HOSPITAL DISINFECTION USING EF-CHLOR BIO-TAB/ EFFI-SEPT/ EFFI- KLEEN FOR STERILIZATION & SURFACE DISINFECTION (1.67gm)

Khushboo Bhati[1*], Aayush Goswami[1], Priti Phadnis[2]

Abstract

Hospital acquired infection (HAI) has recently grown to be a very serious issue that is only becoming worse. Hospitals, clinics, nursing homes, and other healthcare facilities may all experience these illnesses. Because they may increase morbidity, death, and healthcare expenditures, HAIs are a serious problem. Finding a solution that is affordable, easily accessible, user-friendly, and environmentally friendly is a good method to deal with this issue. Sodium Dichloroisocyanurate (NaDCC) is one such remedy. Due to its great efficacy against a variety of pathogens, sodium dichloroisocyanurate (NaDCC), a commonly used disinfectant, has gained importance recently. NaDCC, the active ingredient in Ef- Chlor BIO-TAB/ Effi- Sept/ Effi- Kleen (1.67 mg), is extremely effective in eliminating almost all known microorganisms. The World Health Organization (WHO), the European Union (EU), and the United States Environmental Protection Agency (US EPA) have all given their approval for the use of NaDCC as a disinfectant. Gram's staining method was employed in this experiment to identify the bacteria and to identify fungus lacto phenol cotton blue stain is used that cause HAI, and Agar- well diffusion method was used to determine the antimicrobial activity of the tablet. When tested against diverse surfaces, the product was found to be 99.99% effective against *E. coli*, *Shigella*, MS2 phage, *Staphylococcus*, *Streptococcus*, *Aspergillus*, etc.

Keywords: HAI, NaDCC, WHO, UNICEF, US EPA, Agar- well diffusion method, Ef-Chlor, BIO-TAB, Effi- Sept, Effi- Kleen

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Introduction

Healthcare-associated infections (HAI), also known as hospital-acquired infections (HAI), are a type of infection or contamination that is developed inside a healthcare institution and is often absent or in the incubation stage at the time of a patient's admission. These kinds of acquired infections frequently happen after the patient has been hospitalized and manifest themselves within 48 hours of being there. Hospitals, clinics, nursing homes, and other healthcare facilities may all experience these illnesses. Because they can result in higher rates of morbidity, death, and hospital expenses, HAIs are a critical issue (**Monegro et al., 2022**).

Hospital acquired infections frequently occur in:

1. Catheter-associated urinary tract infections (CAUTIs): These infections arise from improper insertion, maintenance, or removal of urinary catheters, which allows bacteria to colonize the urinary system.

2. Surgical site infections (SSIs): SSIs happen when a surgical incision has been made. Poor sterilization practices, contaminated surgical instruments, and insufficient wound care are some of the causes of SSIs.

3. CLABSIs (central line-associated bloodstream infections): These infections are brought about by the insertion or maintenance of central venous catheters. Through the catheter, bacteria can enter the bloodstream and cause a dangerous bloodstream infection (**Monegro et al., 2022**).

Infections acquired in hospitals are brought on by a number of reasons, such as:

1. Microorganisms: Numerous viruses, bacteria, and fungus that can cause diseases are frequently found in hospitals. It may be difficult to treat some of these organisms since they are resistant to antibiotics.

2. Invasive techniques: Catheters, ventilators, and surgical operations can compromise the body's built-in defensive mechanisms, providing an opportunity for germs to enter and spread infection.

3. Environmental aspects: Healthcare facilities are possible hosts to infectious diseases. The spread of illnesses can be aided by contaminated surfaces, inadequate disinfection procedures, and inadequate ventilation.

Healthcare providers prioritize on preventing illnesses picked up in hospitals. The following are some strategies and measures used to lower HAIs:

1. Hand hygiene: To stop the spread of illnesses, proper hand washing and the use of hand sanitizers are important Healthcare professionals should follow exact hand hygiene guidelines.

2. Surface Cleaning, Sterilization and disinfection:

Keeping medical tools, surfaces, and equipment clean, sterilized, and disinfected helps to get rid of infectious agents (**Monegro et al., 2022**).

It has been shown that Ef-Chlor BIO-TAB/Effi-Sept/Effi- Kleen, which contains 1g of available chlorine and 1.67g of NaDCC, is effective against a wide range of microorganisms (efchlor.com).

With a molecular weight of 219.9g/mol, sodium dichloroisocyanurate (NaDCC) is a white, crystalline or granular powder. It contains about 62% chlorine that is readily available. NaDCC stands for 1, 3, 5-dichloro-1, 3, 5- triazine- 2, 4, 6 (1H, 3H, 5H) - trione sodium salt. Although NaDCC dissolves easily in water and produces hypochlorous acid and other chlorinated species, including hypochlorites, which are powerful disinfectants, it is stable in solid form. Swimming pools, healthcare facilities, and drinking water treatment plants often use NaDCC as a disinfectant. (**Seo et al; 2021**)

Mode of Action: When NaDCC is dissolved in water, free available chlorine (FAC) is released. FAC is a potent oxidizing chemical that destroys microbes by damaging their cell membranes and proteins. The disinfecting activity of NaDCC is additionally aided by the production of hypochlorous acid and other chlorinated species. The biocidal chemical that eliminates bacteria is HOCl. According to **Clasen et al. (2006)**, HOCl splits into the hypochlorite ion (OCl-) and hydrogen ion (H+) in alkaline conditions.

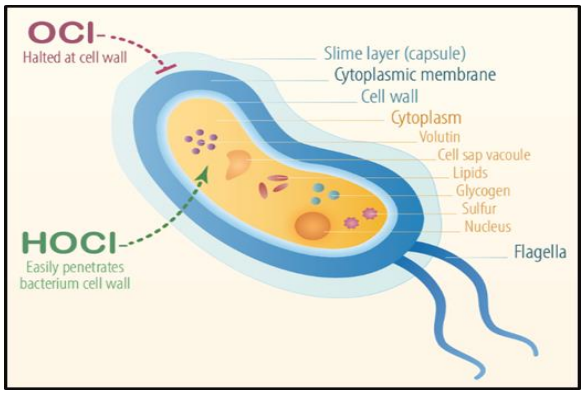


Fig 1: Action mechanism of NaDCC

Chemical action of Ef-Chlor (NaDCC in water):

$NaDCC + H2O \longrightarrow HOCl + Na^{+} + H_2C_4^{-}$

Chlorine Hypochlorous acid hydrolysis formation

$HOCl \longrightarrow H^{+} + ClO^{-}$

Hypochlorous acid stabilizes at water pH

$HOCl \rightarrow HCl + [O]$

Decomposition of HOCl to liberate nascent oxygen

$[O] + Microorganism \rightarrow HCl + Oxidised\ Microbe$

Nascent oxygen oxidises & destroys the microbe (germicidal action)

In contrast to the negatively charged hypochlorite ions (OCl-), hypochlorous acid (HOCl) is electrically neutral. When compared to the negatively charged hypochlorite ion, pathogenic microorganisms frequently have negatively charged cell walls that can be penetrated by neutral hypochlorous acid. Therefore, the free available chlorine produced when NaDCC (and other chlorine donors such chlorinated isocyanurates) are dissolved in a solution, the resulting free avaible chlorine has the ability to oxidize a variety of organic and inorganic substances found in water. Compared to hypochlorites, NaDCC has the ability to target and destroy a wider variety of harmful substances, making it an excellent disinfectant that helps to disinfect surfaces. (**Bajpai et al. 2017**).

An investigation into the hospital's present use of disinfectants was done before this study was started. 1% hypochlorite solution was the current cleaning agent used by the hospital; the government also provided them with other cleaning supplies like phenyl, glutaraldehyde, etc.

The government has switched to disinfectants based on chlorine after COVID. Therefore, the government provided 1% hypochlorite solution which was currently the most popular surface disinfectant in the hospital.

In addition to hypochlorite, the cleaning facility also employed phenyl glutaraldehyde as a disinfectant.

The three bucket system, which is used for cleaning in most places, is as follows:

Fig 2: Three Bucket System for Cleaning

The majority of locations, including hospital beds, ambulances, general wards, locker rooms, etc., follow this standard practices.

The Indian government had recently provided the hospital, an equipment for producing automated disinfectants. Only the maternity ward and O.T. currently make use of the machine. For cleaning purposes, the machine can be configured at various concentrations (ppm).

200 ppm for the floor

400 ppm for the O.T.

600 ppm for the blood and urine spills

For the total eradication of microorganisms, this concentration is insufficient. For cleaning purposes, a higher concentration is necessary, which can be achieved with the use of Ef-chlor BIO-TAB/ Effi- Sept/ Effi- Kleen 1.67gm tablets.

Disadvantages-

- Hypochlorite use has disadvantages, including the following:
  - Rust and decay are caused by hypochlorite use on a regular basis on walls, machinery, and furniture.
  - The expense of using hypochlorite is quite large because it costs twice as much to buy it and to maintain it, thus maintenance must be done on a regular basis.
- The sterilant machine has the following disadvantages:
  - It takes up a lot of room and is expensive to run.
  - The machine needs routine maintenance and servicing.

