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Full Length Research Paper

Susceptibility pattern of cold room bacterial pathogens to locally sold disinfectants in Owerri, Eastern Nigeria

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Efficacy of some locally sold disinfectants on bacterial pathogens isolated from cold rooms was determined. Five milliliters of different dilutions of chloroxylenol (Dettol), 5-chloro-2-hydroxy-diphenyl methane (Septol), and chlorohexidine gluconate (Purit) respectively were used. Organisms were isolated from swab and effluent samples collected in triplicates from 10 major cold rooms under standard microbiological techniques. *Staphylococcus aureus, Streptococcus pyogenes* and *Citrobacter freundii* were isolated. A 0.1ml of each dilution was delivered into a culture plate of individual isolate respectively, and zones of inhibition measured. Zones diameter of inhibition produced by disinfectants on isolates revealed increasing inhibition as concentrations increased with significant difference in inhibition of individual isolates by the different disinfectants used($F_{(117.54)} > F_{crit(4.19)}$) at P<0.05. The trend of the zones of inhibition showed that Dettol>Septol>Purit. Bacterial pathogens present in cold rooms can cause food contamination and poisoning, and infection of exposed individuals. Proper sanitary application of Dettol, Septol and Purit will help prevent public health consequences of food contamination.

Keywords: Disinfectants, cold room, effluent, pathogens, susceptibility, and public health

INTRODUCTION

Potential public health benefits of environmental disinfection are recognized by infection control experts and government agencies, including, the Centre for Diseases Control (CDC) and the Food and Drug Administration (FDA). Both agencies recommended addressing infection control or prevention as a multilayered process, including barrier precautions, personal hygiene, environmental monitoring, facility maintenance, medical device sterilization/disinfection, and environmental disinfection (Centers for Disease Control and Prevention (CDC), 2001). According to a CDC draft guideline, environmental surface disinfection is supported by epidemiologic, clinical, or experimental studies and is important to the control of Methicillin-Resistant *Staphilococcus aureus* (MRSA), vancomycin intermediate resistant *S.aureus*, and Vancomycin-Resistant Enterococci (VRE) transmission (Centers for Disease Control and Prevention (CDC), 2001).

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The risk of infection from pathogenic microorganisms on environmental surfaces derives not only from their presence but also from their ability to survive on many surfaces. The persistence of pathogenic microorganisms has been established in studies of their survival on surfaces in institutional, commercial, and domestic settings (Weber and Rutala WA, 2001; Rusin et al., 1998; Buckalew et al., 1996; Wilson and Heaney, 1999). Bacteria are a major cause of disease and even human death. Disinfectant as an effective agent to kill or eliminate bacteria is widely used in various ways, especially in microbial laboratory. Disinfectants can be mainly divided into five agents: alkylating, sulfhydryl combining, oxidizing, dehydrating and permeable. Mounting concerns over the potential for microbial contamination and infection risks in the food and general consumer markets have also led to increased use of antiseptics and disinfectants by the public. 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 (Mcdonnell and Russell, 1999). A wide variety of active chemical agents (or "biocides") are found in these products, many of which have been used for hundreds of years for antisepsis, disinfection, and preservation (Block, 1991). Preservation is the prevention of multiplication of microorganisms in formulated products, including pharmaceuticals and foods. A number of biocides are also used for cleaning purposes; cleaning in these cases refers to the physical removal of foreign material from a surface (Block, 1991).

Chloroxylenol (4-chloro-3,5-dimethylphenol; p-chloro-m-xylenol) is the key halophenol used in antiseptic or disinfectant formulations (Bruch, 1996). Chloroxylenol is bactericidal, but *P. aeruginosa* and many molds are highly resistant (Bruch, 1996). Surprisingly, its mechanism of action has been little studied despite its widespread use over many years. Because of its phenolic nature, it would be expected to have an effect on microbial membranes. Mcdonnell and Russell (Mcdonnell and Russell, 1999) reported that hexachlorophene (hexachlorophane; 2,2'-dihydroxy-3,5,6,3',5',6'-hexachlorodiphenylmethane) is another bis-phenol whose mode of action has been extensively studied. The primary action of hexachlorophene, based on studies with *Bacillus megatherium*, is to inhibit the membrane bound part of the electron transport chain, and the other effects noted above are secondary ones that occur only at high concentrations (Mcdonnell and Russell, 1999). It induces leakage, causes protoplast lysis, and inhibits respiration. The threshold concentration for the bactericidal activity of hexachlorophene is 10 µg/ml (dry weight), but peak leakage occurs at concentrations higher than 50 µg/ml and cytological changes occur above 30 µg/ml. Furthermore, hexachlorophene is bactericidal at 0°C despite causing little leakage at this temperature. Despite the broad-spectrum efficacy of hexachlorophene, concerns about toxicity (Mcdonnell and Russell, 1999). Chlorhexidine is a bactericidal agent (Denyer, 1995). Its interaction and uptake by bacteria were reported initially by Fitzgerald*et al.* (Fitzgerald et al., 1989), who found that uptake of chlorhexidine by *E. coli* and *S. aureus* was very rapid and depended on the chlorhexidine concentration and pH. More recently, by using (14C) chlorhexidine gluconate, the uptake by bacteria (Fitzgerald et al., 1989) and yeasts (Hiom et al., 1992) were extremely rapid, with a maximum effect occurring within 20s. Chlorhexidine is probably the most widely used biocide in antiseptic products, in particular in hand washing and oral products but also as a disinfectant and preservative.

