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Antimicrobial Efficiency of Commonly Used Disinfectants against *Escherichia coli* and *Staphylococcus aureus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Three different types of disinfectants that are frequently employed in regular laboratories include ethanol, bleach, and phenols. This study used the agar hole diffusion method to examine the effectiveness of these three disinfectants against *Staphylococcus aureus* and *Escherichia coli*. Different bleach, ethanol, and phenolic concentrations were utilized. There were variations in the measured concentrations because the disinfectants' initial concentrations varied. The results after 24 hours of incubation at 37°C demonstrated that all the disinfectants in their concentrated forms

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prevented the development of the test organism. When different quantities of the inhibitors were used, their efficacy varied, and the width of the zone of inhibitions around each well was determined in millimeters.
The findings revealed that bleach had a stronger impact on *Staphylococcus aureus* than *Escherichia coli*, while ethanol showed the least susceptibility. Phenolics had the highest efficiency against both test species.

Keywords: Antimicrobial efficiency; Escherichia coli; Staphylococcus aureus; phenolic concentrations.

1. INTRODUCTION

The reduction in quantity or activity of the overall microbial load is referred to as microorganism control. The following are the main justifications for regulating microorganisms: to stop the spread of illnesses and infections, to stop the growth of unfavorable bacteria, to stop the degradation and rotting of materials by germs. (Pelezar, et al., 2016).Disinfection is the process of controlling dangerous bacteria. The majority of the time, it alludes to the elimination of vegetative (non-endospore producing) infections. The phrase is most frequently used to describe the release of an inert material or surface using a disinfectant. Antisepsis is the term for this treatment when it targets live tissue, while antiseptic refers to the chemical. Therefore, in reality, a substance may be referred to as a disinfectant for one use and an antiseptic for another [1].

“Disinfectants cannot be administered systemically because, according to [2], they are toxic not only to microbial pathogens but also to host cells. As a result, they can only be used to inactivate microorganisms in the inanimate environment or, to a limited extent, on skin surfaces”. These cleaners provide a diversionary effect by either coagulating the bacterial protein, rupturing the cell membrane, or removing a sulphonhydric group from the organisms [3]. Additionally, according to Brooks et al. [4], “although bacteria differ in their susceptibility to chemical germicides, the method of action of disinfectants is assumed to be connected to breakdown of proteins, lipids, or nucleic acids in the cells or its cytoplasmic membrane”.

“A chemical that kills or prevents the growth of germs like bacteria, fungus, protozoa, or viruses is known as an antibiotic. Antibiotics are chemicals generated by microorganisms that either kill or stop the development of other microorganisms” [5]. While antivirals are especially intended to treat viral infections, antibiotics are often used to treat bacterial infections. Antifungal medications are used to treat fungus infections, but some of its adverse effects can be fatal if taken improperly. Antibiotics derived from various microorganisms are currently used to treat a wide range of human diseases, so action must be taken to control their use. New drugs, either synthetic or natural, must be developed, and for a long time, plants have been a valuable source of natural products for preserving human health. India has a long history of using medicinal plants to create pharmaceuticals. Any plant that contains compounds that can be used therapeutically or that is a precursor to chemo-pharmaceuticals semi-synthetic novel medications is referred to as a medicinal plant, according to the World Health Organization [6].

There are several antiseptics available in Nigerian marketplaces today, all of variable potency. Their active components could be to blame for these variances. One of the following substances is present in the majority of antiseptics: chlorhexidine, phenol, chloroxylenol, and cetylpyridinium chloride. (CPC). All are administered topically, with the exception of mouthwash, to stop microbial population growth, especially during baths.

Disinfectants are related to antiseptics in that they kill or stop the development of bacteria in or on living tissue, but antiseptics are used on surfaces or things that are inanimate. In hospitals and other healthcare facilities, substances including alcohols, phenols, iodine, and chlorine are frequently used for infection control and nosocomial infection prevention. The efficiency of these agents may be influenced by PH, detergent base, temperature, organic matter, ionic, and type of surfactants. An optimal disinfectant to combat bacteria with antimicrobial resistance should have a broad range of antimicrobial activity. Disinfectants are antimicrobial substances used to get rid of bacteria residing on non-living objects. Disinfection is less successful than sterilization, which is an extreme physical and/or chemical process that eliminates all forms of life, although

it does not necessarily kill all microbes, particularly resistant bacterium spores. A perfect disinfectant would also be non-corrosive, affordable, and provide complete and total microbiological sterilisation without damaging people or beneficial forms of life.

Disinfectants are typically applied in diluted form, but it has been demonstrated that when certain of these substances are employed, some Gram negative bacteria, such as E coli, can still live, rendering them useless against nosocomial infections. The rise of resistant bacteria in healthcare facilities and the general population is complicating patient care and infection control. Methicillin-resistant *Staphylococcus aureus*, glycopeptide-resistant enterococci, and Pseudomonas generating extended range beta-lactamases are some of the organisms of particular concern. The goals of this study are to determine the antimicrobial activity of various disinfectants sold under various trade names against the test microorganisms *Staphylococcus aureus* and *E. coli*, the concentrations at which they were effective, the susceptibility of the test gram positives and gram negatives to the test disinfectants, and to determine the most effective disinfectants to use for household cleaning.

1.1 Statement of the Problems

One of the largest issues affecting public health is antibiotic resistance. This issue arises as a natural result of pathogenic organisms' adaptation to antimicrobials used in a variety of settings, such as medicine, food animals, crop production, and disinfectants in farms, hospitals, and homes. As a result of microorganisms developing resistance to all known antibiotics, these multidrug-resistant germs carry a heavy economic cost [7]. Applications for various disinfectant compositions vary. Numerous factors, including temperature, contact time, pH and disinfectant concentration, bioburden, organic soil, and the hardness of the water used for dilution, might have an impact on the disinfection process. Therefore, to confirm the disinfectant's efficacy, field testing for the designated application should be done. Users are not always aware of the importance of selecting the right disinfectant, particularly in smaller healthcare facilities. Typically, a broad-spectrum antimicrobial agent is chosen based on the manufacturer-supplied literature. Despite widespread use of phenolic disinfectants being discouraged in developed nations, many hospitals and houses continue use them. Some affluent nations have stopped using gluteraldehydes due to toxicity concerns, however these substances are still widely utilized in developing nations. For many users, the only source of information about the disinfectant's effectiveness is the manufacturer's literature. The majority of manufacturers advertise their disinfection as a versatile, broad-spectrum antibacterial agent. Keeping the aforementioned in mind, the following study was designed with the objective of evaluating and comparing the actual disinfection efficacy of a few locally accessible phenolic disinfectants for the disinfection of surfaces and infectious microbiological and other waste. *Staphylococcus aureus* and E coli isolates that were locally isolated were used to test the effectiveness.

