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COMPARATIVE ANTIMICROBIAL STUDY OF A LOCALLY PRODUCED DISINFECTANT AND SOME COMMERCIALLY AVAILABLE DISINFECTANTS AGAINST SOME CLINICAL ISOLATES

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ABSTRACT

The main goal of the present study was to determine the efficacy of five routinely used disinfectants and a locally produced one against some selected clinical isolates. The disinfectants used in this study were Roberts® antiseptic disinfectant, Tetmosol® Protect Plus antiseptic disinfectant, Izal® liquid germicide disinfectant, Ivy's antiseptic®, Dettol® antiseptic disinfectant and a locally produced antiseptic. The test organisms used in this study were *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Campylobacter jejuni* and *Candida albicans.* Agar well diffusion method was used to test the antibacterial activity of the various antiseptic/disinfectant at different concentrations (100%, 50% and 25%) as described by the Clinical and Laboratory Standards Institute. The results obtained from this study showed that the test organisms vary in their responses to each of the disinfectant, although all five pathogens were sensitive to Izal® and Roberts® antiseptic at different concentrations. On the other hand, Izal® and Tetmosol® Protect Plus gave the highest inhibition zone diameter (IZD) readings of 41mm and 33mm respectively against *Klebsiella pneumoniae* at 100% concentration while Ivy's antiseptic® had no antimicrobial effect against *Escherichia coli* in all the different concentrations used. The locally produced disinfectant gave the highest inhibition zone diameter (IZD) readings for *Candida albicans* making it very suitable to be used as an antifungal agent. This study has revealed that the antimicrobial effects of antiseptics and disinfectants are dependent on their different concentrations and nature of microorganism. Therefore, emphasis should be made on the need for strict adherence to standard disinfection policy for proper use of disinfectants and antiseptics.

KEYWORDS: Disinfectant, antimicrobial study, clinical isolates, inhibition zone diameter.

1.0 INTRODUCTION

Microorganisms can be found virtually everywhere including extreme environment where other groups of living organisms cannot survive (Cho *et al*., 2010). The discovery of microorganisms as the causative agents of infectious diseases (pathogens), has necessitate the use of various methods of microbial growth control to reduce their population or completely eliminate them in order to halt the prevalence of these infectious diseases (Kitis, 2004). Pathogenic microorganisms can grow on surfaces and establish an infection when they find their way into the body of plants and animals including humans (Dasani *et al.*, 2012).

Staphylococcus aureus is one of the main cause of nosocomial infections and toxin mediated diseases which affect the bloodstream, skin, soft tissues and lower respiratory tracts (Heyman, 2004). *Escerichia coli* and *Klebsiella Sp.* are Gram negative pathogens commonly associated with mild diarrhea, severe bloody diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome (HUS) with kidney failure (Kotloff *et al.*, 2013). *Candida Sp.* are leading cause of invasive candidiasis a persistent public health problem (Pfaller and Diekema, 2007).

Disinfection, decontamination and sterilization are usually employed as basic components for infectious disease control and in healthcare settings (Bouzada *et al.*,

2010). Wide varieties of clinical agents are used as disinfectants these include; phenols, alcohols, heavy metals, glutaraldehyde, chlorhexidine, sodium hypochlorite and quaternary ammonium compounds (Rutala et al., 2001). Disinfectants are used to disinfect inanimate objects like floors, tables, bench tops and are important for infection control in hospitals and other medical settings to prevent nosocomial infections (Lindsay and Von Holy, 1999). While Antiseptics are used extensively in health care settings to control the growth of microorganisms on living tissues (Bhat *et al.*, 2011).

Over the years, disinfectants and antiseptics have been used in the control of infectious diseases, microbial food spoilage and unwanted microbes (Lotfipour, *et al.*, 2006). However, the selection of appropriate antiseptics and disinfectants are often difficult because different pathogens response differently to different antiseptics or disinfectants depending on the inherent characteristics and cell composition such as cell envelope, non-susceptible proteins, or the ability to develop resistance either by adaption or by exchange of resistant gene (Cloete, 2003). Some pathogens (e.g. *Staphylococcus aureus* and *Pseudomonas* sp.) continuously acquiring resistance to new antiseptics and disinfectants used over time (Igbinosa *et al.*, 2015). Therefore this study was aimed to determine the efficacy of five routinely used disinfectants and a locally produced one against some selected clinical isolates, so that appropriate antimicrobial agent can easily be selected.

