***Original Research Article***

ANTIMICROBIAL EFFICIENCY OF COMMONLY USED DISINFECTANTS AGAINST *Escherichia coli* and *Staphylococcus aureus*.


## ABSTRACT

*Ethanol, Bleach and Phenolics are three kinds of disinfectants which have been widely used in common laboratories. In this study, a compared experiment on these three disinfectants efficiency was conducted against Staphylococcus aureus and Escherichia coli using agar hole diffusion method. Different concentrations of bleach, ethanol as well as Phenolics were used. Differences in concentrations tested was because, the original concentrations of the disinfectants differs. After 24 hours of incubation at 37$^0$C, the results showed that all the disinfectants inhibited the growth of the test organism in their concentrated forms. The diameter of zone of inhibitions were measured around each well by using a ruler in millimeters, using different concentrations, their efficacy varied. The results showed that Phenolics had the best efficiency against both test organisms and bleach had a better effect on Staphylococcus aureus than Escherichia coli, while ethanol showed least sensitivity.*




## INTRODUCTION

The term control of microorganisms refers to the reduction in number or activity of the total microbial load. The principal reasons for controlling microorganisms are as follow, to prevent transmission of diseases and infection, to prevent contamination by the growth of undesirable microorganisms, to prevent deterioration and spoilage of materials by microorganisms (Pelezar, *et al.*, 2016).The control directed at destroying harmful microorganisms is called disinfection. It usually refers to the destruction of vegetative (non-endospore forming) pathogens. The term most commonly applies to the use of a disinfectant to free an inert surface or substance. When this treatment is directed at living tissue,

it is called antisepsis, and the chemical is then called antiseptic. Therefore, in practice the same chemical might be called a disinfectant for one use and antiseptic for another (Willey, *et al.*,2018).

According to (Tortora, & Funke, 2014), disinfectants are toxic not only for microbial pathogens but for host cells as well and because of this, they can only be used to inactivate microorganisms in the inanimate environment or to a limited extent, on skin surfaces but cannot be administered systemically. These disinfectants cause distraction either by coagulating the protein of the bacteria, by destroying its cell membrane or by removal of a sulphonhydric group from the organisms (Ryan and Ray, 2011). Also according to (Brooks, *et al.*,2014), the mode of action of disinfectants is thought to be linked to destruction of proteins, lipids or nucleic acids in the cells or its cytoplasmic membrane, although microorganisms differ in their sensitivity to chemical germicide.

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa or viruses. Antibiotics are those substances which are produced by microorganism that kills or prevents the growth of another micro-organism (Omoruyi & Idemudia 2011). Antibiotics are generally used against bacteria, antiviral are used specifically for treating viral infections. Antifungal are used to treat fungal infections, some of these side effects can be life threatening if the drug is not used properly. Several microorganisms derived antibiotics are currently in use to treat a variety of human disease, therefore the action must be taken to control the use of antibiotics, develop new drugs either synthetic or natural, for a long period of time, plant have a valuable source of natural products for maintaining human health. India has a rich tradition in use of medicinal plants to develop drugs. According to world health organization (WHO, 2013), any plant which contain substances that can be used for therapeutic purpose or which are precursor of chemo-pharmaceuticals semi synthetic new drugs is referred as medicinal plant.

Many antiseptics in Nigeria markets today have varying degrees of effectiveness. These variations may be attributable to their active ingredients. Most of the antiseptics contain one of the following compounds: chlorhexidine, phenol, chloroxylenol and cetylpyridinium chloride (CPC). All, with the exception of the mouthwash, are applied externally to prevent proliferation of microbial population particularly during bath.

Antiseptics are agents that destroy or inhibit the growth of microorganisms in or on living tissue while disinfectant are similar but are used on inanimate objects or surface. These agents such as alcohols, phenols, iodine and chlorine used extensively in hospitals and other health care settings for infections control and prevention of nosocomial infections. An ideal disinfectant to overcome the antimicrobial resistant

pathogens should have broad spectrum of antimicrobial activity and the efficacy of these agents may be affected by PH, detergent base, temperature, organic matter, ionic and type of the surfactants.

Disinfectants are antimicrobial agents that are applied to non-living objects to destroy microorganisms that are living on the. Disinfection does not necessarily kill all microorganisms, especially resistant bacteria spores; it is less effective than sterilization, which is an extreme physical and/or chemical process that kills all types of life. A perfect disinfectant would also offer complete and full microbiological sterilisation, without harming humans and useful forms of life, be inexpensive, and non-corrosive.

Disinfectants are usually used in dilutions, however it has been shown that when some of these agents are diluted for use, some Gram negative bacteria e.g. *E coli* can still survive making them ineffective against nosocomical infections. The emergence of resistant microorganisms in hospitals and the community is causing problems for both the treatment of patients and infection control. Organisms of particular concern include methicillin- resistant *Staphylococcus aureus*, glycopeptide resistant enterococci and extended spectrum beta-lactamase producing *Pseudomonas*. The aims of this study are to know the effectiveness of different disinfectants under different trade names on selected tests microorganisms: *Staphylococcus aureus*, and *E. coli*; to know if they have any antimicrobial activity against the test microorganisms; to know the concentrations at which they were effective; the susceptibility of the test gram positives and gram negative to the test disinfectants, and to help know the most effective disinfections to use for household and hospital cleanings.

**Statement of the problems**

Resistance to antibiotics is one of the biggest problems that faces public health. This problem is a natural consequence of the adaption of infectious pathogens to antimicrobials used in several areas, including medicine, food animals, crop production and disinfectants in farms, hospital and households. Bacteria have developed resistance to all known antibiotics and, as so, the economic burden associated with these multidrug-resistant bacteria is high (Cheesbrough, 2005). Different disinfectant formulations have different applications. The process of disinfection may be affected by many variables like temperature, contact period, pH and concentration of the disinfectant, bioburden, organic soil and hardness of water used for dilution. Therefore, the disinfectant ought to be tested in the field for the specified application to ensure its effectiveness. There is limited awareness among users about choosing an

appropriate disinfectant, especially in small health care settings. Usually, an agent with broad-spectrum antimicrobial activity is chosen based on the literature provided by manufacturers. Many hospitals and homes are still using phenolic disinfectants, while their use is being discouraged throughout advanced countries.