1.2 Aim of the Study

The main aim of this study is the evaluation of antibacterial strength of selected household disinfectants on *Staphylococcus aureus,* and *E coli*;

The specific objectives of the study will be;

- To isolate *Staphylococcus aureus,* and *E coli* from clinical samples
- To determine the antimicrobial activity of three antiseptics and disinfectants against the *Staphylococcus aureus,* and *E coli* isolated
- To know the concentrations at which the three antiseptics and disinfectants were effective against the test microrganisms
- To determine the susceptibility of the test organisms to the test disinfectants
- To help know the most effective disinfections to use for household and hospital cleanings.

2. LITERATURE REVIEW

2.1 Phenol as Disinfectant

The oldest known disinfectant is probably phenol, which Lister first presented as "carbolic acid." Disinfectants are frequently employed in the medical, food, and pharmaceutical industries today to stop harmful germs from spreading disease. Particularly efficient against gram-positive bacteria and enveloped viruses as BRS, BVD, Coronavirus, IBR, Leukemia, PI3, Pox, Rabies, and Stomatitis virus are phenols. Phenolic compounds are utilized as intermediate

level disinfectants for non-critical medical devices since they have a low risk of spreading infections and typically only come into touch with healthy skin. They are more effective in the presence of organic material than disinfectants that contain iodine or chlorine.

2.2 Application of Phenolic Compounds in Disinfectants and Antiseptic

In hospitals and other health care facilities, antiseptics and disinfectants are widely utilized for a variety of topical and hard-surface applications. They help prevent nosocomial infections in particular and are a crucial component of infection control procedures. Public use of antiseptics and disinfectants has expanded as a result of growing worries about the possibility of microbial contamination and illness hazards in the food and general consumer markets.

Disinfectants are comparable to antiseptics but are typically products or biocides used on inanimate items or surfaces (e.g., surgical scrubs and hand washes for medical workers). Antiseptics are biocides or chemicals that kill or prevent the growth of bacteria in or on living tissue. Antiseptics and disinfectants contain a wide range of active chemical substances (also known as "biocides"). Disinfectants and antiseptics can be divided into a number of classes based on their chemical makeup. They are aldehydes, quaternary ammonium compounds (QACs), halogens, phenolics, and alcohols. Depending on the chemical compound present, disinfectants and antiseptics have quite different modes of action. The choice of the appropriate disinfectant relies on the circumstances. While some disinfectants have a broad spectrum and destroy practically all germs, others only kill a small subset of disease-causing organisms but are nevertheless recommended due to other factors (they may be non-corrosive, non-toxic, or affordable) (Pelczar et al., 2013). The three rounds of testing, which are now generally acknowledged as the core premise, investigate the antimicrobial effectiveness of a disinfectant or an antiseptic (Pelczar et al., 2013). The first step involves laboratory tests to determine whether a substance or preparation has antibacterial action. Suspension tests are taken into consideration for these initial screening exams. In the second round of testing, cleaning methods rather than cleaning agents are looked at testing that mimic real-world circumstances, such as carrier testing for the disinfection of materials by submersion and surface disinfection tests, are used to determine the circumstances and use-dilution at which the preparation is active for a specific application. The final step, which is performed in the field, consists of in-situ testing that determine whether the disinfectant solution continues to destroy germs after being used for a typical amount of time.

2.3 Types of Disinfectants and Antiseptics

One of the most popular types of antiseptics and disinfectants is alcohol. They are hydroxyl functional group-containing colorless hydrocarbons. Alcohols are sporicidal but not bactericidal and fungicidal. Alcohol's manner of action is dependent on its concentration. Alcohol degrades membrane lipids, alters cell surface tension, and jeopardizes membrane integrity at concentrations of 50% and above. Only in alcohol-water solutions of between 50 and 95 percent may a protein that has entered the protoplasm be denatured by an alcohol through coagulation. 100% pure alcohol dehydrates cells and prevents cell development.

Solutions of 70-95% alcohol are used as skin degerming agents. Most frequently used is ethanol (60-90%), 1-propanol (60-70%), and 2-propanol/isopropanol (70-80%) or a mixture of these alcohols. They are commonly referred to as "surgical alcohol" and are used to disinfect the skin before injections. “Some of its effectiveness as surface disinfectants can be attributed to its cleansing or detergent action, which helps in the mechanical removal. One or more aromatic carbon rings with additional functional groups make up phenolics. Alkylated phenols (cresols), chlorinated phenols (chlorophene), and bisphenols (hexachlorophene) are the three key compounds”. (Talaro and Talaro, 2016). “Phenolics have a high level of microbicity and will kill vegetative bacteria, fungi, and the majority of viruses. (not hepatitis B). They are not, however, consistently sporicidal” (Talaro and Talaro, 2016). “They may be either bacteriostatic or bactericidal, depending on the concentrations utilized. Modes of action of phenol depend on the concentrations employed. They are cellular poisons in high quantities, quickly rupturing cell walls and membranes and precipitating proteins. They disrupt the cytoplasmic membrane's regular selective permeability, allowing essential intracellular chemicals to flow out and damaging the cell wall. Lower amounts render the vital

enzyme system inactive". (Pelczar et.al, 2013). Some household disinfectants use phenols as active components. They can also be discovered in various hand washes, disinfecting soaps, and mouthwashes. To clean drains and cesspools, phenol is diluted in water to a concentration of 5%. Phenol can sometimes be hazardous to sensitive people and is quite caustic to the skin.

2.4 Occurrence of Phenolic Compounds

The three types of phenolic chemicals that are frequently found in food materials include simple phenols and phenolic acids, hydroxycinnamic acid derivatives, and flavonoids.

2.4.1 The simple phenols and phenolic acids

Monophenols like p-cresol, isolated from various fruits (such as raspberries and blackberries), 3,4-dimethylphenol and 3-ethylphenol, found to be responsible for the smoky flavor of some cocoa beans, and diphenols like hydroquinone, which is likely the most common simple phenol, are some examples of simple phenols. Sesamol, a common hydroquinone derivative, is present in sesame oil. Sesaminol, one of the sesamol derivatives present in sesame oil, has been found to exhibit potent antioxidant properties.

2.4.2 The flavonoids

Flavonoids, which primarily consist of catechins, proanthocyanins, anthocyanidins, and flavones, flavonols, and their glycosides, are the most significant single group of phenolics in food. Despite appearing to be present throughout plants, catechins are exclusively abundant in tea leaves, where they can make up to 30% of the dry leaf weight. This book's Volume II has several chapters that examine recent studies on the antioxidative and cancer-preventive benefits of tea and its catechin components.

2.5 Phenolic Compounds as Natural Antioxidants and Antimicrobial

To stop the oxidation of lipids from forming different off tastes and other undesirable substances, antioxidants are added to fats, oils, and foods that contain fat. The most extensively used synthetic antioxidants, BHA and BHT, have unmatched effectiveness in a variety of food systems in addition to their high stability, low cost, and other useful benefits. However, their use in food has decreased as a result of both widespread opposition to artificial food additives and speculation that they may accelerate the development of cancer (Thomas et al., 2016). (2012). Tocopherols are the most significant naturally occurring antioxidants that are used commercially. By capturing peroxyl radicals, tocopherols exert a strong inhibitory effect on lipid peroxidation in living organisms. Tocopherols are unfortunately far less efficient as dietary antioxidants.

