Antimicrobial Activities of Some Brands of Household Disinfectants Marketed In Abuja Municipal Area Council, Federal Capital Territory, Nigeria

Aboh M. I.,* Oladosu P. and Ibrahim K.

Department of Microbiology and Biotechnology,
National Institute for Pharmaceutical Research and Development, Idu. P.M.B 21 Garki, Abuja.
*Corresponding author: Aboh Mercy I.
Tel: +2348061586188
E mail: itohan07@yahoo.com
Fax: 234 9 5231043

ABSRACT

The antibacterial activities of five brands of household disinfectants were obtained from different locations within the Federal Capital Territory, Nigeria and comparatively studied using the Rideal Walker Phenol coefficient test and quantitative suspension test. The quantitative suspension test was carried out at the recommended concentrations of the manufacturers for household and utensil disinfection. The active compounds of the products according to their respective labels were: D1-(Chloroxylenol 4.8%), D2-(Dichloroxylenol 2%), D3- (Chlorhexidine gluconate 0.3% and cetrimide 3%), D4 -(Dichlorometaxylenol 2.5%), D5-(Chlorhexidine gluconate 0.3% and cetrimide 3%). The test organisms were clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella aerogenes and Escherica coli*. The phenol coefficient of the disinfectants ranged between 5.0 – 9.0. All the disinfectants showed strong bactericidal effect against the organisms used with exception to *Pseudomonas aeruginosa* to which only D5 and D3 were effective.

Key words: Antibacterial activity, Disinfectants, Neutralizer

{Citation: Aboh M. I., Oladosu P., Ibrahim K. Antimicrobial activities of some brands of household disinfectants marketed in Abuja municipal area council, federal capital territory, Nigeria. American Journal of Research Communication, 2013, 1(8): 172-183} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Disinfectants, antiseptics and preservatives are chemicals which have the ability to destroy or inhibit the growth of microorganisms and which are used for this purpose. Disinfection is the process of removing microorganisms including potentially pathogenic ones, from the surface of inanimate objects. The British Standards Institution further defines 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. Antiseptics and disinfectants have been found useful in many hospitals, clinics, homes and laboratories either as chemical agents to disinfect inanimate objects like cleaning of floors, disinfection of water and surgical instruments.[1]

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 nosocomical infections. [2, 3]

The emergence of resistant microorganisms in hospitals and the community is causing problems for both the treatment of patients and infection control. Organisms of particular concern include methicillin- resistant *Staphylococcus aureus*, glycopeptide resistant enterococci and extended spectrum beta-lactamase producing *Klebsiella* .[4]

All these organisms are transferred from patient to patient on staff hands. A recent major review of antibiotic resistance emphasized the importance of hospital infection control, and the control of these organisms, and many authorities have reiterated the key role of hand washing with disinfectants.[5]

The objective of this study was to assess the antibacterial activities of some common brands of household disinfectants marketed in the Abuja Municipal Area Council (AMAC), of The Federal Capital Territory (FCT), Nigeria and to further determine the time required by the disinfectants to kill these organisms as well as their spectrum of antibacterial activity.

Materials and Methods

Organisms

Clincal isolates of four commonly encountered organisms were used; *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella aerogenes and Escherica coli.*

Collection of samples

Samples of commonly used household disinfectants were procured from different locations within the Abuja Municipal Area Council (AMAC) Federal Capital Territory (FCT), Nigeria. The samples were labelled D1- D5 and transferred to the microbiology laboratory in their original packages and the contents aseptically withdrawn from the bottles for antimicrobial study. The contents of the samples were as follows: D1 (Chloroxylenol 4.8%), D2 (Dichloroxylenol 2%), D3 (Chlorhexidine gluconate 0.3% and cetrimide 3%), D4 (Dichlorometaxylenol 2.5%), D5 (Chlorhexidine gluconate 0.3% and cetrimide 3%).

Preparation of inocula

Organisms were grown overnight at 37°C in Nutrient broth (BHI; Oxoid, Basingstoke, UK). The overnight broth culture of organism was diluted in nutrient broth to an inoculum load of approximately $1x10^6$ cfu/ml. It was standardized according to National Committee for Clinical Laboratory Standards (NCCLS, 2002) by gradually adding

normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0 × 106 cfu/ml[6].

Rideal Walker Phenol Coefficient Test

Rideal-Walker Phenol Coefficient Test was used to determine the efficacy of the disinfectants. 5mls of samples containing different dilutions of phenol (1:90, 1:95, 1:100, 1:105 and 1:110) and the test disinfectants (1:100, 1:500 ,1:640, 1:720 and 1:800) and (1:1000) for D5 each was inoculated with *Staphylococcus aureus.* At 5, 10 and 15 min intervals 0.1ml samples of the dilutions were withdrawn and transferred into fresh nutrient broth and incubated at 37^{o}C for 48 hrs. The phenol coefficient was determined as the ratio of the reciprocal of the highest dilution of disinfectant that prevented growth at 10 min and not 5 min to that of phenol.

