**CHAPTER ONE**

**INTRODUCTION**

Liquid disinfectants are antimicrobial agents that are applied to non-living objects to destroy microorganisms that are living on the objects. The process of killing the microbes is called disinfection which is the killing of some or all of the pathogenic organisms that cause infection. Disinfection does not necessarily kill all microorganisms, especially resistant [bacteria spores](https://en.wikipedia.org/wiki/Endospore); it is less effective than [sterilization](https://en.wikipedia.org/wiki/Sterilization_(microbiology)), which is an extreme physical and chemical process that kills all types of life (U.S Center for Disease Control and Prevention, 2016). Disinfectants are different from other antimicrobial agents such as [antibiotics](https://en.wikipedia.org/wiki/Antibiotic), which destroy microorganisms within the body, and [antiseptics](https://en.wikipedia.org/wiki/Antiseptic), which destroy microorganisms on living [tissue](https://en.wikipedia.org/wiki/Biological_tissue). Disinfectants are also different from [biocides](https://en.wikipedia.org/wiki/Biocide). The latter are intended to destroy all forms of life not just microorganisms. Disinfectants work by destroying the cell wall of microbes or interfering with the metabolism.

Sanitizers are substances that simultaneously clean and disinfect (Mid Sussex District Council 2009). Disinfectants are frequently used in hospitals, dental surgeries, kitchens, and bathrooms to kill infectious organisms. Bacterial [endospores](https://en.wikipedia.org/wiki/Endospore) are most resistant to disinfectants, but some viruses and bacteria also possess some tolerance.

There are arguments for creating or maintaining conditions that are not conducive to bacterial survival and multiplication, rather than attempting to kill them with chemicals. Bacteria can increase in number very quickly, which enables them to [evolve](https://en.wikipedia.org/wiki/Evolution) rapidly. Should some bacteria survive a chemical attack, they give rise to new generations composed completely of bacteria that are resistance to particular chemical used. Under a sustained chemical attack, the surviving bacteria in successive generations are increasingly resistant to the chemical used, and ultimately the chemical is rendered ineffective.

**1.1 AIM**

* To determine the etopic application of disinfectants on wounds using animal model (Albino rats)

**1.2 OBJECTIVES**

* To determine the time duration of healing using animal models
* To determine the efficacy of the chosen disinfectants
* To identify the organism isolated from the wounds

**LITERATURE REVIE**

**CHAPTER TWO**

**2.1 Properties of disinfectants**

A perfect disinfectant will offer complete and full microbiological [sterilization](https://en.wikipedia.org/wiki/Sterilization_(microbiology)) without harming humans and useful forms of life. It can be inexpensive and noncorrosive. However, most disinfectants are also potentially harmful (even [toxic](https://en.wikipedia.org/wiki/Toxic)) to humans or animals by nature. Most modern household disinfectants contain [Bitrex](https://en.wikipedia.org/wiki/Denatonium), an exceptionally bitter substance added to discourage ingestion as a safety measure. Those that are used indoors should never be mixed with other cleaning products as [chemical reactions](https://en.wikipedia.org/wiki/Chemical_reaction) can occur (Department of Health and Senior Services April 23 2016). The choice of disinfectant to be used depends on the particular situation. Some disinfectants have a wide spectrum (kill many different types of microorganisms), while others kill a smaller range of disease-causing organisms but are preferred for other properties where they may be non-corrosive, non-toxic, or inexpensive (State Department of Health., 2015).

**2.2 Types of disinfectant**

1. **Air disinfectants**

Air disinfectants are typically chemical substances capable of disinfecting microorganisms suspended in the air. Disinfectants are generally assumed to be limited to use on surfaces, but that is not the case. In 1928, a study found that airborne microorganisms could be killed using mists of dilute bleach (Robertson *et al*., June 1942). An air disinfectant must be dispersed either as an [aerosol](https://en.wikipedia.org/wiki/Aerosol) or [vapour](https://en.wikipedia.org/wiki/Vapour) at a sufficient concentration in the air to cause the number of viable infectious microorganisms to be significantly reduced.

In the 1940s and early 1950s, further studies showed inactivation of diverse [bacteria](https://en.wikipedia.org/wiki/Bacteria), [influenza virus](https://en.wikipedia.org/wiki/Influenza_virus), and [*Penicillium chrysogenum*](https://en.wikipedia.org/wiki/Penicillium_chrysogenum) (previously *P. notatum*) [mold](https://en.wikipedia.org/wiki/Mold) [fungus](https://en.wikipedia.org/wiki/Fungus) using various glycols, principally [propylene glycol](https://en.wikipedia.org/wiki/Propylene_glycol) and [triethylene glycol](https://en.wikipedia.org/wiki/Triethylene_glycol) (Lester *et al*., 1952). In principle, these chemical substances are ideal air disinfectants because they have both high lethality to microorganisms and low mammalian toxicity (United States Environmental Protection Agency., 2006).

Although glycols are effective air disinfectants in controlled laboratory environments, it is more difficult to use them effectively in real-world environments because the disinfection of air is sensitive to continuous action. Continuous action in real-world environments with outside air exchanges at door and window interfaces, and in the presence of materials that absorb and remove glycols from the air, poses engineering challenges that are not critical for surface disinfection.

1. **Alcohol**

[Alcohol](https://en.wikipedia.org/wiki/Alcohol) and alcohol plus [Quaternary ammonium cation](https://en.wikipedia.org/wiki/Quaternary_ammonium_cation) based compounds comprise a class of proven surface sanitizers and disinfectants approved by the [EPA](https://en.wikipedia.org/wiki/EPA) and the [Centers for Disease Control](https://en.wikipedia.org/wiki/Centers_for_Disease_Control) for use as a hospital grade disinfectant (Guidelines library: Infection control., 28 (2016) Alcohols are most effective when combined with [distilled water](https://en.wikipedia.org/wiki/Distilled_water) to facilitate diffusion through the cell membrane; 100% alcohol typically denatures only external membrane proteins. A mixture of 70% ethanol or [iso-propanol](https://en.wikipedia.org/wiki/Isopropanol) diluted in water is effective against a wide spectrum of bacteria, though higher concentrations are often needed to disinfect wet surfaces (Moorer., 2003). Additionally, high-concentration mixtures (such as 80% ethanol + 5% iso-propanol) are required to effectively inactivate lipid-enveloped viruses (such as [HIV](https://en.wikipedia.org/wiki/HIV), [hepatitis B](https://en.wikipedia.org/wiki/Hepatitis_B), and [hepatitis C](https://en.wikipedia.org/wiki/Hepatitis_C))., (Lages *et al* 2018).