Methodology

1. Collection of samples

Samples were taken from the hospital's most affected locations such as ICUs (beds, floors, and walls), N.I.C.U.s (beds, floors, and walls), general wards (beds, floors, and walls), reception areas (desks, chairs, floors, and walls), surgical tools, restrooms (door handles, walls, and floors), and so forth. Swab rods were used to collect the samples, which were then sent to the lab for analysis. The Civil Hospital in Sant Hirdaram Nagar, Bhopal, Madhya Pradesh, was where the samples were collected.

2. Identification of Microbes

The process of identifying microorganisms typically combines a number of approaches and methodologies.

Bacteria were identified by Gram's staining technique, which entailed making slides from

broth or Petri plate cultures and an inoculating loop in a sterile environment with laminar air flow.
Similar procedures were used to identify fungi on light microscope slides. Spores were collected from petri plate cultures using an inoculating loop, and the slides were then stained under sterile conditions using lactophenol cotton blue stain.

3. Antimicrobial activity of Ef-Chlor BIO-TAB/ Effi- Sept/ Effi- Kleen 1.67gm
The Agar- Well Diffusion Method was used for the antimicrobial evaluation of Ef-Chlor BIO TAB/ Effi- Sept/ Effi- Kleen. The main idea behind this technique is to place an antibiotic-impregnated disc on agar that has already been contaminated with the test microorganism. The antibiotic diffuses radially outward into the agar medium as a result of the disc absorbing moisture, resulting in a gradient of antibiotic concentration. The antibiotic concentration is highest right at the disc's edge and gradually drops down as you move away from it until it stops inhibiting the organism. The bacterium can now grow without restriction. After incubation, a clear zone or ring will be visible around the antibiotic disc if the antibiotic successfully limits bacterial growth. (**Tendencia, E. et al. 2004**). For this method, commonly used agar called Nutrient Agar Media (NAM) for bacteria and Sabouraud Dextrose Agar Media (SDA) for fungi culture.

Sterile petri plates were prepared and in the standardized bacterial and fungal suspension, pour the broth cultures (both bacteria and fungi) on to the petri plate and then pour the sterilized agar media (NAM and SDA) (cooled at normal room temperature) in the respective petri plates .

Each plate was divided into 4 equal portions in accordance with the 4 distinct NaDCC concentrations (10,000 ppm, 1000 ppm, 500 ppm, and 100 ppm). Subsequently, a sterile cork borer or tip is used to make hole measuring 6 to 8 mm in diameter. A volume of 20 to 100 µL of the antimicrobial agent or extract solution at the specified concentration is then added to the well. After that, agar plates are incubated in the appropriate environment for the test microorganism. After incubation, the plates were checked and observed if zones of inhibition have formed. If so, a zonal scale or a Vernier calliper was used to measure each zone. Graph was plotted after recording your observations in a table.

Results

1. Identification of Microbes

1.1. Bacterial Identification

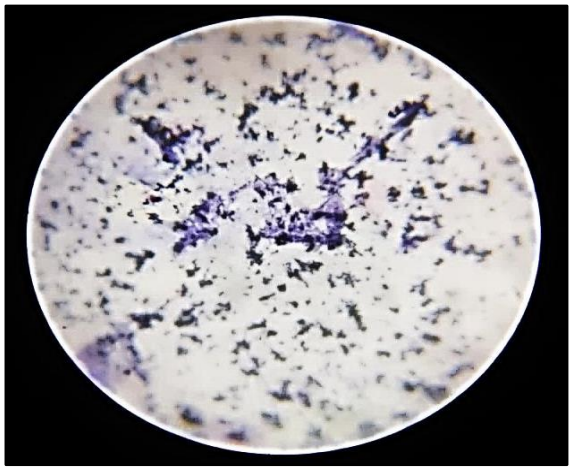


Fig. 3; ICU Floor

Microbe Identified:

- *Staphylococcus*

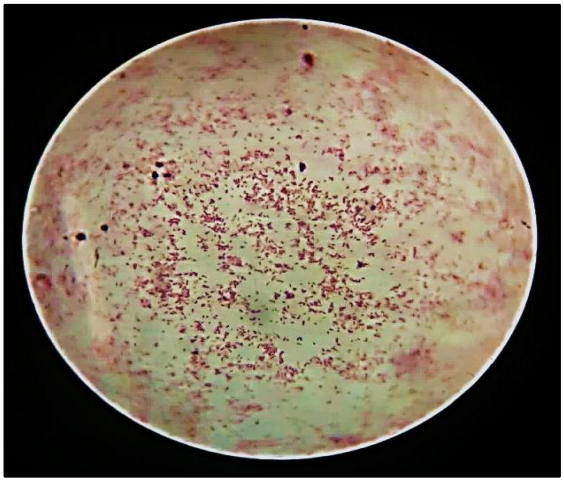


Fig. 4; ICU Wall

Microbe Identified:

- *Bacillus*

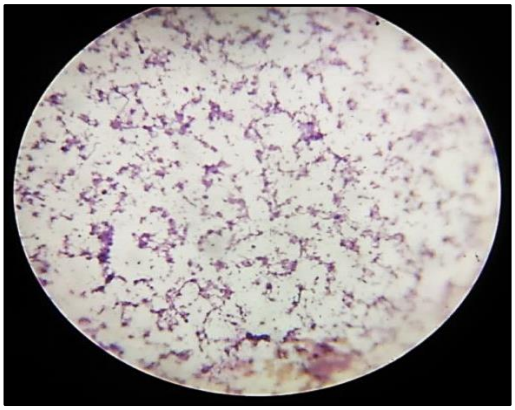


Fig. 5; NICU Bed

Microbe Identified:

- *Streptococcus*
- *Clostridium*
- *Vibrio cholerae*

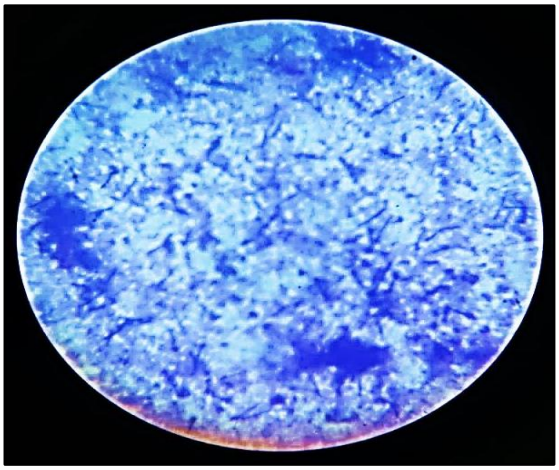


Fig. 6; NICU Floor

Microbe Identified:

- *Bacillus*
- *Streptococcus*
- *Vibrio cholerae*

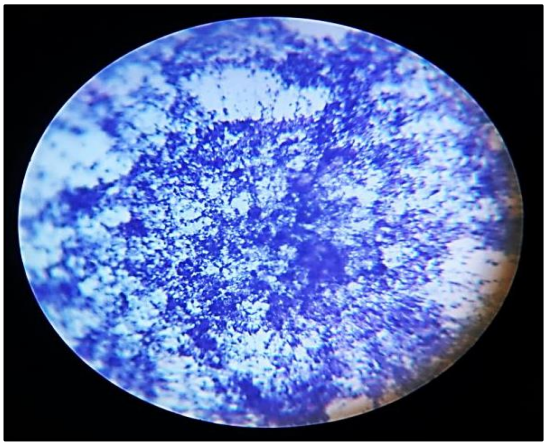


Fig. 7; NICU Wall

Microbe Identified:

- *Streptococcus*
- *Vibrio cholerae*

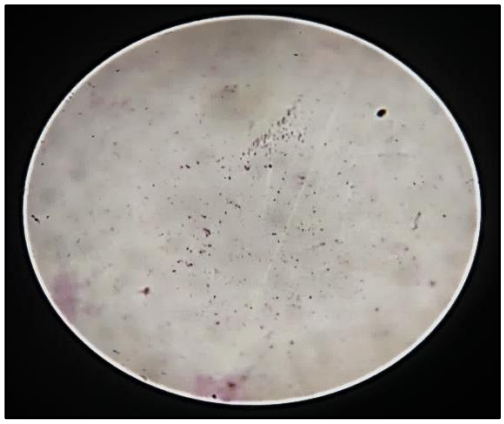


Fig. 8; Reception Area Desk

Microbe Identified:

- *Vibrio cholera*
- *Cocci*
- *Diplococci*

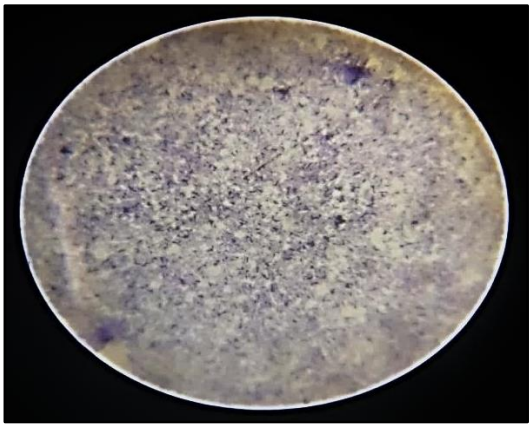


Fig. 9; Reception Area Floor

Microbe Identified:

- *Baqcillus species*
- *Cocci*
- *Clostridium*

Fig. 10; Reception Area Wall

Microbe Identified:

- *Streptococci*

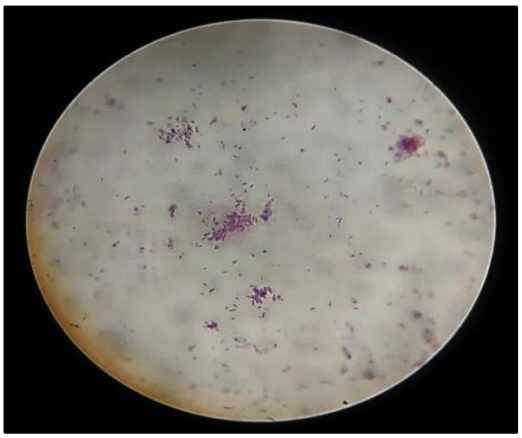


Fig. 11; Drip Stand

Microbe Identified:

- *Klebseilla*
- *E.coli*

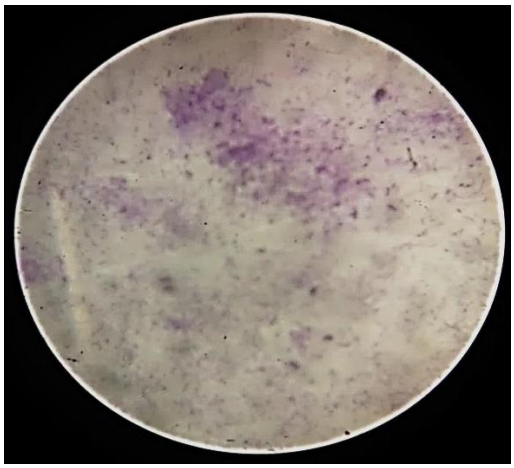


Fig. 12; General Ward Bed

Microbe Identified:

- *Cocci*
- *Diplococci*
- *Clostridium*

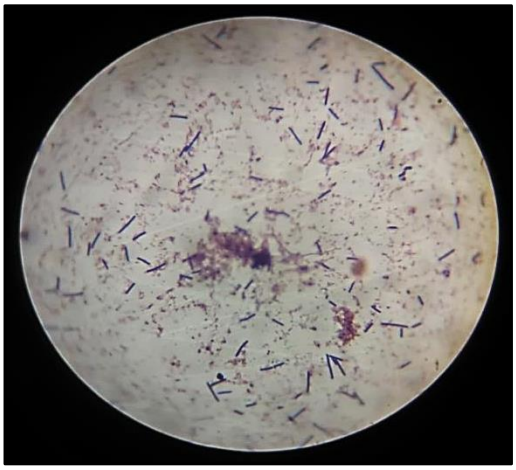


Fig. 13; General Ward Floor

Microbe Identified:

- *Bacillus species*

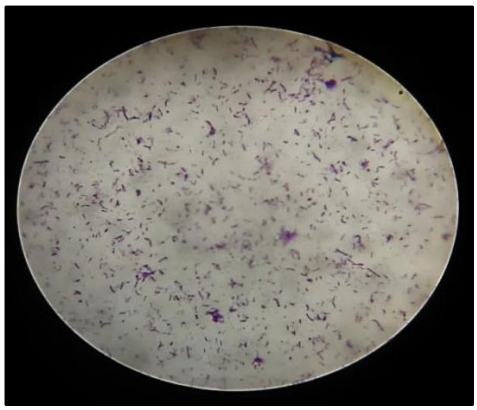


Fig. 14; General Ward Food Table

Microbe Identified:

- *Cocci*
- *Clostridium*
- *Vibrio cholerae*

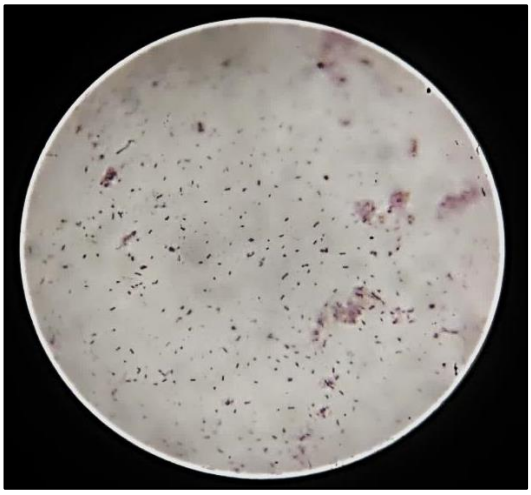


Fig. 15; General Ward Wall

Microbe Identified:

- *Cocci*
- *Diplococci*
- *Clostridium*

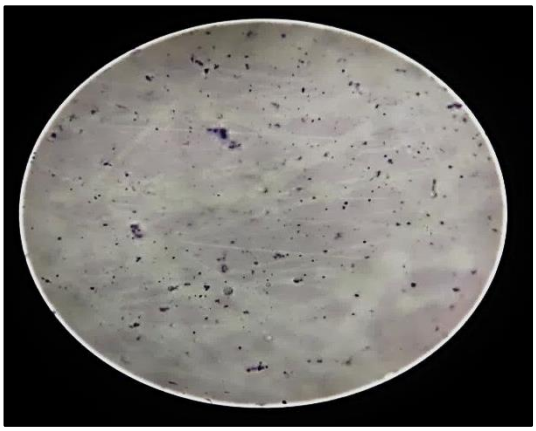


Fig. 16; Wheel Chair

Microbe Identified:

- *Streptococcus*
- *Vibrio cholerae*

1.2. Fungal Identification

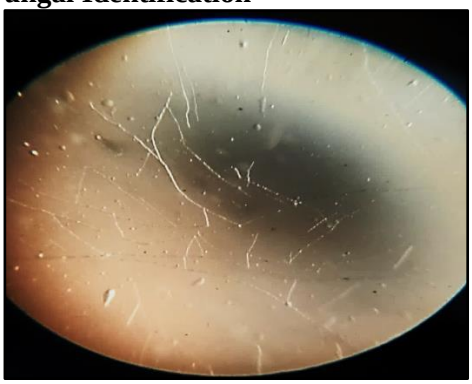


Fig. 17; ICU Bed

Microbe Identified:

- *Cladosporium*

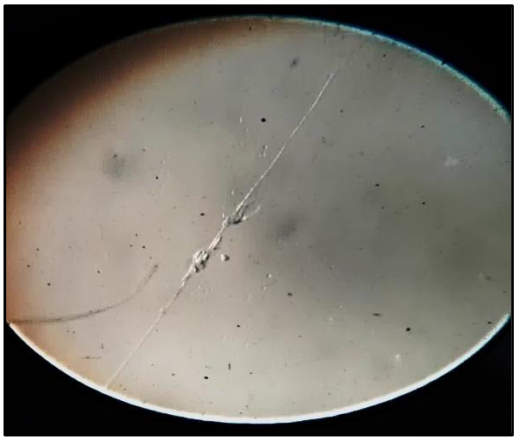


Fig. 18; ICU Floor

Microbe Identified:

- *Rhizopus*

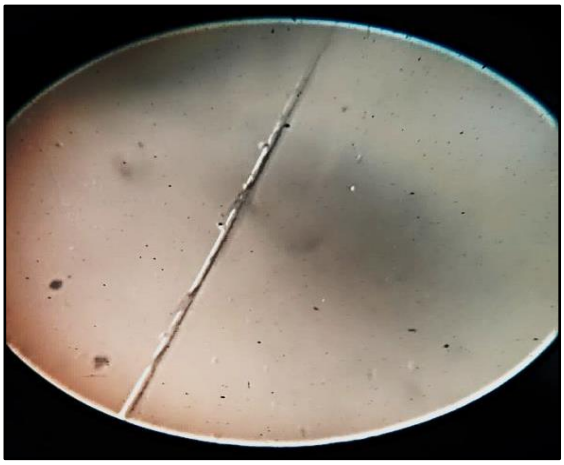


Fig. 19; ICU Wall

Microbe Identified:

- *Nigrospora*

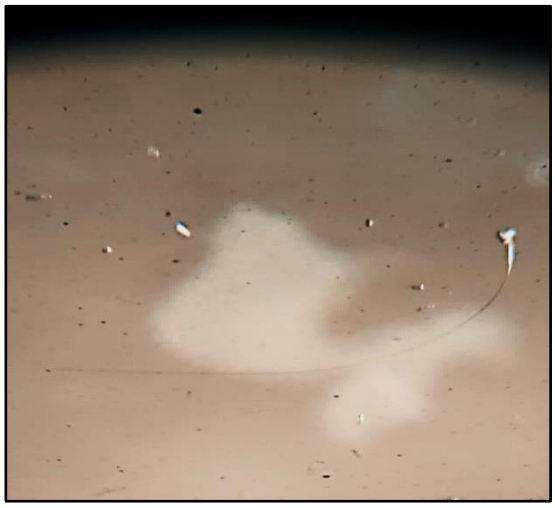


Fig. 20; NICU BED

Microbe Identified:

- *Mucor*

Fig. 21; NICU Floor

Microbe Identified:

- *Mucor*

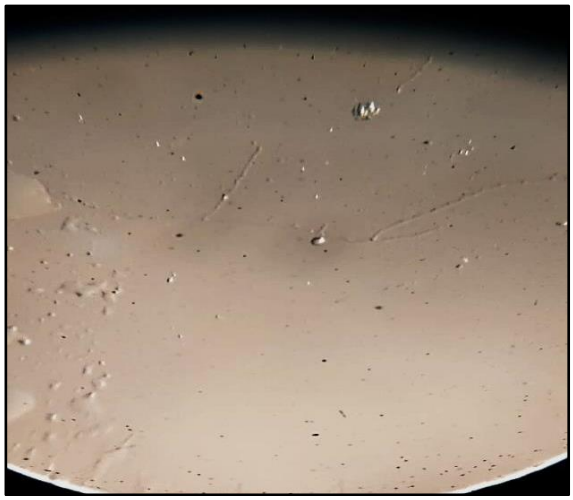


Fig. 22; NICU Wall

Microbe Identified:

- *Mucor*

Fig. 23; Reception Area- Desk

Microbe Identified:

- *Mucor*

Fig. 24; Reception Area- Floor

Microbe Identified:

- *Rhizopus*

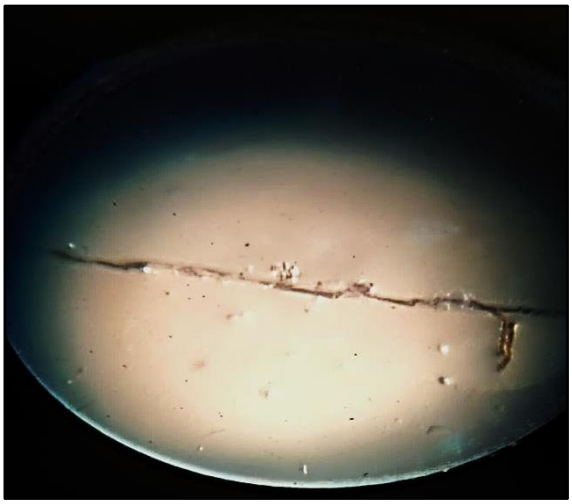


Fig. 25; Reception Area- Wall

Microbe Identified:

- *Mucor*

Fig. 26; General Ward- Bed

Microbe Identified:

- *Mucor*

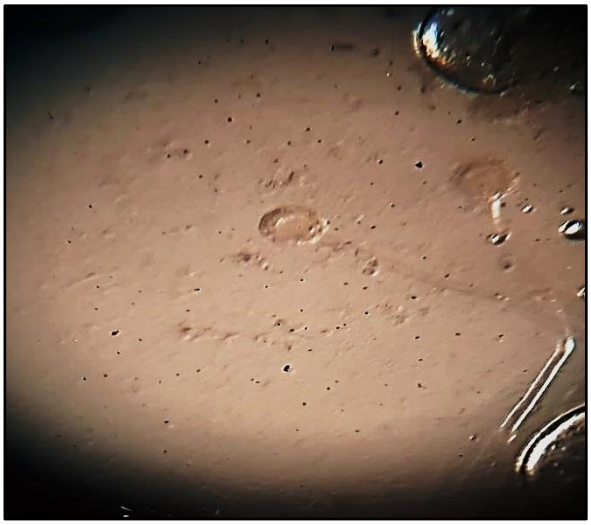


Fig. 27; General Ward- Floor

Microbe Identified:

- *Mucor*

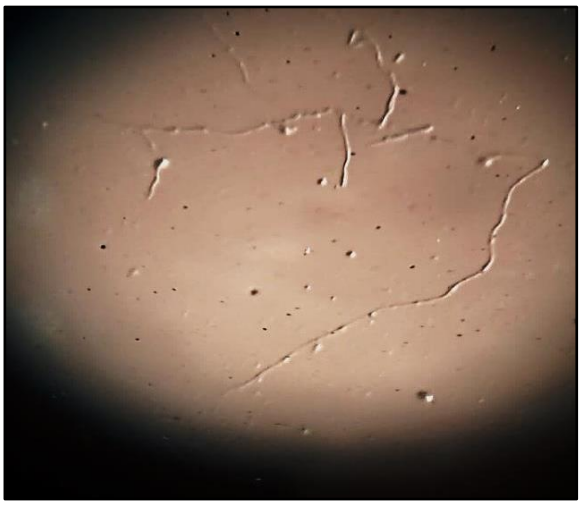


Fig. 28; General Ward- Food Table

Microbe Identified:

- *Mucor*

Fig. 29; General Ward- Wall

Microbe Identified:

- *Nigrospora*

Fig. 30; Drip Stand

Microbe Identified:

- *Mucor*

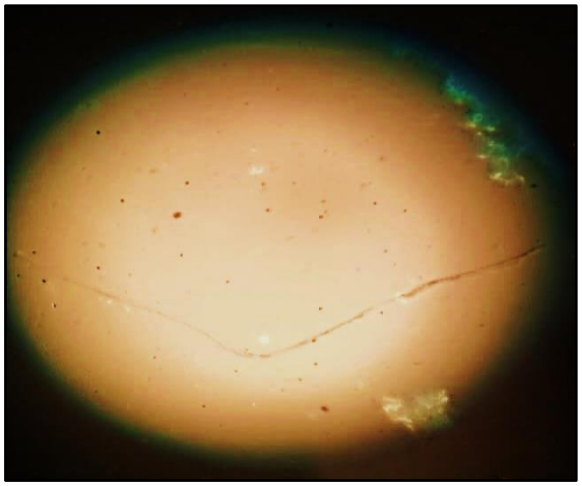


Fig. 31; Wheel Chair

Microbe Identified:

- *Mucor*

Note:

The microbes identified were shown in Table 1.

Table 1: Identification of Microbes

S. No.	Sub Category	Area	Method of sanitation	Microorganism Found	
				Bacteria	**Fungi**
1	**General ward**	Drip(IV)stands	Wiping	*Klebseilla, E. coli*	*Mucor*
		Bed	Wiping	*Cocci, Diplococci, Clostridium*	*Mucor*
		Floor	Mopping	*Bacillus species*	*Mucor*
		Walls	Mopping	*Cocci, Diplococci, Clostridium,*	*Nigrospora*
		Food table	Wiping	*Cocci, Clostridium, Vibrio cholerae*	*Mucor*
2	**N.I.C.U**	Bed	Wiping	*Streptococcus, Clostridium, Vibrio cholera,*	*Mucor*
		Floor	Mopping	*Bacillus, Streptococcus, Vibrio cholerae*	*Mucor*
		Walls	Mopping	*Staphylococcus, Vibrio cholera*	*Mucor*
3	**I.C.U.**	Bed	Wiping		*Cladosporium*
		Floor	Mopping	*Staphylococcus*	*Rhizopus*
		Walls	Mopping	*Bacillus*	*Nigrospora*
4	**Reception area**	Desk	Wiping	*Vibrio cholera, Cocci, Diplococci*	*Mucor*
		Walls	Mopping	*Streptococci*	*Mucor*
		Floor	Mopping	*Bacillus species, Cocci, Clostridium*	*Rhizopus*
		Wheel chairs	Wiping	*Streptococci, Vibrio cholerae*	*Mucor*

2. Antimicrobial activity of Ef-Chlor BIO-TAB/ EFFI- SEPT/ EFFI- KLEEN 1.67gm

According to the results, after 24 hours of incubation, a clear disc was obtained. The zone of inhibition can be denoted as a point at which no growth is observed to the naked eye.

Zones of different sizes were observed for different concentrations. There was no zone of

inhibition at 100 ppm and 500 ppm. A slight zone of inhibition was seen at 1000ppm and a much bigger zone of inhibition was seen at 10,000ppm.

