

Comparative Studies of the Efficacy of Some Disinfectants on Human Pathogens

Mbajiuka Chinedu¹, Onuoha Stephen² and Ugah Uchenna^{3*}

¹Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture Umudike

²Department of Biological Sciences, Faculty of Science and Technology Federal University Ndufu-Alike Ikwo

³Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University Ndufu-Alike Ikwo

Abstract

The anti microbial activities of three commonly used disinfectants Septol, Dettol and Z-germicides against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were investigated. Their efficacies were determined using the agar diffusion method at different concentrations of the test disinfectants and their potency was tested against a standard (phenol) by using phenol coefficient tests. The use of Septol on *E.coli* and *S.aureus* showed weak bactericidal efficacy with a zone of inhibition of 30mm and 29mm at 100% concentrations as compared to the others, even in their use in dilutions, although it possesses a high efficacy against *C.albicans* with a zone of inhibition of 75mm. Dettol and Z-germicides had equal inhibitory effects of 45mm on *E.coli* and *S.aureus* at 100% concentrations but Dettol was more effective, as compared to Z-germicides, although *S.aureus* was observed to be the most susceptible to Z-germicides when used in higher concentrations. It was observed that as the concentration reduces the susceptibility rates of the test organisms increases. The potency of the disinfectants against a standard disinfectant (phenol) showed that they were all more effective than phenol with Dettol being the most potent disinfectant as compared to both Z-germicide and Septol. To ensure disinfectants efficacy, tests should be carried out on new disinfectant products and also further studies should be carried out about disinfectants.

Keywords: Disinfectants; Pathogens; Efficacy

***Corresponding Author:** Ugah Uchenna, Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University Ndufu-Alike Ikwo, Ebonyi, Nigeria; Tel: +2347062154353; E-mail: ugahuchennaiyioku@gmail.com

Introduction

Infectious disease and its physical, physiological and economical impact remains a significant problem in today's society. Through research, we have learnt that by limiting the number of infectious agents to which people are exposed, the chances of disease transmission can be reduced. One important control measure to help prevent the spread of infectious diseases is through disinfection. Disinfectants are substances that are applied to inanimate surfaces and objects to destroy harmful microorganisms, although they may not kill bacteria spores, and they are categorized by their spectrum of microbial activity. According to [1] they are chemicals used to inhibit or prevent the growth of microorganisms on inanimate objects usually disinfectants are "cidal" in action in that they kill susceptible potential pathogenic microbes. Major consideration in selecting disinfectants compounds should be based on the job they are expected to do not necessary on the sales pitch or on what one has always used. Considerations such as health risk, potential damage to the skin surface and the scope of effectiveness should

be considered. Disinfectants used in hospitals, industries, laboratories and in the homes must be tested periodically to ascertain their potency validation which is defined as establishing documented evidence that a disinfection process will consistently remove or inactivate known or possible pathogens from inanimate objects [2]. Most infections caused by pathogenic microbes are important cause of morbidity and mortality all over the world, according to Wilson et al. [3] wound infections represents an important cause of morbidity and accounts for 70 – 80% mortality, all wounds regardless of their origin can be contaminated by microbes or foreign bodies or both and are likely to contain a significant amount of necrotic tissues [4].

Disinfectants are used extensively in the homes, healthcare settings, laboratories and industries for a variety of topical and hard surface application particularly as an essential component of infection control practice and aids in prevention of other infections [5]. Although there has been an interest in improving the sterilization and disinfection procedures to reduce the infection risk for hospitalized patients and health care workers because disinfectants resistant bacteria stains have arisen as a result of lack in standardization of some factors such as criteria for use of chemical agents, specification in the label of available products and scarcity of well trained personnel [5]. The prevalence of disinfectant-resistant hospital bacteria have increased significantly in the world including Brazil and have become a serious public health problem [6]. Methicillin resistant *Staphylococcus aureus* has been tested for susceptibility to disinfectant with disagreeing results. The widespread use of disinfectant products have prompted some speculation on the development of microbial resistance, in particular cross-resistance to antibiotics, in that sense according to Moorer, [6] disinfection does not necessarily kill all microorganisms especially resistant bacteria spores and they are less effective than sterilization, but the selection, use and control of effectiveness of disinfectants have been emphasized, since environmental surfaces and medical and surgical instruments can serve as a vehicle in infectious agents in susceptible host