The efficacy of alcohol is enhanced when in solution with the wetting agent [dodecanoic acid](https://en.wikipedia.org/wiki/Dodecanoic_acid) (coconut soap). The synergistic effect of 29.4% ethanol with dodecanoic acid is effective against a broad spectrum of bacteria, fungi, and viruses. Further testing is being performed against [*Clostridium difficile*](https://en.wikipedia.org/wiki/Clostridium_difficile_(bacteria)) spores with higher concentrations of ethanol and dodecanoic acid, which proved effective with a contact time of ten minutes.

1. **Aldehyes**

[Aldehydes](https://en.wikipedia.org/wiki/Aldehyde), such as [formaldehyde](https://en.wikipedia.org/wiki/Formaldehyde) and [glutaraldehyde](https://en.wikipedia.org/wiki/Glutaraldehyde), have a wide microbiocidal activity and are [sporicidal](https://en.wikipedia.org/wiki/Sporicidal) and [fungicidal](https://en.wikipedia.org/wiki/Fungicidal). They are partly inactivated by organic matter and have slight residual activity. Some bacteria have developed resistance to glutaraldehyde, and it has been found that glutaraldehyde can cause asthma and other health hazards, hence [ortho-phthalaldehyde](https://en.wikipedia.org/wiki/Ortho-phthalaldehyde) is replacing glutaraldehyde.

1. **Oxidizing Agent**

[Oxidizing agents](https://en.wikipedia.org/wiki/Redox) act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell [lysis](https://en.wikipedia.org/wiki/Lysis) and death. A large number of disinfectants operate in this way. [Chlorine](https://en.wikipedia.org/wiki/Chlorine) and [oxygen](https://en.wikipedia.org/wiki/Oxygen) are strong oxidizers, so their compounds figure heavily here.