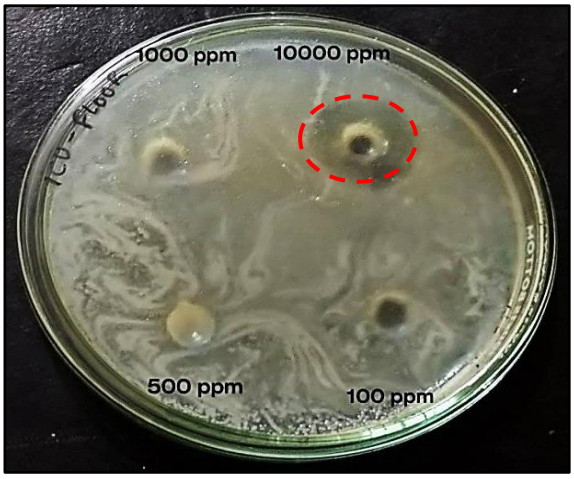


Fig. 38; ICU Floor

2.1 Against Bacteria:

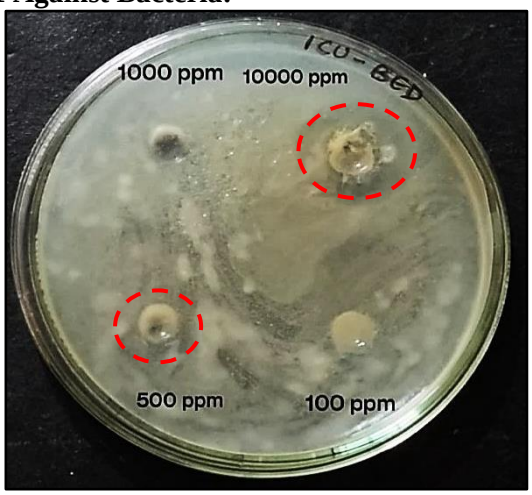


Fig. 37; ICU Bed

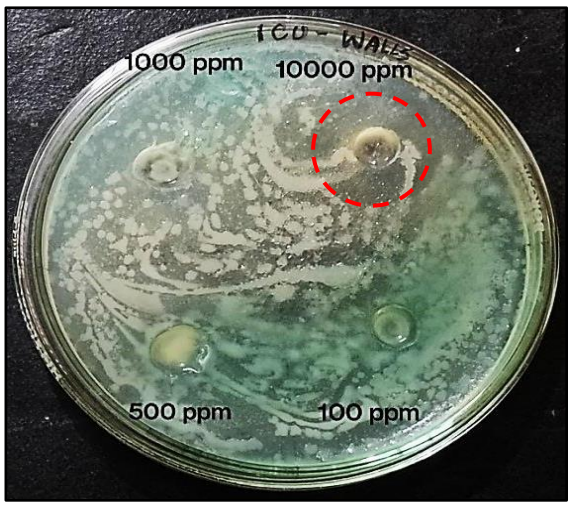


Fig. 39; ICU Wall

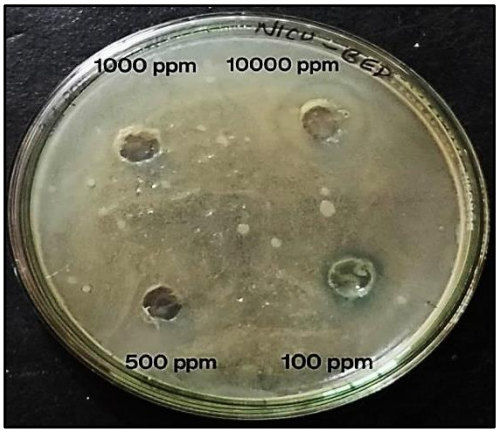


Fig. 40; NICU Bed

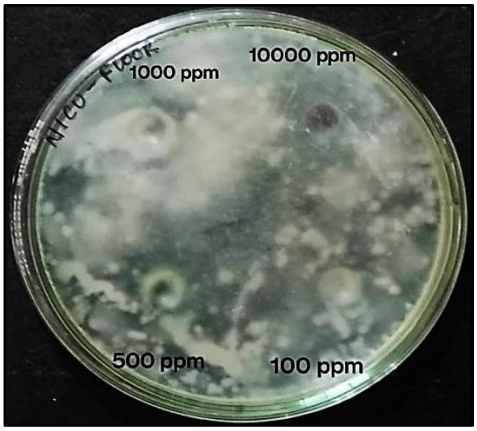


Fig. 41; NICU Floor

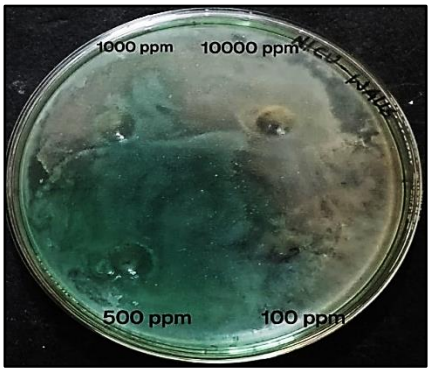


Fig. 42; NICU Wall

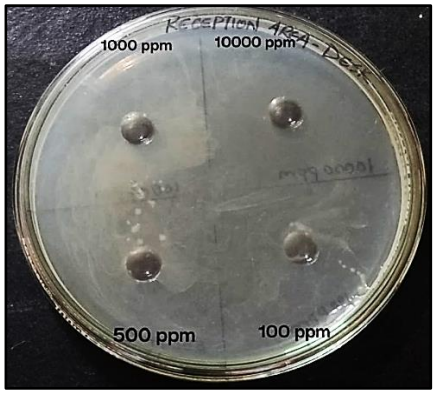


Fig. 43; Reception Area- Desk

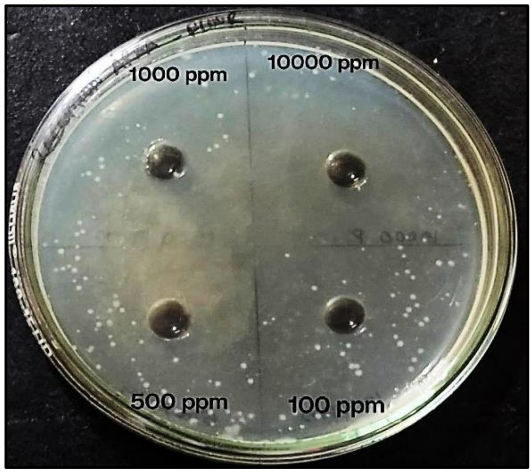


Fig. 44; Reception Area- Floor

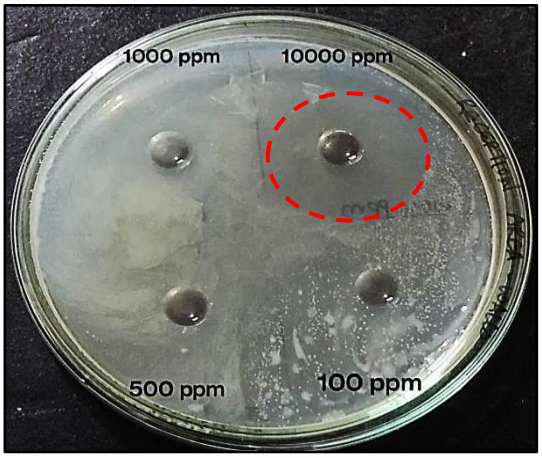


Fig. 45; Reception Area- Wall

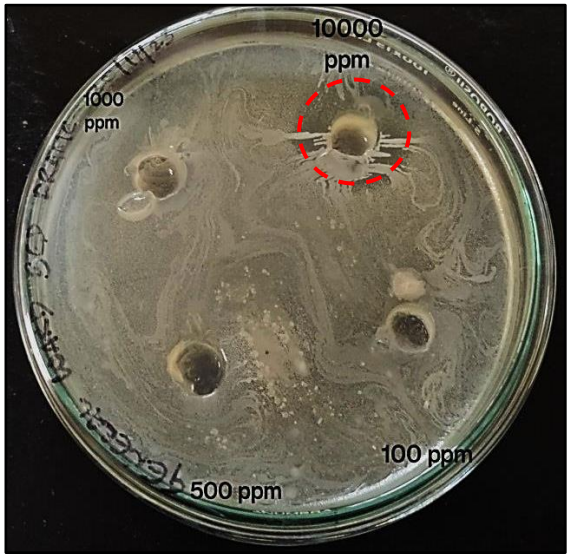