It would be ideal to find and produce additional antioxidants with natural origins. Such novel antioxidants would be beneficial in the fight against aging and carcinogenesis. The majority of natural antioxidants are phenolic in composition. The following food items have been examined and reported here for their phenolic antioxidant content: Osbeckia chinensis, Chili pepper, Ginger, Green tea, Pepper, and Oregano.

2.5.1 Application of Phenolic Compounds

- Phenols have a significant role as industrial raw materials and additives for: laboratory procedures; the chemical industry; and chemical engineering operations.
- processing of polymers and wood
- In the tanning business, tannins are used.
- Some organic phenols have potential as biopesticides.As an insecticide or acaricide, furanoflavonoids such rotenoids and karanjin are utilized. Enological tannins play a significant role on wine flavor.

2.5.2 Review of Test Microorganisms

2.5.2.1 Staphylococcus aureus

Gram-positive cocci belonging to the genus Staphylococcus (staphylococci) typically cluster together in grape-like formations (Ryan and Ray, 2004).

Domain bacteria class Staphylococcaceae
Family Staphylococci Firmicutes
Class Bacilli
Order Bacillales
Genus Staphylococcus
Variety aureus

2.5.3 Morphology and identification

Round cells with a diameter of around 1 m make up staphylococci, which are organized in haphazard clusters. Liquid cultures can also contain single cocci, pairs, tetrads, and chains of cocci. When cocci are young, they strongly stain

gram-positive; as they age, many cells turn gram-negative. Staphylococci are not spore-producing, non-motile bacteria (Brooks et al., 2007). The ideal temperature and pH for *Staphylococcus aureus*, a facultative anaerobe, are 37 degrees Celsius and 7, respectively. The origin of the species name aureus is the white colonies that *S. aureus* creates, which have a propensity to turn a buff-golden hue over time (golden). Most strains, but not all, exhibit a visible rim of -hemolysis around the colony. (Ryan and Ray, 2004). Colonies on nutritional agar are 1 to 3 mm in diameter, have a smooth, glistening surface, an entire edge, and an opaque pigmented appearance after aerobic incubation for 24 hours at 37 °C. The majority of types have golden coloring with orange, yellow, and cream variants. Colonies are tiny to medium in size and pink to pink-orange in color on MacConkey agar.

Staphylococci have a great deal of success colonizing both people and animals. Their primary habitat is the skin, particularly moist places like the axilla, groin, and anterior nares (nose). These organisms are carried by between one-third and three-quarters of people at any given moment. Staphylococcal infections happen everywhere, and newly discovered multiresistant or hypervirulent strains disperse quickly over a large geographic area.

The bacteria can contaminate places (like hospitals) and spread for extended periods of time since they can survive for days in the air, on objects, or in dust. The organism may be shed by certain people more than others. The origins of staphylococcal infections are either internal (endogenous) or external (exogenous).

2.5.3.1 Staphylococcus aureus infections

Serious infections of the skin, soft tissues, bones, lungs, heart, brain, or blood are brought on by *S. aureus*. Other infections include pneumonia, bacteremia that can cause endocarditis, secondary pneumonia, and osteomyelitis, as well as septic arthritis, which is more common in youngsters and people with a history of rheumatoid arthritis. Scalded skin syndrome and toxic shock syndrome are two diseases brought on by staphylococcal toxins.

Antimicrobial A positive test for -lactamase can indicate penicillin G susceptibility; 90% of S aureus strains express this enzyme. About 35% of *S. aureus* isolates and over 75% of S. epidermidis isolates are resistant to nafcillin (as well as oxacillin and methicillin) (Brooks et al., 2007). Vancomycin, erythromycin, and gentamicin are additional medicines for resistant pathogens (like MRSA). Multiple antibiotics can cause certain bacteria to develop resistance.

2.5.3.2 Background information on Escherichia coli

The bacteria that can cause diarrhea include Escherichia coli, sometimes known as *E. coli*. Young Austrian pediatrician Dr. Theodor Escherich made the first *E. coli* isolate in Munich in 1885. He conducted research on the intestinal flora of children as a potential source of diarrhea outbreaks while having clinical assistantships at Hunters Children's Hospital and Children's Polyclinic. For the first time, the name of the Bacterium coli commune, or B. coli, was read. He rose to prominence as the top bacteriologist in pediatrics and an expert in infant feeding.

1- Several veterinary professionals who were examining calves' scours in the late 1800s and early 1900s first hypothesized that *E. coli* may cause diarrhea in animals (Cheesbrough, 2020). The organism and other microbes were isolated from the newborn babies' feces. It was discovered to go together with nursing. It was characterized by Escherich as a small, chubby rod that rapidly grew on gelatin or agar. It developed into a slimy mass with the development of acid on potato and coagulated milk. The organism was given various names in its early years, including Bacillus escherichii in 1889 and Bacillus coli in 1895. Its numerous names in 1900 included Aerobacter coli, Bacterium verus, and Bacillus coli communes. The genus Escherichia was first introduced by Migula in 1895 and became solidly recognized in 1919 by castellani and charmers in the third edition of the Manual of Tropical Medicine. At present eight types of *E. coli* are recognized Enterotoxigenic *E. coli*, Enteropathogenic *E. coli*, Enteroinvasive *E. coli*, Enteroaggregative *E. coli*, Diffuselly adhering *E. coli*, Uropathogenic *E. coli*, Enterohaemorrhagic *E. coli* and *E. coli* that causes sepsis and meningitis .
2- Classification of Escherichia
3- The genus Escherichia belongs to family enterobacteriaceae (Barrow and Feltham, 1993). The following tribes make up the enterobacteriaceae:
4- 1-Escherichеae.

5- 2-Klebsielleae.
6- 3. Proteusae.
7- Yersinia 4-.
8- 5-Erwineae.

There are five genera in the tribe Eschericheae:

1. Escherichia.
2. Edwardsiella.
3. Citrobacter.
4. Salmonella.
5. Shiglla.

The following species are found in the genus:

1. *E. coli:* Like many other enterobacteria, it has a wide variety of serotypes, some of which are linked to specific diseases in humans and animals; others, meanwhile, cause a wide range of other intestinal infections. Some of these serotypes are particularly linked to diarrheal sickness.