The efficacy of some locally available disinfectants like Dettol, Purit and Septol available in major markets in Nigeria and some West African countries, and application methods should be confirmed for sanitary usage in cold rooms. This research therefore aimed at isolating pathogenic organisms from the cold rooms and the wastewater, to ascertain the potency, efficacy, and applicability of some locally available disinfectants against the isolates, which will help improve the efficiency of sanitary approaches in cold rooms to prevent public health consequences of diseases transmission.

MATERIALS AND METHODS

Isolation of organisms

Ten cold-room test organisms were isolated from triplicate sterile swab sticks used on the surfaces of tables, slabs, metal and plastic containers and walls, and serial dilutions of waste water discharged from the cold-rooms collected with sterile water sampling bottles. The samples were plated out on blood agar, nutrient agar, and MacConkey agar respectively incubated at 35^0C for 24 hour. The organisms were identified as described by Cheesbrough (Cheesbrough, 1984) while serological test confirmed the pathogenicity of isolates based on the methods described by Vandepitte *et al.* (Vandepitte et al., 2003). Stock cultures of individual isolate were prepared with nutrient agar slants and incubated at 23^0C according to Cheesbrough (Cheesbrough, 1984).

Preparation of disinfectants

Non-expired triplicate bottles of Chloroxylenol (Dettol), 5-Chloro-2-hydroxy-diphenylmethane (Septol) and Chlorhexidine gluconate (Purit) selected from different supermarkets in Owerri, were the disinfectants used for this study. The in-use concentrations of each disinfectant was prepared by diluting to 5ml with sterile distilled water at 0.5ml (10%), 1.5ml (30%), 2.5ml (50%), 3.5ml (70%)

and 4.5ml (90%) respectively alongside with recommended manufacturer's concentration of each disinfectant.

Susceptibility test

This was carried out using Kirby-Bauer method as described by Lalitha (Lalitha, 2008). Fresh plates of individual isolate were prepared and incubated at 35^{0}C for 24hours. A discrete colony of each isolate was transferred into a test tube of 5ml sterile saline respectively, which resulted in suspension containing approximately 1 - 2 x 10^8 cfu/ml of microbial isolate and the culture broth incubated at 35^{0}C for 2 – 6 hours to allow the broth to achieve a suitable turbidity. After achieving suitable turbidity of the inoculum, a sterile cotton swab each for each isolate in test tube was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removed excess inoculums from the swab. The dried surface of the nutrient agar plates were then inoculated by streaking the swabs over the entire sterile nutrient agar surface respectively for each inoculum. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculums. As a final step, the rim of the agar was swabbed. The lid was left ajar for 3 to 5 minutes to allow any excess surface moisture to be absorbed before introducing the disinfectant. The potency was carried out using Well-in-agar technique as described by Lalitha (Lalitha, 2008). The butt of a glass Pasteur pipette was flamed over a Bunsen burner cooled in air and used to make uniform wells on the inoculated plates. Automatic pipettes (micropipette) were used to deliver 0.1ml of each dilution of different disinfectants into the respective wells. Each plate was properly labeled. The plates were incubated at 35^{0}C for 16 to 18 hours and were observed for growth inhibition. After 16 to 18 hours of incubation, each plate was examined and circular zones of inhibition observed.

The diameters of the zones of inhibition (as judged by the unaided eye) were measured. Zones were measured to the nearest whole millimetre, using sliding calipers, which was held on the back of the inverted plates. The zones were measured from the upper surface of the blood agar illuminated with reflected light, with the cover removed. The zone margin was taken as the area showing no obvious, visible growth that can be detected with the unaided eye. The results of zones of inhibition on individual isolates by different concentration of Detol, Septol and Purit were subjected to Analysis of Variance (ANOVA) as described by Statistical Package for Social Sciences (SPSS) at 95% confidence interval to compare isolates at different concentrations and within and among different disinfectants.