2.0 MATERIALS AND METHOD

Sterilization Method

All glass wares were properly washed and rinsed with clean water and sterilized in hot air oven at 160^{o}C for 2hours. The media used were all sterilized by autoclaving at 121^{o}C for 15 minutes. The inoculating loop was sterilized using the flame from a Bunsen burner. The flaming of the wire loop was repeatedly done at the end of every inoculation and the working bench was disinfected with swabs soaked in ethanol (Otter, *et al.*, 2013).

Sample Collection

The disinfectants used in this study were purchased from different commercial stores within Auchi, Edo State, Nigeria. The samples were kept at room temperature before use.

Table 2.1: Samples Of Disinfectants And Antiseptic With Their Active Ingredients.

Disinfectants Samples	Disinfectants	Ingredients
A	Roberts Antiseptic disinfectant	Aqua, isopropyl alcohol, terpineol, castor soap, dichloro-meta-xylenol, benzophenone, perfume, acid vibracolor yellow, C I 174180 Active ingredient: dichloro-meta-xylenol(2%w/w)
B	Tetmosol Protect Plus antiseptic disinfectant	Chloroxylenol (4.85%), castor oil, sodium hydroxide, isopropyl alcohol, fragrance, colour, water
C	Izal	Ortho Benzyl chloro phenol (15%), meta para cresol (4%) (saponated)
D	IVY's	dichloro-meta-xylenol, IPA, terpineol, Colour (caramel)
E	Dettol Antiseptic Disinfectant	Chloroxylenol B.PC. (4.8%w/v), oleum pini aromaticum, IPA, sapo vegtalis, sacccharum ustum q.s., Aqua

Test Organisms

The pure clinical isolates which includes: *Escherichia coli, Klebsiella spp, Staphylococcus aureus, Campylobacter spp* and *Candida spp*. were obtained from Microbiology Laboratory, Auchi Polytechnic Auchi, Edo State and were maintained in appropriate agar slants at 4°C.

Culture Media

The media were prepared according to manufacturer's instruction (Lab M Limited 1 Quest Park, Moss Hall Road, Heywood, and Lancashire BL9 7JJ, United Kingdom) and sterilized according to the method described by Rutala *et al.*, (2000).

Preparation of Samples

The disinfectants purchased were removed from their wrappings and each were labeled with letters respectively (Table2.1). The samples were diluted in 2 folds to get 50% and 25% respectively.

Antimicrobial Screening Of The Samples

The test isolates were streaked unto the agar plates using a sterile swap stick and labeled for easy identification. A sterile cork borer was used to make three (3) well-spaced holes (wells) on each of the inoculated glass petri dishes and the wells were labeled with respect to the dilution (100%, 50% and 25%) of the disinfectant solution. 0.02 ml of each dilution was introduced into appropriately labeled wells using the Pasteur pipette. This was allowed to stand to allow diffusion and was later incubated at 37^{O}C for 24 hours. The average diameter of inhibition zone was measured and recorded in mm according to Clinical and Laboratory Standards (2006).

3.0 RESULT AND DISCUSSION

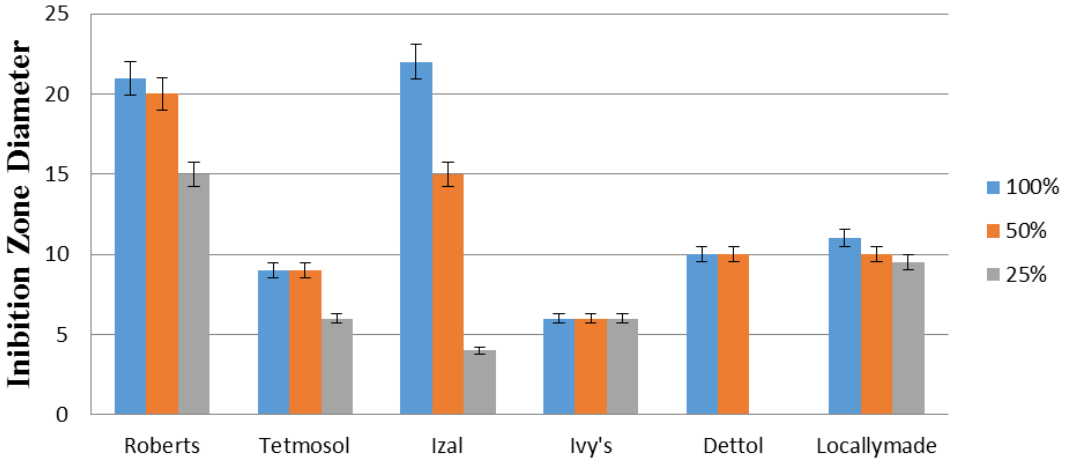

Fig. 1: Chart showing the inhibition zone diameter of the Different Disinfectants Against *Staphylococcus aureus* at different concentrations.