Adecarboxylata, 2E
E. fergusonii three
E. hermanii 4
E. blattae 5
E. vulneris six

2.5.3.3 The meaning of E. coli

E. Escherichia coli are nonsporing, straight Gram negative rods. It can be found alone or in pairs, and it grows well on cheap nutrient media. With peritrichus flagella, the majority of the organisms are mobile. There are a large number of serotypes overall. All warm-blooded animals' lower intestine tracts normally contain the bacterium as a resident. Fish and other cold-blooded animals' intestines typically do not contain t. In the stomach and first part of the intestines, there may be a few numbers or none at all. Compared to herbivores, it is more prevalent in omnivores and carnivores. It is one of the most common bacteria on the surface of the earth since it is one of the main components of feces. In the feces of cows and horses, the bacteria are frequently present in extremely small concentrations. The majority are benign saprophytes, but some are aggressive pathogens that injure the colon and other locations outside of the intestinal tract. Enteric infections, septicemia, urinary tract infections, and mastitis are the main illnesses brought on by *E. coli*. Under specific circumstances, the populations of these organisms experience a sharp and quick rise in vivo, which may be accompanied by overt symptoms of sickness and occasionally even death. It is both facultatively aerobic and anaerobic.

The sterile intestine of the fetus is seeded with bacteria from the mother and the surroundings, which causes F.E.coli to become established in the gut shortly after birth. Because the pH of the stomach in newborn animals and humans is almost neutral, *E. coli* can readily pass through and enter the intestine. The intestine of an adult remains home to *E. coli*, which is typically the dominating isolate in an aerobic culture of feces or intestinal contents. Some *E. coli* strains are severe pathogens that attack the colon or extra-intestinal locations, however the majority of *E. coli* strains are benign commensals.

2.5.4 Biochemical tests

The biochemical assays used to distinguish *E. coli* from other bacterial groups that are closely related to it must be based on the reactions that take place in various mediums. All *E. coli* strains ferment glucose and lactose, producing acid and gas in the process, although only a few strains are slow to ferment lactose or may frequently fail to do so. The majority of strains have a positive methyl red test result, give a negative voges-proskaur reaction, and do not produce urease. Milk is acidified and coagulated. In the Ejkman test, fecal *E. coli* may grow at 44°C in MacConkey's lactose bile broth while also producing gas. Water bacteriologists can use this test to ascertain the presence of faecal *E. coli*. Generally, there is no single biochemical feature which is particularly characteristic of Escherichia group. A comparison of various reactions is required for its classification.

2.5.5 Method of evaluating effectiveness of phenolic compound

The Phenol Coefficient Test, which compares a disinfectant's potency to that of phenol, is the most well-known disinfectant screening test, can be used to assess the efficacy of a particular disinfectant. The phenol and the disinfectant being tested are made in a series of dilutions. Each dilution receives a standard dose of the test organism before being placed in a 20°C or 37°C water bath. Samples from each dilution are obtained every five minutes, inoculated in a growth medium, and then incubated at 37°C for 24 to 48 hours. We'll check the tubes for growth. The dilution at the moment of sampling kills the bacteria if there is no growth in the growing

media. The phenol coefficient is calculated using the highest dilution (or lowest concentration) that kills the bacteria after 10 minutes of exposure but not after 5 minutes. This is accomplished by dividing the reciprocal of the suitable phenol dilution by the reciprocal of the appropriate disinfectant dilution that is being tested. When the value is greater than 1, the disinfectant is more efficient than phenol.

"Phenol coefficient can be misleading if used as a direct indicator of disinfectant potency in everyday use, despite being a helpful initial screening method. This is because disinfectants are typically used on complex populations in the presence of organic matter and with significant variations in environmental factors like pH, temperature, and the presence of salts, whereas the phenol coefficient is determined under carefully controlled conditions with pure bacteria strains" [8-11].

3. MATERIALS AND METHODS

3.1 Materials

Petri dish, autoclave, inoculating wire loop, forceps, Bunsen burner, Conical flask, Antibiotic discs, Weighing balance, Test tube rack, plastic pipette, wire loop, Microscope, Incubator, beakers, glass slide, sterile cotton wool, test tube rack, universal container.

3.1.1 Media used

Tryptic soy agar, Plate count agar, selenite F agar and muller hinton agar

3.1.2 Reagent

Pepton water, pepton broth

3.1.3 Collection of samples

Dettol, Izal, and Phenol were obtained from Eke Awka market in Anambra State, Nigeria.

3.1.4 Source of microorganisms

Cultures of the test organisms *Staphylococcus aureus,* and *E coli* were isolated from clinical isolate.

3.1.5 Preparation of media and plating

The medium was set up in accordance with the manufacturer's recommendations. 100 ml of sterile, distilled water was combined with 27 g of Muller Hinton Agar, and the mixture was autoclaved at 121°C for 15 minutes. After cooling, the mixture was added to a sterile petri dish, which was then let to sit at room temperature before being used.

3.1.6 Test organism suspension

Suspension of each of the test organisms was made by collecting a loopful of colony from each plate and inoculating in a nutrient broth. The tubes of the subcultured organisms were incubated at 37°C for 24 hours.

3.2 Identification of Microorganisms

3.2.1 Morphological identification

The isolated bacteria were identified on the basis of negative staining and Gram's-staining.

3.2.2 Gram's staining

The Gram stain is by far the most popular method for coloring bacteria and classifying them into Gram (+) positive and Gram (-) negative groups. Over a spotless, grease-free slide, apply a thin layer of specimen and let it air dry. Fix it by putting it three times through a Bunsen flame. Crystal violet should be applied liberally and left to sit for 60 seconds. Using lugol's iodine and (mordant), saturate the stain on the slide, then let it sit for 60 seconds. Iodine must be removed, the slide must be briefly decolored with acetone (a decolourizer), the slide must then be stained for 60 seconds with safranin (a counterstain), and finally the stain must be removed. After that, let the slide air dry and dry the back. Examine with the oil immersion, x 100 lens. A purple colour signifies Gram (+) positive while the colour of the safranin which is red signifies Gram (-) Negative.

3.2.3 Motility test

The purpose of this test is to recognize motile vibranaceae and enterobacteriaceae members. A needle was used to inject the test organism into the mobility medium five times at a depth of 1-2 cm from the tube's bottom. The tube was incubated for 24 hours at 37 °C. Examining the line of incubation for cloudiness that indicates the organisms are motile [7].

3.2.4 Methyl red test

Which of the isolates could develop and maintain a steady acid product from glucose fermentation

was determined using this assay. Typically, the test is performed to help identify and differentiate the Enterobacteriaceae. According to Cheese Brough [7], this test was conducted. (2005). Incubate the suspected organism for 24 hours at 37°C after inoculating it with a sterile buffered glucose-peptone broth. After 24 hours, whisk the mixture with five drops of methyl red indicator before observing. A vivid red color indicates a successful outcome.

3.2.5 Citrate utilization test

In this experiment, as reported by Cheese Brough [7]. Which isolates can use citrate as their exclusive source of carbon for metabolism was determined using the test. The test is typically used to help distinguish between distinct Enterobacteriacea species of organisms. Insert a loopful of the culture into sterile test tubes containing Simmon's citrate media. tube for 24 hours at 37 degrees. A color shift from green to blue is an advantage. Negative reaction is indicated by the absence of any growth and by the color remaining unchanged.