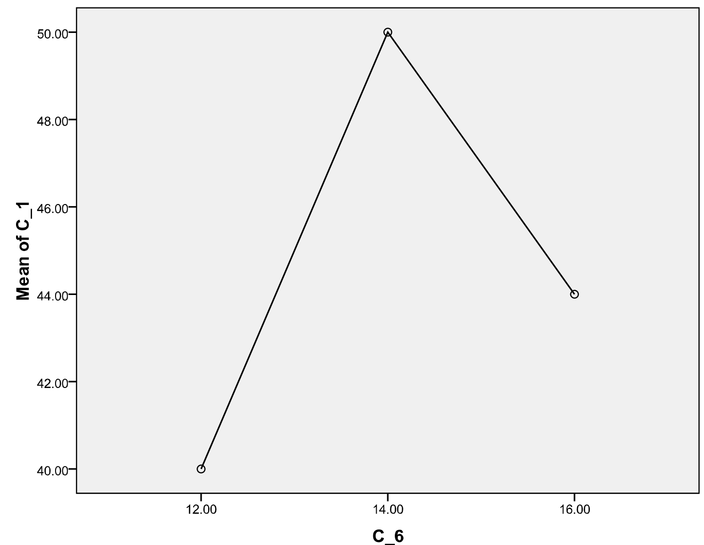

Figure 1. Means plot of growth inhibitions of isolates with 90% Dettol dilution

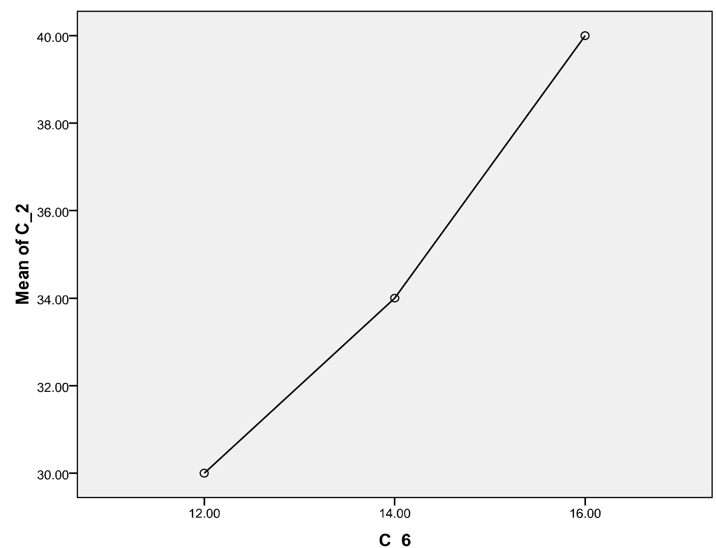

Figure 2. Means plot of growth inhibitions of isolates with 70% Dettol dilution

RESULT

The bacterial pathogens isolates include *Streptococcus pyogenes, Citrobacter freundii* and *Staphylococcus aureus.* The test of homogeneity in mean variance of zones of inhibition of the microbial isolates by the various concentrations of Dettol, revealed significant difference ($F_{(109.78)}>F_{crit(4.13)}$) at $P<0.05$. A post-hoc structure of group means revealed that with the 90% concentration inhibitions in *Streptococcus pyogenes* (14.00) (Figure 1), and with the 70%, 50%, 30% and 10% concentrations, inhibitions in *Citrobacter freundii* (16.00) (Figures 2, 3, 4,