* [**Sodium hypochlorite**](https://en.wikipedia.org/wiki/Sodium_hypochlorite) is very commonly used. Common household [bleach](https://en.wikipedia.org/wiki/Bleach) is a sodium hypochlorite solution and is used in the home to disinfect drains, [toilets](https://en.wikipedia.org/wiki/Toilet), and other surfaces. In more dilute form, it is used in swimming pools, and in still more dilute form, it is used in drinking water. When pools and drinking water are said to be chlorinated, it is actually sodium hypochlorite or a related compound (not pure chlorine) that is being used. Chlorine partly reacts with proteinaceous liquids such as blood to form non-oxidizing N-chloro compounds, and thus higher concentrations must be used if disinfecting surfaces after blood spills (Weber *et al* 1999). Commercial solutions with higher concentrations contain substantial amounts of [sodium hydroxide](https://en.wikipedia.org/wiki/Sodium_hydroxide) for stabilization of the concentrated hypochlorite, which would otherwise decompose to chlorine, but the solutions become strongly basic as a result.
* [**Calcium hypochlorite**](https://en.wikipedia.org/wiki/Calcium_hypochlorite) are used as specially as a swimming pool additive. Hypochlorites yield an aqueous solution of [hypochlorous acid](https://en.wikipedia.org/wiki/Hypochlorous_acid) that is the true disinfectant. Hypobromite solutions are also sometimes used.
* [**Electrolyzed water**](https://en.wikipedia.org/wiki/Electrolyzed_water) **or "Anolyte"** is an oxidizing, acidic hypochlorite solution made by [electrolysis](https://en.wikipedia.org/wiki/Electrolysis) of [sodium chloride](https://en.wikipedia.org/wiki/Sodium_chloride) into [sodium hypochlorite](https://en.wikipedia.org/wiki/Sodium_hypochlorite) and hypochlorous acid. Anolyte has an oxidation-reduction potential of +600 to +1200 mV and a typical pH range of 3.5––8.5, but the most potent solution is produced at a controlled pH 5.0–6.3 where the predominant oxychlorine species is [hypochlorous acid](https://en.wikipedia.org/wiki/Hypochlorous_acid).
* [**Chloramine**](https://en.wikipedia.org/wiki/Chloramine) is often used in drinking water treatment. The particular type of chloramine used in drinking water disinfection is called monochloramine. Monochloramine is mixed into water in levels that kill germs but are still safe to drink., (Cdc Gov 2 2018).
* [**Chloramine-T**](https://en.wikipedia.org/wiki/Chloramine-T) is antibacterial even after the chlorine has been spent, since the parent compound is a [sulfonamide](https://en.wikipedia.org/wiki/Sulfonamide_(medicine)) [antibiotic](https://en.wikipedia.org/wiki/Antibiotic).
* [**Chlorine dioxide**](https://en.wikipedia.org/wiki/Chlorine_dioxide) is used as an advanced disinfectant for drinking water to reduce waterborne diseases. In certain parts of the world, it has largely replaced chlorine because it forms fewer byproducts. [Sodium chlorite](https://en.wikipedia.org/wiki/Sodium_chlorite), [sodium chlorate](https://en.wikipedia.org/wiki/Sodium_chlorate), and [potassium chlorate](https://en.wikipedia.org/wiki/Potassium_chlorate) are used as precursors for generating chlorine dioxide.
* [**Hydrogen peroxide**](https://en.wikipedia.org/wiki/Hydrogen_peroxide) is used in [hospitals](https://en.wikipedia.org/wiki/Hospital) to disinfect surfaces and it is used in solution alone or in combination with other chemicals as a high level disinfectant. Hydrogen peroxide is sometimes mixed with [colloidal silver](https://en.wikipedia.org/wiki/Colloidal_silver). It is often preferred because it causes far fewer [allergic](https://en.wikipedia.org/wiki/Allergic) reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic.
* [**Hydrogen peroxide vapour**](https://en.wikipedia.org/wiki/Vaporized_hydrogen_peroxide) is used as a [medical sterilant](https://en.wikipedia.org/wiki/Sterilization_(microbiology)) and as room disinfectant. Hydrogen peroxide has the advantage that it decomposes to form oxygen and water thus leaving no long term residues, but hydrogen peroxide as with most other strong oxidants is hazardous, and solutions are a primary irritant. The vapor is hazardous to the respiratory system and eyes and consequently the [OSHA](https://en.wikipedia.org/wiki/Occupational_Safety_and_Health_Administration) permissible exposure limit is 1 ppm (29 CFR 1910.1000 Table Z-1) calculated as an eight-hour time weighted average and the [NIOSH](https://en.wikipedia.org/wiki/National_Institute_for_Occupational_Safety_and_Health) immediately dangerous to life and health limit is 75 ppm., Cdc gov 31(2009). Therefore engineering controls, personal protective equipment, gas monitoring etc. should be employed where high concentrations of hydrogen peroxide are used in the workplace. Vaporized hydrogen peroxide is one of the chemicals approved for decontamination of anthrax spores from contaminated buildings, such as occurred during the [2001 anthrax attacks](https://en.wikipedia.org/wiki/2001_anthrax_attacks) in the U.S. It has also been shown to be effective in removing exotic animal viruses, such as avian influenza and [Newcastle disease](https://en.wikipedia.org/wiki/Newcastle_disease) from equipment and surfaces.
* The antimicrobial action of hydrogen peroxide can be enhanced by [surfactants](https://en.wikipedia.org/wiki/Surfactant) and organic acids. The resulting chemistry is known as Accelerated Hydrogen Peroxide. A 2% solution, stabilized for extended use, achieves high-level disinfection in 5 minutes, and is suitable for disinfecting medical equipment made from hard plastic, such as in [endoscopes](https://en.wikipedia.org/wiki/Endoscope) (Omidbakhsh *et al* 2006). The evidence available suggests that products based on Accelerated Hydrogen Peroxide, apart from being good germicides, are safer for humans and benign to the environment (Sattar *et al* 1998).
* [**Iodine**](https://en.wikipedia.org/wiki/Iodine) is usually dissolved in an organic solvent or as [Lugol's iodine](https://en.wikipedia.org/wiki/Lugol%27s_iodine) solution. It is used in the [poultry](https://en.wikipedia.org/wiki/Poultry) industry. It is added to the birds' drinking water. In human and veterinary medicine, iodine products are widely used to prepare incision sites prior to surgery. Although it increases both scar tissue formation and healing time, [tincture of iodine](https://en.wikipedia.org/wiki/Tincture_of_iodine) is used as an antiseptic for skin cuts and scrapes, and remains among the most effective antiseptics known. Also used as an [iodophor](https://en.wikipedia.org/wiki/Iodophor)
* [**Ozone**](https://en.wikipedia.org/wiki/Ozone) is a gas used for disinfecting water, laundry, foods, air, and surfaces. It is chemically aggressive and destroys many organic compounds, resulting in rapid decolorization and deodorization in addition to disinfection. Ozone decomposes relatively quickly. However, due to this characteristic of ozone, tap water chlorination cannot be entirely replaced by ozonation, as the ozone would decompose already in the water piping. Instead, it is used to remove the bulk of oxidizable matter from the water, which would produce small amounts of [organochlorides](https://en.wikipedia.org/wiki/Organochloride) if treated with chlorine only. Regardless, ozone has a very wide range of applications from municipal to industrial water treatment due to its powerful reactivity.
* [**Peracetic acid**](https://en.wikipedia.org/wiki/Peracetic_acid) is a disinfectant produced by reacting hydrogen peroxide with acetic acid. It is broadly effective against microorganisms and is not deactivated by [catalase](https://en.wikipedia.org/wiki/Catalase) and [peroxidase](https://en.wikipedia.org/wiki/Peroxidase), the enzymes that break down hydrogen peroxide. It also breaks down to food safe and environmentally friendly residues (acetic acid and hydrogen peroxide), and therefore can be used in non-rinse applications. It can be used over a wide temperature range (0-40 °C), wide [pH](https://en.wikipedia.org/wiki/PH) range (3.0-7.5), in [clean-in-place](https://en.wikipedia.org/wiki/Clean-in-place) (CIP) processes, in [hard water](https://en.wikipedia.org/wiki/Hard_water) conditions, and is not affected by protein residues.
* [**Performic acid**](https://en.wikipedia.org/wiki/Performic_acid) is the simplest and most powerful perorganic acid. Formed from the reaction of hydrogen peroxide and formic acid, it reacts more rapidly and powerfully than peracetic acid before breaking down to water and carbon dioxide. One must take caution when using, as it can be irritating to the skin, eyes, and mucous membranes.
* [**Potassium permanganate**](https://en.wikipedia.org/wiki/Potassium_permanganate) (KMnO4) is a purplish-black crystalline powder that colours everything it touches, through a strong oxidising action. This includes staining "stainless" steel, which somehow limits its use and makes it necessary to use plastic or glass containers. It is used to disinfect [aquariums](https://en.wikipedia.org/wiki/Aquarium) and is also widely used in community swimming pools to disinfect ones feet before entering the pool. Typically, a large shallow basin of KMnO4/water solution is kept near the pool ladder. Participants are required to step in the basin and then go into the pool. Additionally, it is widely used to disinfect community water ponds and wells in tropical countries, as well as to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution.
* [**Potassium peroxymonosulf ate**](https://en.wikipedia.org/wiki/Potassium_peroxymonosulfate), the principal ingredient in [Virkon](https://en.wikipedia.org/wiki/Virkon), is a wide-spectrum disinfectant used in laboratozries. [Virkon](https://en.wikipedia.org/wiki/Virkon) kills bacteria, viruses, and fungi. It is used as a 1% solution in water, and keeps for one week once it is made up. It is expensive but very effective, its pink colour fades as it is used up so it is possible to see at a glance if it is still fresh.