Antimicrobial activity testing

The quantitative suspension test as developed for bactericidal evaluation of various disinfectants in timed experiments (Bohdan et. al. 1987)[7] was the adopted method. A 2.5mls of the overnight broth culture of organism was mixed with 37.5mls of disinfectant solution yielding 40 mls of the use dilution concentration containing viable cells. For controls, 2.5 mls of the cell suspension was added to 37.5 mls of sterile distilled water. The use dilutions of the disinfectants were as recommended by manufacturer for household and utensil disinfection. The bacterial count in the solution at zero time was 1x10^{6} CFU/mL. After exposure time of 5, 10, 15, 20 and 30 min, 4mls of suspension was withdrawn and mixed with 9mls of neutralizer (0.5% Tween80 in nutrient broth) [8] , the mixture was vortex mixed for 10s and allowed to stand for 1min in order to inactivate any residual disinfectant. 0.1 ml of suspension was inoculated on nutrient agar

plate. The plates were made in duplicates and incubated aerobically at 37 °C for 24 h. The experiment was repeated twice and viable count calculated to give cfu/ml.

Result

The results of the phenol coefficient test of the disinfectants against S*taphylococcus aureus* are presented in tables 1 – 6. D5 had the highest phenol coefficient of 9. It was the nine times more effective than phenol. This was followed by D3 with phenol coefficient of 8. D1 and D2 were 6.4 times more effective than phenol. D4 had the least phenol coefficient of 5. (Table 7)

Rideal Walker Phenol Coefficient Test

Table 1: Bactericidal efficiency of Phenol against staphylococcus aureus

Dilution	Time (min)		
	5	10	15
1:90	-	-	-
1:95	-	-	-
1:100*	+	-	-
1:105	+	+	-
1:110	+	+	+

+ = growth, - = no growth and * critical dilution for phenol coefficient

Table 2: Bactericidal efficiency of D1 against staphylococcus aureus

Dilution	Time (min)		
	5	10	15
1:100	-	-	-
1:500	-	-	-
1:640*	+	-	-
1:720	+	+	-
1:800	+	+	+

+ = growth, - = no growth and * critical dilution for phenol coefficient

Table 3: Bactericidal efficiency of D2 against staphylococcus aureus

Dilution	Time (min)		
	5	10	15
1:100	-	-	-
1:500	-	-	-
1:640*	+	-	-
1:720	+	+	-
1:800	+	+	+

+ = growth, - = no growth and * critical dilution for phenol coefficient

Table 4: Bactericidal efficiency of D3 against staphylococcus aureus

Dilution	Time (min)		
	5	10	15
1:100	-	-	-
1:500	-	-	-
1:640	-	-	-
1:720	-	-	-
1:800*	+	-	-
1:900	+	+	-

+ = growth, - = no growth and * critical dilution for phenol coefficient

Table 5: Bactericidal efficiency of D4 against staphylococcus aureus

Dilution	Time (min)		
	5	10	15
1:100	-	-	-
1:500*	+	-	-
1:640	+	+	-
1:720	+	+	+
1:800	+	+	+

+ = growth, - = no growth and * critical dilution for phenol coefficient

The results of the quantitative suspension test are presented in Tables 8 – 11. The disinfectants (D1-D5) were found to be very active against growing cells of *Escherica coli* and *Staphylococcus aureus.* Within 5 minutes of exposure, there was a total reduction in the viable bacterial counts (Tables 8 and 11). This was also achieved by D1,

D2, D3, and D5 on *Klebsiella aerogenes*, as no viable cell survived after 5min of exposure to the disinfectants. D4 on the other hand, did not eliminate all the cells until after 10min, as $1x10^3$ cells survived after 5min exposure time. (Table 9)

Table 6: Bactericidal efficiency of D5 against staphylococcus aureus

Dilution	Time (min)		
	5	10	15
1:100	-	-	-
1:500	-	-	-
1:640	-	-	-
1:720	-	-	-
1:800	-	-	-
1:900*	+	-	-
1:1000	+	+	-

+ = growth, - = no growth and * critical dilution for phenol coefficient

Table 7: Phenol Coefficient of disinfectants

Sample	Phenol co efficient
D1	6.4
D2	6.4
D3	8.0
D4	5.0
D5	9.0

Pseudomonas aeruginosa was not susceptible to most of the disinfectants (Table 10). D1 was ineffective against the cells at 5min and 10min of exposure however after 15min all the cells were eliminated. D3 had similar behaviour like D1 as the cells were totally destroyed after 15min. D2 was totally ineffective on *Pseudomonas aeruginosa* even after 30min. D2 and D4 were the most effective on *Pseudomonas aeruginosa*, as all the cells were destroyed after 5min of interaction time.