Toxicity issues have led to discontinued use of gluteraldehydes in some developed countriesbut, in developing countries, they are used very frequently. Many users has to solely rely upon the literature provided by the manufacturer regarding the efficiency of the disinfectants. Almost all the manufacturers claim their disinfectant as a broad-spectrum antimicrobial agent suitable for diverse applications. Keeping in view the above, the following study was planned with an aim to evaluate and compare the practically achieved disinfection efficacy of some locally available phenolic disinfectants for disinfection of surfaces and infectious microbiological and other waste keeping their cost-effectiveness in mind. The efficacy was tested against locally isolated isolates of *E coli*, and *Staphylococcus aureus.*

**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.

## LITERATURE REVIEW

### 2.1 Phenol as disinfectant

Phenol is probably the oldest known disinfectant introduced by Lister as "carbolic acid". Today disinfectants are widely used in the health care, food and pharmaceutical sectors to prevent unwanted microorganisms from causing disease. Phenols are effective especially against gram positive bacteria and enveloped viruses which include BRS, BVD, Coronavirus, IBR, Leukemia, PI3, Pox, Rabies and Stomatitis virus. Phenolic compounds are used as intermediate level disinfectants used to treat non critical medical devices which pose the lowest risk of transmission of infection, usually contact only intact skin. They retain more activity in the presence of organic material than iodine or chlorine-containing disinfectants.

**Application of phenolic compounds in disinfectants and antiseptic**

Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections. Mounting concern over the potential for microbial contamination and infection risks in the food and general consumer markets have also led to increased usage of antiseptics and disinfectants by the general public.

Antiseptics are biocides or products that destroy or inhibit the growth of microorganisms in or on living tissue (e.g. health care personnel hand washes and surgical scrubs); and disinfectants are similar but generally are products or biocides that are used on inanimate objects or surfaces. A wide variety of active chemical agents (or "biocides") are found in antiseptics and disinfectants. Depending on the chemical nature of disinfectants and antiseptics they can be categorized into several groups. They are alcohols, phenolics, halogens, Quaternary Ammonium Compounds (QACs) and aldehydes. The mode of action of disinfectants and antiseptics differ greatly according to the chemical substance present. The choice of the disinfectant to be used depends on a particular situation. Some disinfectants have a wide spectrum (kill nearly all microorganisms), whilst others kill a smaller range of disease-causing organisms, but are preferred for other properties (they may be non-corrosive, non-toxic, or inexpensive) (Pelczar *et al.*, 2013). The basic principle now widely accepted is that, the antimicrobial efficiency of a disinfectant or an antiseptic is examined at three stages of testing (Pelczar *et al.*, 2013). The first stage concerns laboratory tests in which it is verified whether a chemical compound or a preparation possesses antimicrobial activity. For these preliminary screening tests, suspension tests are considered. In the second stage of tests, disinfection procedures and not disinfectants are examined. It is determined under which conditions and at which use-dilution for a given application the preparation is active: the tests simulate real-life situations; such tests are carrier tests for the disinfection of materials by submersion and surface disinfection tests. The last stage takes place in the field, and comprises the in-situ tests which examine whether, after a normal period of use, germs are still killed by the disinfectant solution.

**Types of disinfectants and antiseptics**

Alcohols are among the most widely used disinfectants and antiseptics. They are colourless hydrocarbons with one or more hydroxyl functional groups. Alcohols are bactericidal and fungicidal but not sporicidal. The mode of action of alcohol depends upon its concentration. Alcohol with a concentration of 50% and higher dissolves membrane lipids, disrupts cell surface tension and compromises membrane integrity. An alcohol that has entered the protoplasm denatures protein through coagulation but only in alcohol-water solution of 50-95%. Absolute alcohol (100%) dehydrates cells

and inhibits their growth. Some of its effectiveness as surface disinfectants can be attributed to its cleansing or detergent action, which helps in the mechanical removal of micro-organisms. Solutions of 70-95% alcohol are used as skin degerming agents. Most commonly 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" which is used to disinfect the skin before injections. Ethyl alcohol is used to disinfect surgical instruments, face masks, thermometers etc. Alcohol effectively kills the Staphylococcus aureus (70% Ethyl alcohol concentration in 10min), the *Escherichia coli* (70% Ethyl alcohol concentration in 2 min) and the Polio virus (70% Ethyl alcohol concentration in 10min).

Phenol was the first widely used antiseptic and disinfectant. Phenolics consist of one or more aromatic carbon rings with added functional groups. The three important substances are alkylated phenols (cresols), chlorinated phenols (chlorophene) and bisphenols (hexachlorophene) (Talaro and Talaro, 2016). Phenolics are strongly microbicidal and will destroy vegetative bacteria, fungi and most viruses (not hepatitis B). However, they are not reliably sporicidal (Talaro and Talaro, 2016). They may be either bacteriostatic or bactericidal, depending on the concentrations used. Modes of action of phenol depend on the concentrations used. In high concentrations they are cellular poisons, rapidly disrupting cell walls and membranes and precipitating proteins. They damage the cell wall by altering the normal selective permeability of the cytoplasmic membrane, causing leakage of vital intracellular substances. In lower concentrations they inactivate the critical enzyme system (Pelczar et.al,2013). Phenolics are active ingredients in some household disinfectants. They are also found in some mouthwashes and in disinfectant soap and hand washes. A 5% aqueous solution of phenol is used for disinfecting drains and cesspools. Phenol is rather corrosive to the skin and sometimes toxic to sensitive people.

### 2.2.1 Occurrence of Phenolic Compounds

The phenolic compounds which occur commonly in food material may be classified into three groups, namely, simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids.

**a. The Simple Phenols and Phenolic Acids.**

The simple phenols include monophenols such as p-cresol isolated from several fruits (e.g. raspberry, blackberry), 3-ethylphenol and 3,4-dimethylphenol found to be responsible for the smoky taste of certain cocoa beans and diphenols such as hydroquinone which is probably the most widespread simple phenol. A typical hydroquinone derivative, sesamol, is found in sesame oil. Several derivatives of sesamol, such as sesaminol, found in sesame oil have been evaluated to have strong antioxidant activity.

b. **The Flavonoids.**

The most important single group of phenolics in food are flavonoids which consist mainly of catechins, proanthocyanins, anthocyanidins and flavons, flavonols and their glycosides. Although catechins seem to be widely distributed in plants, they are only rich in tea leaves where catechins may constitute up to 30% of dry leaf weight. A number of chapters in Volume II of this book discuss current research on antioxidative and cancer chemopreventive properties of tea and its catechin components.