1. **Phenols**

[Phenolics](https://en.wikipedia.org/wiki/Phenols) are active ingredients in some household disinfectants. They are also found in some mouthwashes and in disinfectant soap and hand washes. Phenols are toxic to cats and newborn babies.

* [Phenol](https://en.wikipedia.org/wiki/Phenol) is probably the oldest known disinfectant as it was first used by [Lister](https://en.wikipedia.org/wiki/Joseph_Lister,_1st_Baron_Lister), when it was called carbolic acid. It is rather corrosive to the skin and sometimes toxic to sensitive people. Impure preparations of phenol were originally made from [coal tar](https://en.wikipedia.org/wiki/Coal_tar), and these contained low concentrations of other [aromatic hydrocarbons](https://en.wikipedia.org/wiki/Aromatic_hydrocarbon) including [benzene](https://en.wikipedia.org/wiki/Benzene), which is an [IARC](https://en.wikipedia.org/wiki/International_Agency_for_Research_on_Cancer) [Group 1](https://en.wikipedia.org/wiki/List_of_IARC_Group_1_carcinogens) [carcinogen](https://en.wikipedia.org/wiki/Carcinogen).
* [***o*-Phenylphenol**](https://en.wikipedia.org/wiki/2-Phenylphenol) is often used instead of [phenol](https://en.wikipedia.org/wiki/Phenol), since it is somewhat less corrosive.
* [**Chloroxylenol**](https://en.wikipedia.org/wiki/Chloroxylenol) is the principal ingredient in [dettol](https://en.wikipedia.org/wiki/Dettol), a household disinfectant and [antiseptic](https://en.wikipedia.org/wiki/Antiseptic).
* [**Hexachlorophene**](https://en.wikipedia.org/wiki/Hexachlorophene) is a phenolic that was once used as a germicidal additive to some household products but was banned due to suspected harmful effects.
* [**Thymol**](https://en.wikipedia.org/wiki/Thymol) derived from the herb thyme, is the active ingredient in some "broad spectrum" disinfectants that often bear ecological claims. It is used as a stabilizer in pharmaceutic preparations. It has been used for its antiseptic, antibacterial, and antifungal actions, and was formerly used as a vermifuge (a drug that causes the death of intestinal worm).
* [**Amylmetacresol**](https://en.wikipedia.org/wiki/Amylmetacresol) is found in [Strepsils](https://en.wikipedia.org/wiki/Strepsils), a throat disinfectant.

1. **Quaternary ammonium compounds**

[Quaternary ammonium compounds](https://en.wikipedia.org/wiki/Quaternary_ammonium_compounds) ("quats"), such as [benzalkonium chloride](https://en.wikipedia.org/wiki/Benzalkonium_chloride), are a large group of related compounds. Some concentrated formulations have been shown to be effective low-level disinfectants. Quaternary Ammonia at or above 200ppm plus Alcohol solutions exhibit efficacy against difficult to kill non-enveloped viruses such as [norovirus](https://en.wikipedia.org/wiki/Norovirus), [rotavirus](https://en.wikipedia.org/wiki/Rotavirus), or [polio virus](https://en.wikipedia.org/wiki/Polio_virus).[[11]](https://en.wikipedia.org/wiki/Disinfectant#cite_note-cdc.gov-11) Newer synergous, low-alcohol formulations are highly effective broad-spectrum disinfectants with quick contact times (3–5 minutes) against bacteria, enveloped viruses, pathogenic fungi, and [mycobacteria](https://en.wikipedia.org/wiki/Mycobacteria). Quats are biocides that also kill algae and are used as an additive in large-scale industrial water systems to minimize undesired biological growth.

### Silver

[Silver](https://en.wikipedia.org/wiki/Silver) has antimicrobial properties, but compounds suitable for disinfection are usually unstable and have a limited shelf-life. [Silver dihydrogen citrate](https://en.wikipedia.org/w/index.php?title=Silver_dihydrogen_citrate&action=edit&redlink=1) (SDC) is a chelated form of silver that maintains its stability. SDC kills microorganisms by two modes of action

1. The silver ion deactivates structural and metabolic membrane proteins, leading to microbial death;
2. The microbes view SDC as a food source, allowing the silver ion to enter the microbe. Once inside the organism, the silver ion denatures the DNA, which halts the microbe's ability to replicate, leading to its death. This dual action makes SDC highly and quickly effective against a broad spectrum of microbes. SDC is non-toxic, non-caustic, colorless, odorless, and tasteless, and does not produce toxic fumes. SDC is non-toxic to humans and animals: the [United States Environmental Protection Agency](https://en.wikipedia.org/wiki/United_States_Environmental_Protection_Agency) classifies it into the lowest toxicity category for disinfectants, category IV.

A meta-analysis of 26 studies by the [Cochrane Collaboration](https://en.wikipedia.org/wiki/Cochrane_Collaboration) found that, most were small and of poor quality, and that there was not enough evidence to support the use of silver-containing dressings or creams, as generally these treatments did not promote wound healing or prevent wound infections. Some evidence suggested that silver sulphadiazine had no effect on infection, and actually slowed healing.[[25]](https://en.wikipedia.org/wiki/Disinfectant#cite_note-25)