associated with the hospital settings. Disinfectants are of different types and may include alcohols, quaternary ammonium compounds, hypochlorides, iodine, bromines, pine oils, peroxides or phenolic compounds. The scope of the organisms controlled and the mechanism of performances varies widely between these agents. Some puncture the cell walls of the microorganisms, allowing the contents to leak out, while others permeate and enters the cell destroying the microorganism from within [6]. To activate optimal efficiency, shelf life and safety, disinfectant agents are carefully formulated with other essential ingredients such as buffers, solubilisers, detergents, bulders, stabilizers, synergist and fragrances. Proper balancing of test formula compounds will ensure good wetting properties, minimal toxicity emulsification of fatty matters and penetration of organic soil. This ultimately helps deliver the disinfecting agents to the infectious source for maximum impact minimal concentration. This study aimed at determining the phenol coefficient test and the efficacy of some disinfectants against a Gram positive (*Staphylococcus aureus*), a Gram negative (*Escherichia coli*) bacteria and a fungi (*Candida albicans*).

Materials and Methods

Materials

All the materials used for this work were available in the laboratory of National Agency for Food and Drugs Administration and Control (NAFDAC), Area laboratory, Port-Harcourt, Rivers State, Nigeria except the disinfectants used that were purchased from the open market and the test organisms (*Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*) were obtained from Beacon and Guide Diagnostics limited (BMD Laboratories) Abia State, Nigeria.

Methods

Sterilization of Glass Wares

All glass wares such as test tubes and beakers were sterilized using hot air oven at a temperature of 160°C for 1 hour prior to use.

Media Preparation

The media used for testing the growth of *Escherichia coli* and *Staphylococcus aureus* was Nutrient agar (Lab M Ltd) while Potato dextrose agar was used for *Candida albicans* (Lab M Ltd). They were all cultured first in Buffered peptone water which is a pre-enrichment broth before subculture onto the solid media. All the media were prepared according to manufacturer's instruction.

Disinfectant dilution methods

A series of increasing concentration of the disinfectants were obtained using serial dilution method in which 5mls of distilled water was first transferred into each test tube and 5mls of the concentrated disinfectants was transferred to the first tube containing 5mls of distilled water mixed thoroughly and from that same dilution 5ml also was also transferred to the next serially in this order 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and the control (c). The control was sterile distilled water.

Agar Diffusion Method

A wire loop was used to pick a colony from a culture of nutrient agar; the colony was then transferred into a test tube containing 10ml of buffered peptone water and was incubated for 24hours at 37°C. After that, a sterile swab thick was dipped into the broth culture of the organism, gently squeezed inside the tube to remove excess fluid to streak the nutrient agar plate and also the Potatoes dextrose agar plate then the plates was allowed to dry for 5 minutes. A sterile core borer was used to make a standard well (of about 0.5mm diameter) on the surface of the inoculated plate. A total of four wells were made on each plate with each plate containing two different concentrations of each disinfectant with their replicate. Micropipettes were used to deliver the disinfectants to respective wells. The plates were incubated at 37°C for 24 hours and zone of growth inhibition were measured in millimeters using transparent meter rule and was recorded.

Phenol Coefficient Test

1% phenol concentration was prepared by dissolving 1g of phenol crystals in 100ml of sterilized distilled water in a conical flask, a series of dilution of the phenol and the test disinfectants were prepared in sterile test tubes and were all equalized to 5ml each in the order 1:1, 1:20, 1:40, 1:60, 1:80, 1:100. 0.5ml of viable test bacteria *Staphylococcus aureus* which had been inoculated in buffered peptone water for 24hours were inoculated into each test tube containing the disinfectant and phenol and incubated at 37°C. After 10 minutes, samples of inoculated tubes were transferred into fresh culture medium without disinfectants using pour plate method (in which 1ml of the inoculums was transferred into the Petri dish and immediately the respective media was poured into it and was gently mixed and allowed to gel and it was incubated for 72hours at 37°C. The inoculated plates was evaluated for growth on the plate, the highest dilution of the test antimicrobial agent and of phenol that efficiently kill the test organisms within 10 minutes exposure was used to compute the phenol coefficient as follows;

$\frac{1}{(\text{effective dilution of test disinfectant})}$

$\frac{1}{(\text{effective dilution of phenol})}$

Disinfectants with a phenol coefficient greater than 1 were more effective than phenol. The higher the phenol coefficient values the more the efficacy of the disinfectant was compared to phenol.

Results

A wide divergence was observed in the responses of disinfectant agents among the test organisms. The effect of disinfectants against the organisms is shown below in the tables showing the different dilution test microorganisms and their respective zones of inhibitions. At 100% concentration, *E. coli* was more resistant to Septol than Dettol and Z – germicide which had equal zones of inhibition of 45mm while *S. aureus*, is much more resistant to Septol, followed by Dettol and then Z –

germicide for *C. albicans*. Dettol was resistant to it followed by Z – germicide and lastly Septol which had the highest zone of inhibition as seen in figure 1.