3.2.6 Oxidase test

The suspicious organisms were picked up using a sterile wire loop and mixed with the freshly made oxidase (p- aminodimethylanine) reagent before being added to the filter paper for the test. Deep purple instead of the usual color denotes a successful outcome, while the typical color denotes an unsuccessful outcome.

3.2.7 Vogas Proskaeur test

“This test was used to detect which of the isolates were able to produce a neutral red end point acetyl methyl carbinol (acetion) from glucose fermentation or its reductive product butylenes glycerol. The test is usually used to differentiate between Gram negative organisms especially members of the *Enterobacteriaceae”* [7]. “Inoculate the suspected organism into a test tube containing buffered glucose peptone water and incubate at 37°C for 24 hours. Into the incubated medium, add 0.6% w/v solution of A and 0.2ml of solution B Shake the mixture and live to stand. A red colour is a positive result. While the development of a yellow colour indicates a negative reaction. Solution A Contains 5g of - naphlho100ml absolute ethyl alcohol Solution B contains100ml Distilled water 40g potassium hydroxide. The alkalis oxidize the acetyl methyl carbonyl (acetone) to diacetyl which gives the pink colour” [7].

3.2.8 Coagulate test

“This test was done according to Cheese Brough, [7] to differentiate *staphylococcus aureus* and other *staphylococcus* species. Add 2 - 3 drops of normal saline on a grease free slide to the normal saline mix the suspected organism and add 1 – 2 drops of plasma and Rock, the presence of agglutination means a positive result while no agglutination means a negative results”.

3.2.9 Indole test

Escherichia coli is indole positive and only some *shigella* strain are indole positive. The test organism was inoculated in a test tube containing 3ml of sterile trytone water. Incubation was done at 37°c for 24hrs. The test for indole was done by adding 0.5ml of kovac’s reagent and shaken gently. Examination for a red colour in the surface of the layer within 10minutes means positive, while no colour change means negative.

3.2.10 Urease test

This test was used to demonstrate the ability of the isolates to produce the enzyme urease which splits urea forming ammonia. The test is usually used to differentiate organisms like proteus from other non urease positive organisms. A loop full of the isolates was used to inoculate a tube of urea-agar. The tubes were incubated at 37°C. a change in colour from yellow to red confirmed the presence of urease.

3.2.11 Catalase test

This test was used to demonstrate which of the isolates could produce the enzyme catalase that release oxygen from hydrogen peroxide. This test is usually used as an aid to differentiate *Staphylococci* from *Streptococci* and to differentiate other catalase positive organism from catalase negative. A loopful of the pure colony was transferred into a plane, clean glass slide. The sample was then mixed with a drop of 3% v/v hydrogen peroxide. The reaction was observed immediately Gas production indicated by the production of gas bubbles confirmed the presence of catalase.

3.2.12 Sugar fermentation

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially Gram negative bacteria utilize different sugars as source of carbon and energy with the production of both acid and gas, or acid only the

test is used as an aid in their differentiation. Peptone water was prepared in a conical flask and the indicators bromocresol purple was added. The mixture was dispensed into test tubes containing Durhams tubes. The tubes with their content were sterilized by autoclaving at 121°C for 15 minutes. 1% solution of the sugar was prepared and sterilized separately at 115°C for 10 minutes. This was then aseptically dispensed in 5ml aliquot volume into the tubes containing the peptone water and indicator. The tubes were inoculated with young culture of the isolates and 1ncubated at 37°C. Acid and gas production or acid only were observed after about 24 hours of incubation. Acid production was indicated by the change of the medium from light green to yellow colour, while gas production was indicated by the presence of gas in the Durham's tubes. The control tubes were not incubated.

3.3 Antimicrobial Screening Tests

3.3.1 Standardization of the inoculums

The inoculum will be prepared by inoculating colonies of fresh test cultures into sterile distilled water. The turbidity will be compared to 0.5McFarland standard prepared according to method of Cheesbrough, (2004).

3.3.2 Antibiotics sensitivity test

Antibiotic susceptibility test of the isolated test organisms against commonly prescribed antibiotics was determined using standard microbiological protocol.

3.3.2.1 Inoculation of the test organisms

"Using different sterile swab sticks, 24 hour old culture of each of the test organisms was collected. The swab sticks containing the different bacterial cultures were swirled into different test tubes containing 10ml of sterile water. The content of each of the tubes was properly homogenized before the inoculation. Another set of sterile swab sticks were dipped into each of the bacterial solution and were used to inoculate the solidified Nutrient agar plates ensuring that the plates were completely covered for uniform growth" [12].

3.3.2.2 Preparation of the disinfectants

The disinfectants were poured into different sterile test tubes and these became the stock solutions. A 2-fold serial dilution of each of the disinfectant was prepared as follow:

"3 sterile test tubes were placed in a test tube rack; 1ml of distilled water was pipetted into each of the 3 test tubes using a sterile pipette; 1ml of disinfectant was pipetted from the stock into test tube 1, and this was labelled 2-1, the content was properly mixed; 1 ml of solution was collected from tube 1 (2^{-1}) and transferred into tube 2 (2^{-2}) and the content was properly mixed; 1ml was collected from tube 2(2^{-2}) and transferred to tube 3 (2^{-3}) and the content was properly mixed; 1ml was collected from tube 3 (2^{-3}) and discarded. This procedure was repeated for all the disinfectants" [12].

3.3.2.3 Paper disc diffusion method

"This involves a heavy inoculation of an agar plate with the test organism. Sterile colour coded filter paper discs were impregnated with the different antiseptics or disinfectants and equally spaced on the inoculated plate. Following incubation, the agar plate was examined for zones inhibition (areas of no growth) surrounding the discs" [12].

A zone of inhibition is indicative of microbial activity against the organism. Absence of zone of inhibition indicates that the antiseptic or disinfectant was ineffective against the test organism.

3.3.2.4 Impregnation of the discs

The sterile filter paper discs were impregnated with 0.1ml each of the dilutions of the disinfectant using different sterile pipettes.

3.3.2.5 Inoculation of impregnated disc

"Using sterile forceps, the different discs impregnated with different dilution of the disinfectants were placed on each of the plates inoculated with the test organisms. The forceps was used to press down each of the disc gently against the agar surface so as to ensure good contact. The plates were incubated in an inverted position at 37°C for 24 hours. The zones of inhibition were observed, and then measured accurately" [12].

4. RESULTS

The nature of growth and mean bacterial and fungal counts are presented in Table 1. The bacterial count ranged from *4.70 x10^4 cfu/ml* which occurred in sample from temp site Awka, to 6.1x10^4 cfu/ml in sample from Eke Awka while the mean Fungal counts ranged from a 2.7 x10^4

cfu/ml in sample from Eke Awka to 4.50 x10^4 cfu/100 ml in sample from Amenyi Awka.