Fig. 46; General Ward Bed

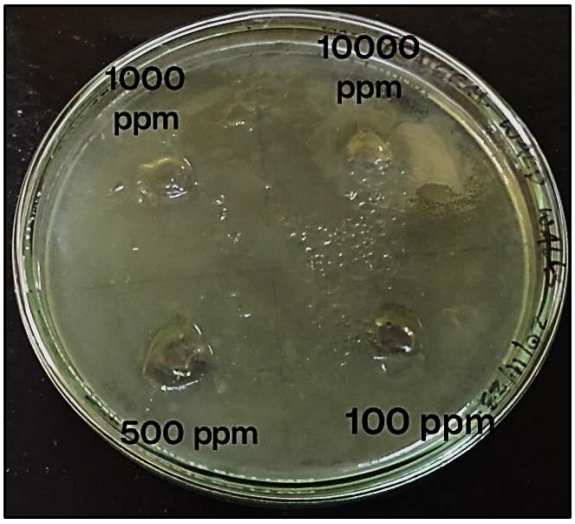


Fig. 47; General Ward Wall

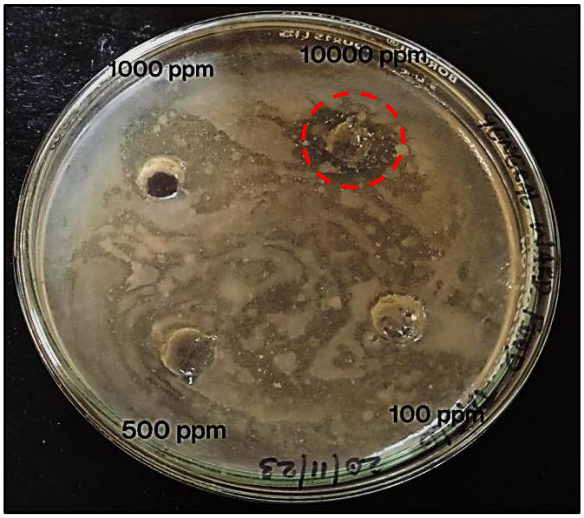


Fig. 48; General Ward food Table

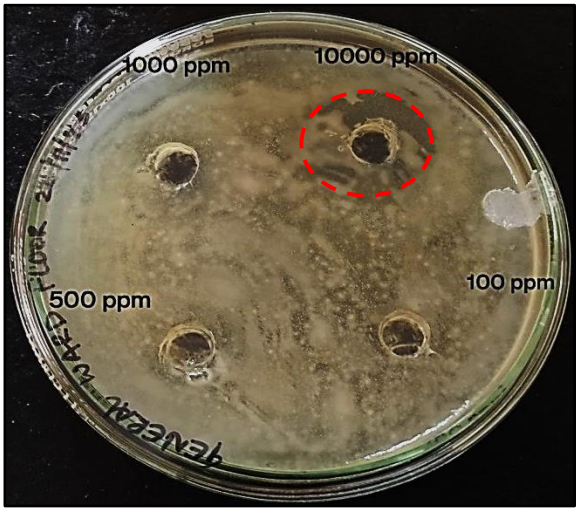


Fig. 49; General Ward Floor

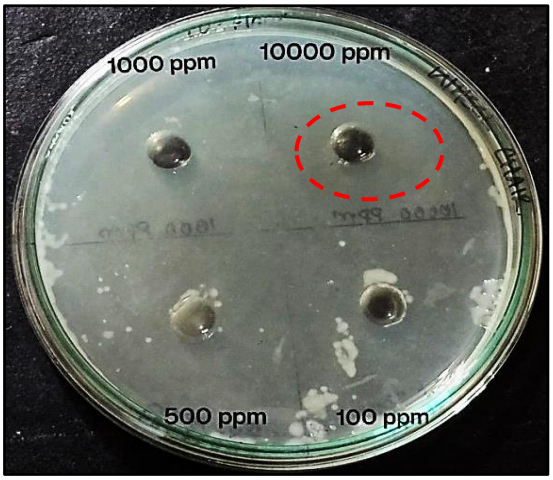


Fig. 50; WHEEL CHAIR

2.2 Against Fungi:

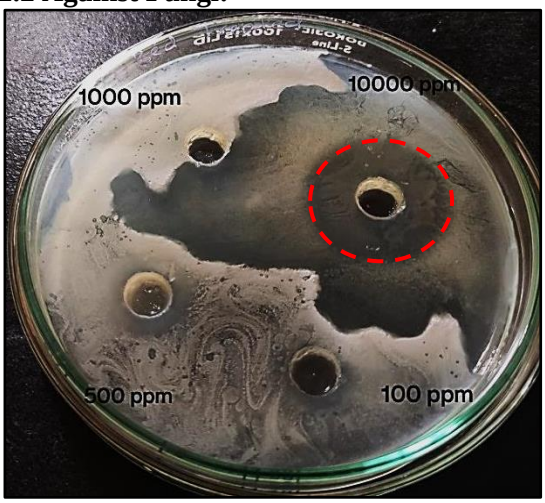


Fig. 51; ICU- Bed

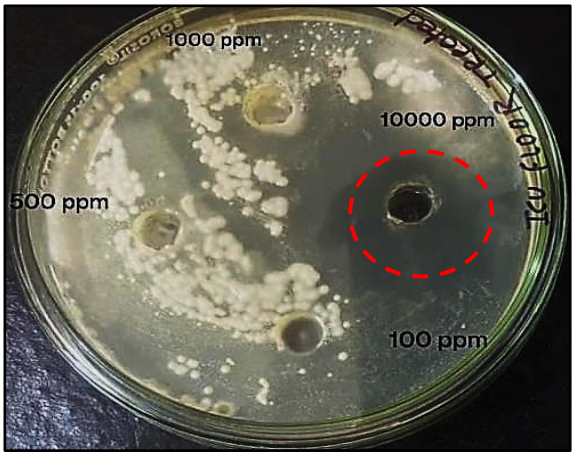


Fig. 52; ICU- Floor

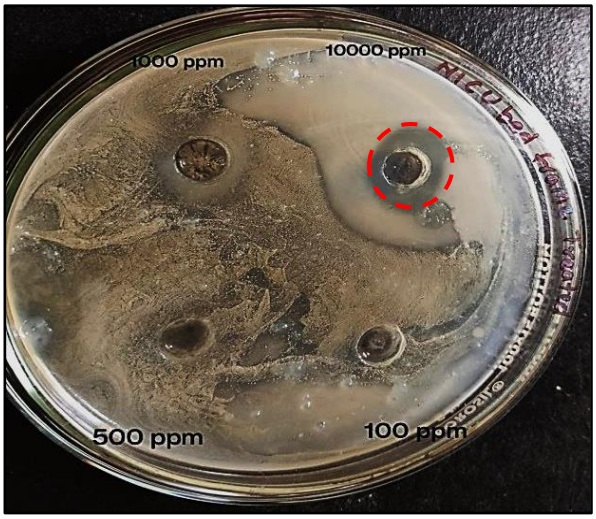


Fig. 53; NICU Bed

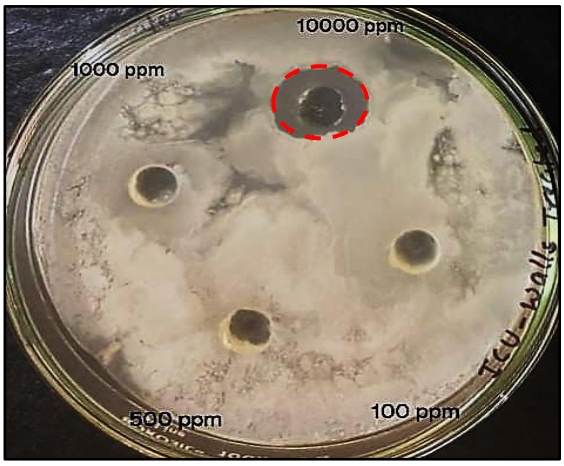


Fig. 54; ICU- Wall

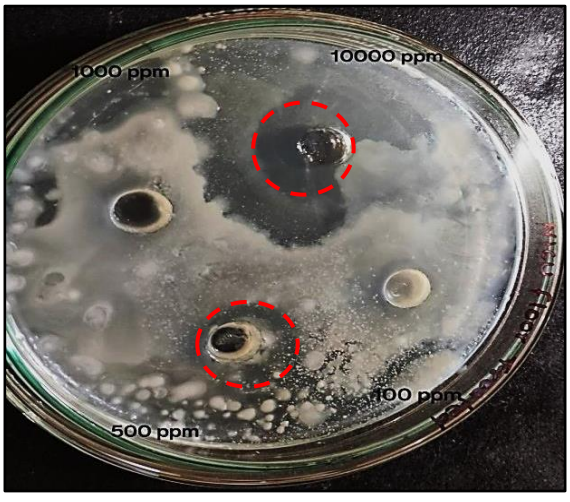


Fig. 55; NICU Floor