**Phenolic Compounds as Natural Antioxidants and Antimicrobial**

Antioxidants are added to fats and oils or foods containing fats to prevent the formation of various off-flavors and other objectionable compounds that result from the oxidation of lipids. BHA and BHT, the most widely used synthetic antioxidants, have unsurpassed efficacy in various food systems besides their high stability, low cost, and other practical advantages. However, their use in food has been falling off due to their suspected action as promoters of carcinogenesis as well as being due to a general rejection of synthetic food additives (Thomas *et al.,* (2012). The most important natural antioxidants which are commercially exploited are tocopherols. Tocopherols have a potent ability to inhibit lipid peroxidation in vivo by trapping peroxyl radicals. Unfortunately, tocopherols are much less effective as food antioxidants. The search and development of other antioxidants of natural origins is highly desirable. Such new antioxidants would also be welcome in combatting carcinogenesis as well as the aging process. Most natural antioxidants are phenolic in nature. Some of the food materials containing phenolic antioxidants studied and reported herein include: *Osbeckia chinensis*, Chili pepper, Ginger, Green tea, Pepper, Oregano.

**Application of phenolic compounds**

Phenols are important raw materials and additives for industrial purposes in:

- laboratory processes
- chemical industry
- chemical engineering processes
- wood processing
- plastics processing
- Tannins are used in the tanning industry.
- Some natural phenols can be used as biopesticides. Furanoflavonoids like karanjin or rotenoids ar e used as acaricide or insecticide. Enological tannins are important elements in the flavor of wine.

**Review of test microorganisms**

***Staphylococcus aureus:***

Members of the genus Staphylococcus (staphylococci) are Gram-positive cocci that tend to be arranged in grape-like clusters (Ryan and Ray, 2004).

Domain Bacteria
Phylum Firmicutes
Class Bacilli
Order Bacillales
Family Staphylococcaceae
Genus *Staphylococcus*
Species *aureus*

**Morphology and identification**

*Staphylococci* are spherical cells about 1 m in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on aging, many cells become gram-negative. Staphylococci are non-motile and do not form spores (Brooks et al, 2007). *Staphylococcus aureus* is a facultative anaerobe that grows at an optimum temperature of 37ºC and an optimum pH of 7,5. *S. aureus* produces white colonies that tend to turn a buff-golden color with time, which is the basis of the species epithet *aureus* (golden). Most, but not all, strains show a rim of clear β-hemolysis surrounding the colony (Ryan and Ray, 2004). On nutrient agar, following aerobic incubation for 24 hours at 37ºC, colonies are 1 – 3mm in diameter, have a smooth glistening surface, an entire edge and an opaque pigmented appearance. In most strains, pigmentation is golden with orange, yellow and cream varieties. On MacConkey agar, colonies are small to medium in size and pink or pink-orange in colour.

**Epidemiology**

*Staphylococci* are highly successful colonizers of humans and animals. They reside mainly on the skin, particularly in moist areas such as the anterior nares (nose), axilla and groin. Between one-third and three-quarters of individuals carry these organisms at any one time. Staphylococcal infections occur worldwide and newly emerging hypervirulent or multiresistant strains spread rapidly over wide geographical areas. The bacteria survive in the air, on objects or in dust for days, therefore they can contaminate environments (such as hospitals) and continue to be transmitted over long periods of time. Some individuals may shed the organism more heavily than others. Staphylococcal infections are acquired from either self (endogenous) or external (exogenous) sources.

**Infections of *Staphylococcus aureus***

*S. aureus* causes serious infections of the skin, soft tissues, bone, lung, heart, brain or blood. Other infections include pneumonia, bacteremia leading to secondary pneumonia and endocarditis, osteomyelitis secondary to bacteremia and septic arthritis, seen in children and in patients with a history of rheumatoid arthritis. Diseases caused by staphylococcal toxins include scalded skin syndrome and toxic shock syndrome.

Antimicrobial Susceptibility to penicillin G can be predicted by a positive test for β-lactamase; approximately 90% of *S aureus* produce β-lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 35% of *S aureus* and approximately 75% of *S epidermidis* isolates (Brooks *et al.*, 2007). Alternative antibiotics for resistant organisms (e.g. MRSA) include vancomycin, erythromycin and gentamicin. Some strains become resistant to multiple antibiotics.

**Historical background of *Escherichia coli***

*Escherichia coli* commonly called *E. coli*, is just one of many bacteria that can cause diarrhea. The first isolation of *E. coli* was mode by a young Austrian Pediatrician, Dr. Theodor Escherich in Munich 1885. He was holding clinical assistantships at children's Polyclinic and Hunters children s Hospital; he carried out researches in the intestinal flora of children as a possible cause of epidemics of diarrhea. The name of the Bacterium coli commune was read for the first time, which is now known as B. coli. He became the leading bacteriologist in the field of pediatrics and an authority on infant nutrition.

In animal the ability of *E. coli* to cause diarrhea was first suggested in late 1800 and early 1900 by several veterinary workers studying the calves scours (Cheesbrough, 2020) . The organism was isolated along with other bacteria from the faeces of newborn babies. It was found to be concomitant with breastfeeding. Escherich described it as a short plump rod, growing readily on gelatin or agar. On potato and coagulated milk, it grew as a slimy mass with the production of acid. In the early days different names have been applied to the organism such as Bacillus escherichii in 1889, Bacillus coli in 1895. In 1900 it was known variously as Bacterium verus, Bacillus coli communes and Aerobacter coli. The genus Escherichia was first proposed by Migula in 1895 and became firmly established 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 .

**Classification of *Escherichia***

The genus *Escherichia* belongs to family enterobacteriaceae (Barrow and Feltham, 1993). The enterobacteriaceae includes the following tribes:

1- Eschericheae.
**2-** Klebsielleae.
**3-** - Proteusae.
**4-** Yersinieae.
**5-** Erwineae.