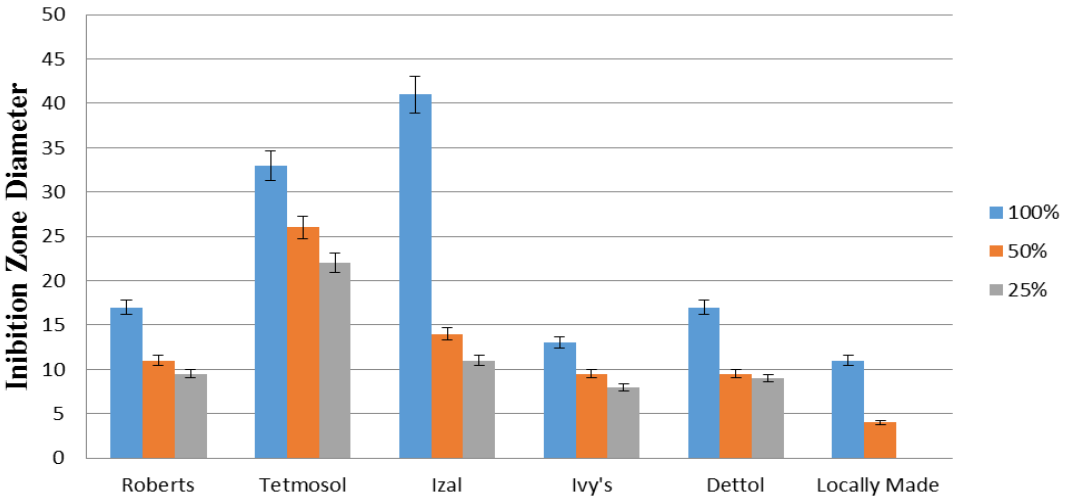

Fig. 2: Chart showing the inhibition zone diameter of the Different Disinfectants Against *Klebsiella pneumoniae* at different concentrations.

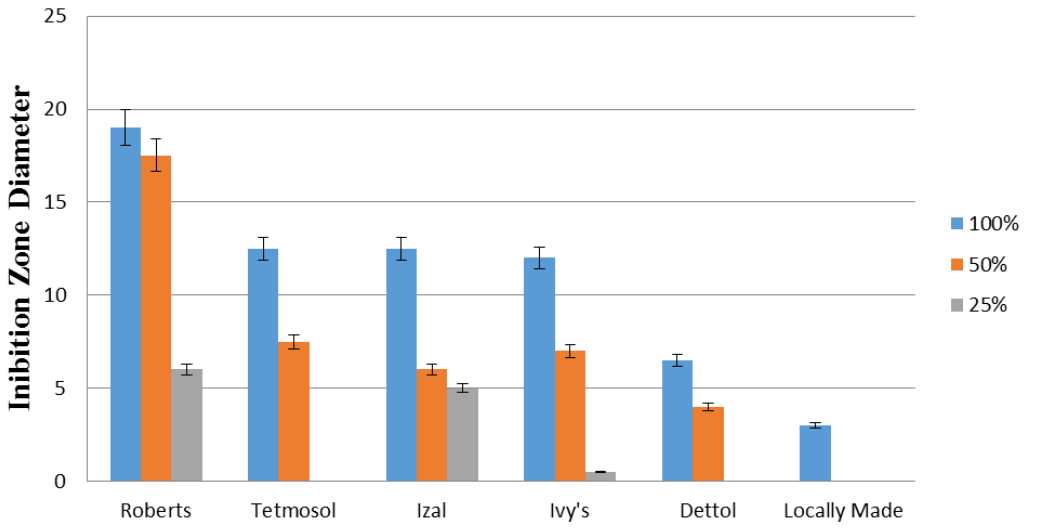

Fig. 3: Chart showing the inhibition zone diameter of the Different Disinfectants Against *Campylobacter jejuni* at different concentrations.