Figure 1

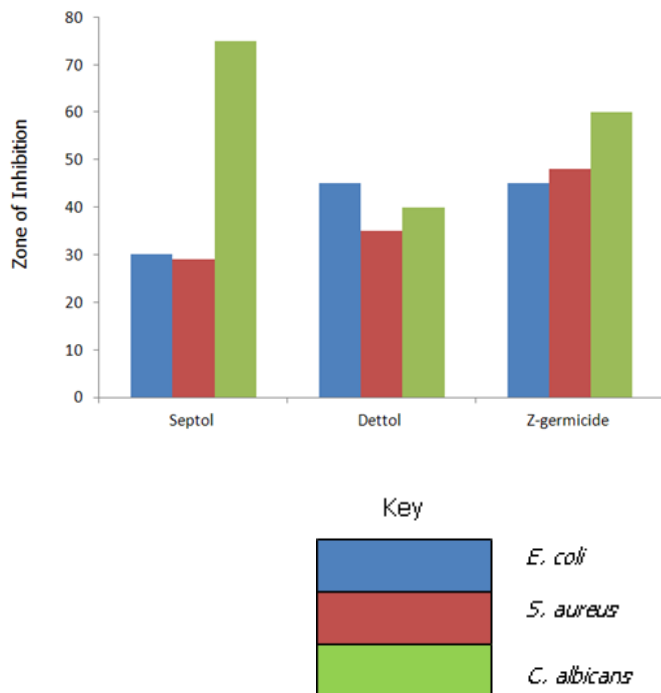


Figure 1: The Efficacy of the Test Disinfectants At 100% Concentration

At varying dilutions of Septol, it was observed that the sum of all the zone of inhibitions exhibited on the test organisms reduced as the dilution reduced in that at dilution 1:64 downwards only *E.coli* was susceptible to it with a zone of inhibition of 9mm while the other two organisms showed resistance. Likewise in all the dilutions that showed susceptibility *C.albicans* was observed to have the highest zone of inhibition as compared (figure 2). In figure 3, in which represents the efficacy of Dettol at different concentration, *E. coli* was observed to be the most susceptible organism as compared to others even at the dilution of 1:512, and *S. aureus* also was observed to be the most resistant.

Figure: 2

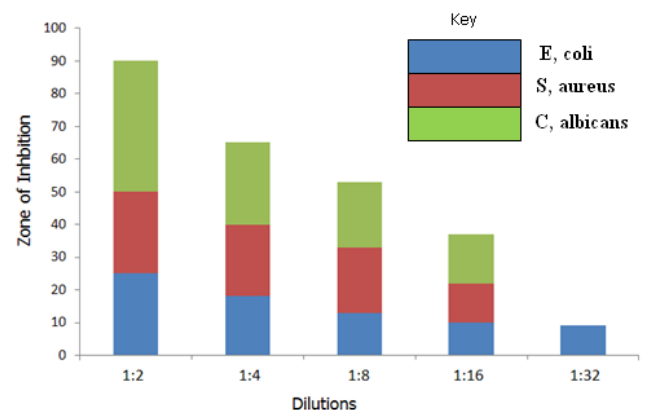


Figure 2: The Efficacy of Septol at Different Concentrations

Figure: 3

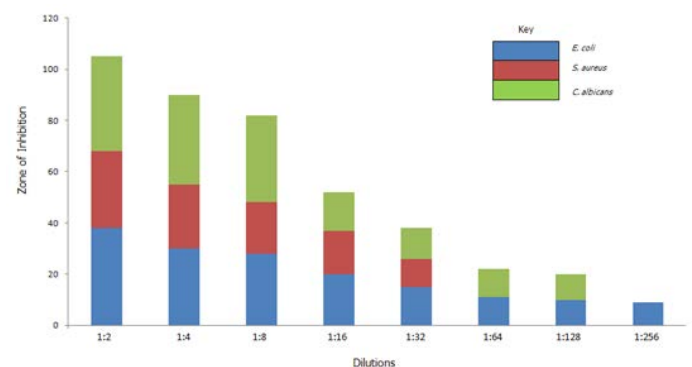


Figure 3: The Efficacy of Dettol at Different Concentrations

Z-germicide at different concentration of the test organisms indicated that at lower concentration, it was not effective against the pathogens but only effective when the dilution was very high. Although *S. aureus* and *C. albicans* were both susceptible up to the dilution of 1:32 but *S. aureus* had a wider zone of inhibition as compared to *C. albicans* and *E. coli* showed most resistance to it as compared to the two organisms (figure 4). The phenol coefficient was gotten by that dilution of the disinfectant in which the disinfectant suspension in a given time is divided by that dilution of phenol which disinfects the suspension in the

same time. The phenol coefficient result was presented in table 1 below.

