

ANTIMICROBIAL ACTIVITIES OF SOME HOUSEHOLD DISINFECTANTS ON SELECTED HUMAN PATHOGENS IN UMUAHIA, ABIA STATE, NIGERIA.

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ABSTRACT

Susceptibility of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans* to each of five selected household disinfectants (Dettol, Izal, Z-Germicide, Jik and EDN[®] Hand sanitizer) was determined using agar diffusion method. Phenol coefficients were used to compare the effectiveness of these disinfectants against phenol. Jik disinfectant was inhibitory against *Salmonella typhi* ($P < 0.05$) and *C. albicans* at 1:2 dilution respectively with MIC value of 1:64 dilution against *E. coli* and 1:4 dilution for *C. albicans*. Dettol was biocidal to *E. coli*, *S. typhi* and *C. albicans* with MIC value of 1:512 dilution against *E. coli* and 1:256 dilution for *C. albicans*. Z-germicide was inhibitory against *S. typhi* ($P < 0.05$) with a bacteriocidal MIC of 1:64 dilution and fungicidal MIC of 1:32 dilution. Izal inhibited *Candida albicans* at MIC of 1:32, but inhibited *S. typhi* and *E. coli* at 1:4

dilution respectively but did not inhibit *S. aureus* even at 1:2 dilution. EDN[®] Hand Sanitizer had MIC values of $< 1:1024$ for both the bacterial isolates and *C. albicans* and at the lowest dilution inhibited *S. aureus* ($P < 0.05$). This MIC value was the lowest recorded against the four disinfectants analysed in the work. At 100% concentration, EDN[®] Hand Sanitizer has the greatest inhibition against *S. typhi*, *S. aureus* and *E. coli* ($53.00^a \pm 1.41\text{mm}$, $53.00^a \pm 0.71\text{mm}$ and $48.00^b \pm 0.71\text{mm}$ respectively: $P < 0.05$) while Jik has the highest inhibition against *C. albicans* ($76.00 \pm 1.41\text{mm}$: $P < 0.05$). Among all the tested disinfectants, Izal gave the least inhibition ($18.00 \pm 0.71\text{mm}$) against *E. coli* and $10.00 \pm 0.71\text{mm}$ against *C. albicans*. EDN[®] Hand Sanitizer also has the highest Phenol coefficient (Pc) value of 7 against *E. coli* and *C. albicans* respectively while Izal had zero Pc value against *S. aureus* and

C. albicans respectively. All the disinfectants inhibited the four test isolates thus confirming their broad spectrum activity, but only EDN[®] Hand Sanitizer, Jik, Dettol and Z-germicide did so upon dilution thus confirming their broad spectrum activity. EDN[®] Hand Sanitizer is encouraged to be used in homes to prevent the transfer of pathogens at close contacts with persons.

KEYWORDS: Bacteria, *Candida albicans*, disinfectants, phenol coefficient, susceptibility.

1.0 INTRODUCTION

Disinfectants, antiseptics and preservatives are chemicals which have the ability to destroy or inhibit the growth of microorganisms and process of removing microorganisms including potentially pathogenic ones, from the surface of inanimate objects. The British Standards Institution further define disinfection as not necessarily killing all of the organisms, but reducing them to a level which is neither harmful to health nor to the quality of perishable goods (Wilson *et al.*, 2004). Disinfectants are essential parts of infection control practices and aid in the prevention of nosocomial infections. But a common problem is the selection of disinfections, because different pathogens vary in their response to different disinfectants.

Most infections caused by pathogenic microbes are important cause of morbidity and mortality all over the world, according to (Wilson *et al.*, 2004), 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 (Bellcham *et al.*, 1999).

With prior cleaning before application, the number of microorganisms colonizing the skin, objects and surfaces are greatly reduced (Ratula, 1997). The antimicrobial properties of the disinfectant against some pathogen bacteria have earlier been reported. Moreover, microorganisms are continuously acquiring resistance to new antiseptic or disinfectants, as a result, no single disinfectant will be appropriate for all pathogens (Tortora *et al.*, 2004). Therefore, it is necessary to evaluate the effectiveness of any disinfectant against a specific pathogen so that an appropriate agent can be easily selected.