The tribe Eschericheae includes five genera:

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

The genus includes the following species:

**1- *E. coli* :** Like many other enterobacteria contains numerous serotypes some of which are associated with certain infections in man and animals, some are particularly associated with diarrheal disease while others causes avareity of extra intestinal infections.
**2- *E. Adecarboxylata***
**3- *E. fergusonii***
**4- *E. hermanii***
**5- *E. blattae***
**6- *E. vulneris***

**Definition of *E.coli***

*Escherichia coli* are a straight Gram negative rod and nonsporing rod. It grows readily on simple nutrient media, occurring singly or in pairs. Most of the organisms are motile with peritrichus flagella. The total number of serotypes is very high.

The organism is a normal inhabitant of the lower part of the intestinal tract of all warm-blooded animals. Usually t is not found in the intestines of fish or other cold-blooded animals. A few numbers or none are found in the stomach and anterior portion of the bowel. It is found in greater abundance in carnivores and omnivores than herbivores. Being a primary components of faeces, it is therefore one of the most ubiquitous bacteria on the surface of the earth. Frequently there are very few numbers of the bacteria in the faeces of cows and horses. Most are harmless saprophytes but others are virulent pathogens that affect the intestine and extra intestinal sites. The major diseases caused by *E.coli* are enteric infections, septicemia, urinary tract infection, and mastitis. Under certain condition the numbers of these organisms undergo a marked and rapid increase in vivo, and this

may be associated with definite signs of illness and some times death. It is aerobic and facultatively anaerobic.

**Normal habitat of *E.coli***

*E. coli* is a world wide in distribution. Many *E. coli* are part of normal flora of the intestinal tract of human and animal. Some species are free living occurring in soil, water and vegetation. *E.coli* becomes established in the intestine shortly after birth when the sterile intestine of the fetus is seeded with bacteria derived from the mother and the environment. *E. coli* passes easily and reaches the intestine because in newborn animal and human the stomach pH is nearly neutral. *E. coli* continues throughout adult live as the intestine and is usually the dominant isolate on aerobic culture of feaces or intestinal contents. Most strains of *E. coli* are harmless commensals but others are virulent pathogens that affect intestine or extra- intestinal sites.

**Characteristics of *E.coli***

**a. Morphology**

*Escherichia coli* is straight rods measuring 1.1-1.5 by 2.0-6.0μm (living) or 0.4 - 0.7 by 1.0 - 3.0 μm (dried and stained) with parallel sides and rounded ends. It is a Gram-negative rod, that may form chains under unfavorable condition (exposure to penicillin) capsules or microcapsules are produced by many stains. The organism is non acid fast and non – spore former. Many strains possess peritrichous flagella but may be sluggish motile, some strains are non-motile or only feebly motile.

**b. Capsules**

Certain strains of *E.coli* that cause diarrhea in calves and a subset of strains that cause diarrhea in pigs produce abundant capsular polysaccharide that may aid in colonization of the intestine. The polysaccharide capsule is produced in vivo and appears to be a virulence factor in these strains. Spontaneous a capsular mutant of these strains failed to colonize the intestine and to produce diarrhea in experimental infected calves. It is uncertain, however, whether the acapsular mutants were deficient in structures and/or products other than capsular polysaccharide. Studies on the ultra structure of the capsulated *E.coli* in association with the intestine of calves suggest that the bacteria attached to the intestinal epithelium.

**c. Cultural characteristics**

The organism is aerobic and facultative an aerobic in the presence of a fermentable carbohydrate. Growth occur between 14-45ºC (optimum temperature is 37ºC). Optimum pH for growth is 7 but growth occurs within a wide pH range. It grows readily on ordinary laboratory media. Uniform clouding is produce in broth after 12-18 hours incubation. Colonies on nutrient agar have slightly raised surfaces. Pigments are

not produced. Growth on agar slants is in confluent with a turbid water of syneresis. Wide zones of beta type of haemolysis around colonies are produced by some strains. Colonies on agar medium are usually 2-3 mm in diameter.

**Isolation and cultural characterization of *E. coli***

**a. Media for isolation of *E. coli***

Three types of media can be used for primary isolation of *E*. coli:

**Differential or selective media**

**A- MacConkey's agar media**

It is used to detect coli form and enteric pathogens from faecal samples based on their ability to ferment lactose. Lactose fermenting bacterial species like *E. coli* gives pink to red colonies while other non lactose fermenting organisms give colourless to transparent colonies after an overnight incubation at 37ºC on this medium.

**B- Eosin and methylen blue medium (EMB)**

It is used for the isolation of lactose fermenting Gram negative organism like *E. coli*. Eosin and, methylen blue medium consist of peptone base with lactose, sucrose, eosin and methylen blue. Eosin and methylen blue serve as indicators for fermentation as well as inhibiting gram-positive organisms. On EMB media *E. coli* like lactose fermenting organisms produces a black precipitate. Colonies will be either black or posses dark center with transparent colourless peripheries after an overnight incubation at 37ºC.

**Enrich medium**

Blood agar is used for first isolation for *E. coli* from systemic infection. Blood agar is constituted of tryptose, sodium chloride, heart infusion, agar and 5% sheep blood. *E. coli* is an aerobe and facultative anaerobe. On blood agar *E. coli* produce (1-4) mm in diameter colonies after an overnight incubation at 37ºC. The colonies may appear mucoid and some strains are haemolytic due to production of haemolysin.

**Basic media**

Nutrient agar is used for sub culturing of *E. coli* from differential selective or storage media(slant) prior to perform biochemical and serological identification.

**Maintenance and preservation of *E. coli***

*E. coli* can survive well in holding media as modified Carey-Blair medium for several weeks to month without losing its plasmids. Storage of strains in liquid broth media supplemented with 15% glycerol as cryopreservative at -70ºc gives good stability of the enterotoxin properties as well as of the surface adhesion. Storage of strains on Dorset egg medium at 4ºc is a good alternative for liquid

broth media. Lypholization of *E.coli* strains also give a good stability of plasmid for years.

**Resistance to physical and chemical agents**

*E.coli* is relatively susceptible to physical and chemical agents. In the majority of instances a temperature of 55ºC for one hour or 60ºC for 20 minutes is lethal to these organisms. They are killed rapidly by autoclaving at 120ºC.Under natural conditions; *E.coli* may survive for weeks or months in water, faeces and dust in animal houses. They are highly susceptible to the lethal action of phenol and cresol, but the efficacy of these disinfectants is reduced in the presence of mucus and faeces.