Figure: 4

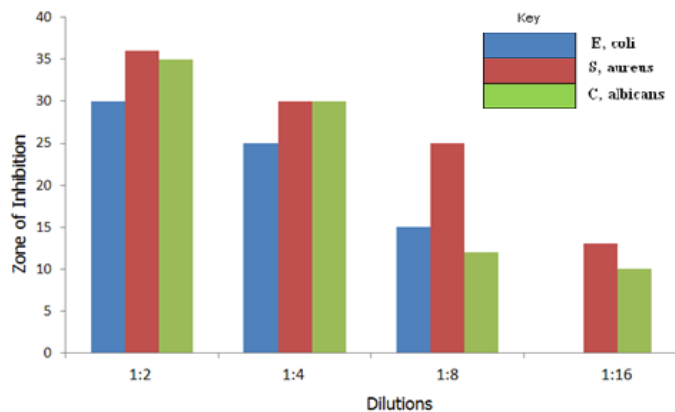


Figure 4: The Efficacy of Z-Germicides at Different Concentrations

Table 1: Phenol Coefficient of disinfectants

Dilutions	Phenol	Septol	Dettol	Z-Germicide
1:1	-	-	-	-
1:20	+	-	-	-
1:40	+	+	-	+
1:60	+	+	+	+
1:80	+	+	+	+
1:100	+	+	+	+

+	Growth Occurred
-	No Growth Occurred

From the table 1, the phenol coefficient for each disinfectant was calculated as follows

$$\begin{aligned}
 \text{For Septol} &= \frac{1/(\text{effective dilution of test disinfectant})}{1/(\text{effective dilution of phenol})} \\
 &= \frac{20}{1} \\
 &= 20
 \end{aligned}$$

This indicated that the test disinfectant Septol can be diluted 20 times as much as phenol and still possess equivalent killing power for the test organism (*Staphylococcus aureus*).

$$\begin{aligned}
 \text{For Dettol} &= \frac{1/(\text{effective dilution of test disinfectant})}{1/(\text{effective dilution of phenol})}
 \end{aligned}$$

$$\begin{aligned}
 &= \frac{40}{1} \\
 &= 40
 \end{aligned}$$

This also indicated that Dettol can be diluted 40 times as much as phenol and still possess equivalent killing power for the test organism *Staphylococcus aureus*.

$$\begin{aligned}
 \text{For Z - germicides} &= \frac{1/(\text{effective dilution of test disinfectant})}{1/(\text{effective dilution of phenol})} \\
 &= \frac{20}{1} \\
 &= 20
 \end{aligned}$$

This indicated that Z-germicides can be diluted 20 times as much as phenol and still possess equivalent killing power for the test organism *Staphylococcus aureus*.

Discussion

The efficacy activities of the test disinfectants against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* was investigated in this study. All test disinfectants at 100% concentration showed their widest zone of inhibition as compared to their various dilutions, this conforms to the study by Kortenbout [8] in that the higher the concentration of the solution, the more potent and effective it would be. It was observed that Septol exhibited the greatest antimicrobial effect on *C. albicans* with a zone of inhibition of 75mm as compared to the other two disinfectants in which Z-germicide had a zone of 60mm and Dettol 40mm, although Dettol and Z-germicides was observed to have equal inhibitory effect on *E. coli* as compared to Septol. The Septol disinfectant at different concentrations showed no inhibitory activity against *S. aureus* at the dilution of 1:64 downward except for *E. coli*, only which