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. *Pseudomonas aeruginosa* can still survive, making them ineffective against nosocomial infections. The emergence of

resistant microorganisms in the community is causing problems for infection control. Organisms of particular concern include methicillin resistant *Staphylococcus aureus*, extended spectrum beta-lactamase producing *Klebsiella* and glycopeptide resistant enterococci (Aboh *et al.*, 2013).

Disinfectants are of different types and may include alcohols, quaternary ammonium compounds, hypochlorides, iodine, bromines, pine oils, peroxide or phenolic compounds. The scope of the organisms controlled and the mechanism of performances varies widely between these agents (Moorer, 2003). These disinfectants cause destruction either by coagulating the protein of the bacteria, by destroying its cell membrane or by removal of a sulphhydryl group from the organisms (Omoruyi and Idemudia, 2011). Also, according to Chioma *et al.*, (2014) and Cheesbrough (2006), the mode of action of disinfectants is thought to be linked to destruction of protein, lipids or nucleic acids in the cells or its cytoplasmic membranes, although microorganisms differ in their sensitivity to chemical germicide. The Gram positive and Gram negative microorganisms are implicated in different diseases and infections. The content of many chemical agents can be expressed by more than one notation. The effectiveness of a given disinfectant can be evaluated using the phenol coefficient test which is the best known disinfectant screening test in which the potency of a disinfectant is compared with that of phenol.

Disinfectants agents are carefully formulated with other solubilizers, detergents, stabilizers 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 infectious source for Maximum impact at minimal concentration (Moorer, 2003).

1.1 Objectives of the Study

- i. To know the antimicrobial potentials of different disinfectants under different trade names on selected tests isolates: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans*.
- ii. To determine the minimum inhibitory concentrations of the selected disinfectants against the test isolates.

2.0 MATERIALS AND METHODS

2.1 Collection of samples

Samples of six commonly used household disinfectants (Izal, JIK, Dettol, Z-germicide, EDN® Hand sanitizer and phenol) were procured from supermarket in Umuahia and taken to the microbiology laboratory for analyses.

2.2 Test isolates

Pure cultures of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans* were collected from The National Veterinary Institute, Umudike in slant bottles. They were transported in Buffered peptone water which is a pre-enrichment broth to the laboratory (Cheesbrough, 2010). The test isolates were sub-cultured on appropriate media (Mannitol Salt agar for *Staphylococcus aureus*, MacConkey and Eosin Methylene blue agar for *Escherichia coli*, Salmonella-shigella agar for *Salmonella typhi* and SDA for *Candida albicans*) and later subjected to biochemical tests to confirm their identity. *Candida albicans* was confirmed using Germ tube test. The isolates were re-stored in a slant bottles in a fridge for future use.

2.3 Preparation of inocula

Organisms were grown overnight at 37°C in Nutrient broth (Oxoid, UK). The overnight broth culture of organism was diluted in nutrient broth to an inoculum load approximately 1.0×10^6 cfu/ml. It was standardized according by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 cfu/ml.

2.4 ANTIBIOTIC SUSCEPTIBILITY TESTING

2.4.1 Preparation of Turbidity Standard Equivalent to McFarland 0.5

One percent (1%) v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water. 1% of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride in 50ml of distilled water. 0.6ml of Barium chloride solution was added to 99.4ml of the sulphuric acid solution and mixed properly. The solution was preserved in the fridge.

2.4.2 Serial dilution of disinfectants

A series of increasing concentration of the disinfectants was obtained using serial dilution method in which 5mls of sterile distilled water was first transferred into each test tubes and 5mls of the concentrated disinfectant was transferred to the first tube containing 5mls of

distilled water mixed thoroughly to give a concentration of 1:1. From this tube, 5ml aliquot was successively transferred to other test tubes to give concentrations of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:512 and 1:1024. Sterile distilled water was used as a control (Sridhar, 2012).