### Copper alloy surfaces

Copper-alloy surfaces have natural intrinsic properties to destroy a wide range of [microorganisms](https://en.wikipedia.org/wiki/Microorganisms) (e.g., [*E. coli*](https://en.wikipedia.org/wiki/Escherichia_coli) O157:H7, [methicillin](https://en.wikipedia.org/wiki/Methicillin)-resistant [*Staphylococcus aureus*](https://en.wikipedia.org/wiki/Staphylococcus_aureus) ([MRSA](https://en.wikipedia.org/wiki/Methicillin-resistant_Staphylococcus_aureus)), [*Staphylococcus*](https://en.wikipedia.org/wiki/Staphylococcus), [*Clostridium difficile*](https://en.wikipedia.org/wiki/Clostridium_difficile_(bacteria)), [influenza A virus](https://en.wikipedia.org/wiki/Influenza_A_virus), [adenovirus](https://en.wikipedia.org/wiki/Adenoviridae), and [fungi](https://en.wikipedia.org/wiki/Fungus)). In addition, extensive tests on [E. coli](https://en.wikipedia.org/wiki/E._coli) O157:H7, [methicillin](https://en.wikipedia.org/wiki/Methicillin)-resistant [*Staphylococcus aureus*](https://en.wikipedia.org/wiki/Staphylococcus_aureus) ([MRSA](https://en.wikipedia.org/wiki/Methicillin-resistant_Staphylococcus_aureus)), [*Staphylococcus*](https://en.wikipedia.org/wiki/Staphylococcus), *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* sanctioned by the [United States Environmental Protection Agency](https://en.wikipedia.org/wiki/United_States_Environmental_Protection_Agency) (EPA) using Good Laboratory Practices found[] that when cleaned regularly, some 355 different copper alloy surfaces:

* Continuously reduce bacterial contamination, achieving 99.9% reduction within two hours of exposure;
* Kill greater than 99.9% of Gram-negative and Gram-positive bacteria within two hours of exposure;
* Deliver continuous and ongoing antibacterial action, remaining effective in killing greater than 99.9% of bacteria within two hours;
* Kill greater than 99.9% of bacteria within two hours, and continue to kill 99% of bacteria even after repeated contamination;
* Help inhibit the buildup and growth of bacteria within two hours of exposure between routine cleaning and sanitizing steps.

These copper alloys were granted [EPA registrations](https://en.wikipedia.org/wiki/Antimicrobial_copper-alloy_touch_surfaces#USEPA_registrations_of_antimicrobial_copper_alloy_touch_surfaces) as "antimicrobial materials with public health benefits which allows manufacturers to legally make claims regarding the positive public health benefits of products made with [registered antimicrobial copper alloys](https://en.wikipedia.org/wiki/Antimicrobial_copper-alloy_touch_surfaces). EPA has approved a long list of antimicrobial copper products made from these alloys, such as bedrails, [handrails](https://en.wikipedia.org/wiki/Handrails), over-bed tables, [sinks](https://en.wikipedia.org/wiki/Sinks), [faucets](https://en.wikipedia.org/wiki/Faucets), [door knobs](https://en.wikipedia.org/wiki/Door_knobs), [toilet](https://en.wikipedia.org/wiki/Toilet) hardware, [computer keyboards](https://en.wikipedia.org/wiki/Computer_keyboards), [health club](https://en.wikipedia.org/wiki/Health_club) equipment, [shopping cart](https://en.wikipedia.org/wiki/Shopping_cart) handles, etc.. Antimicrobial copper alloy products are now being installed in healthcare facilities in the U.K., Ireland, Japan, Korea, France, Denmark, and Brazil and in the subway transit system in Santiago, Chile, where copper-zinc alloy handrails will be installed in some 30 stations between 2011 and 2014 (Samuel., 2011).

### 2.3 Thymol-based disinfectant

[Thymol](https://en.wikipedia.org/wiki/Thymol) is a [phenolic](https://en.wikipedia.org/wiki/Phenol) chemical found in [thyme](https://en.wikipedia.org/wiki/Thyme), can be as effective as bleach in terms of disinfecting as both are considered an intermediate level disinfectant. Thyme essential oils have bacteriostatic activity against a variety of microorganisms including [*E. coli*](https://en.wikipedia.org/wiki/E._coli) and [*S. aureus*](https://en.wikipedia.org/wiki/Staphylococcus_aureus) (Marino *et al* 1999).

**2.4** [**Biguanide**](https://en.wikipedia.org/wiki/Biguanide)[**polymer**](https://en.wikipedia.org/wiki/Polymer)

[Polyaminopropyl biguanide](https://en.wikipedia.org/wiki/Polyaminopropyl_biguanide) is specifically bactericidal at very low concentrations (10 mg/l). It has a unique method of action: The polymer strands are incorporated into the bacterial cell wall, which disrupts the membrane and reduces its permeability, which has a lethal effect to bacteria. It is also known to bind to bacterial DNA, alter its transcription, and cause lethal DNA damage (Allen *et al* 2006). It has very low toxicity to higher organisms such as human cells, which have more complex and protective membranes.

Common [sodium bicarbonate](https://en.wikipedia.org/wiki/Sodium_bicarbonate) (NaHCO3) has antifungal properties (Zamani *et al* 2007) and some antiviral and antibacterial properties (Malik., 2006) though those are too weak to be effective at a home environment (William *et al* 2000).

[Lactic acid](https://en.wikipedia.org/wiki/Lactic_acid) is a registered disinfectant. Due to its natural and environmental profile, it has gained importance in the market.

### 2.5 Non-chemical

[Ultraviolet germicidal irradiation](https://en.wikipedia.org/wiki/Ultraviolet_germicidal_irradiation) is the use of high-intensity shortwave [ultraviolet light](https://en.wikipedia.org/wiki/Ultraviolet_light) for disinfecting smooth surfaces such as dental tools, but not porous materials that are opaque to the light such as wood or foam. Ultraviolet light is also used for municipal [water treatment](https://en.wikipedia.org/wiki/Water_treatment). Ultraviolet light fixtures are often present in [microbiology](https://en.wikipedia.org/wiki/Microbiology) labs, and are activated only when there are no occupants in a room (e.g., at night).