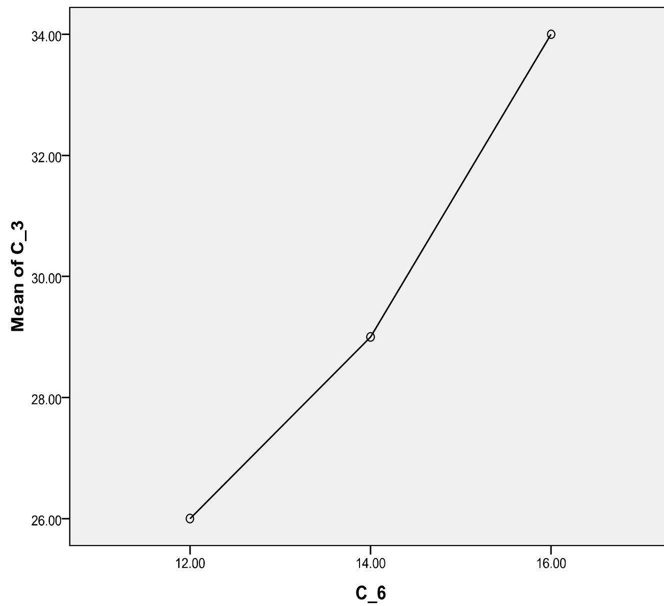

Figure 3. Means plot of growth inhibitions of isolates with 50% Dettol dilution

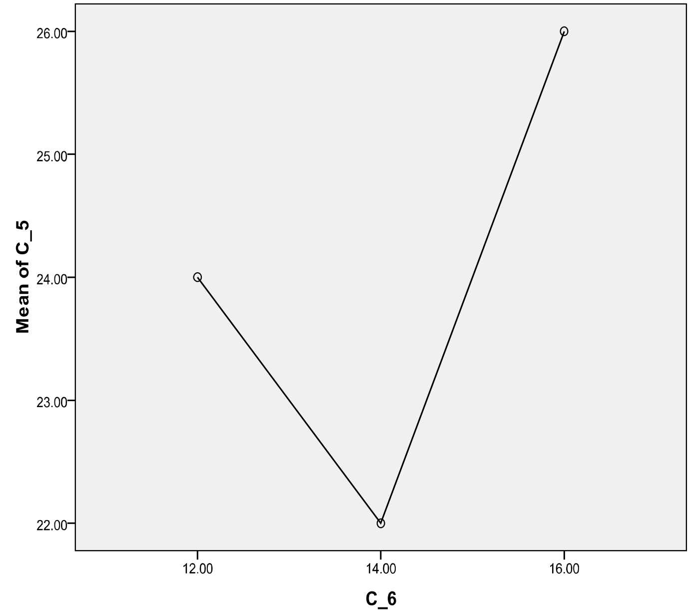

Figure 5. Means plot of growth inhibitions of isolates with 10% Dettol dilution

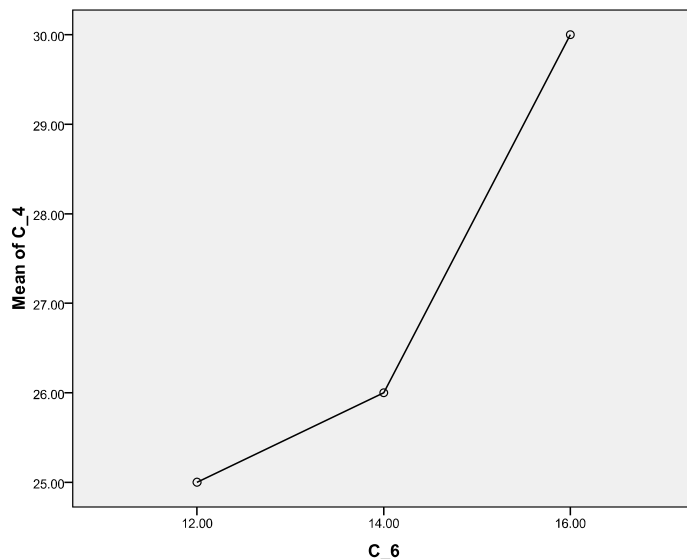

Figure 4. Means plot of growth inhibitions of isolates with 30% Dettol dilution

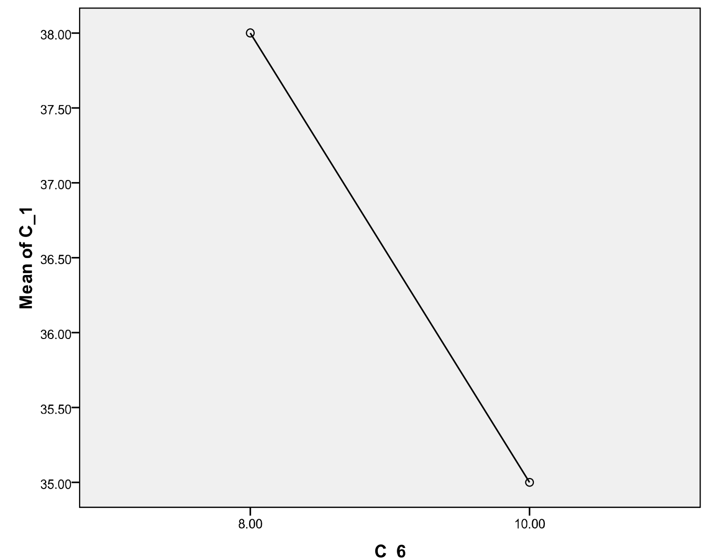

Figure 6. Means plot of growth inhibitions of isolates with 90% Septol dilution

5), were more responsible for the observed heterogeneity. While the test of homogeneity in mean variance of zones of inhibition of the microbial isolates by the various concentrations of Septol, revealed significant difference ($F_{(71.09)} > F_{crit(4.13)}$) at $P<0.05$.A post-hoc structure of group means revealed that with the 90% and 10% concentrations, inhibitions in *Staphylococcus aureus* (8.00) (Figure 6) and (Figure 10) respectively, and with the 70% concentration, inhibitions in *Streptococcus pyogenes* (10.00) (Figure 7), 50% and 30% (Figures 8, 9) showed no significant differences in inhibition, were more responsible for the observed heterogeneity. For Purit, the test of homogeneity in mean variance of zones of inhibition of the microbial isolates by the various concentrations revealed significant difference ($F_{(33.82)} > F_{crit(4.13)}$) at $P<0.05$. While a post-hoc structure of group means revealed that with the 90%, 70%, 50%, 30% and 10% concentrations, inhibitions in *Citrobacter freundii* (10.00) (Figures 11, 12, 13, 14, 15) were more responsible for the observed heterogeneity.