2.4.3 Preparation of sensitivity paper discs

Sterile filter paper discs were impregnated with 0.1ml each of the dilutions of the disinfectants using different sterile pipettes. Using sterile forceps, the discs impregnated with different dilution of the disinfectants were placed on plates of Mueller Hinton agar inoculated with the test organisms using sterile swab sticks. The discs were equally spaced on the inoculated plate and allowed to stay for fifteen minutes before they were incubated at 37°C for 24hrs. Following incubation, the zones of inhibition were measured and recorded (Quinn and Markey, 2001). Absence of zone of inhibition indicated resistance from the test organism (Tortora *et al.*, 2004).

2.4.4 Determination of Phenol Coefficient (Pc) of the Disinfectants

The phenol coefficient (Pc) of the disinfectants was determined using standard microbiological method. Different dilutions of the phenol stock solution were made (1:80, 1:90 and 1:100) in sterile test tubes. 0.1ml each of 24hrs old the suspension of the test organisms (adjusted to McFarland standard) was introduced into each of the phenol dilutions and mixed properly. 0.1ml aliquot was then inoculated into tubes of 2ml of sterile nutrient broth after 5 minutes and 15 minutes for each of the dilutions. The same procedure was repeated for each of the test disinfectants using dilutions 1:400, 1:450 and 1:500. The tubes were incubated for 24 hours at 37°C and then observed for growth (turbidity).

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

$$Pc = \frac{\text{Highest dilution of the test disinfectant that killed the organism in 10 minutes}}{\text{Highest dilution of the phenol that killed the same organism in 10 minutes}}$$

(Ewart, 2001).

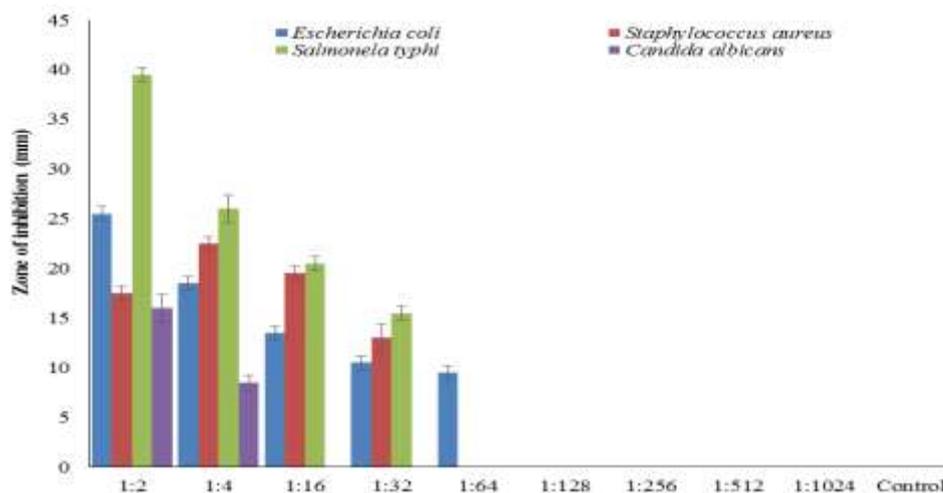


Fig. 1: Zone of inhibitions of JIK at different concentrations.