showed susceptibility at the same dilution. This result corresponds with the findings of Ratula and Weber [9] in which a great number of disinfectants that were used were considered germicidal, when used in appropriate concentrations. *E. coli* was the most susceptible to Septol, but the organism that was most susceptible at the dilution of 1:2 was *C. albicans* which had the highest zone of inhibition as compared to *S. aureus* and *E. coli* but showed resistance at the i dilution of 1:32. Despite the high zone of inhibitions *E. coli* still showed susceptibility to Septol as compared to *S. aureus* and also *S. aureus* was susceptible at the in use dilution of 1:32 as compared to *E. coli* which showed susceptibility at the in use dilution of 1:64, also Dettol at different concentrations showed that *E. coli* was most susceptible to it even to the dilution of 1:512 as compared to the other two microbes even to the extent that it still showed the highest zone of inhibition at the concentration of 1:2 dilution as compared to others. *S. aureus* was observed to be the most resistant, which showed no inhibition at the dilution of 1:128 as compared to *C. albicans* which showed susceptibility at the dilution of 1:256. According to the work done by Mamman, et al. [10] which showed that gram negative bacteria were resistant to effects by disinfectant than gram positive bacteria probably due to their having a more complex cell wall. However, this does conform with our findings, in that the Gram negative bacteria (*E. coli*) did not show much resistance to Dettol and Septol and yet showed resistance to Z-germicide when compared to the gram positive bacteria (*Staphylococcus aureus*) and the fungi (*C. albicans*) this could be attributed to the differences in their active components, the differences in the activity of the disinfectants, as well as the differences in their mode of action or likewise the media components could also have affected the outcome of the activity testing, because the presence of organic matter has been identified as a factor that affects the action of disinfectants [1]. Disinfectants that are more effective than phenol have a coefficient greater than one (>1) those that are less effective have a coefficient less than one (<1), and also the higher the phenol coefficient, the more potent is the disinfectant [11]. From the result obtained from the phenol coefficient all the disinfectants used for this study were

more effective than phenol. Z-germicide and Septol had the same phenol coefficient of 20 while Dettol had a phenol coefficient of 40, hence, Dettol was the most effective and potent disinfectant as compared to Septol and Z-germicide.

Conclusion

The test disinfectants used in this study have been confirmed to be very effective when compared to the antimicrobial action of a standard phenol by having a high phenol coefficient value, also it has demonstrated that they have both antibacterial and antifungal efficacy but their rates of efficiency varies due to the differences in their chemical composition and mechanism of action, such cases can be observed mainly in *C. albicans* which was observed to be the most susceptible to Septol.

Recommendation

In view of the importance of disinfection in clinical practices and domestic hygiene, and the danger of development of resistance by the organisms exposed to the disinfectant, it will be in the best interest of all to ensure that only fresh preparations of disinfectants should be used routinely and dilution should be restricted to the concentration ranges that have been found to have definite activity against the organism, also the newly produced disinfectant products should be tested periodically in order to ensure the efficiency standard of the disinfectant, and finally the toxicities study of the active ingredients of the disinfectants should be determined so as to ensure safety when the product is used.

References

- [1]. Kennedy, J. and Bek, J. (1998). Selection and use of Disinfectants. Braska Cooperatibe extension, 40:5-8.
- [2]. Sridhar, R. P. N. (2012). The testing of disinfectants. Indian journal of Medical Sciences, 30: 50 – 80.
- [3]. Wilson, A.P.R., Gibbons, C., Raeves, B.C., Hogson, B., Lius, M and Plummer, D. (2004). Surgical wound infections as a performance adicator agreement of

- common disinfection of wound infections in patients. *American Journal of Infection Control*, 329: 720 – 925.
- [4]. Bellcham, J., Haris, J.M., Cullinam, P.J., Gaya, H and Pepper, J.R. (1999). A prospective study of wound infection coronary. *Journal of Cardiothorac Surgery*, 15(1): 45 – 50.
- [5]. Guimaraes, M.A., Tibana, A., Nunes, M.P. and Netto dos Santos, K.R. (2000). Disinfectant and antibiotic activities: a comparative analysis in Brazilian hospital bacterial isolates. *Brazilian Journal of Microbiology*, 31:193 – 199.
- [6]. Moorer, W. R. (2003). Antiviral activity of alcohol for surface disinfection. *International Journal of Dental Hygiene*, 3:138 – 142
- [7]. Dazzo, F. (2005). Control of Microbial Growth: Chemical antimicrobial. *Science*, 15:1-10
- [8]. Kortenbout, W.P. (1982). Some Factors influencing the effective use of disinfectants and cleaning agent. *Lancet infectious diseases*, 1: 29 – 33
- [9]. Ratula, W.A and Weber, D.J. (1997). Use of Inorganic hypochlorite (bleach) in health care facilities. *Centre for disease control health care infection control practices advisory committee*, 10; 597 – 610.
- [10]. Mamman, P.H., Kazeem, H.M and Kwanashie, C.N. (2005). Disinfectant effect of carcil (Alkyl-benxyldimethyl ammonium chloride) on bacteria. *Sciences*, 1:33 – 136.
- [11]. Williams, A. R., Susan, L. B., Newman, C. A., Mark, D. S and Weber, D.J. (2000). Antimicrobial activity of home disinfectants and natural products against potential human pathogens. *Infection Control and Hospital Epidemiology*, 21: 33 – 38.