4.1 Antimicrobial Sensitivity Results for the Identified Isolates on Household Disinfectants

The antimicrobial sensitivity results were presented in Table 2 and showed that the antiseptic 1 gave the highest collective zones of inhibition, followed by antiseptic 2 while antiseptic 3 gave the least collective zones of inhibition.

5. DISCUSSION

From the different diameters of zones of inhibition of the three disinfectants under study, it was discovered that all the disinfectants inhibited the growth of the test organisms in their concentrated forms. On dilutions, their activities varied. Disinfectant C at 30% concentration showed the highest activity on *Staphylococcus aureus,* whereas Disinfectant. B and A showed the least. The distribution of the activities in decreasing order is as shown phenolics > bleach > ethanol.

Table 1. Mean bacterial and fungal counts in food samples

Sample site	Total Bacterial count (x 10^4 cfu/ml)	Total fungi count (x 10^4 cfu/ml)
Eke awka	6.10 ± 0.32	2.70 ± 1.00
Temp site	4.70 ± 0.11	3.18 ± 0.21
Amenyi market	5.60 ± 0.03	4.50 ± 0.33

Table 2. The zone of inhibitions (mm) shown by the bacterial isolates

Isolate	Antiseptic 1 100%	Antiseptic 2 100%	Antiseptic 3 100%	Std antibiotics 30µg/ml
Staphylococcus sp.	27.00 ± 0.32	14.90 ± 1.11	6.00 ± 1.00	34.85 ± 0.20
Escherichia coli	43.00 ± 0.10	35.00 ± 0.10	30.00 ± 0.20	34.83 ± 0.30
Klebsiella sp.	18.00 ± 0.11	15.70 ± 0.03	11.00 ± 0.320	19.16 ± 0.50