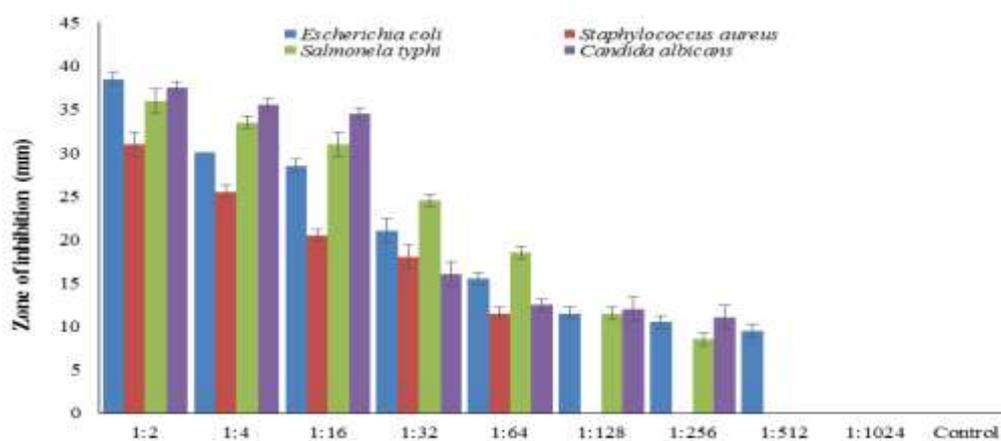


Fig. 2: Zone of inhibitions of Dettol at different concentrations.

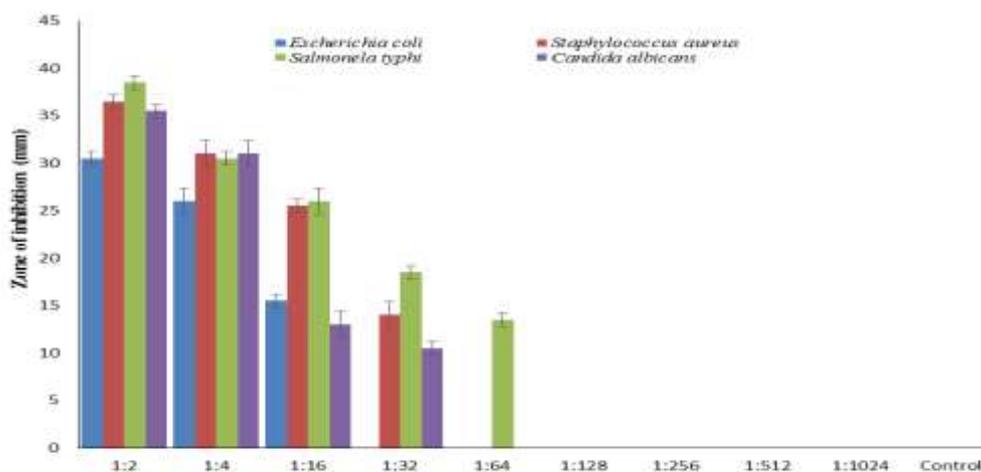


Fig. 3: Zone of inhibitions of Z-Germicide at different concentrations.

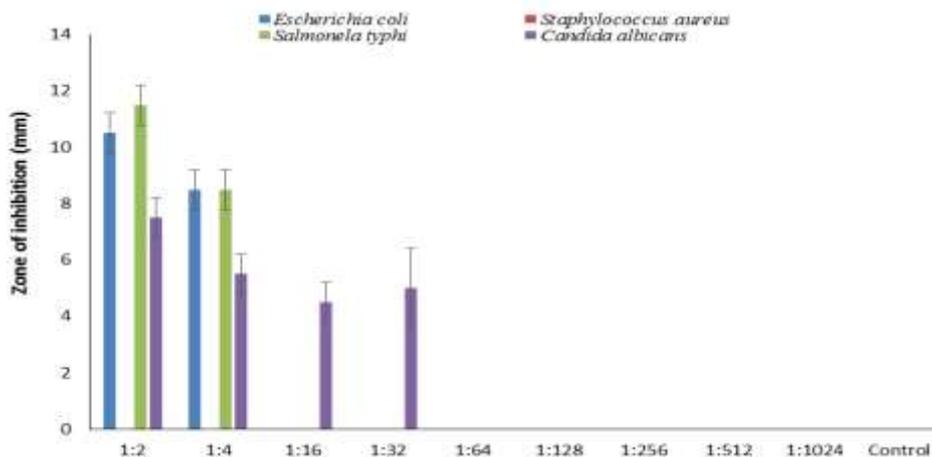


Fig. 4: Zone of inhibitions of Izal at different concentrations.

