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Antimicrobial Activity of Disinfectants and Comparative Study with Phenol

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A B S T R A C T

Disinfectants are substances that are applied to inanimate surfaces and objects to destroy harmful microorganisms. Although they may not kill bacteria spores, they are categorized by their spectrum of microbial activity. Disinfectants are of different types and may include alcohols, quaternary ammonium compounds, hypo chlorides, iodine, bromines, pine oils, peroxides and phenolic compounds. Some puncture the cell walls of the microorganisms, allowing the contents to leak out, while others permeate and enter the cell destroying the microorganism from within. To activate optimal efficiency, shelf life and safety, disinfectant agents are carefully formulated with other essential ingredients such as buffers, solubilizers, detergents, builders, stabilizers, synergists and fragrances. Phenol type antimicrobial agents have long been used for their antiseptic, disinfectant and preservative properties. It has been known for many years that although they have often been referred to as "general protoplasmic poisons," they have membrane-active properties which also contribute to their overall activity. The present study is aimed at studying the antimicrobial activity of commonly used disinfectants and compare its efficiency with Phenol.

Keywords: Disinfectants, Bacteria, shelf life, Phenol and Antimicrobial activity

A R T I C L E I N F O

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1. Introduction

Disinfectant is a chemical substance which is used to kill microorganisms which cannot be applied on living tissues (**Hammond SA, *et.al.*, 1987**). They play a major role in water treatment and in public health sanitation. These are

commonly applied to inanimate objects such as floors, instruments, etc (**Bloomfield S F, 1996).** The same substance can act as disinfectant as well as antiseptic depending upon its concentration. For example, a 0.2% solution of **phenol** acts as antiseptic and its 1% solution acts as disinfectant. A study indicates Phenol to induce progressive leakage of intracellular constituents, including the release of K^+, the first index of membrane damage and of radioactivity from C-labeled *E. coli* . Low concentrations of phenols (0.032%, 320 µg/ml) and other (non-phenolic) agents lysed rapidly growing cultures of *E. coli*, *Staphylococci*, and *Streptococci* and concluded that autolytic enzymes were not involved. Thus phenol acts only at the point of separation of pairs of daughter cells with young bacterial cells being more sensitive than older cells (**Sagripati JL and Bonifacino A, 1996)**. Phenols possess antifungal and antiviral properties. Their antifungal action probably involves damage to the plasma membrane resulting in leakage of intracellular constituents. Phenol does not affect the transduction of *P. aeruginosa* and bacteriophage has no effect on phage DNA within the capsid, and has little effect on several of the phage band proteins unless treatments of 20 min or longer are used (**Chioma C. Okore *et. al.,* 2014)**. Health is one of the most important factors in human life. Contracting an illness can be bad, and can even cause death. Using a disinfectant that will effectively kill harmful bacteria can help people stay healthy (**Hussong, D *et. al.,* 1985).**

There are seven main types of disinfectants. They include:

- Alcohols
- Formaldehyde and Glutaraldehyde
- Hypo-Chlorites
- Iodophors
- Phenols
- Pine Oil Disinfectants
- Quaternary Ammonium Compounds

Alcohols:

There are two different types of alcohol disinfectants. They are ethyl and isopropyl alcohols. These disinfectants are used to clean plastic and rubber. They also clean thermometers used to take a person's body temperature.

Formaldehyde and Glutaraldehyde: Formaldehyde and glutaraldehyde are fast-acting disinfectants. They disinfect quickly and effectively. They are used mostly by hospitals to clean the surgical tools and other medical devices.

Hypochlorites: Hypochlorites are disinfectants that have chlorine bleach and chlorinated lime, the usual ingredients in disinfectants and deodorizers. They are used to treat water and sewage systems and to clean eating utensils.

Iodophors: Iodophors are disinfectants that include iodine. They are used to clean hospital surfaces like tables and beds, and also to disinfect food preparation equipment.