**Biochemical tests**

Biochemical tests for the differentiation of *E.coli* from other closely related bacterial groups must be based on the reactions which occur in a variety of media. All strains of *E.coli* ferment glucose and lactose with the production of acid and gas but few strains are late lactose fermenters or may often fail to ferment this sugar. Most strains do not develop urease, give a negative voges-proskaur reaction and are positive to the methyl red test. Milk is coagulated and acidified. Faecal *E.coli* is able to grow in MacConkey's lactose bile broth at 44ºC (Ejkman's test) with the production of gas. This test is of value for water bacteriologists in presumptive identification 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.

***E.coli* infection in animals and man**

The organism produce colibacillosis in all species of newborn farm animals, it is a major cause of loss in this age group. Infection also occurs in man and poultry with various manifestation.

**Childhood Diarrhea**

Diarrhea is a leading cause of morbidity and mortality among children in developing countries. The bacterial pathogen most commonly associated with endemic forms of childhood diarrhea is *Escherichia coli*. At least six categories of diarrheagenic *E. coli* have been described: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E.coli* (ETEC), enteroinvasive *E.coli* (EIEC), enterohemorrhagic *E.coli* (EHEC), enteroaggregative *E.coli* (EAEC) and diffusely adherent *E.coli* (DAEC). Two additional categories cell detaching *E.coli* (CDEC) and cytolethal distending toxin producing *E.coli* (CLDTEC), have been proposed. The epidemiological significance of each *E.coli* category in childhood varies with geographical area. Epidemiological evidence and human challenge studies have demonstrated unequivocally that EPEC, ETEC, EIEC and EHEC are important causes of diarrhea world wide.

***Ecoli* O157:H7**

*E.coli* O157:H7 is a common cause of a variety of illnesses including bloody diarrhea and the hemolytic uremic syndrome (HUS). The O157:H7 serotype was first described in the literature in 1983 following two outbreaks of hemorrhagic colitis in a fast-food restaurant chain in Organ and Michigan in 1982. The most frequent mode of transmission for *E.coli* O157:H7infections is through consumption of contaminated food and water, and several outbreaks have been caused by ground beef. Approximately 1% of healthy cattle may have the organism in their intestines.

**Mechanism of Actions of Natural Antimicrobials**

Phenolic compounds are the main antimicrobial agents in plants. Even though the exact antimicrobial mechanism of phenolic compounds is not clear, phenolic compounds are commonly known for their antimicrobial effects. The ability of phenolic compounds to alter microbial cell permeability, thereby permitting the loss of macromolecules from the cell interior, could help explain some of the antimicrobial activity. Another explanation might be that phenolic compounds interfere with membrane function and interact with membrane proteins, causing deformation in structure and functionality. A combination of phenolic compounds can provide synergistic antimicrobial effects and can contribute to a better antimicrobial reaction as compared to the reaction of an individual compound. In addition, the effect of phenolic compounds can be concentration dependent; at low concentration, phenols affect enzyme activity while at high concentrations they cause protein denaturation. It has been reported that the antimicrobial activity of isothiocynates derived from onion and garlic is related to the inactivation of extracellular enzymes through oxidative cleavage of disulfide bonds and that the formation of the reactive thiocyanate radical was proposed to mediate the antimicrobial effect.

For peptides, the mechanism of action of antimicrobial peptides seems to involve multiple targets. The plasma membrane is the most cited target by peptides whereas recent studies have suggested intracellular targets to be more likely for some peptides. Most antimicrobial peptides have nonspecific mechanisms and they may display some selectivity between different microorganisms. Antimicrobial peptides can assume amphipathic structures, which are able to interact directly with the microbial cell membrane. This action rapidly disrupts the membrane in several locations and result in the leaching out of vital cell components. Studies on the mechanism of action of pleurocidin revealed that this peptide exhibits a strong membrane translocation and pore-formation ability reacting with both neutral and acidic anionic phospholipid membranes.

**Factors Influencing the Antimicrobial Activity of Natural Products**

The antimicrobial activity of natural compounds could be influenced by number of factors including botanical source, time of harvesting, stage of development, and method of extraction in addition to the composition, structure, and functional groups of the natural compounds.

**Method of evaluating effectiveness of phenolic compound**

The effectiveness of a given disinfectant can be evaluated using the Phenol co-efficient test which is the best known disinfectant screening test in which the potency of a disinfectant is compared with that of phenol. The series of dilutions of phenol and the disinfectant being tested are prepared. A standard amount of the test organism is added to each dilution, the dilutions are then placed in a 200C or 370C water bath. At interval of 5 minutes, samples are taken from each dilution and inoculated in a growth medium and then incubated for 24- 48 hours at 370C. The tubes will be examined for growth. If there is no growth in the growth medium, the dilution at that particular time of sampling killed the bacteria. The highest dilution (i.e. the lowest concentration) that kills the bacteria after 10 minutes of exposure, but not 5 minutes, is used to calculate the phenol coefficient. This is done by dividing the reciprocal of the appropriate dilution for the disinfectant being tested, by the reciprocal of the appropriate dilution of the phenol. A value greater than one means that the disinfectant is more effective than phenol.

Phenol coefficient, though a useful initial screening procedure, can be misleading if taken as a direct indication of disinfectant potency during normal use. This is because the phenol coefficient is determined under carefully controlled conditions with pure bacteria strains whereas disinfectants are normally used on complex populations in the presence of organic matter and with significant variations in environmental factors like pH, temperature and presence of salts (Otokunefor & Usoh 2009; Iruoha *et al.*,2016; Ghotaslou & Bahrami 2012; Rutala *et al.*,2010).

## MATERIALS AND METHODS

**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.

**Media used**

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

**Reagent**

Pepton water, pepton broth,

**Collection of samples**

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

**Source of Microorganisms**

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

**Preparation of media and plating**

The medium was prepared according to manufacturers instructions. 27g of muller Hinton Agar was added to 100ml of sterile distilled water and autoclaved at 121°C for 15minutes. After cooling, the content was mixed and poured into sterile petri dish and was allowed to set at ambient temperature and used.

**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.

**Identification of Microorganisms**

**a) Morphological identification:** The isolated bacteria were identified on the basis of negative staining and Gram's-staining.