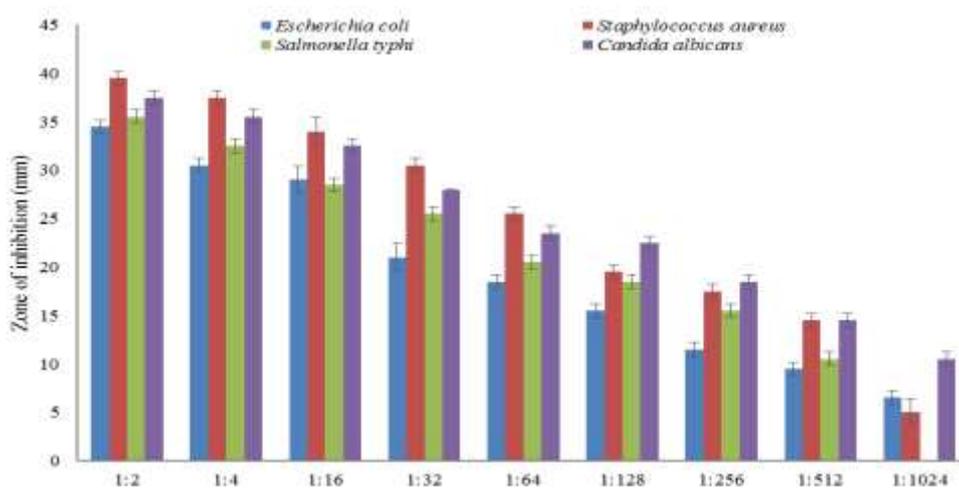


Fig. 5: Zone of inhibitions of Hand sanitizer at different concentrations.

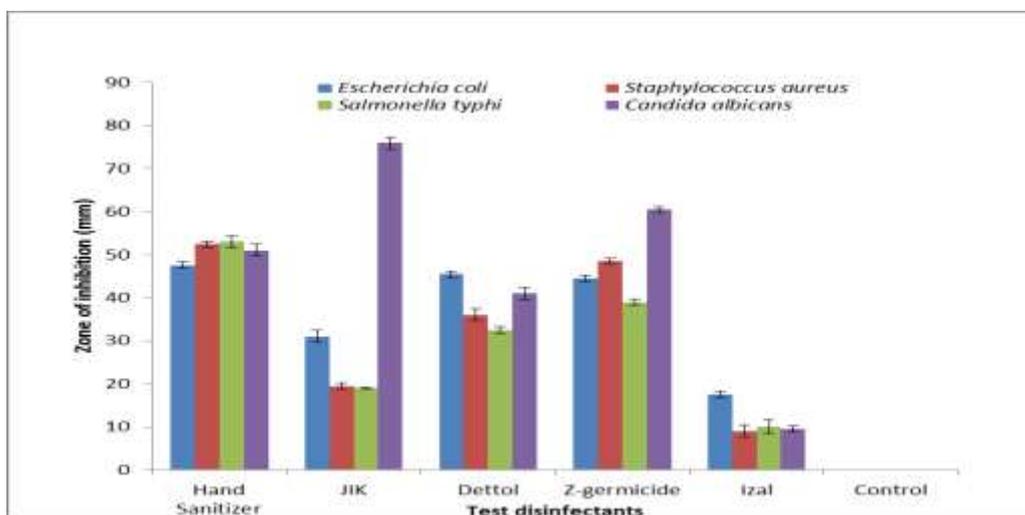


Fig. 6: Zone of inhibitions of test disinfectants at 100% concentration.

Table 1: Phenol Coefficients (Pc) of the disinfectants against the test organisms.