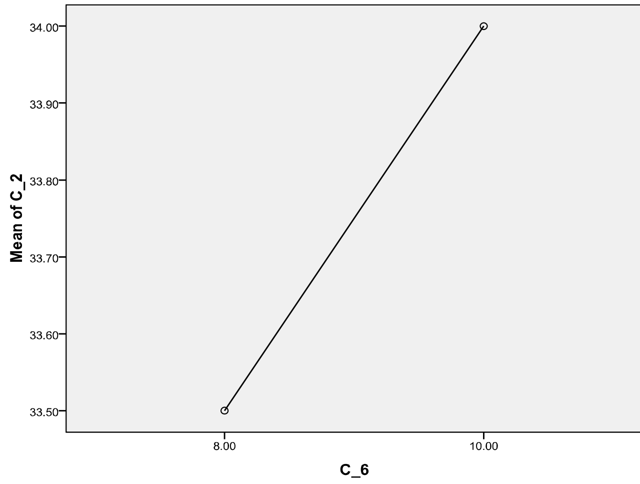

Figure 7. Means plot of growth inhibitions of isolates with 70% Septol dilution

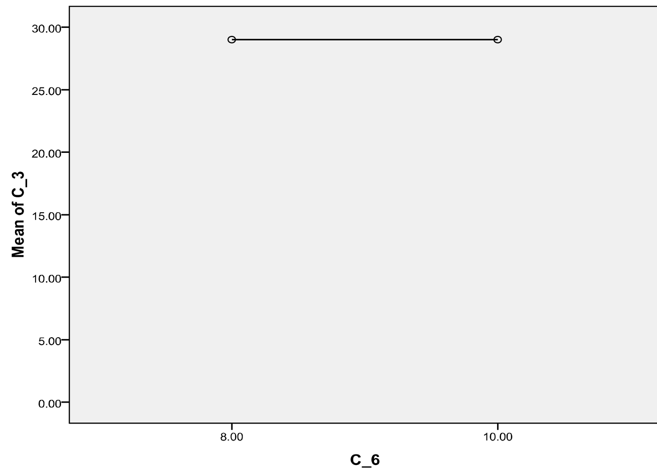

Figure 8. Means plot of growth inhibitions of isolates with 50% Septol dilution

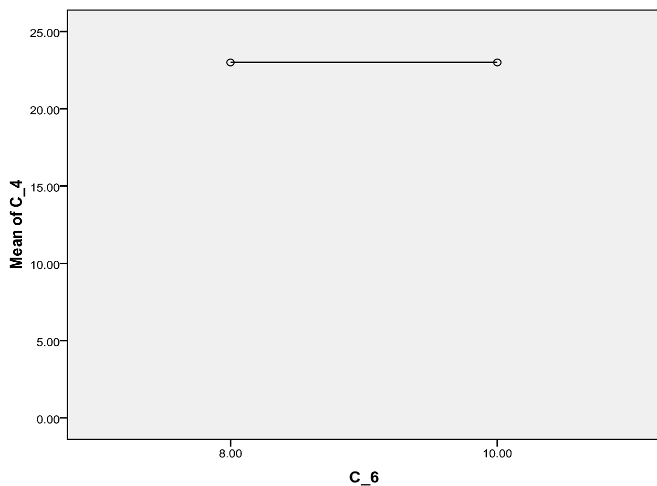

Figure 9. Means plot of growth inhibitions of isolates with 30% Septol dilution

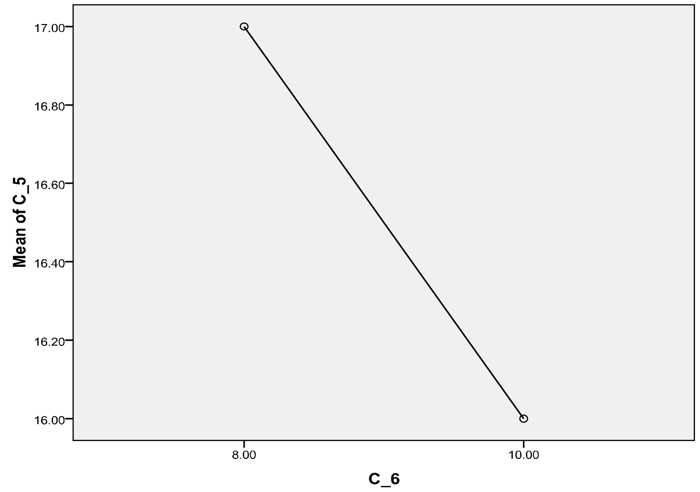