**Gram's staining**

The Gram stain is by far the most widely used procedure for staining bacteria and separating it into two major groups: Gram (+) positive and Gram (-) negative. Spread thin film of specimen over a clean grease free slide and allow to air dry. Fix it by passing it over a Bunsen flame thrice. Flood the film with crystal violet and leave for 60 seconds. To the slide wash off and flood the stain with lugol's iodine and (mordant) and leave for 60 seconds. Wash off iodine and decolorized the slide with acetone (decolourizer) for a second, wash the slide and train with safranin (counter stain) for 60 seconds and wash off. Then dry the back of the slide and air dry. 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.

**Motility Test**

This test is to identify members of vibranaceae and must members of the enterobacteriaceae which are also motile. The mobility medium was inoculated using a needle to make 5 stabs of the test organism to the depth of 1-2cm of the bottom of the tube. The tube was incubated at 37°C for 24hrs The line of incubation was examined for cloudiness showing the organisms is motile (Cheese Brough, 2005).

**Methyl Red Test**

This test was used to detect which of the isolates could produce and maintain sufficiently a stable acid product from glucose fermentation. The test is usually used as an aid in the identification and differentiation of the *Enterobacteriaceae* This test was performed according to Cheese

Brough (2005). Inoculate the suspected organism into a sterile buffered glucose- peptone broth and incubate at 37℃ for 24 hours. After 24 hours add five drops of methyl red indicator and shake the mixture and observed. A bright red colour is a positive result.

**Citrate Utilization Test**

This test was done according to Cheese Brough (2005). The test was used to identify which of the isolates can utilize citrate as the sole sources of carbon for metabolism. The test is usually used as an aid in the differentiation of organisms in the *Enterobacteriacea* group. Inoculate simmon's citrate medium in sterile test tubes with a loopful of culture. Incubate tube at 37℃ for 24 hours. A colour change from green to blue is a positive result. The absence of any growth as well as no change in the colour indicates a negative reaction.

**Oxidase Test**

This test was done by dropping 2 – 5 drops of a freshly prepared oxidase (p- aminodimethylanine) reagent on a filter paper, the suspected organisms is picked using a sterile wire loop and mix with the oxidase reagent. A change from the normal colour to deep purple means a positive result, while no change means negative.

**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. C*heese Brough (2005). Inoculate the suspected organism into a test tube containing buffered glucose peptone water and incubate at 37oc 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.

**Coagulate Test**

This test was done according to Cheese Brough, (2005) 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.

**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 37oc 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.

**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$^{o}$C. a change in colour from yellow to red confirmed the presence of urease.

**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.

**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.

**Antimicrobial screening tests**

**Standardization of the inoculum**

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).

**Antibiotics sensitivity test**

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

**a. 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.

**b.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.

**c. 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.

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.