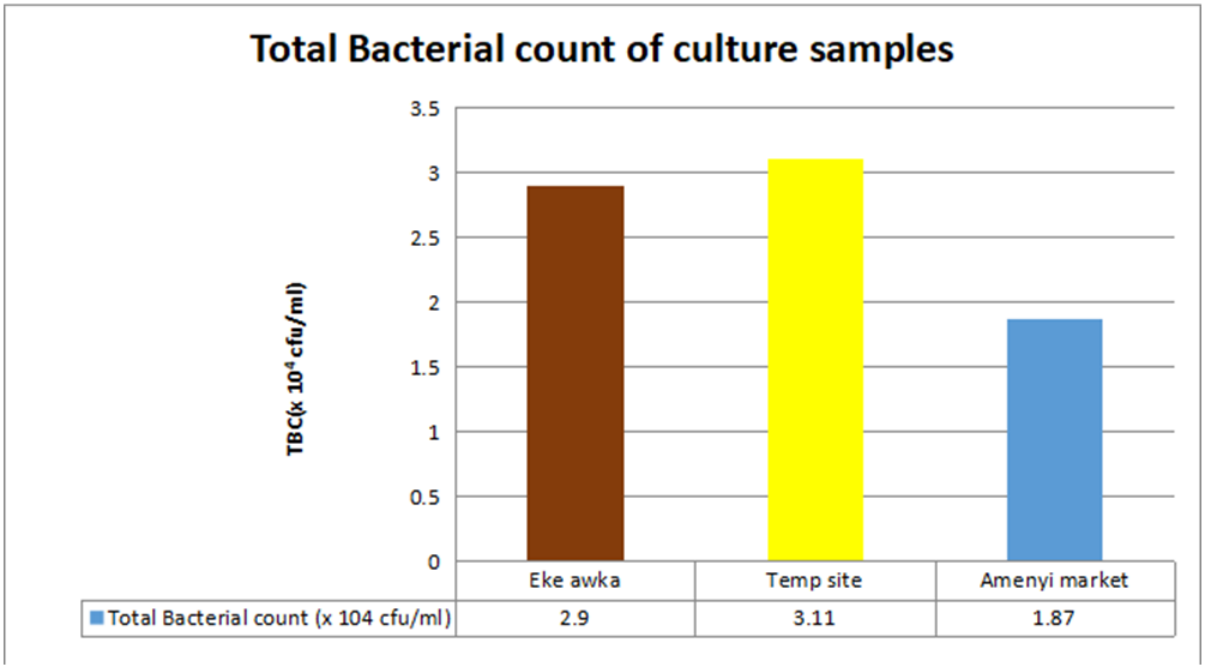

Fig. 1. Mean bacterial counts in food samples

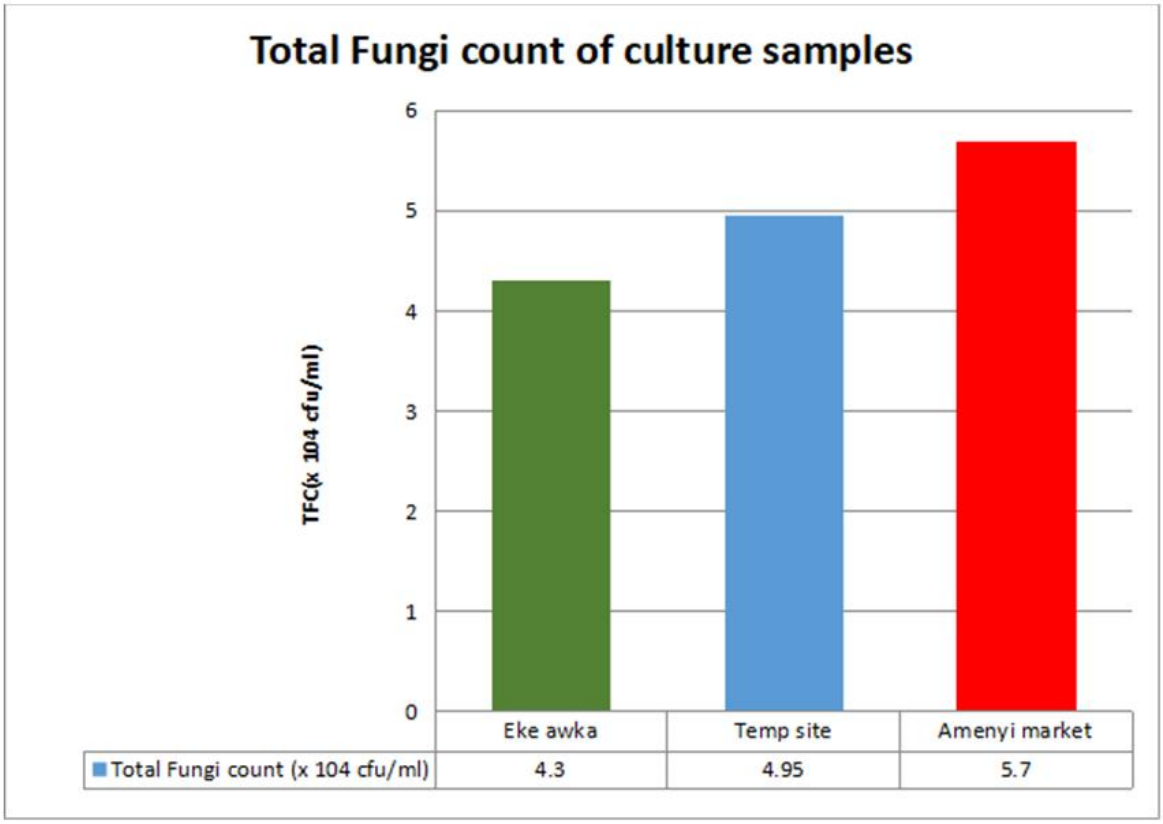

Fig. 2. Mean fungal counts in food samples

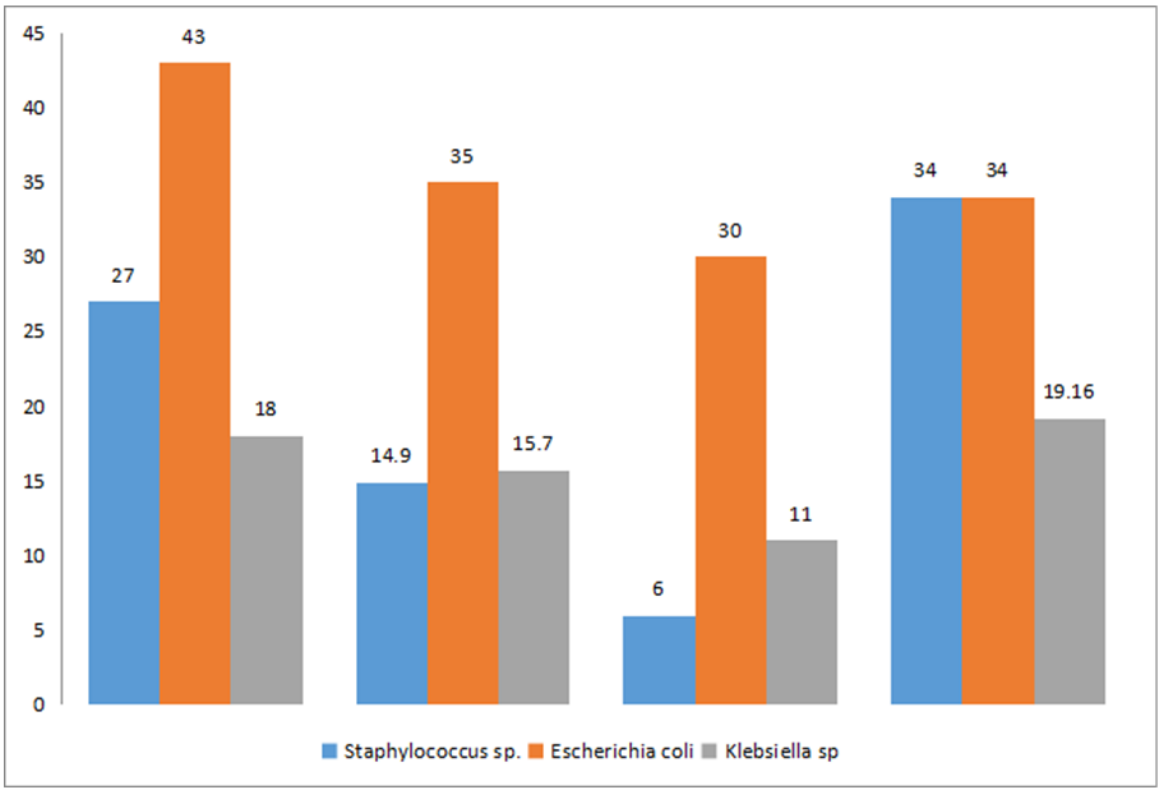

Fig. 3. Zone of inhibition shown by bacterial isolates

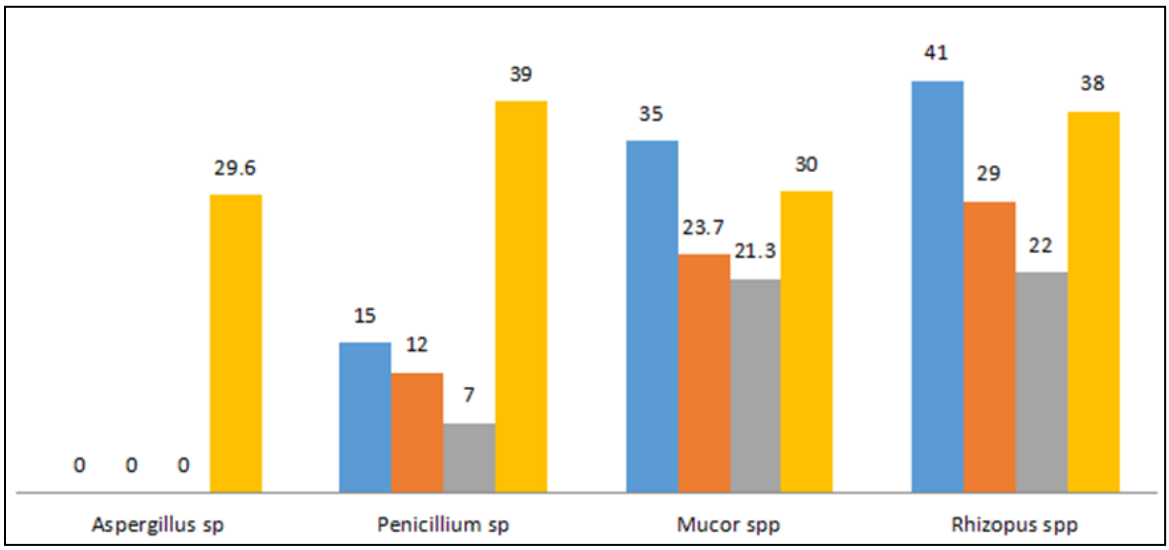

Fig. 4. *In vitro* antifungal activity of anticeptic

Table 3. The zone of inhibitions (mm) shown by the fungi isolates

Isolate	Antiseptic 1 100%	Antiseptic 2 100%	Antiseptic 3 100%	Std antibiotics 30µg/ml
Penicillium sp	15.00 ± 0.10	12.00 ± 0.11	7.00 ± 0.10	39.00 ± 0.10
Mucor spp	35.00 ± 0.10	23.70 ± 0.15	21.30 ± 0.11	30.00 ± 0.20
Rhizopus sp	41.00 ± 0.21	29.00 ± 0.10	22.00 ± 0.10	38.00 ± 0.00

Disinfectants B and C showed the highest activities at the concentrations of 5% 30% on *E coli*, whereas disinfectant A showed the least on the same organism. The distribution of their activities in decreasing order is as shown, bleach > phenolics > ethanol.