Figure 10. Means plot of growth inhibitions of isolates with 10% Septol dilution

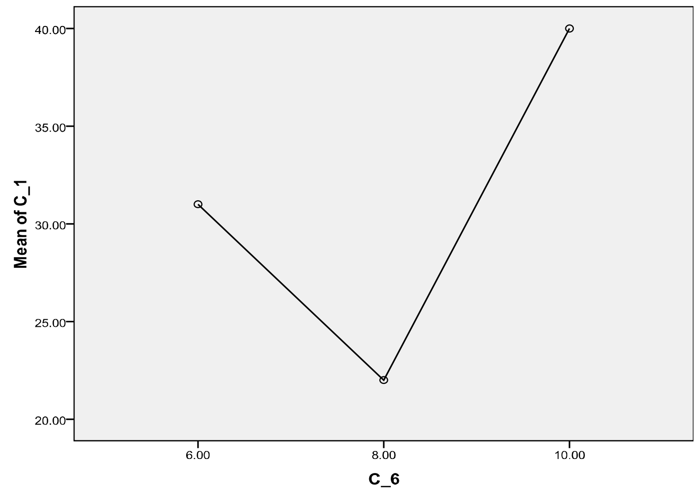

Figure 11. Means plot of growth inhibitions of isolates with 90% Purit dilution

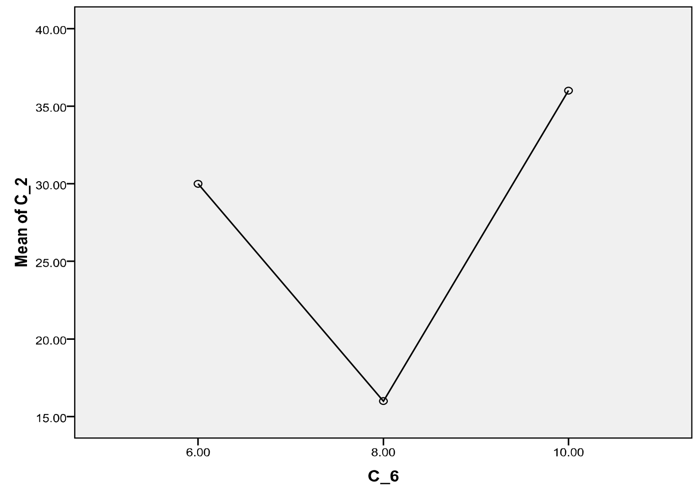

Figure 12. Means plot of growth inhibitions of isolates with 70% Purit dilution

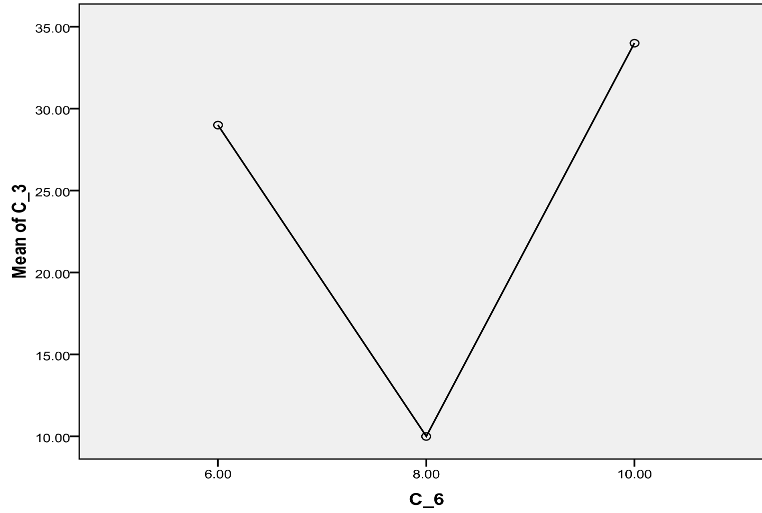

Figure 13. Means plot of growth inhibitions of isolates with 50% Purit dilution