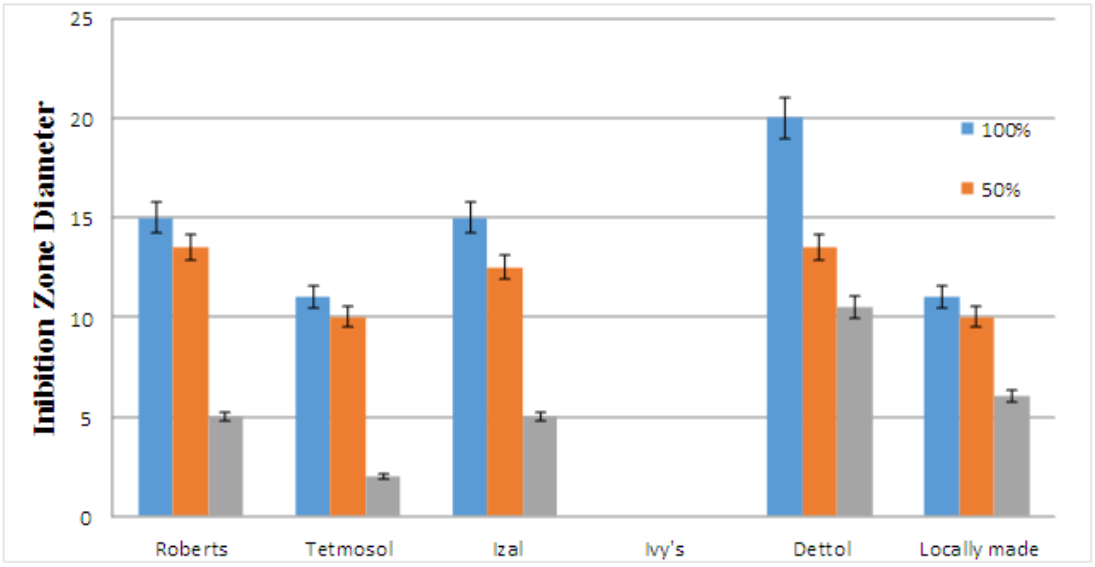

Fig. 4: Chart showing the inhibition zone diameter of the Different Disinfectants Against *Escherichia coli* at different concentrations.

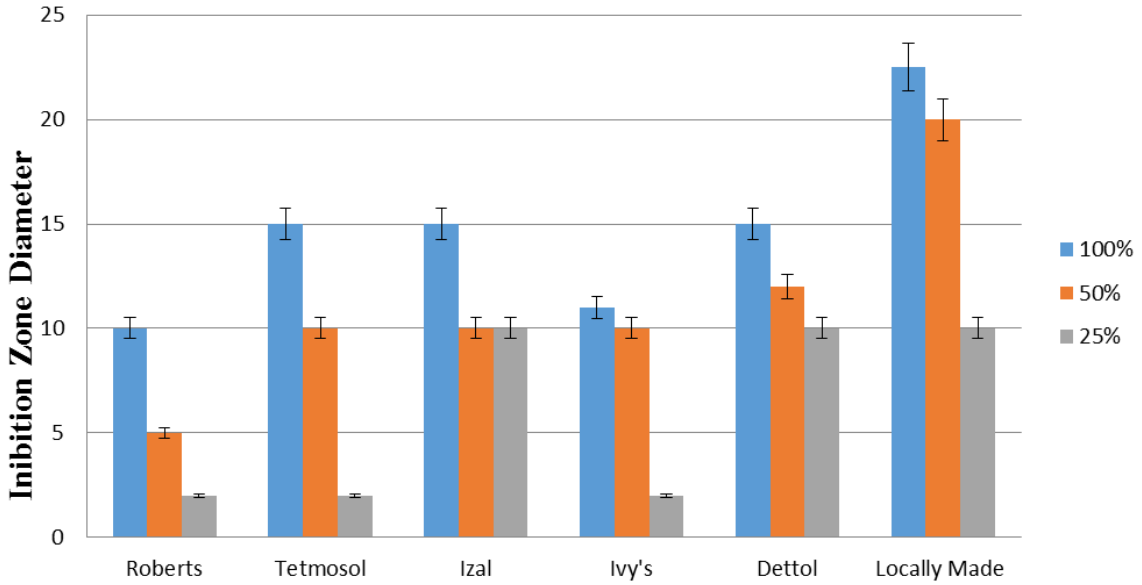

Fig. 5: Chart showing the inhibition zone diameter of the Different Disinfectants Against *Candida albicans* at different concentrations.