However, on the contrary, disinfectant A has the lowest antimicrobial effect as compared to others on both organisms. From Table 3, disinfectant C had the highest inhibitory activity and can be deduced to be highly bactericidal on both organisms. Phenolics which is active ingredient for disinfectant C are active against bacteria (especially gram positive bacteria). This tallies with my findings, a phenolics proves highest inhibition against *Staphylococcus aureus*. Owing to their high activity level, disinfectants C maintain their activities in the presence of organic material (milk) as they last long on surfaces unlike ethanol which evaporates easily. Also since the mode of action of phenols in mainly by protein penetration and cell disruption, this extrapolates the bactericidal action of phenols.

Moreover, from the results, it indicated that bleach had an ideal bactericidal effect against both *E coli and Staphylococcus aureus* at 55 and 5% Concentrations as seen in Table 2. According to Busca et al., [13], "former study, it found that oxidation reactions will occur when bleach is dissolved in water, which can destroy organisms fold structure leading to sterilization". "Another study also found similar result that bleach is rapidly bactericidal achieving a 5log10 kill of *E coli* and other vegetative organisms in one minute" [14].

The data's in Figs. 1, 2, 3 and 4 generally showed that diameters of zone of inhibition decreases as the concentrations of disinfectant decreases, but the observation was stable in disinfectant A. from the results in Figs. 1 and 4, it was shown that as the concentration of ethanol increased, the diameter decreased. Ethanol are rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria (gram $_{+}$ve and gram-ve), but their cidal activities drop sharply when diluted below 60% concentration and optimum bactericidal concentration in the range of 60% - 90% solution in water, volume/volume (Moorer, 2003). The result showed that 70% ethanol gave better effect on both test organisms than other ethanol concentrations. According to Nair, et al. [15], "70% ethanol had been found to be most effective to

denature protein thereby killing bacteria, because of its diffusion rate and transportation into the cells organism. It evaporates at a slow rate and less harmful to the hand, this is the reason why it's been used in the laboratories for disinfection. Below 70% does not denature protein, while 85%-absolute ethanol evaporates fast and leave the protein untouched. They leave traces on the applied surfaces thus, adding unwanted reagents. Also, they are harmful to the skin thereby making it dry and may not be effective".

"From this study, it confirmed Calabrase and Kenyon [16], study which showed similar result that higher concentrations are less effective as the action of denaturing proteins is inhibited without the presence of water. They also evaporate rapidly which makes extended exposure time difficult to achieve unless items are immersed in the ethanol" [16].

According to Talaro and Talaro, [17] researches, "it also found that some kinds of bacteria cannot be billed easily and have some characteristics of resistance on ethanol. Its sterilization in mainly due to dehydration of protein enzyme deactivation and prevent bacteria growth. Different proteins have different biological characters which cause selectivity in ethanol deactivation of organisms". However, this conforms with Talaro, and Talaro, [17,18] as *E. coli* are more resistant to disinfectant A.

In addition, disinfectant C and B are both effective disinfectants for sterilization against *pseudomonas* aerations and *Staphylococcus aureus* but disinfection C has the highest inhibitory effect.

6. CONCLUSION

The main goal of this study is to compare the efficiency of three disinfectants at five different concentrations. Conclusively, among the three common disinfectants tested in this project, disinfectant C in all its concentration had this best efficiency against both *E coli and Staphylococcus aureus.*

When these antimicrobial agents are used to disinfect sites suspected to be contaminated with gram positive bacteria, they should be used in their concentrated forms. Any dilution above this will only succeed in providing the user with a false sense of security

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Willey LM, Sherwood, Woolverton CJ. Prescott, Harley, and Klen's Microbiology, 7th ed., New York: McGraw-Hill Higher Education. 2008;158-165.
2. Tortora BR, Funke CJ. Case, Microbiology: An Introduction, 8th ed., New York: Pearson Education and Dorling Kindersley Pvt, Ltd. 2004;224-225.
3. Ryan and Ray CG. Bacteria, In: Sherris Medical Microbiology, 4th ed., Mc Gram Hill, ISBN; 2011.
4. Brooks SJ, Butel, Morse AS. Jawetz, Melnick and Adelberg's Medical Microbiology, 23rd Ed., United States: McGraw-Hill Companies. 2004;161-165.
5. Omoruyi MI, Idemudia MI. Comparative analysis of the antiseptic properties of some disinfectants on bacteria and fungi of public health importance isolated from barbing clippers. Journal of Asian Scientific Research. 2011;2(1): 65-68.
6. World Health Organization. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world; 2003.
7. Cheesbrough C. District Laboratory Practice in Tropical Countries, 2nd ed., New York: Cambridge University Press. 2005;38-39.
8. Otokunefor TV, Usoh CU. Microbial contamination of inuse disinfectants in small private medical facilities in Owerri, in south east Nigeria. Scientia Africana. 2009; 8(2):17-25.
9. Iruoha IR, Oji AE, Nwosu OK, Amadi ES. Antimicrobial activity of Savlon, Izal and Z-germicide against clinical isolates of *E. coli* from hospital wards. European Journal Dentistry and Medicine. 2016; 3(1):32-35.
10. Ghotaslou R, Bahrami N. Antimicrobial activity of chlorhexidine, peracetic acid/peroxide hydrogen and alcohol based compound on isolated bacteria in Madani Heart Hospital, Tabriz, Azerbaijan, Iran. Advanced Pharmaceutical Bulletin. 2012; 2(1):57-59.

11. Rutala WA, Barbee SL, Agular NC, Sobsey, MD, Weber DJ. Antimicrobial activity of home disinfectants and natural products against potential human pathogens. Infection Control and Hospital Epidemiology. 2010;21(1):33-38.
12. Okore CC, Mbanefo ON, Onyekwere BC, Onyewenjo SC, Ozurumba AU, Abba-Father CA. Antimicrobial efficacy of selected disinfectants. American Journal of Biology and Life Sciences. 2014;2(2):53-7.
13. Busca G, Berardinelli S, Resini C, Arrighi L. Technologies for the removal of phenol from fluid streams: A short review of recent developments. Journal of Hazardous Material. 2008;160:265- 288.
14. El-Mahmood AM, Doughari JH. Bacteriological examination of some diluted disinfectants routinely used in the specialist hospital Yola, Nigeria. African Journal of Pharmacy and Pharmacology. 2009;3(5):185-190.
15. Nair D, Okiemen H, Neuwinger JD. Translated from by Porter A. African ethnobotany poison and drugs. Chapman and Hall, Weinheim. 2008;495-499.
16. Calabrase EJ, Kenyon EM. Air toxics and risk assessment. Lewis Publishers, Chelsea, Michigan; 2011.
17. Talaro and Talaro R. Foundation in Microbiology, 5th ed, New York: McGraw-Hill companies. 2005;324-335.
18. Pelczar E, Chan CS, Krieg RN. Microbiology, 5th Ed, New Delhi: Tata McGraw-Hill publishing Company Ltd. 2006;469-490.

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