Phenols: Phenols are disinfectants that include carbolic acid, creosote, and hexachlorophene. They are used to clean floors, trash cans, bathrooms, and other large surfaces.

Pine Oil Disinfectants: Pine oil disinfectants are mixed with detergents. They are most commonly used to clean floors, walls, and bathroom fixtures, like toilets and sinks, and have a pine-like smell to them.

Quaternary Ammonium Compounds:

Quaternary ammonium compounds are used in lots by common household cleaners. They are used as disinfectants and as detergents. Antiseptics and disinfectants are used extensively in hospitals and other health care centers to control the growth of microbes on both living tissues and inanimate objects. They are essential parts of infection control practices and aid in the prevention of nosocomial infections. But a common problem is the selection of disinfectants and antiseptics because different pathogens vary in their response to different antiseptics or disinfectants**.** Dettol is widely used in homes and healthcare settings for various purposes including disinfection of skin and objects (**Karabit MS, *et.al.,* 1985)**.

In an analysis of the action of a disinfectant, it may often be difficult to distinguish between the primary stage (characteristic of the mode of action) and the secondary stage (merely a consequence of the action). A bacterium is protected from its environment by a membrane, the integrity of which is essential for the survival of the bacterium. This membrane consists of basic compounds such as phospholipids and lipopolysaccharides, and is stabilised by Mg + + and Ca+ + cations. Thus, if ionized disinfecting molecules are absorbed or repelled by electrical charges at the initial contact and absorption stage, the following means of action will theoretically be possible. Non-polar molecules may dissolve and enter the lipid phase- specific carrying systems and will lead other molecules through the membrane to other molecules and will be able to disturb the organization of the membrane by remaining bound to certain sites. The bacterial wall is important, as this confers rigidity and differs considerably between Gram-positive and Gram-negative bacteria. This diversity leads to great variation in the affinities of the hydrophilic disinfectants. An active molecule, such as a nutrient, may penetrate the cytoplasmic membrane in the following ways: a) passive diffusion (non-specific and slow) and b) active transport (specific, enabling the accumulation of products in bacteria after either transformation or binding to a membrane protein). Some disinfectants acting on adenosine triphosphatase (ATP) production were studied. The disinfectant mechanism may operate on the cytoplasm and nucleus at the chromosome level. The impermeability and the presence of dipicolinic acid in bacterial spores make these forms much more resistant to disinfectants than vegetative forms. The active disinfectants include highly oxidising products, such as hydrogen peroxide and chlorine which can destabilize this structure in spores (**Walsh S, *et.al.,* 1997)**.

2. Materials and Methods

Collection of Samples

The sample was taken from sink, floor and bathroom with sterile swab. It was then streaked in the nutrient agar in petridish. The inoculates were then incubated at 37°C for 48 hours and observed for changes.

Preparation of Culture Plates

The sample were inoculated in Mannitol salt agar medium, Cetrimide agar medium, Macconkey agar medium, MRS

agar medium and blood agar and was incubated for 24 hours at room temperature. The microorganism was identified using Gram staining method. Biochemical characterization was done using Indole, MR-VP, Oxidase, Catalase and Gelatinase Tests.

Antimicrobial Activity

Sensitivity disc diffusion method was employed for checking the antimicrobial activity of the disinfectant samples. 24 hour cultures of *Staphylococcus, Pseudomonas, Bacillus* and *Aeromonas* were used for the sensitivity test. The Mueller Hinton Agar was prepared and autoclaved at 121^{o}C for 15 minutes. The plates were swabbed with respective organisms and marked according to the organism. Sterile disc was placed and different concentration (25%, 50%,75%,100%) of the disinfectants were poured onto to each disc. After the disc is placed the plate were incubated at 37^{o}C for 24 hours. A zone of inhibition is indicative of microbial activity against the organism. Presence of zone of inhibition indicates that the antiseptic or disinfectant is effective and was measured and recorded in millimeters using transparent meter rule.