The phrase "sunlight is the best disinfectant" was [popularized in 1913](https://en.wikipedia.org/wiki/Other_People%27s_Money_and_How_the_Bankers_Use_It) by [United States Supreme Court](https://en.wikipedia.org/wiki/United_States_Supreme_Court) Justice [Louis Brandeis](https://en.wikipedia.org/wiki/Louis_Brandeis) and later advocates of [government transparency](https://en.wikipedia.org/wiki/Government_transparency). While sunlight's ultraviolet rays can act as a disinfectant, the Earth's [ozone layer](https://en.wikipedia.org/wiki/Ozone_layer) blocks the rays' most effective wavelengths. Ultraviolet light-emitting machines, such as those used to disinfect some hospital rooms, make for better disinfectants than sunlight (McCarthy., 2013).

## 2.6 Measurements of effectiveness

One way to compare disinfectants is to compare how well they do against a known disinfectant and rate them accordingly. Phenol is the standard, and the corresponding rating system is called the "[Phenol coefficient](https://en.wikipedia.org/wiki/Phenol_coefficient)". The disinfectant to be tested is compared with phenol on a standard microbe (usually [*Salmonella typhi*](https://en.wikipedia.org/wiki/Salmonella_typhi) or [*Staphylococcus aureus*](https://en.wikipedia.org/wiki/Staphylococcus_aureus)). Disinfectants that are more effective than phenol have a coefficient > 1. Those that are less effective have a coefficient < 1.

The standard European approach for disinfectant validation consists of a basic suspension test, a quantitative suspension test (with low and high levels of organic material added to act as ‘interfering substances’) and a two part simulated-use surface test ( Sandle., 2012).

A less specific measurement of effectiveness is the [United States Environmental Protection Agency](https://en.wikipedia.org/wiki/United_States_Environmental_Protection_Agency) (EPA) classification into either *high*, *intermediate* or *low* levels of disinfection. "High-level disinfection kills all organisms, except high levels of bacterial spores" and is done with a chemical germicide marketed as a sterilant by the U.S. [Food and Drug Administration](https://en.wikipedia.org/wiki/Food_and_Drug_Administration) (FDA). "Intermediate-level disinfection kills mycobacteria, most viruses, and bacteria with a chemical germicide registered as a 'tuberculocide' by the Environmental Protection Agency. Low-level disinfection kills some viruses and bacteria with a chemical germicide registered as a hospital disinfectant by the EPA (Centers for Disease Control and Prevention., 2012)

An alternative assessment is to measure the Minimum inhibitory concentrations (MICs) of disinfectants against selected (and representative) microbial species, such as through the use of microbroth dilution testing (Vijayakumar., 2012).

**2.7 Home disinfectants**

By far the most cost-effective home disinfectant is the commonly used chlorine bleach (a 5% solution of [sodium hypochlorite](https://en.wikipedia.org/wiki/Sodium_hypochlorite)), which is effective against most common [pathogens](https://en.wikipedia.org/wiki/Pathogens), including difficult organisms such as [tuberculosis](https://en.wikipedia.org/wiki/Tuberculosis) ([mycobacterium tuberculosis](https://en.wikipedia.org/wiki/Mycobacterium_tuberculosis)), [hepatitis](https://en.wikipedia.org/wiki/Hepatitis) B and C, [fungi](https://en.wikipedia.org/wiki/Fungi), and antibiotic-resistant strains of [staphylococcus](https://en.wikipedia.org/wiki/Staphylococcus) and [enterococcus](https://en.wikipedia.org/wiki/Enterococcus). It even has some disinfectant action against parasitic organisms.

Positives are that it kills the widest range of pathogens of any inexpensive disinfectant, is extremely powerful against viruses and bacteria at room temperature, is commonly available and inexpensive, and breaks down quickly into harmless components (primarily table salt and oxygen).

Negatives are that it is caustic to the skin, lungs, and eyes (especially at higher concentrations); like many common disinfectants, it degrades in the presence of organic substances; it has a strong odor; it is not effective against [*Giardia lamblia*](https://en.wikipedia.org/wiki/Giardia_lamblia) and [*Cryptosporidium*](https://en.wikipedia.org/wiki/Cryptosporidium); and extreme caution must be taken not to combine it with ammonia or any acid (such as [vinegar](https://en.wikipedia.org/wiki/Vinegar)), as this can cause noxious gases to be formed. The best practice is not to add anything to household bleach except water.

To use chlorine bleach effectively, the surface or item to be disinfected must be clean. In the bathroom or when cleaning after pets, special caution must be taken to wipe up urine first, before applying chlorine, to avoid reaction with the ammonia in urine, causing toxic gas by-products. A 1-to-20 solution in water is effective simply by being wiped on and left to dry. The user should wear rubber gloves and, in tight airless spaces, goggles. If parasitic organisms are suspected, it should be applied at 1-to-1 concentration, or even undiluted. Extreme caution must be taken to avoid contact with eyes and mucous membranes. Protective goggles and good ventilation are mandatory when applying concentrated bleach.

The use of some antimicrobials such as [triclosan](https://en.wikipedia.org/wiki/Triclosan), in particular in the uncontrolled home environment, is controversial because it may lead to the germs becoming resistant. Chlorine bleach and alcohol do not cause resistance because they are so completely lethal, in a very direct physical way.

**2.8 Albino Rat ( *Rattus norvergicus*)**

Laboratory rats share origins with their cousins in domestication, the [fancy rats](https://en.wikipedia.org/wiki/Fancy_rat). In 18th century Europe, wild Brown rats ran rampant and this infestation fueled the industry of rat-catching. [Rat-catchers](https://en.wikipedia.org/wiki/Rat-catcher) would not only make money by trapping the rodents, but also by selling them for food, or more commonly, for [rat-baiting](https://en.wikipedia.org/wiki/Rat-baiting). The first time one of these albino mutants was brought into a laboratory for a study was in 1828, in an experiment on [fasting](https://en.wikipedia.org/wiki/Fasting). Over the next 30 years rats were used for several more experiments and eventually the laboratory rat became the first animal [domesticated](https://en.wikipedia.org/wiki/Domesticated) for purely scientific reasons. (krinke and George., 2000).