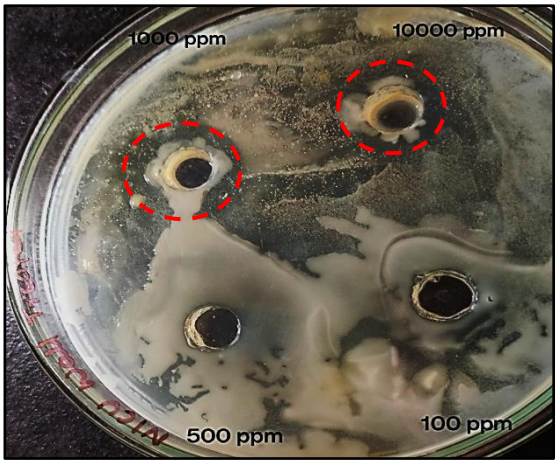


Fig. 56; NICU Wall

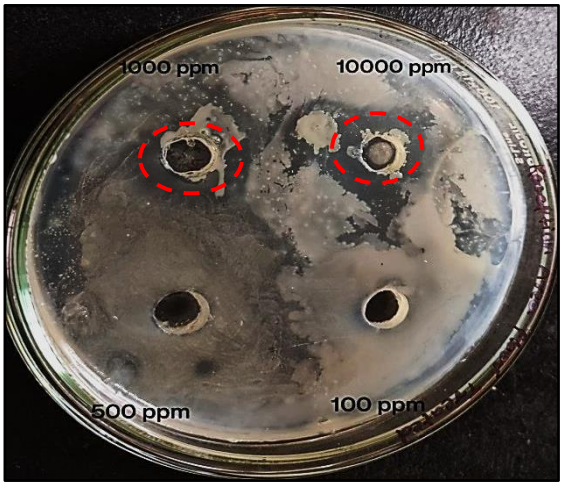


Fig. 57; RECEPTION AREA- Floor

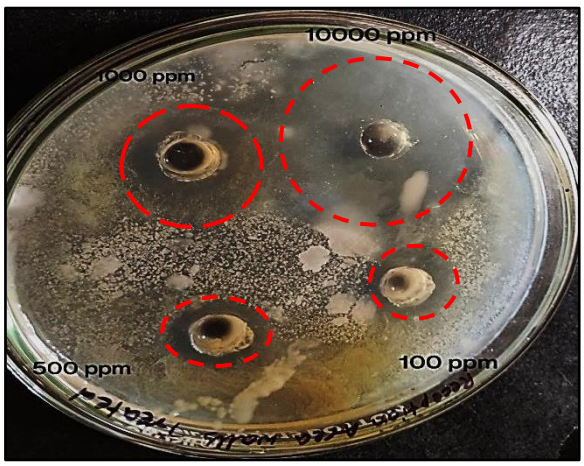


Fig. 58; RECEPTION AREA- Wall

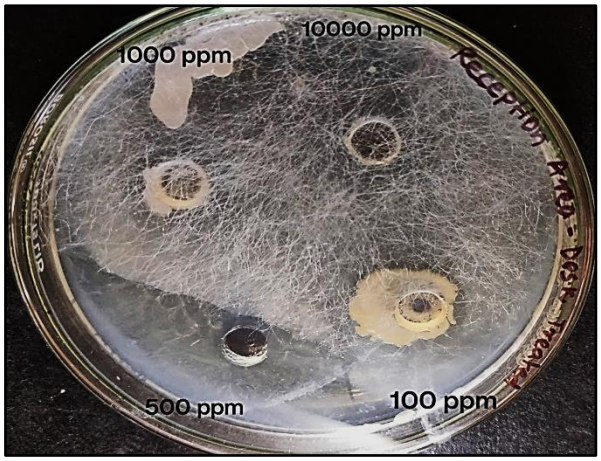


Fig. 59; RECEPTION AREA- Desk

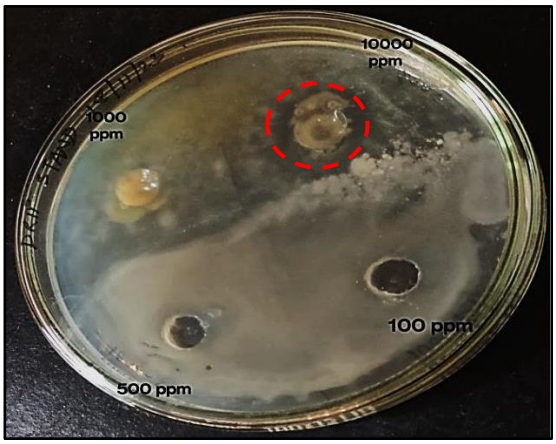


Fig. 60; GENERAL WARD- Drip Stand

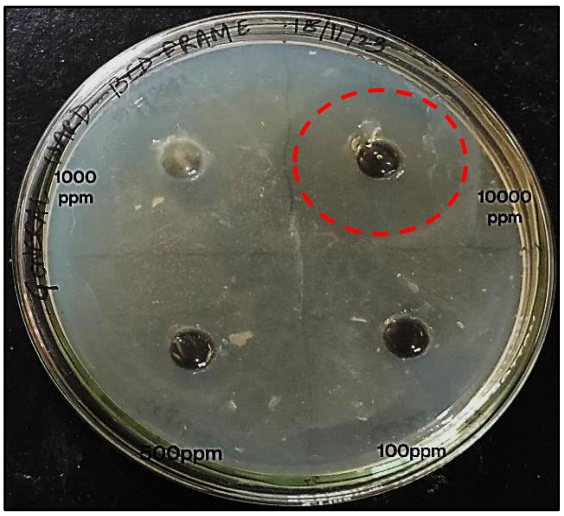


Fig. 61; GENERAL WARD- Bed

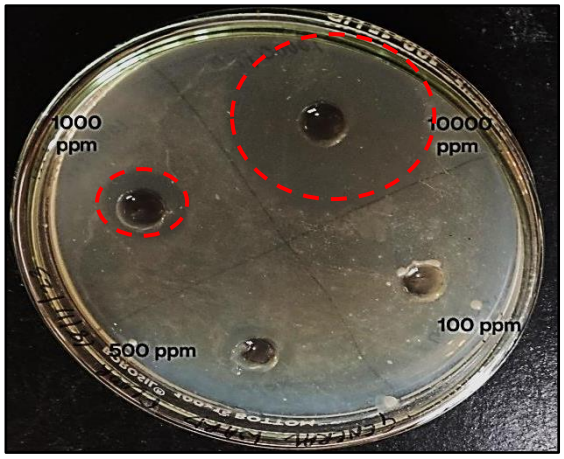


Fig. 62; GENERAL WARD- Floor

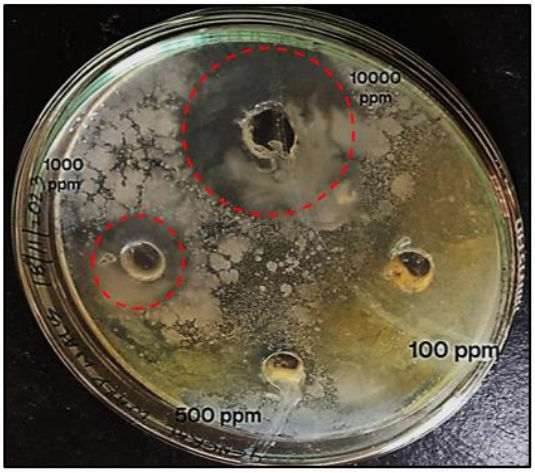


Fig. 64; GENERAL WARD- Wall

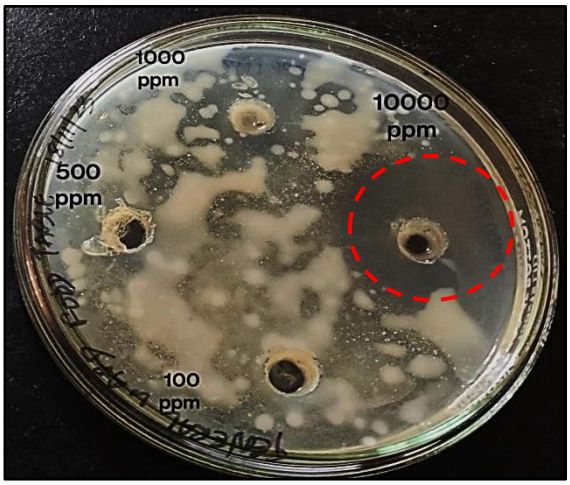
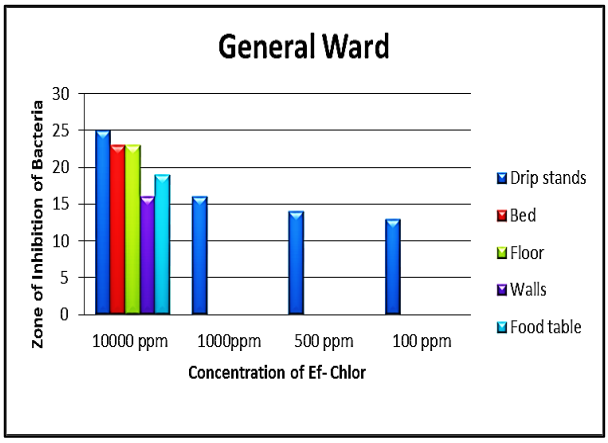


