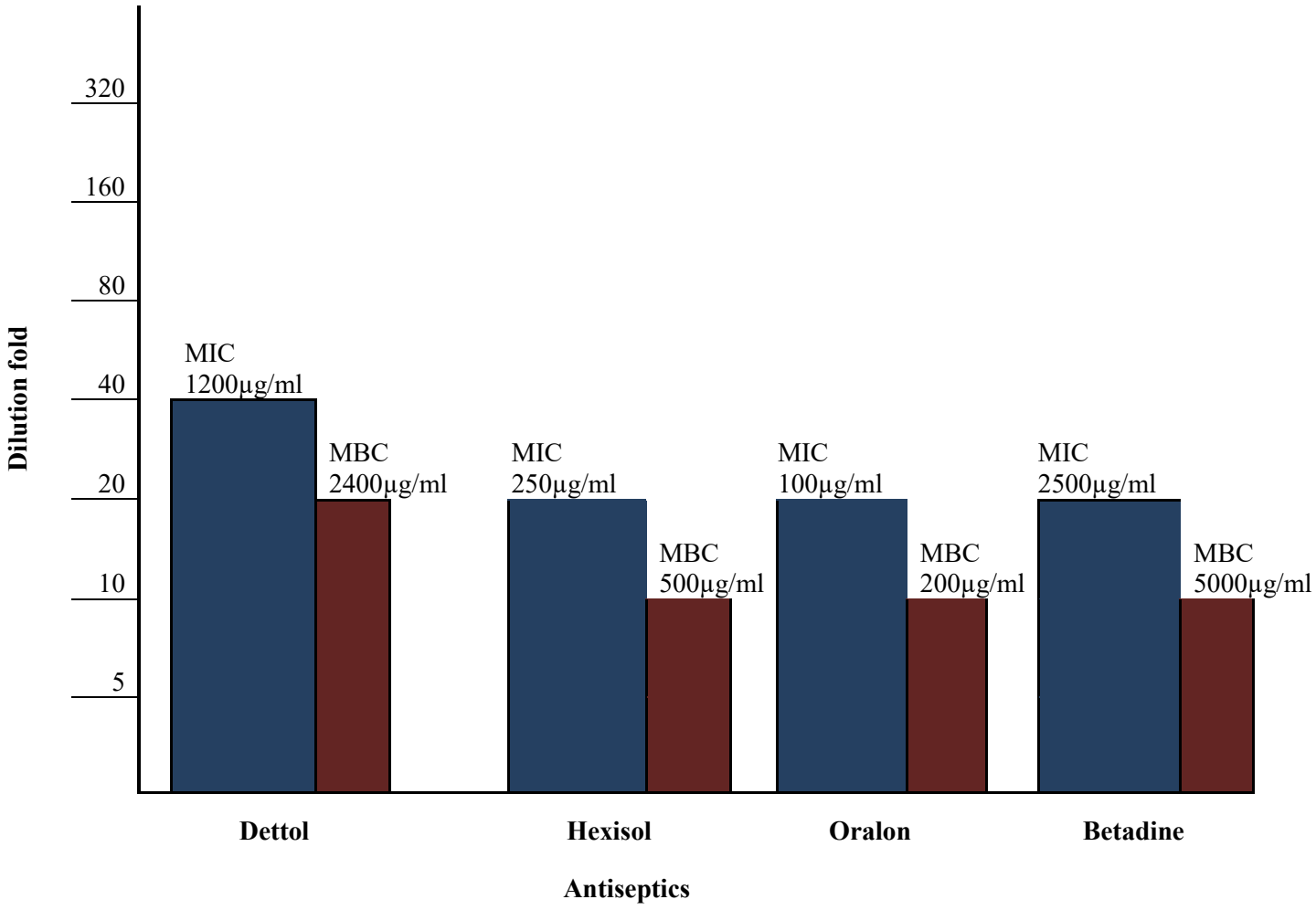



**Fig: Comparative Chart of MIC & MBC of the Antiseptics against *Bacillus cereus***

# Discussion

The present study is the comparison of antibacterial activities of four commercially available antiseptics, (Dettol, hexisol, oralon and betadine) that are used highly in Bangladesh and other countries as well. Antiseptics should have a broad microbicidal spectrum and potent germicidal activity with rapid onset and long-lasting effects (Müller and Kramer, 2008). Mainly, disinfection includes techniques of microorganisms control by chemical means and of their mechanical removal. Most, but not all, bacteria die during these activities. Antiseptics and disinfectants are essential parts of infection control and aid in the prevention of nosocomial infections (Larson et al., 1991). Dettol is widely used in homes and healthcare settings for various purposes including disinfection of skin, objects, equipment, as well as environmental surfaces (Rutala 1996). It is claimed that the new Dettol Multi-Surface Cleaner is the first of its kind in the surface-cleaning category providing 10 times better cleaning and germ kill vs detergents (Samguine, 2016) and it can kill 98% of microbes in just 15 seconds as shown in agar patch studies (Mellefont et al., 2003). Hexisol Hand rub works by killing as well as preventing bacterial growth (Gothner et al., 2007). Dr Ishtiaq Mannan, Save the Children's director for Health Nutrition and HIV/AIDS sector, termed the introduction of this new solution a "game changer" to bring down neonatal deaths in Bangladesh and this game changer antiseptic is chlorhexidine. Chlorhexidine gluconate is a germicidal mouthwash that reduces bacteria in the mouth, it is usually prescribed by a dentist (Multum, 2012). Betadine antiseptic is mainly used for cuts wounds and abrasions and is suggested to the treatment of Blisters, Tinea (Athletes Foot), Paronychia (Infected skin around the nails), and Ringworm (another name for tinea) (Fogorv, 1999). Although these four antiseptics are used in many purposes and are thought to be very effective but their standard concentration of MIC and MBC are not found specifically. However, many studies were done to detect the effectiveness of these antiseptics against various organisms and their results of effectiveness were varied from organism to organism.

As those antiseptics are very much used in many ways in our country and also almost every country, an effort was given to study the antibacterial activity or effectiveness of these four antiseptics against four well-known bacteria *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and for the effectiveness testing purpose the