The rat found was used in laboratory research in five areas: [W. S. Small](https://en.wikipedia.org/wiki/W._S._Small) suggested that the rate of learning could be measured by rats in a maze; a suggestion employed by [John B. Watson](https://en.wikipedia.org/wiki/John_B._Watson) for his Ph.D. dissertation in 1903, John B (1903). The first rat colony in America used for nutrition research was started in January 1908 by [Elmer McCollum](https://en.wikipedia.org/wiki/Elmer_McCollum), ( Day Harry., 1974) and then nutritive requirements of rats were used by [Thomas Burr Osborne](https://en.wikipedia.org/wiki/Thomas_Burr_Osborne_(chemist)) and [Lafayette Mendel](https://en.wikipedia.org/wiki/Lafayette_Mendel) to determine the details of [protein nutrition](https://en.wikipedia.org/wiki/Protein_(nutrient)). The [reproductive function](https://en.wikipedia.org/wiki/Reproduction) of rats was studied at Institute for Experimental Biology at [University of California, Berkeley](https://en.wikipedia.org/wiki/University_of_California,_Berkeley) by [Herbert McLean Evans](https://en.wikipedia.org/wiki/Herbert_McLean_Evans) and Joseph A. Long, (Evans *et al*., 2005). The [genetics](https://en.wikipedia.org/wiki/Genetics) of rats was studied by [William Ernest Castle](https://en.wikipedia.org/wiki/William_Ernest_Castle) at the [Bussey Institute](https://en.wikipedia.org/wiki/Bussey_Institution) of [Harvard University](https://en.wikipedia.org/wiki/Harvard_University) until it closed in 1994. And rats have long been used in [cancer research](https://en.wikipedia.org/wiki/Cancer_research). The Wistar rat is an outbred albino rat. This breed was developed at the [Wistar Institute](https://en.wikipedia.org/wiki/Wistar_Institute) in 1906 for use in biological and medical research, and is notably the first rat developed to serve as a [model organism](https://en.wikipedia.org/wiki/Model_organism) at a time when laboratories primarily used the common [house mouse](https://en.wikipedia.org/wiki/House_mouse) (*Mus musculus*). More than half of all laboratory rat strains are descended from the original colony established by [physiologist](https://en.wikipedia.org/wiki/Physiology) [Henry Donaldson](https://en.wikipedia.org/w/index.php?title=Henry_Donaldson&action=edit&redlink=1), scientific administrator Milton J. Greenman, and [genetic researcher](https://en.wikipedia.org/wiki/Genetics)/[embryologist](https://en.wikipedia.org/wiki/Embryologist) [Helen Dean King](https://en.wikipedia.org/wiki/Helen_Dean_King), (Clause B.T., 1998).

The Wistar rat is currently one of the most popular rats used for laboratory research. It is characterized by its wide head, long ears, and a tail length that is always less than its body length. The Sprague Dawley rat and Long–Evans rats were developed from Wistar rats. Wistar rats are more active than others like Sprague Dawley rats.

**CHAPTER THREE**

**MATERIALS AND METHODS**

**MATERIALS**

Animal model (10 albino rats),Disinfectants i.e Dettol disinfectant,1zal,Ivy disinfectant, normal saline, Hand gloves, Surgical blades, Ethanol ,Cotton wool, Swab sticks ,Nutrient agar, Petri-dish, Wire loop, incubator, Bunsen burner.

**3.1 Case study:** This research was done in Godfrey Okoye University animal house.

**3.2 Sterilization**: The work bench was sterilized to get rid of the microorganisms present there and the practical was set to be carried out.

**3.3 Labelling**: The animals were divided into five (5) groups where two albino rats are put in 5 cages. Each of them was labelled for easy identification.

**3.4 Weighing:** The animals were weighed before the practical was carried out.

**3.5 Wound infliction**: The animals were given cuts on the right hind limb of their leg. The wound was allowed to be infected for 48 hours before it was cultured

**3.6 Sample collection**: Swab sticks were used to swab the infected area before being cultured on the nutrient agar for 24 hours. The organisms were sub-cultured into a fresh media to get a single colony of the organisms present on the wound.

**3.7 Gram staining**:

With the aid of a sterile wire loop the organisms that grew on the sub-cultured plate were smeared on clean grease slides containing a drop of normal saline. The slides were passed over flame three (3) times and kept to air dry.

Crystal violet was poured on the slide for 60 seconds and washed off with water.

Lugol’s iodine was poured on the slides for 60 seconds and washed off with water.

Acetone was used to decoulorize and washed immediately with water

Safranin was poured on the slide and allowed to stand for 30 seconds to counter stain the smear and it was washed off with water.

The slide was allowed to dry, oil immersion was put on the slide and viewed on the microscope with x100.

**3.8 Treatment**: with the aid of the disinfectants the wounds on the rats are disinfected. The disinfectants are labelled as thus:

Names of the disinfectants used on the animals were as follows

|  |  |  |
| --- | --- | --- |
| S/N | Specimen | Samples |
|  | Specimen A | Control |
|  | Specimen B | Dettol |
|  | Specimen C | Izal |
|  | Specimen D | Ivy |
|  | Specimen E | Normal saline |

These disinfectants were applied everyday and the healing process on the wound was observed daily after the treatment.

**CHAPTER FOUR**

**RESULTS**

Table 1 shows the result for the swab collected from the wound which was cultured, incubated and gram stained to identify the probable isolates.

Table 1: The result for the organisms isolated, cultured and gram stained were as follows

|  |  |  |  |
| --- | --- | --- | --- |
| Specimen A | Gram reaction | Shape | Probable Isolates |
| Specimen A blue-tail | - | Cocci | *Escherichia coli* |
| Specimen A plain | + | Cocci | *Staphylococcus spp* |
| Specimen B blue-tail | + | Cocci | *Staphylococcus spp* |
| Specimen B plain | + | Cocci | *Staphylococcus spp* |
| Specimen C blue-tail | + | Cocci | *Staphylococcus spp* |
| Specimen C plain | + | Cocci | *Staphylococcus spp* |
| Specimen D blue-tail | + | Cocci | *Staphylococcus spp* |
| Specimen D plain | + | Cocci | *Staphylococcus spp* |
| Specimen E blue-tail | + | Cocci | *Staphylococcus spp* |
| Specimen E plain | + | Cocci | *Staphylococcus spp* |

Table 2 shows the result of the weight of the animals. The weight of the container and the animals were recorded.