Method for Determining the Phenol Coefficient of the Disinfectants: The phenol coefficient of the disinfectants was determined using standard microbiological method. Different dilutions of the phenol stock solution were made (that is 25%, 50%,75%, 100%) in sterile test tubes. 0.1ml each of the suspension of the test organisms was introduced into each of the dilutions and mixed properly. 0.1ml was inoculated into tubes of (2ml each) sterile nutrient broth after 5 minutes, 10 minutes for each of the dilutions. The same procedure was repeated for each of the test disinfectants using dilutions 25%, 50%, 75%, 100%. The tubes were incubated for 24 hours at 37^{o} C and then observed for growth (turbidity).

Phenol coefficient for each of the test disinfectants was calculated using the formula:

$$\frac{\text{Highest dilution of chemical being tested that destroyed the microorganisms in 10min}}{\text{Highest dilution of phenol that destroyed the microorganisms in 10min}}$$

3. Results and discussions

The sample was taken from sink, floor and bathroom, with sterile swab. It was streaked in nutrient agar in a petridish. The inoculates were then incubated at 37°C for 48 hours and was observed for changes (**Figure 1**).

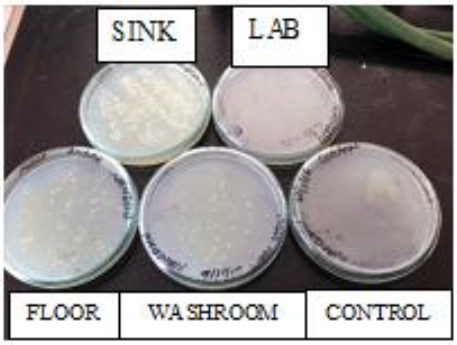


Figure 1: Isolation of organism in nutrient agar

Subculture from nutrient agar was carried out in selective media such as mannitol salt agar, cetrimide agar, MRS *lactobacillus* agar, Macconkey agar and SDA (Sabouraud Dextrose Agar medium) (**Figure 2**).

Figure 2: Subcultures from nutrient agar in selective media

Gram staining revealed the following results (**Figure 3**). *Staphlococcus aureus* was gram positive and *Pseudomonas aeraginosa, Bacillus* and *Aeromonas* were gram negative.

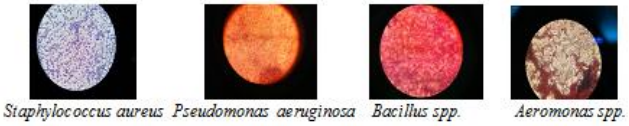


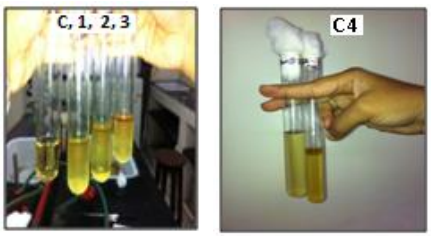
Figure 3: Gram Staining

Oxidase Test: The disc which showed no color change was negative and the disc with violet color indicated positive result (**Figure 4.1. & 4.2**).

Figure 4.1: Negative

Figure 4.2: Positive

Indole Test: The cherry red colouration indicates positive result and negative result indicates no colour change. All the organisms were negative. Control, *1. Staphylococcus 2. Pseudomonas 3.Bacillus 4. Aeromonas* (**Figures 5 & 6**).



Figures 5 & 6: Indole Test

VP Test: The distinct red formation indicates positive result and yellow colour negative. All the organisms were negative. Control, *1.Staphylococcus 2.Pseudomonas 3.Bacillus 4.Aeromonas* (**Figure 7**).

Figure 7: VP Test

Catalase Test: Bubble formation was positive result and absence of bubble negative (**Figure 8**).