MIC, MBC, and the disk diffusion method was done and the results indicate the antibacterial activity or effectiveness of the antiseptics or disinfectant. All the methods were followed step by step and before starting the dilution method, the selective isolation and biochemical test of the stock cultured bacteria were done properly.

The MIC is the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism in tubes. Dilution methods were the most appropriate ones for the determination of MIC values (Balouiri et al., 2016). The MBC is the lowest concentration of antibiotic required to kill a particular bacterium. At the end of 24 h of incubation, the tubes were read for the MIC and then the MBC was determined by sampling all the macroscopically clear tubes (1 dilution below the MIC was used for the levels to be assessed in the MBC assay). The suspension was inoculated onto plates of blood agar or nutrient agar (here in the study the nutrient agar is used). The plates were incubated for 24h at 37°C (Yilmaz, 2012). Working bacteria culture was adjusted to be equal to 0.5 McFarland standards ($1 \times 10^8$ CFU/ml) (Zainol et al., 2013).

For the project, Dilution was done in this way: 5 fold, 10 fold, 20 fold, 40 fold, 80 fold, 160 fold, 320 fold, and so on. After 24 hours incubation of the dilution tubes, 100 µl solution from each dilution tube was transferred to the large Petri dish through a pipette and then it was spread with the help of a spreader. After the incubation period, the bacterial growth was observed for each plate and the MIC and MBC were also identified through the observation of the growth of bacteria. MIC was the plate that had very less or minimum growth and 1 dilution below the MIC was used for the levels to be assessed in the MBC assay. After the MIC, MBC test, and Agar disk-diffusion testing were done, it is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing (Balouiri et al., 2016). For this test, any two antiseptics and any specific bacteria were chosen. Also, a positive control and a negative control were chosen. For the Positive control (that can inhibit bacterial growth) the ciprofloxacin was chosen and as a negative control normal psychological saline was chosen, which cannot inhibit bacterial growth. The agar plate was spread properly through that cotton swab which contained the bacterial suspension then each of the two filter paper discs was soaked with different antiseptics and one filter paper disc was soaked with saline solution and an antibiotic disc (ciprofloxacin) was taken.