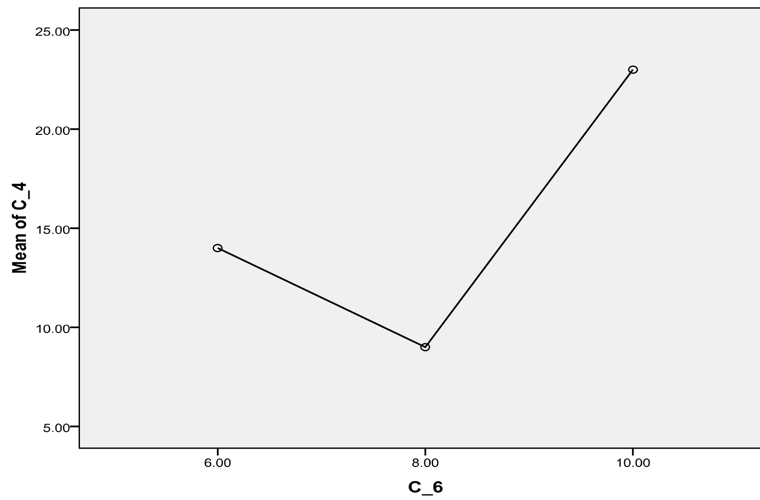

Figure 14. Means plot of growth inhibitions of isolates with 30% Purit dilution

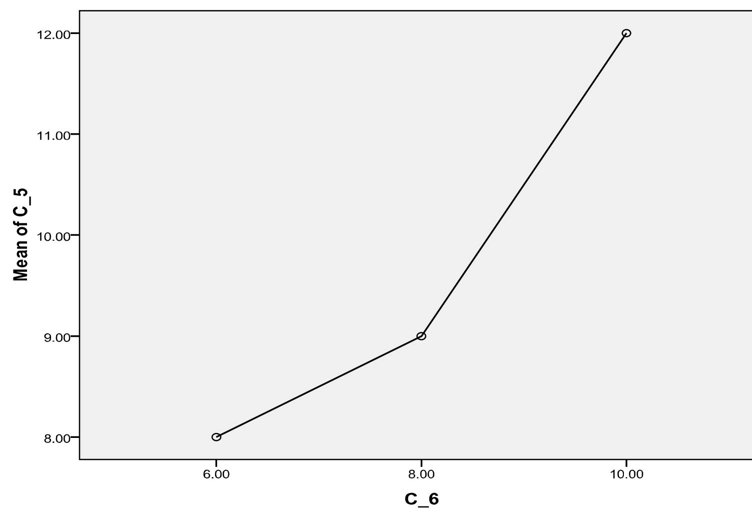

Figure 15. Means plot of growth inhibitions of isolates with 10% Purit dilution

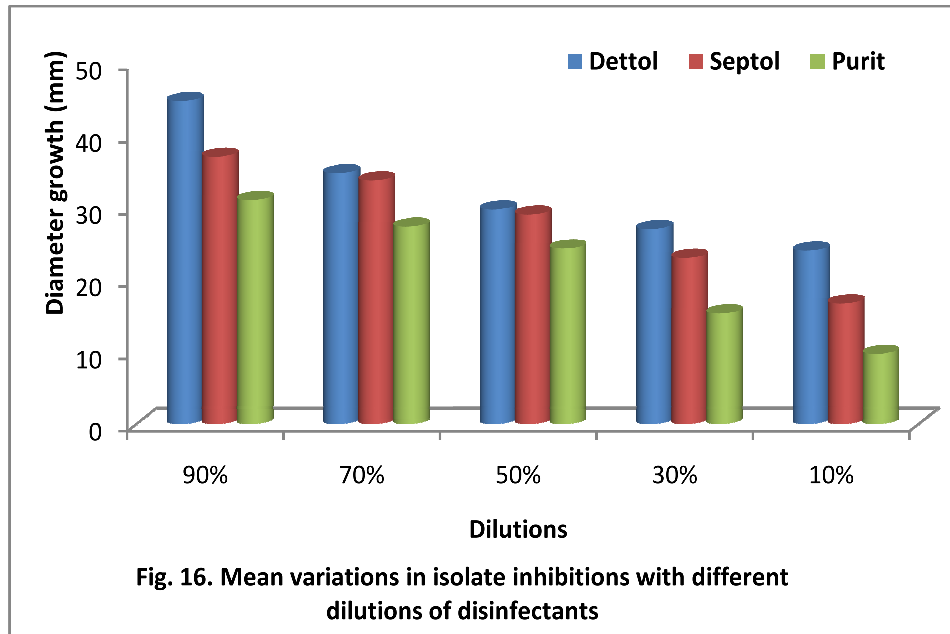

Figure 16. Variations in isolate inhibitions with different dilutions of disinfectants