Figure 8: Catalase Test

MR Test: The pink or red formation indicates positive result and absence of colour change negative. All the organisms were negative. Control, *1.Staphylococcus 2.Pseudomonas 3.Bacillus 4.Aeromonas* (**Figure 9**).

Figure 9: MR Test

Gelatin Test

Total liquefied formation indicates positive result. All the organisms were positive *1.Staphylococcus 2.Pseudomonas 3.Bacillus 4.Aeromonas* (**Figure 10**).

Figure 10: Gelatin test

Antimicrobial Activity of *Staphylococcus aureus* (**Table 2, Figure 11, Graph 1**)

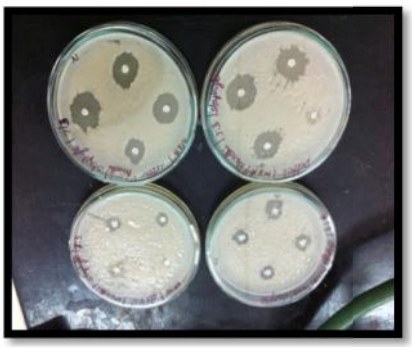
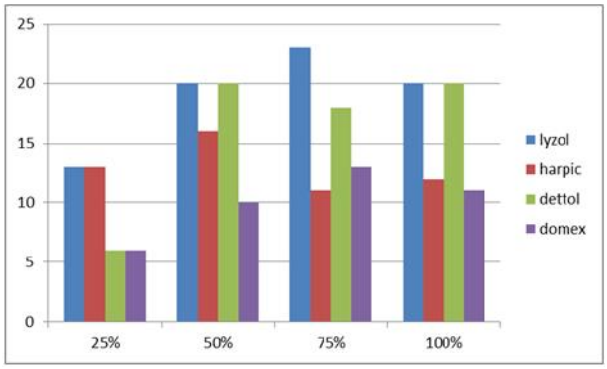


Figure 11: Antimicrobial activity of *Staphylococcus aureus*



Graph 1: Antimicrobial activity of *Staphylococcus aureus*

Antimicrobial Activity of *Pseudomonas aeruginosa* (Table 3, Figure 12, Graph 2)

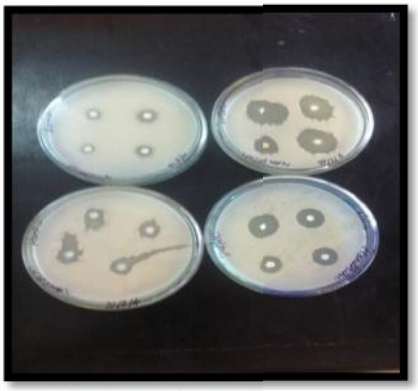
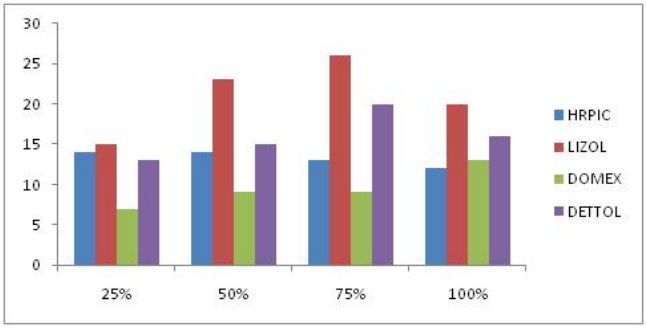


Figure 12: Antimicrobial activity of *Psuedomonas aerogenosa*



Graph 2: Antimicrobial activity of *Pseudomonas aeruginosa*

Antimicrobial Activity of *Pseudomonas aeruginosa* (Table 4, Figure 13, Graph 3)