The Petri dishes were incubated under suitable conditions. Generally, an antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism, and then the diameters of inhibition growth zones were measured.

In the case of MIC and MBC test, after observing the turbidity and also observing the colonies of the plate, it was detected that MIC of Dettol against *Bacillu .cereus* was 1200µg/ ml and MBC was 2400 µg/ml. For Hexisol against *Bacillus .cereus* was 250µg /ml, and MBC was 500 µg/ml. MIC of Oralon against *Bacillus .cereus* 100µg /ml, and MBC was 200 µg/ml. MIC of Oralon against *Bacillus .cereus* was 2500µg/ml, and MBC was 5000 µg/ml. MIC of Dettol against *Pseudomonas aeruginosa*was 600µg/ml and MBC was also same 600 µg/ml. MIC of Hexisol against *Pseudomonas aeruginosa* was 125µg/ml, and MBC was 250 µg/ml. MIC of Oralon against *Pseudomonas aeruginosa* was 50µg/ml, and MBC was 100 µg/ml. MIC of Oralon against *Pseudomonas aeruginosa* was 50µg/ml, and MBC was 100 µg/ml. MIC and MBC of Betadine against *Pseudomonas aeruginosa* was same that was 2500µg/ml, MIC of Dettol against *Klebsiella pneumoniae* was 300µg/ml and MBC was contain 600 µg/ml. MIC for Hexisol against *Klebsiella pneumoniae*was 62.5µg/ml, and MBC was contain 125 µg/ml. MIC for Oralon against *Klebsiella pneumoniae*was 50µg/ml, and MBC was 100 µg/ml. MIC for Betadine against *Klebsiella pneumoniae* was 2500µg/ml, and MBC was 5000 µg/ml. MIC for Dettol against *Staphylococcus aureus* was 150µg/ml and MBC was 300 µg/ml. MIC for Hexisol against *Staphylococcus aureus*was 31.25µg/ml, and MBC was 62.5 µg/ml. MIC of Oralon against *Pseudomonas aeruginosa* was 50µg/ml, and MBC was 100 µg/ml. MIC for Betadine against *Staphylococcus aureus* was 59.5µg/ml, and MBC was 312.5 µg/ml.

So, overall, through this study it was found that the four antiseptics (Dettol, Hexisol, Oralon and Betadine) have almost very good effectiveness (99.999 %) against *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumonia.*

In the case of disk diffusion test it was found that against *Staphylococcus aureus*, Dettol had the highest diffusion rate among the other three antiseptics that were used, against *Klebsiella pneumonia* Hexisol had the highest diffusion rate among the others, also against *Pseudomonas aeruginosa* Hexisol had the highest diffusion rate comparing the other three antiseptics, against *Bacillus .cereus* it was found that Dettol had the highest diffusion rate among the other three antiseptics that were used.