Test microorganism	Disinfectants	Pc comparism with phenol
<i>Escherichia coli</i> :	Dettol	6.25
	Izal	4
	Jik	4.5
	Z-Germicide	4
	Hand Sanitizer EDN ^(R)	7
<i>Staphylococcus aureus</i> :	Dettol	6.25
	Izal	0 (R)
	Z-Germicide	4
	Jik	0 (R)
	Hand sanitizer EDN ^(R)	6.75
<i>Salmonella typhi</i> :	Dettol	6.25
	Izal	4
	Z-Germicide	4.5
	Jik	4
	Hand Sanitizer EDN ^(R)	6.5
<i>Candida albicans</i> :	Dettol	5
	Izal	0 (R)
	Jik	4
	Z-Germicide	6.25
	Hand Sanitizer EDN ^(R)	7

R= Resistance

3.0 DISCUSSION

The antimicrobial activities of selected household disinfectants (Jik, Dettol, Z-germicide, Izal and EDN[®] Hand Sanitizer) against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans* were analyzed in this study. Antimicrobial activities of Jik disinfectant showed that it was bacteriocidal against *Salmonella typhi* (40.00±0.71mm: P<0.05) at 1:2 dilution followed by *Escherichia coli* (26.00±0.71mm: P≤0.05). However, as the dilution increased, the sensitivity of the test pathogens to the disinfectant decreased progressively. Jik was effective against *Candida albicans* at 1:2 dilution, thus proving that it has broad spectrum activities against Gram positive, Gram negative bacteria and yeast. The MIC of Jik disinfectant was 1:64 dilution against *E. coli* (for the bacteria) and 1:4 dilution for *C. albicans* implying that the later was most resistant to the disinfectant that the bacterial isolates because it was not inhibited beyond 1:4 dilution (Fig. 1). The MBC for the bacteria was 1:16 dilution while 1:2 dilution was the Minimum fungicidal concentration (MFC) for *C. albicans* proving that the later was most resistant to Jik disinfectant than all the test bacterial isolates. Okore *et al.*, (2014) reported that Jik disinfectant had an inhibitory zone of 25mm (undiluted) while 1:8 dilution (their lowest) had 12mm zone of inhibition against *E. coli*. They also reported

that undiluted Jik had 12mm zone of inhibition against *Mucor* spp and this activity of Jik against *E. coli* were lower than we found in this work.

Result of antimicrobial activities of Dettol disinfectant against the test isolates showed that it was biocidal to *E. coli*, *S. typhi* and *C. albicans* (statistically the same at $P < 0.05$) followed by *S. aureus* (31.00 ± 1.41 mm). The susceptibility of the test isolates to this disinfectant progressively decreased as the dilution increased. Dettol was effective against the four test isolates up to 1:64 dilution. Beyond this point, *S. aureus* was not inhibited again. The MIC of Dettol was 1:512 dilution for *E. coli* and 1:64 dilution for *S. aureus*, but both values were lower than that observed for Jik disinfectant. The MBC for the three bacteria was 1:256 dilution (against *E. coli*) and 1:128 dilution was the MFC for *C. albicans*. Result also proved that Dettol has broad spectrum activities against the two Gram's groups of bacteria and yeast. Okore *et al.*, (2014) reported that Dettol (undiluted) had 21mm inhibition against *E. coli* which result is lower than our findings against same pathogen.

The antimicrobial activities of Z-germicide was recorded against *S. typhi* (39.00 ± 0.71 mm, $P < 0.05$), followed by *S. aureus* and *C. albicans* (statistically the same at $P < 0.05$) and then against *E. coli*. The four test isolates were inhibited by 1:16 dilution, but, beyond this point, *E. coli* was not inhibited again. The MIC for the test bacteria was 1:64 dilution against *S. typhi* followed by 1:32 dilution (against *S. aureus* and *C. albicans* respectively). Z-germicide also exhibited broad spectrum activity against the four isolates and the MIC was also the same with Jik disinfectant for the bacterial isolates though against *S. typhi*. This broad spectrum activity agrees with the inscription on the body of the container of this disinfectant that the product has a broad spectrum activity. The MBC for the bacterial isolates was 1:32 dilution (against *S. typhi*) and 1:16 dilution was the MFC for *C. albicans*.