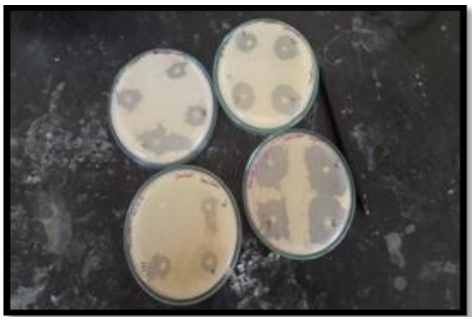
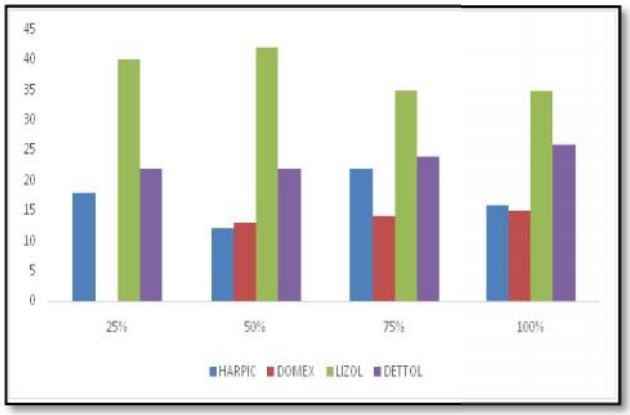


Figure 13: Antimicrobial activity of *Bacillus* sps.



Graph 3: Antimicrobial activity of *Bacillus spp.*
Antimicrobial Activity of *Aeromonas* (Table 5, Figure 14, Graph 4)

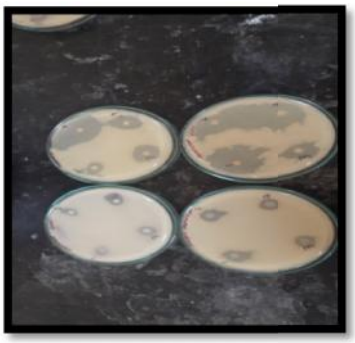
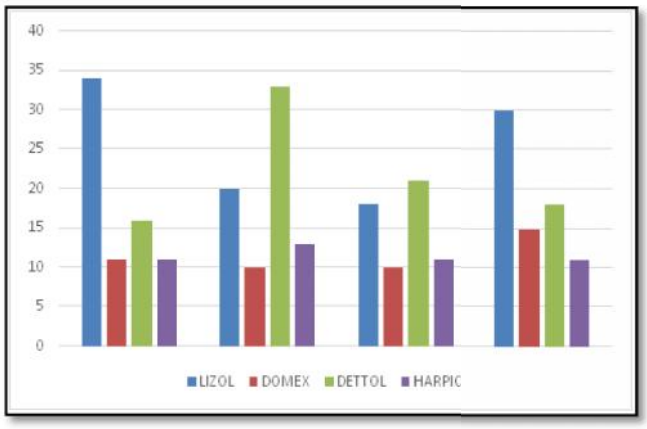


Figure 14: Antimicrobial activity of *Aeromonas*



Graph 4: Antimicrobial activity of *Aeromonas*

Phenol Coefficient Testing

Disinfectant sensitivity was determined by Kirby- Bauer's method whereas phenol coefficient test (PCT) was carried out to compare the antimicrobial activity of chemical compound to that of phenol under experimental condition so as to determine the disinfectant efficacy. The lab contaminants identified were *Pseudomonas and Bacillus.* Disinfectant sensitivity was assessed in terms of zone of inhibition (ZOI). *Pseudomonas* Species showed the following pattern, Lysol>Savlon>Dettol>Betadine>Phenol while Alcohol showed nil response. *Bacillus spp*. showed the following pattern Savlon>Dettol> Lysol> Phenol> Alcohol> Betadine. The efficacy of disinfectant assessed by testing them against standard culture of *Staphylococcus aureus* appear as Lisol>Savlon>Dettol>Betadine>Alcohol and the same pattern was found in all the three bacteria (Tables 6 – 9 & Figure 15).