# Conclusion

The study was done to find out the antibacterial activities or effectiveness of the commercially available antiseptics (Dettol, Hexisol, Oralon and Betadine) and also compare to their effectiveness. Basically, their antibacterial activities were tested against four challenging and well-known bacteria (*Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa*, *Bacillus.cereus*). MIC and MBC were detected through dilution method. For the study, mainly the MIC, MBC and Disk diffusion method was done and the results indicate the antibacterial activity or effectiveness of the antiseptics or disinfectant. In the case of Dettol, it had the best antibacterial activity or effectiveness against *Staphylococcus aureus,* also in case of Hexisol, it has the best effectiveness against *Staphylococcus aureus.* Not only but also, Oralon had the best antibacterial activity against *Staphylococcus aureus* and Betadine also had the best antibacterial activity against *Staphylococcus aureus* comparing the other three bacteria's (according to MIC and MBC test). Beside of this, according to disk diffusion test, the results also supported the MIC and MBC test in most of the cases. Both of the two tests showed that Dettol, Hexisol, Oralon and Betadine have the best antibacterial activity or effectiveness against *Staphylococcus aureus* compared to *Klebsiella pneumonia, Pseudomonas aeruginosa*, *Bacillus .cereus.* Against *Bacillus cereus, comparatively* dettol was more effective but hexisol had the almost similar type of effectiveness as like dettol. Both the oralon, betadine had more or less same type of effectiveness and their effectiveness was less than hexisol and dettol for *Bacillus cereus*. Against *Pseudomonas aeruginosa,* dettol was very effective comparing to Hexisol, Oralon and Betadine. Also, against *Klebsiella pneumoniae*, Dettol was very effective but good effectivity was also found for Hexisol although Betadine and Oralon are less effective in that cases. So, the overall study tried to follow the goal of detecting the antibacterial activity or effectiveness of the four selected antiseptics through the result of MIC, MBC and disk diffusion method and found that overall, through this study the four antiseptics (Dettol, Hexisol, Oralon and Betadine) have almost very good effectiveness (99.999 %) against *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumonia.*

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# Appendices

## Nutrient Agar

| | |
|---|---|
| Beef Extract | 3.0 g |
| Peptone | 5.0 g |
| Agar | 15.0 g |
| Distilled Water | 1000 ml |

## Nutrient Broth

| | |
|---|---|
| Peptone | 10.000gm/l |
| Beef extract | 10.000gm/l |
| Sodium chloride | 5.000gm/l |

## Psychological saline

| | |
|---|---|
| NaCl | 0.85g |
| Water | 100ml |

## Mannitol Salt Agar

| | |
|---|---|
| Pancreatic Digest of Casein | 5.0gm |
| Peptic Digest of Animal Tissue | 5.0gm |
| Beef Extract | 1.0gm |
| Sodium Chloride | 75.0gm |
| D-Mannitol | 10.0gm |
| Phenol Red | 0.025gm |
| Agar | 15.0 gm |

## Cetrimide Agar

| | |
|---|---|
| Enzymatic Digest of Gelatin | 20 g |
| Magnesium Chloride | 1.4 g |
| Potassium Chloride | 10 g |
| Cetrimide (Cetyltrimethylammonium Bromide) | 0.3 g |
| Glycerol | 10 mL |
| Agar | 13.6 g |

## BC Agar

| | gm/litre |
|---|---|
| Peptone | 1.0 |
| Mannitol | 10.0 |
| Sodium chloride | 2.0 |
| Magnesium sulphate | 0.1 |
| Disodium hydrogen phosphate | 2.5 |
| Potassium dihydrogen phosphate | 0.25 |
| Bromothymol blue | 0.12 |
| Sodium pyruvate | 10.0 |
| Agar | 15.0 |

## Macconkey Agar

| | |
|---|---|
| Peptone (Pancreatic digest of gelatin) | 17gm |
| Proteose peptone (meat and casein) | 3 gm |
| Lactose monohydrate | 10gm |
| Bile salts | 1.5gm |
| Sodium chloride | 5 gm |
| Neutral red | 0.03gm |
| Crystal Violet | 0.001 g |
| Agar | 13.5gm |

# TSI Agar

| | |
|---|---|
| Beef extract | 3.0 gram |
| yeast extract | 3 gram |
| peptone | 15 gram |
| protease peptone | 5 gram |
| lactose | 10.0 gram |
| saccharose | 10.0 gram |
| glucose | 1.0 gram |
| ferrous sulphate0.2 gram | |
| sodium chloride | 5.0 gram |
| sodium thiosulphate | 0.3 gram |
| phenol red | 0.024 gram |
| agar | 12 gram |
| distilled water | 1000ml |