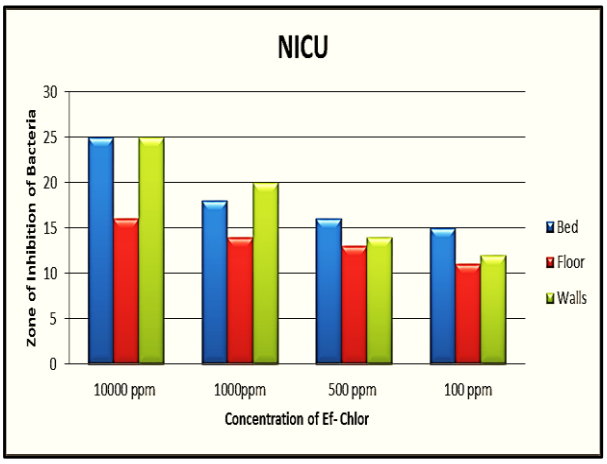
Fig. 63; GENERAL WARD- Food Table

Table 2; Zone of Inhibition

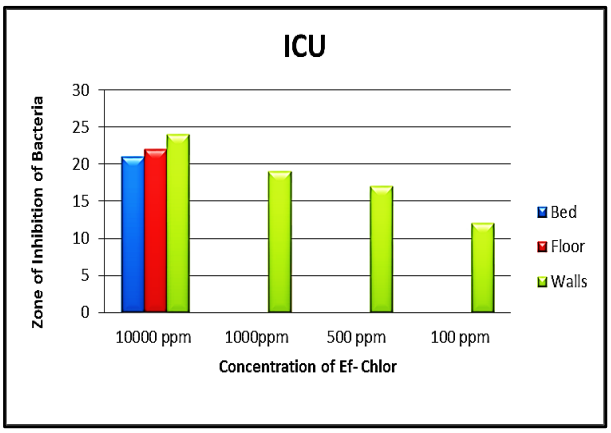
S. No.	Sub Category	Area	Zone of Inhibition (in mm)							
			Bacteria				Fungi			
			10000 ppm	1000 ppm	500ppm	100ppm	10000 ppm	1000 ppm	500ppm	100ppm
1	General ward	Drip stands	25	16	14	13	32	26	19	12
		Bed	23	-	-	-	33	22	16	13
		Floor	23	-	-	-	35	16	14	10
		Walls	16	-	-	-	33	20	13	-
		Food table	19	-	-	-	29	20	16	13
2	N.I.C.U	Bed	25	18	16	15	17	14	12	-
		Floor	16	14	13	11	28	19	14	-
		Walls	25	20	14	12	16	16	-	-
3	I.C.U.	Bed	21	-	-	-	29	21	15	14
		Floor	22	-	-	-	15	-	-	-
		Walls	24	19	17	12	14	-	-	-
4	Reception area	Desk	34	15	12	12	30	19	17	13
		Walls	34	12	-	-	36	27	18	14
		Floor	28	19	15	13	20	18	13	-
		Wheel chairs	30	16	14	13	30	18	16	14



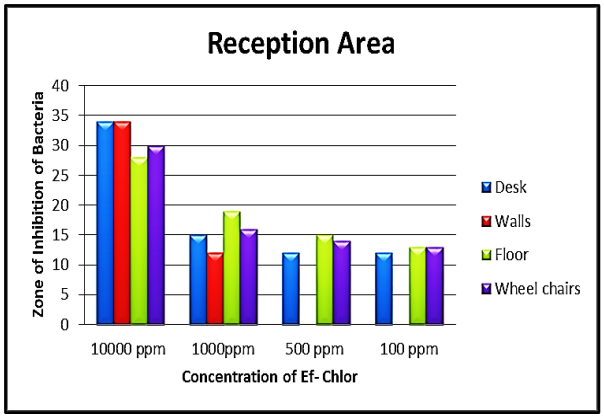
Graph 1; Antimicrobial Activity of Ef- Chlor Against Bacterial Samples of General Ward



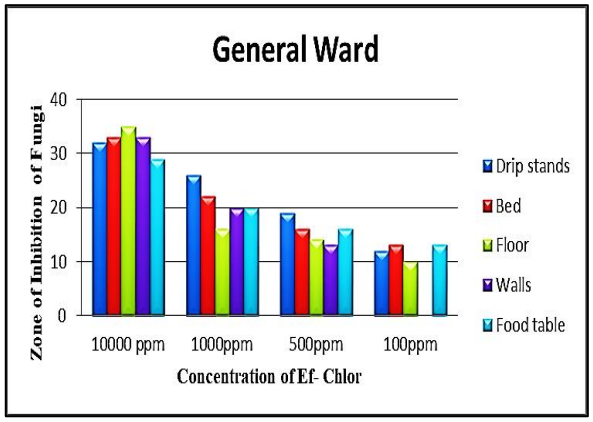
Graph 2; Antimicrobial Activity of Ef- Chlor Against Bacterial Samples of NICU



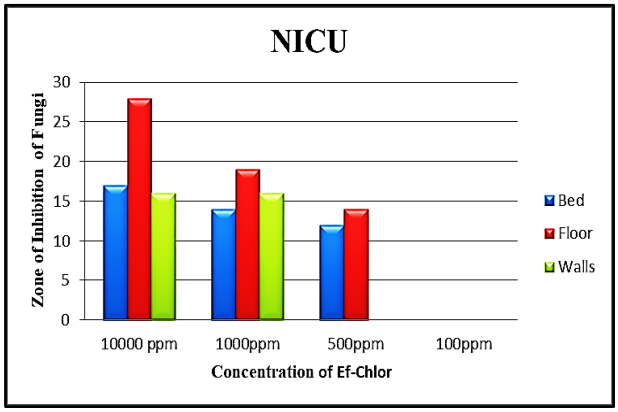
Graph 3; Antimicrobial Activity of Ef- Chlor Against Bacterial Samples of ICU



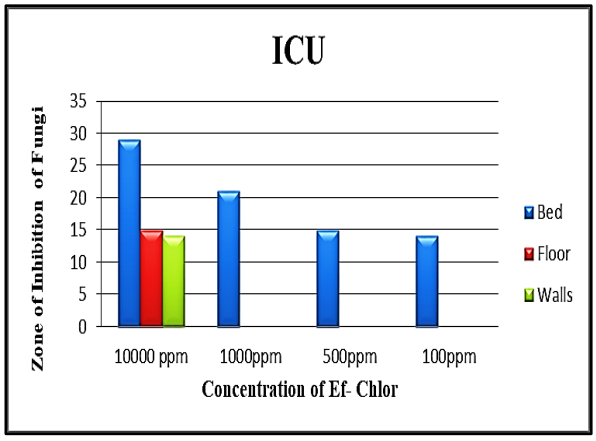
Graph 4; Antimicrobial Activity of Ef- Chlor Against Bacterial Samples of Reception Area



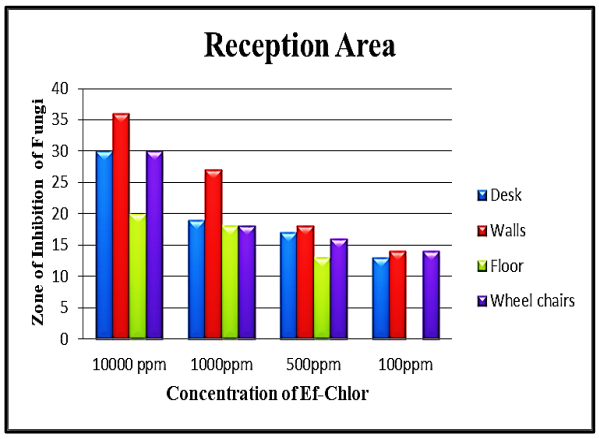
Graph 5; Antimicrobial Activity of Ef- Chlor Against Fungal Samples of General Ward



Graph 6; Antimicrobial Activity of Ef- Chlor Against Fungal Samples of NICU



Graph 7; Antimicrobial Activity of Ef- Chlor Against Fungal Samples of ICU



Graph 8; Antimicrobial Activity of Ef- Chlor Against Fungal Samples of Reception Area

Discussion

Gram's staining was used to identify a wide range of bacteria and lactophenol cotton blue staining method for fungi from the swab samples that were taken from various locations. However, bacteria such as *Staphylococcus, Streptococcus, Cocci, Diplococci,* ect; and fungi *Mucor* was the most often discovered microbe in the bulk of the locations. These bacteria have a long history of colonizing hospitals and producing nosocomial infections; as a result, they were termed "Hospital staphylococci" (**Ananthanarayan, R. et al., 2006**) whereas the fungi *Mucor* can cause nasal congestion, headache, fever, etc.

The administration of Ef-Chlor BIO-TAB/ Effi-Sept/ Effi- Kleen 1.67gm at varying doses results in the formation of a significant zone of inhibition (as shown in table 2) at 500 ppm, which increases between 1000 ppm and 10000 ppm.

The antimicrobial activity of Ef-Chlor at varying concentrations against samples of bacteria and fungi from various areas of hospitals is displayed in Graphs 1-8. The highest peak, which is determined to be at 10,000 ppm, indicates a larger zone of inhibition.

Conclusion

The administration of Ef-Chlor BIO-TAB/ Effi-Sept/ Effi- Kleen (1.67gm) produced positive outcomes, as seen by the distinct zones of inhibition. The application of NaDCC produced positive results, as the rates of microbial growth inhibition increased significantly at all tested doses. A notable reduction in microbial growth was seen at the maximum concentration of 10,000 ppm or more, indicating that NaDCC inhibits the microbial growth as concentrations rise. Positive antimicrobial effects of Ef-Chlor were clearly visible. The floor, work area, and equipment can all be cleaned using Ef-Chlor dissolved in water.

Use of a concentration greater than or equal to 10,000 ppm is recommended due to the antimicrobial activity of Ef-Chlor BIO-TAB/ Effi-Sept/ Effi- Kleen, as seen in graphs 1–8.

Acknowledgement

- We appreciate the staffs of Civil Hospital, Sant Hirdaram Nagar, Bhopal, Madhya Pradesh who kindly agreed to participate in the study. We especially appreciate them enabling us to share private information regarding their hygiene procedures.
- We would like to give a special thanks to Dr. Abhishek Gupta, director of the Center for Microbiology and Biotechnology (CMBT) Research and Training Institute, Nehru Nagar, Bhopal, Madhya Pradesh, 462003, for giving us the necessary time and space to complete the task on time.

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