Izal disinfectant showed selective killing effect against the test isolates as it did not inhibit *S. aureus* (a Gram positive bacterium) at the highest dilution (1:2) and was the only disinfectant that showed such effect in this work. However, it had killing effect against *S. typhi* and *E. coli* (12.00 ± 0.71 mm and 11.00 ± 0.71 mm respectively) which values had no statistical difference ($P < 0.05$) at 1:2 dilution. This is followed by *C. albicans* (8.00 ± 0.71 mm) and was the only isolate inhibited beyond 1:4 dilution and up to 1:32 dilution which incidentally was the MIC for Izal. The MIC for the two inhibited bacteria was 1:4 dilution (9.00 ± 0.71 mm respectively) and this was the highest bacterial MIC recorded in the work. Izal has its MBC at 1:2 dilution while the MFC for *C. albicans* was 1:16 dilution. Comparatively though, Jik

showed the highest MIC for *C. albicans* (1:4 dilution; Fig. 1). This means that Izal showed the weakest antibacterial activity among the four disinfectants tested and this low performance could be attributed to development of resistance by microbes over decades this disinfectant has been in use in homes, schools, hospitals. Therefore, it is important that the manufactures of Izal should upgrade the chemical constituents of the disinfectants thereby improving its antimicrobial potentials and thus compete very well with present and on coming disinfectants. Okore *et al.*, (2014) reported that Izal (undiluted) had 17mm against *E. coli* but no inhibition at 1:8 dilution. They also reported that Izal did not inhibit *S. aureus* and *Streptococcus* spp even at zero dilution. We also found that Izal did not inhibit *S. aureus* even at 100% concentration.

The four test isolates were completely inhibited by EDN[®] Hand Sanitizer at 1:512 dilution indicating that it also has broad spectrum activity. It's wide range activity was shown at lower dilutions. However, *S. aureus* was inhibited at 1:2 dilution (statistically different at $P < 0.05$) followed by *C. albicans* (38.00 ± 0.71 mm). The lowest MIC recorded against the bacterial isolates was at $< 1:1024$ dilution for *E. coli* and *S. aureus* while that for *C. albicans* was also $< 1:1024$ dilution. These two MIC values were the lowest and the best recorded against the four disinfectants analysed in the work. The MBC and MFC was $< 1:1024$ respectively. The wide range of sensitivity of EDN[®] Hand Sanitizer could be attributed to its newness in the disinfectant market in Nigeria. Actually, this disinfectant was popularized in Nigeria market in July, 2014 at the wake of the outbreak of Ebola Fever in the country. So far, microbes have not been able to develop resistance to its activity and this may be due to the complex nature of its chemical ingredients.

Antimicrobial assessment of the four disinfectants at 100% concentration showed that EDN[®] Hand Sanitizer has the highest inhibition against *S. typhi*, *S. aureus* and *E. coli* ($53.00^a \pm 1.41$ mm, $53.00^a \pm 0.71$ mm and $48.00^b \pm 0.71$ mm respectively: $P < 0.05$) compared with other disinfectants. However, Jik disinfectant has the highest inhibition against *C. albicans* (76.00 ± 1.41 mm: $P < 0.05$) while Z-germicide was second in inhibition against *C. albicans*. Izal was least in antimicrobial activity with 18.00 ± 0.71 mm as highest value for the bacterial isolates (against *E. coli*) and 10.00 ± 0.71 mm for *C. albicans*. Although Izal killed *S. aureus* at 100% concentration, it did not do so at 1:2 dilution (Fig. 4). This low inhibition shown by Izal could be due to the development of resistance by the test microbial isolates over time.

It's noteworthy that the four disinfectants showed antimicrobial activities against the four test isolates at 100% concentration.