Figure 15: Phenol coefficient test

4. Conclusion

Staphylococcus, Pseudomonas, Bacillus and Aeromonas were isolated from Sink, Floor and Wash room. Antimicrobial activity for *Staphylococcus, Pseudomanas, Bacillus* and *Aeromonas* was done. Lizol was found to be most effective in all the organisms. Phenol coefficient test was used for *Staphylococcus, Pseudomonas, Bacillus and Aeromonas.* The phenol and disinfectant killed the organisms in 10 minutes for 24 hours incubation. Hence the Disinfectant sensitivity determined by Kirby- Bauer's method and Phenol Coefficient Test (PCT) carried out to compare the antimicrobial activity of chemical compounds to that of phenol under experimental condition revealed the efficacy of the disinfectants used.

Table 1: Biochemical Characterization

Tests	Microorganisms			
	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Bacillus sp.***	***Aeromonase sp.***
Indole Test	Negative	Negative	Negative	Negative
Methyl Red Test (MR)	Positive	Negative	Positive	Positive
Vouges Proskauer TEST (VP)	Negative	Negative	Negative	Negative
Catalase test	Positive	Positive	Negative	Positive
Oxidase test	Negative	Positive	Positive	Positive
Gelatin test	Positive	Positive	Positive	Positive

Table 2: Zone of Inhibition

Staphylococcus aureus	**25%**	**50%**	**75%**	**100%**
HARPIC	**13mm**	**16mm**	**11mm**	**12mm**
LIZOL	**13mm**	**20mm**	**23mm**	**20mm**
DETTOL	**6mm**	**20mm**	**18mm**	**20mm**
DOMEX	**6mm**	**10mm**	**13mm**	**11mm**

Table 3: Zone of Inhibition

Pseudomonas aeruginosa	**25%**	**50%**	**75%**	**100%**
HARPIC	14mm	14mm	13mm	12mm
LIZOL	15mm	23mm	26mm	20mm
DETTOL	13mm	15mm	20mm	16mm
DOMEX	7mm	9mm	9mm	13mm

Table 4: Zone of Inhibition

Bacillus	**25%**	**50%**	**75%**	**100%**
HARPIC	18mm	12mm	22mm	16mm
LIZOL	40mm	42mm	35mm	35mm
DETTOL	20mm	22mm	24mm	26mm
DOMEX	0	13mm	14mm	15mm

Table 5: Zone of Inhibition

Aeromonas	**25%**	**50%**	**75%**	**100%**
LIZOL	34mm	20mm	18mm	30mm
DOMEX	11mm	10mm	10mm	15mm
DETTOL	16mm	33mm	21mm	18mm
HARPIC	11mm	13mm	11mm	11mm

Table 6: Phenol coefficient test – *Staphylococcus aureus*

Disinfectant Name	**Pc/D**	**Final Value**
LIZOL	**25/25**	**1**
DETTOL	**25/0**	**0**
DOMEX	**25/25**	**1**
HARPIC	**25/25**	**1**

Table 7: Phenol coefficient test - *Pseudomonas aeruginosa*

Disinfectant Name	**Pc/D**	**Final Value**
LYZOL	**25/25**	**1**
DETTOL	**25/0**	**0**
DOMEX	**25/50**	**0.5**
HARPIC	**25/50**	**0.5**

Table 8: Phenol coefficient test - *Bacillus*

Disinfectant Name	**Pc/D**	**Final Value**
LYZOL	**25/75**	**0.33**
DETTOL	**25/0**	**0**
DOMEX	**25/25**	**1**
HARPIC	**25/25**	**1**

Table 9: Phenol coefficient test- *Aeromonas*

Disinfectant Name	**Pc/D**	**Final Value**
LYZOL	**25/25**	**1**
DETTOL	**25/0**	**0**
DOMEX	**25/50**	**0.5**
HARPIC	**25/25**	**1**

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