DISCUSSION

Microorganisms can be transmitted from one place to another through person-to-person transmission or through contact with contaminated objects (Boyce, 2007). Disinfectants and antiseptics have been used in the control of infectious diseases, microbial food spoilage and unwanted microbes (Lambert and Johnston, 2001). The determination of the efficacy of five routinely used disinfectants and a locally produced one against some selected clinical isolates showed that different types of microorganisms vary in their response to different types of disinfectants. This agreed with the work of Cloete, (2003) which stated that pathogens depending on the inherent characteristics and cell composition such as cell envelope, non-susceptible proteins, or the ability to develop resistance either by adaption or by exchange of resistant gene response differently to different disinfectants.

Ivy's antiseptic was the least effective against all the pathogens under study because the zone of inhibition ranged from 0 mm to 11 mm. On the other hand, Izal was found highly effective against all pathogens where the zones of inhibition ranged from 10 mm to 41mm while Roberts was also highly effective to all the selected pathogens. All the five pathogens were sensitive to Izal and Roberts antiseptic at different concentrations. Antibacterial effect of Dettol showed a better zone of inhibition against *Escherichia coli* compared to other microorganisms tested and Similarly, Tetmosol was

more effective against *Klebsiella* sp. and *Candida* sp. than the other pathogens.

The locally made antiseptic showed better antifungal efficacy against *Candida* sp. than the other selected pathogens. This study also showed that the antibacterial and antifungal effects of the selected antiseptics and disinfectants are dependent on their concentrations as shown in figure1 to 5. The antifungal activity of the locally formulated antiseptic showed in figure 5 revealed that the higher the concentration of the disinfectants and antiseptic, the higher the diameter of zone of inhibition against the *Candida sp*. Which agrees with the work of Bouzada, *et al.,* (2010) in Tertiary Care Hospital That showed that the susceptibility of the selected microorganisms increased with increased in the concentration of the tested disinfectants.

Ivy's was less effective against *Staphylococcus aureus* strain used in the research study as showed in figure1. This could be as a result of acquired resistance gene against the active ingredient used in the formulation (Falagas and Bliziotis 2007) or the over-used of the disinfectant over a long period of time. This also agrees with the findings of Igbinosa *et al.,* (2015) which stated that some pathogens (such as *Staphylococcus aureus* and *Pseudomonas* sp.) continuously acquiring resistance to antiseptics and disinfectants used over time However, exposing bacteria to sub-lethal dose of disinfectant and other biocides, destroy and damage only minor cells (Saha *et al.,* 2009) and consequently may lead to changes in their phenotype or induction of gene expression, thereby resulting to a more resistant population (Araujo *et al.,* 2011). *Escherichia coli* was completely resistant to Ivy as showed in figure 4. Although Gram-negative bacteria are usually less susceptible to biocides agents due to the nature of their outer membrane that acts as a barrier for the permeability of antimicrobial agents which enables them to withstand the effect of antiseptics, disinfectants and some antibiotics (Jansen *et al.,* 2006). Thus, these bacteria species continues to be an important pathogen in hospital acquired infections (Lotfipour *et al.,* 2006).

The result of this study also shows that the commercially available disinfectants (Roberts, Tetmosol, Izal and Dettol) are highly effective against all the tested clinical isolate and provide a scientific evidence to support the use of disinfectants as part of a program to control infectious disease through surface decontamination, their use in healthcare facilities as recommended by the Centres for Disease Control and Prevention, Occupational Safety and Health Administration and Professional Organizations such as the Association for Professionals in Infection Control and Epidemiology (Bouzada *et al.,* 2010).

4.0 CONCLUSION

In conclusion, the reduced activity of the Ivy's disinfectants from this study may be due to indiscriminate use in sub-optimal concentrations over a long period of time which may have led to the development of resistant strains of *E. coli*. Therefore the use of disinfectant concentrations lower than that recommended by the manufacturers might have serious consequences in the management of patients in hospitals thus there is need for strict adherence to standard disinfection policy for proper use of disinfectants and antiseptics.

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