The zones diameter of inhibition produced by Dettol, Septol and Purit on *Staphylococcus aureus, Streptococcus pyogenes,* and *Citrobacter freundii* as shown in Figure 16 revealed that zones of inhibition of bacterial isolates increased as the concentrations of the disinfectants increased. There was significant difference in inhibition of individual isolates by the different disinfectants used ($F_{(117.54)} > F_{crit(4.19)}$) at $P<0.05$. The trend of the zones of inhibition showed that Dettol>Septol>Purit.

DISCUSSION

The organisms isolated are established pathogens. This is in accordance with the reports of Ihejirika *et al.* (Ihejirika et al. 2011a, b, and c). These organisms might have been present on surfaces of inanimate objects and waste water from the cold room and therefore might pose serious public health risks. This was supported by the reports of Manangan *et al.* (Manangan et al., 2001) and CDC (Centers for Disease Control and Prevention (CDC), 1996), which stated that the role of the inanimate environment in disease transmission has been considered. The CDC stated that contact transmission-direct from body surface to body surface or indirect transmission via contaminated inanimate objects is one of the main routes of microorganism transmission. Environmental contamination is recognized as a source of infection by federal and international agencies. This opinion on infection control illustrates the need to understand the role of environmental surface disinfection in infection control.

The appearances of zones of inhibition on the microbial growths were indications of the efficacy of the test disinfectants on the inhibition of bacterial growth (Lalitha, 2008).

Members of the genus *Citrobacter* occur not only in feces of humans and animals with no disorder but also in water, sewage, soil, and food. *Citrobacter freundii* contamination and/or infections have been linked to carpets, ventilation systems, arm boards, walls and moist surfaces.

For an antiseptic or disinfectant molecule to reach its target site, the outer layers of a cell must be crossed. The nature and composition of these layers depend on the organism type and may act as a permeability barrier, in which there may be a reduced uptake (Russell, 1995). Alternatively but less commonly, constitutively synthesized enzymes may bring about degradation of a compound (Ogase et al., 1992).

Dettol is a chloroxylenol and is bactericidal. It is used as an antiseptic or disinfectant formulation. This is in agreement with the reports of Bruch (Bruch, 1996). As a phenolic compound, its major mechanism of action would be on microbial membranes. Septol is a hexachlorophene and as abis-phenol, its mode of action is to inhibit the membrane bound part of the electron transport chain, and might have secondary effects at high concentrations. This is in accordance with the reports of Mcdonnell and Russell (Mcdonnell and Russell, 1999). Purit as a chlorhexidine is a bactericidal agent. This is supported by the work of Denyer (Denyer, 1995). It has shown broad-spectrum efficacy. This is in agreement with the reports

of Gardner and Gray (Gardner and Gray, 1991).

These organisms are not immediately killed and might be due to disinfectant concentrations and contact times (C·T values) that control coliforms and viruses (Hoff, 1986). Disinfection is also less effective on a variety of environmental organisms that include spore-forming organisms (Clostridia), acid-fast bacteria, gram-positive organisms, pigmented bacteria, fungi, yeast, and protozoan cysts.

While being discharged along with the wastewater through pipe network, persistence and growth of these organisms might be influenced by the same factors that also affect disinfectant effectiveness: habitat locations, water temperature, pH, and assimilable organic carbon concentrations (Rittmann and Snoeyink, 1984). Following flushing, disinfectant should be introduced into the wastewater collection containers and the water held for hours to optimize line sanitation. This was supported by Gerba (Gerba, 1997), which stated that the germicide-surfactant system, germicide concentration, and contact time also could significantly affect antimicrobial activity.

Likewise, the improper use of disinfectants and the limited spectrum of certain germicides might affect their efficacy (de Andrade et al., 2000). The nature of the surface to be disinfected may influence the degree of disinfection that can be achieved (Gangar et al., 2000).

CONCLUSION

The efficacy of the disinfectants was well established. Their applications should be based on the quality of wastewater from cold room activities as well as their concentrations, retention times and spectrum of activities.

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