**d. 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.

**e. 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.

## 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.

**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** |

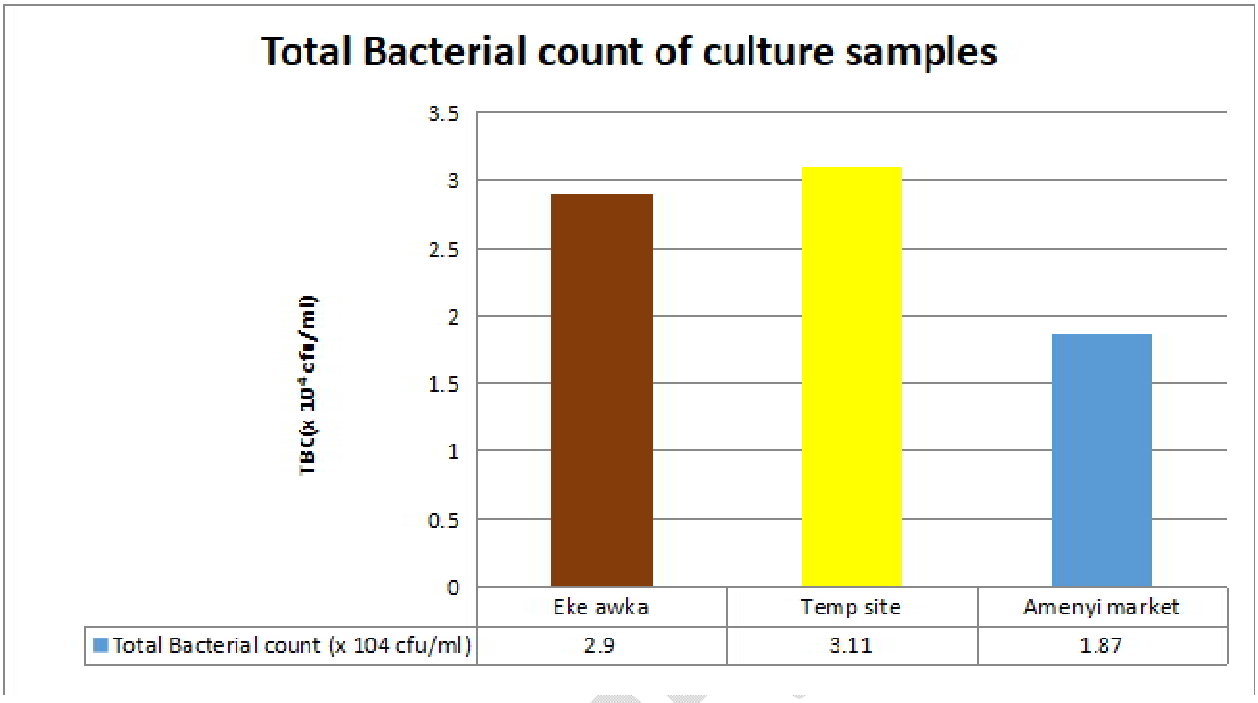


**Fig 1 Mean bacterial counts in food samples**

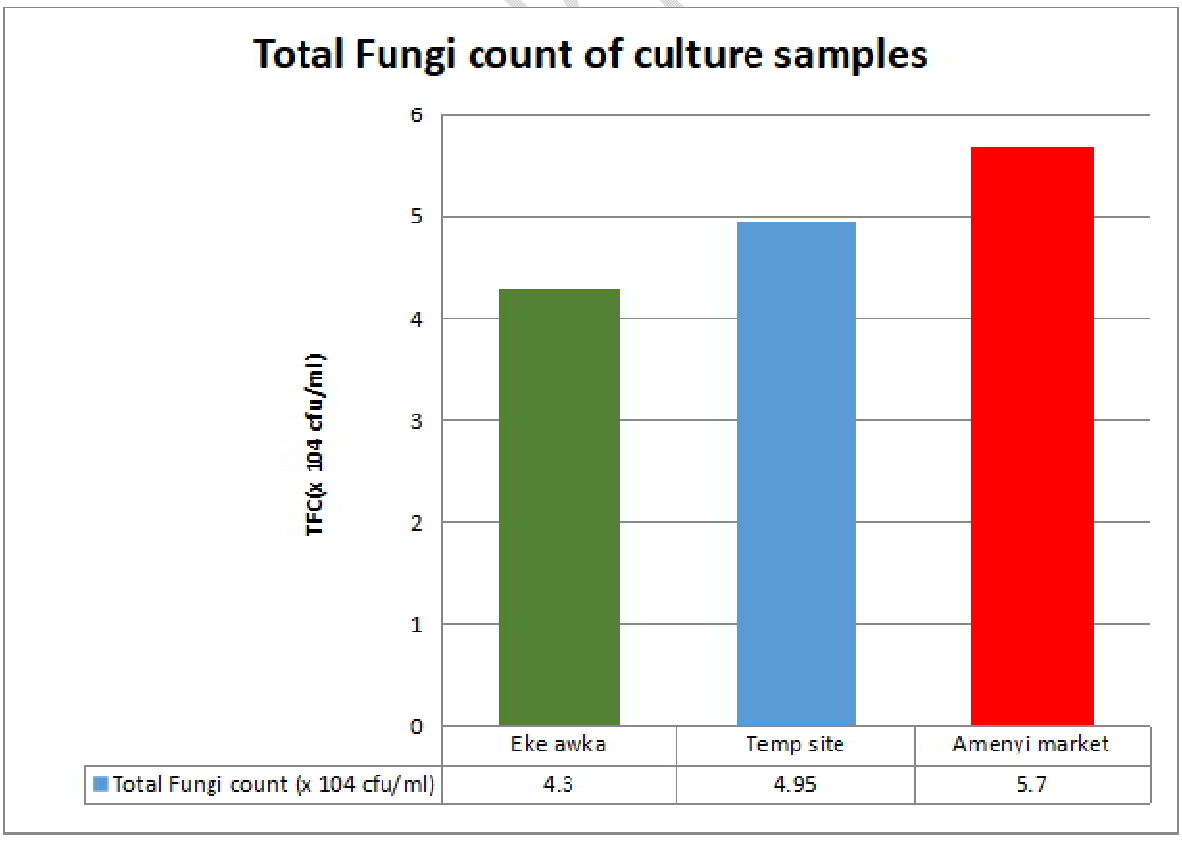


**Fig 2 Mean fungal counts in food samples**

**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.

**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 |

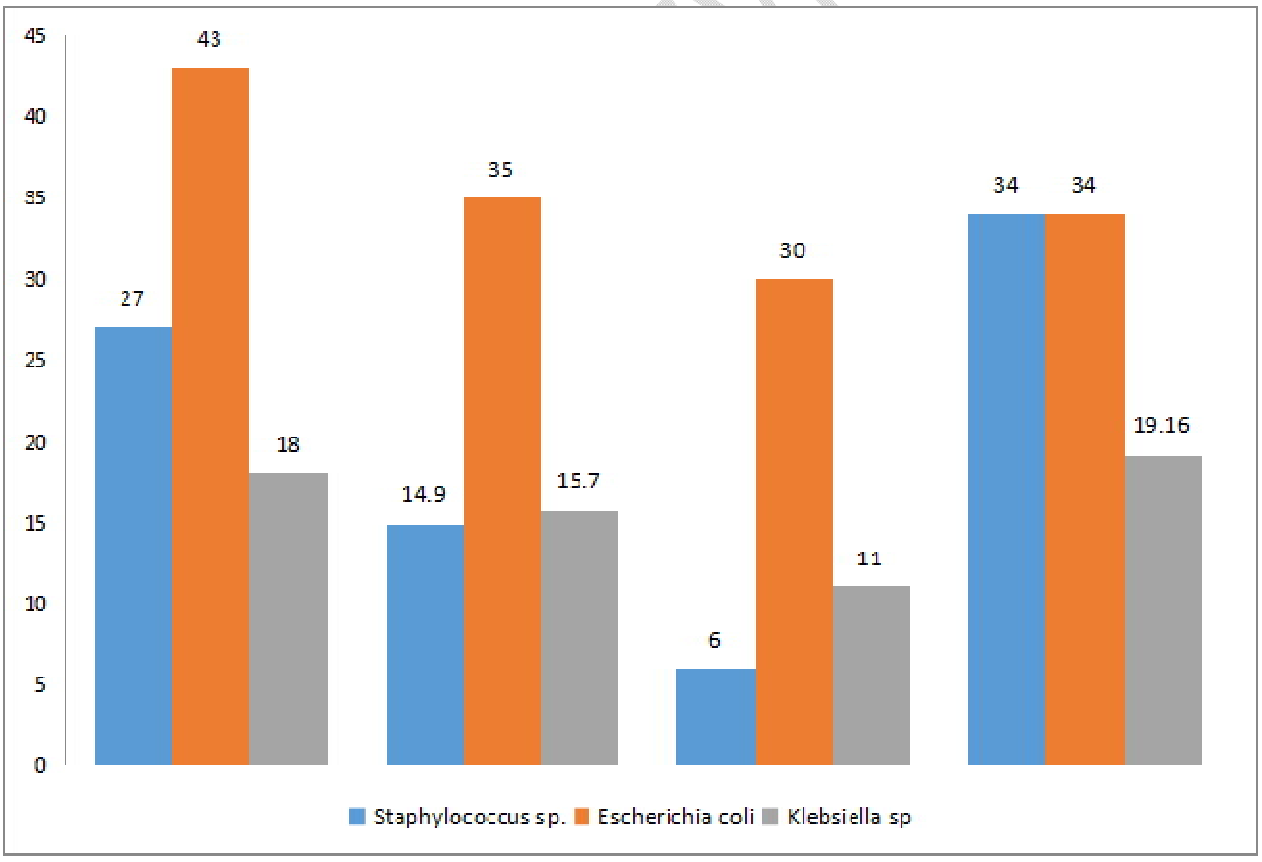


**Fig. 3. inhibitions zone shown by the bacterial isolates**

**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 |

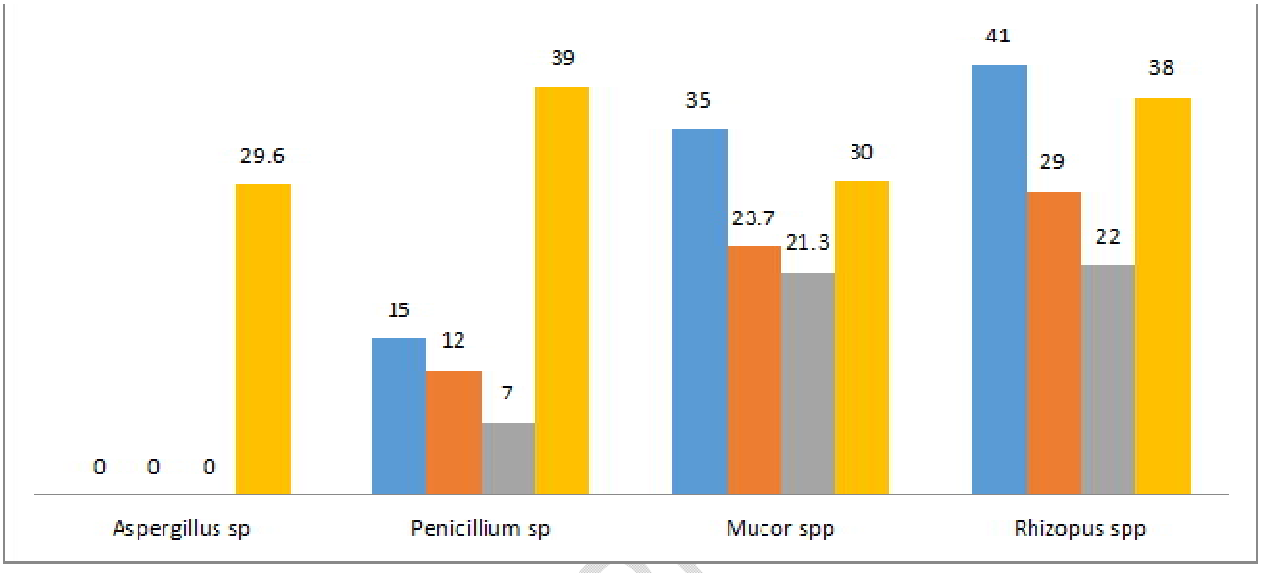


**Fig. 4 . In vitro antifungal activity of antiseptic**



## 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.

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 4, 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 p[roves 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 tables 2 and 3. According to Busca *et al.,* (2008), 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 (El-Mahmood and Doughari, 2009).

The data's in figures 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 figures 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.* (2008), 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 (2011), 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 (Calabrase and Kenyon, 2011).

According to Talaro and Talaro, (2005) 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, (2005) 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.

## 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



**References**

Brooks, S.J., Butel, and Morse, A.S. (2004). *Jawetz, Melnick and Adelberg's Medical Microbiology,* 23rd ed., United States: McGraw-Hill Companies, pp.161-165.

Busca, G., Berardinelli, S., Resini, C. and Arrighi, L. (2008). Technologies for the removal of phenol from fluid streams: A short review of recent developments. *Journal of Hazardous Material* 160, 265- 288.

Calabrase, E.J. and Kenyon, E.M. (2011). *Air Toxics and Risk Assessment*. Lewis Publishers, Chelsea, Michigan.