The Phenol coefficients (Pc) of the four disinfectants against the four test isolates showed that EDN[®] Hand Sanitizer has the highest Pc value for the four isolates with the greatest value of "7" against *E. coli* and *C. albicans* respectively and the lowest Pc of 6.5 against *S. typhi*. Jik has "0" Pc against *S. aureus* while Izal had "0" Pc against *S. aureus* and *C. albicans* respectively. It was only *S. aureus* (Gram positive bacterium) that two disinfectants (Jik and Izal) had zero Pc against. This could be attributed to the thick cellwall of the isolate with characteristic thick peptidoglycan layer which offers strong resistance to the penetration of antimicrobials like disinfectants.

4.0 CONCLUSION

The potency of disinfectants is very important to enhance their antimicrobial activities toward controlling microbial population which includes prevention of diseases transmission and infection. The test disinfectants in this study have been confirmed to be very effective when compared with a standard phenol by high phenol coefficient value; also the results of the work now demonstrate that they have both antibacterial and antifungal activities. 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 is observed to the most susceptible to JIK even when compared with other dilutions used. Izal was found to be lowest in antimicrobial potential ranking. The use of good disinfectants should be encouraged to reduce cases of skin and wound infections caused by most microorganisms.

4.1 RECOMMENDATIONS

In view of the importance of disinfection in domestic hygiene, and the danger of development of resistance by the organisms exposed to the disinfectant, it will be in the overall interest to ensure that only fresh preparations of disinfectants should be used routinely and dilution should be restricted to the concentrations ranges that have been found to have definite activity against the organisms. It is recommended for further studies the determination of antimicrobial activities of these disinfectants in various combination ratios with the aim of finding out the possibility of synergy in their actions.

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REFERENCES

1. Aboh, M. I., Oladosu P., Ibrahim, K (2003) Antimicrobial activity of some brands of household disinfectants marketed in Abuja municipal area council, federal capital territory, Nigeria. *American Journal of Research communication*, 1(8): 172-183.
2. Bellcham, J., Haris, J.M., P. J. Gaya, H and Pepper, J. R. (1999) A prospective study of wound infection. *Journal of cardiothorac Surgery*. 15(1): 45-50.
3. Cheesbrough, M (2006) *District Laboratory Practice in Tropical Countries*, 2nded, New York: Cambridge University Press, 38-39.
4. Cheesbrough, M (2010) *District Laboratory Practice in Tropical Countries*, part 2, 5th edition update, New York. Cambridge University press, 157-266.
5. Ewart, S. L (2001) Disinfectants and control of environmental contamination. In: (Smith BL. Editor) *Large Animal Internal Medicine: diseases of horses, cattle, sheep and goats*. 3rd edition. St. Louis: Mosby. 1371-1380.
6. Moorer, W. R (2003) Antiviral activity of alcohol for surface disinfection. *International Journal of Dental Hygiene*, 3: 138-142.
7. Okore, C. C., Mbanefo, O. N., Onyekwere, B. C., Onyewajo, S. C., Ozurumba, A. U and Abba-Father, C. A. M (2004) Antimicrobial Efficacy of Selected Disinfectants, *American Journal of Biology and Life Science*. 2(2): 53-57.
8. 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. *J. of Asian Scientific Research*, 2(1): 65-68.
9. Quinn, P. J and Markey, B. K (2001) Disinfection and disease prevention in veterinary medicine. In: Block SS, editor. *Disinfection, sterilization and preservation*. 5th edition. Philadelphia: Lippincott, Williams & Wilkins. 1069-1103.
10. 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.
11. Sridhar, R. P. N. (2012) Testing of disinfectants. *India Journal of medical Sciences*, 30: 50-80.

12. Tortora, G. J., Funke, B. R and Case, C. J (2004) *Microbiology: An Introduction*, 8th ed., New York: Pearson Education and Dorling Kindersley, Ltd, 224-225.
13. Wilson, A.P.R., Gibbons, C., Raeves, B. C., Hogson, B., Lius, M and Plummer, D. (2004). Surgical wound infections as a performance abdicator agreement of common disinfection of wound infections in patients. *American Journal of Infection Control*, 329: 720-925.