Table 2: The result of the weight of the animals were as follows

|  |  |
| --- | --- |
| ANIMAL | WEIGHT (g) |
| Specimen A blue-tail | 154.2g |
| Specimen A plain | 148.1g |
| Specimen B blue-tail | 171.2g |
| Specimen B plain | 130.1g |
| Specimen C blue-tail | 171.5g |
| Specimen C plain | 141.2g |
| Specimen D blue-tail | 146.7g |
| Specimen D plain | 133.6g |
| Specimen E blue-tail | 132.7g |
| Specimen E plain | 141.6g |

Table 3 shows the result for the time duration of healing between the animals that were treated with disinfectants.

Table 3: Result for time duration of healing

|  |  |  |
| --- | --- | --- |
| Specimen | Samples | Nature of wound |
| Specimen A | Control | Scarred |
| Specimen B | Dettol | Healed |
| Specimen C | Izal | Healed |
| Specimen D | Ivy’s | Healed |
| Specimen E | Normal saline | Healed |

Table 4 shows result for the efficacy of the used disinfectants. This table shows the days of healing between the animals and the disinfectants used on them

Table 4: result for the efficacy of the used disinfectants

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Disinfectants | Efficacy | Days  1 | 2 | 3 | 4 | | 5 | 6 | 7 | 8 | 9 | | 10 |
| Control | Very Effective | - | - | + | | + | ++ | +++ |  |  | |  |  |
| Dettol | Very Effective | - | - | - | | + | + | ++ | +++ |  | |  |  |
| Izal | Very Effective | - | - | - | | + | + | ++ | +++ |  | |  |  |
| Ivy | Slowly Effective | - | - | - | | - | - | - | - | + | | ++ | +++ |
| Normal saline | Slowly Effective | - | - | - | | - | - | - | - | + | | ++ | +++ |

**CHAPTER FIVE**

**5.1 DISCUSSION**

From the result above it was noticed that specimen A being the control healed six (6) days after the infection but there was still a scar of the wound; specimen B and specimen C healed seven (7) days later after being treated with Dettol and Izal as the effective disinfectants that aided in the healing of the wound. Specimen D and specimen E healed three (3) days later with the application of Ivy and Normal saline and this can be seen as the disinfectant that was slow in healing the wound. There were arguments for creating or maintaining conditions that are not conducive to bacterial survival and multiplication, rather than attempting to kill them with chemicals. Bacteria can increase in number very quickly, which enables them to [evolve](https://en.wikipedia.org/wiki/Evolution) rapidly. Should some bacteria survive a chemical attack, they give rise to new generations composed completely of bacteria that are resistance to particular chemical used. Under a sustained chemical attack, the surviving bacteria in successive generations are increasingly resistant to the chemical used, and ultimately the chemical is rendered ineffective (Hunt and Hopt, 1997). The progression of a colonized wound to an infected state cannot be predicted by the presence of a specific type of bacterium or a specific patho-physiological condition, because a multitude of factors are likely to simultaneously influence wound pathogenesis. Microbiological factors such as the population density, the types of microorganisms present, and microbial interactions and host factors such as the efficacy of the immune response and the condition of the tissue are all critical and must be considered collectively as factors predisposing to infection. As well as the need to control microbial populations that exist in wounds in order to minimize the likelihood of wound infection, there is a need to control the dissemination of potentially pathogenic members of the wound micro-flora into the surrounding environment in order to minimize the opportunity for cross-infection. In this respect, wound dressings should be considered an important. During normal wound healing, the deposition of a collagen matrix forms a scaffold that supports new blood vessels and these, in turn, enable the delivery of oxygen and nutrients required to maintain the healing process (Pfeifer *et al* 1997). There was widespread debate regarding when and how infected wounds should be treated, whether non-infected, non-healing wounds should be treated with antimicrobial agents, what agents should be used, and whether topical or systemic antimicrobial agents should be administered. In summary, a continuous dialogue between the microbiology department and the wound care practitioner is essential to ensure that only wounds that are likely to benefit from a microbiological investigation are sampled (i.e., those with clinical signs of infection or those that are failing to heal), the microbiologist had a thorough understanding of the clinical presentation of the wound, the microbiologist has an understanding of the method of wound sampling, the microbiologist is aware of the requirements of the practitioner and the urgency of the results. The value of Gram's stain in facilitating early and appropriate treatment of a wound infection by the clinician was questionable and was primarily dependent on the type of wound (Meislin et al., 1977) reported that the Gram stain reliably indicates sterile and mixed abscesses, as well as those containing pure S. aureus. Similarly, this procedure may also facilitate identification of the etiological agent of wound infection, where there was a higher probability of one microorganism being involved (e.g., clusters of gram-positive cocci). However, in most other wound types that are characterized by a complex aerobic-anaerobic micro-flora, the Gram stain had little value, although the combined presence of leukocytes and bacteria is likely to be a good indicator of infection, as reported by Hussey et al. In wound management, Gram staining of a known volume of tissue biopsy specimen homogenate has been used to rapidly estimate the microbial load of a wound and thus facilitate successful closure of surgical wounds (Hegger *et al,* 1969). Also, the presence of microorganisms in a Gram-stained smear prepared from a wound swab has been shown to consistently reflect a microbial load of organisms isolated by a quantitative swab technique from open wounds (Levine *et al*, 1976).

**5.2 CONCLUSION**

From the result above it can be said that Dettol and Izal which were used in the treatment of wound of these animal model (albino rats) proved effective and thus can be used to treat cuts or wound on animals this is because the chemical contents in the disinfectant are able to kill the bacteria and encourage healing whereas ivy and normal saline which were also used could heal the wounds and kill microorganisms. This concludes that with the aid of Dettol and Izal the organisms can be killed and the infected wound can be healed within a period of seven (7) days.

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