Cheesbrough, C. (2005). *District Laboratory Practice in Tropical Countries*, 2nd ed., New York: Cambridge University Press, pp. 38-39,

El-Mahmood, A.M. and Doughari, J.H. (2009). Bacteriological examination of some diluted disinfectants routinely used in the specialist hospital Yola, Nigeria. *African Journal. of pharmacy and Pharmacology*; 3(5):185-190,

Ghotaslou, R. and Bahrami, N. (2012). 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*; 2(1):57-59.

Iruoha, I.R., Oji, A.E., Nwosu, O.K. and Amadi, E.S. (2016). Antimicrobial activity of Savlon, Izal and Z-germicide against clinical isolates of *E.*

*coli* from hospital wards. *European Journal Dentistry and Medicine* 3(1):32-35.

Nair, D., Okiemen, H. and Neuwinger, J.D. (2008). Translated from by Porter A. African ethnobotany poison and drugs. *Chapman and Hall, Weinheim*, pp. 495-499.

Omoruyi, M.I. and Idemudia, M.I. (2011). 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* 2(1):65-68.

Otokunefor, T.V. and Usoh, C.U. (2009). Microbial contamination of inuse disinfectants in small private medical facilities in Owerri, in south east Nigeria," *Scientia Africana*; 8(2):17-25,

Pelczar, E., Chan, C.S. and Krieg, R.N. (2006). *Microbiology*, 5th ed, New Delhi: Tata McGraw-Hill publishing Company Ltd, pp. 469-490.

Rutala, W.A, Barbee, S.L., Agular, N.C., Sobsey, M.D., and Weber, D.J. (2010). Antimicrobial activity of home disinfectants and natural products against potential human pathogens. *Infection control and hospital epidemiology* 21(1): 33-38.

Ryan and Ray, C.G. (2011). Bacteria, In: *Sherris Medical Microbiology*, 4th ed., Mc Gram Hill, ISBN.

Talaro, and Talaro, R. (2005). *Foundation in Microbiology*, 5th ed, New York: McGraw-Hill companies, pp. 324-335.

Tortora, B.R. and Funke, C.J.(2004). Case, Microbiology: *An Introduction*, 8th ed., New York: Pearson Education and Dorling Kindersley Pvt, Ltd, pp. 224-225.

Willey, L.M., Sherwood and Woolverton, C.J. (2008). Prescott, Harley, and Klen's Microbiology, 7th ed., New York: McGraw-Hill *Higher Education*, pp. 158-165.

World Health Organization (2003). "Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world."