Antimicrobial Activity Testing

Table 8: Survival of *Staph. aureus* in the presence of disinfectants (in-use dilution)

Sample	Colony counts after minutes (viable cells/ml)				
	5	10	15	20	30
D1	0	0	0	0	0
D2	0	0	0	0	0
D3	0	0	0	0	0
D4	0	0	0	0	0
D5	0	0	0	0	0
Control	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$

Table 9: Survival of *Klebsiella aerogenes* in the presence of disinfectants (in-use dilution)

Sample	Colony counts after minutes (viable cells/ml)				
	5	10	15	20	30
D1	0	0	0	0	0
D2	0	0	0	0	0
D3	0	0	0	0	0
D4	$6.0x10^1$	0	0	0	0
D5	0	0	0	0	0
Control	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$

Table 10: Survival of *Pseudomonas aeruginosa* in the presence of disinfectants (in-use dilution

Sample	Colony counts after minutes (viable cells/ml)				
	5	10	15	20	30
D1	$2.0x10^1$	$1.0x10^1$	0	0	0
D2	$8.8x10^2$	$6.5x10^2$	$4.8x10^2$	$6.1x10^2$	$2.3x10^2$
D3	0	0	0	0	0
D4	$5.0x10^1$	$2.0x10^1$	0	0	0
D5	0	0	0	0	0
Control	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$

Table 11: Survival of *Escherica coli* in the presence of disinfectants (in-use dilution)

Sample	Colony counts after minutes (viable cells/ml)				
	5	10	15	20	30
D1	0	0	0	0	0
D2	0	0	0	0	0
D3	0	0	0	0	0
D4	0	0	0	0	0
D5	0	0	0	0	0
Control	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$

All the disinfectants had bactericidal activity against both Gram positive and Gram negative bacteria. D3 and D5 had the broadest spectrum of activity when compared to others as they totally killed all the bacteria cells after 5min interaction time. D1 and D4 had intermediate spectrum of activity while D2 had the least spectrum of activity as it had no activity on *Pseudomonas aeruginosa* within the 30min exposure time used in the experiment.

Discussion

In this study, the quantitative suspension test method was used as described above to test the bacteria against disinfectants at their recommended use dilution concentrations in timed experiments.[6]

D1 (Chloroxylenol 4.8%), a phenolic compound was very effective against viable cells of *Escherica coli, Klebsiella aerogenes* and *Staphylococcus aureus.* These organisms were killed after 5min interaction time. It also reduced the viable counts of *Pseudomonas aeruginosa* from $1x10^6$ to $1x10^1$ cfu/ml after 10min interaction time but by the 15^{th} min all the cells were killed as the viable count dropped to zero. D1 had activity against both Gram positive and Gram negative bacteria. D1 can be said to have an intermediate spectrum of activity, when compared to other disinfectants assayed in this study.

D2 (Dichloroxylenol 2%), a phenolic compound had the least activity on the bacteria cells. It was ineffective against *Pseudomonas aeruginosa* even after 30min of exposure time. Although it was effective against *Escherica coli, Staphylococcus aureus* and

Klebsiella aerogenes after 5min interaction time, its inability to destroy viable cells of *Pseudomonas aeruginosa* makes its use questionable in disinfection if a 30min interaction time is desired. Several workers have reported the resistance of *Pseudomonas aeruginosa* to some known disinfectants which has raised concern in the choice of disinfectants.[8] The poor activity of this product could be due to an inadequacy of the in use dilution (0.3%) recommended by the manufacturer for utensil disinfection. It is therefore advisable to either reduce the dilution factor or increase the disinfectant – surface interaction time.

D4 (Dichlorometaxylenol 2.5%), another phenolic compound effectively killed viable cells of *Escherica coli* and *Staphylococcus aureus* within 5min of interaction time. *Klebsiella aerogenes* and *Pseudomonas aeruginosa* was not totally eliminated till after 15min of interaction between the bacteria and disinfectant. After 10min the viable count was reduced from $1x10^6$ to $2x10^1$ for *Pseudomonas aeruginosa* and $6x10^1$ for *Klebsiella aerogenes* after 5min of interaction time.

D3 and D5 (Chlorhexidine gluconate 0.3% and cetrimide 3%), quaternary ammonium compounds, were the most effective with the broadest spectrum of activity against the bacteria tested. They effectively killed all the bacteria within 5min of interaction. Compared to the phenolics these products can be recommended for proper disinfection of household utensils. Soliman *et al* (2009) [9], evaluated the antibacterial activities of some disinfectants and reported 100% effectiveness of TH4 (combination of a quaternary

ammonium and glutaraldehyde) against *Staphylococcus aureus*, *Escherica coli*, *Klebsiella oxytoca* and *Psuedomonas aeruginosa* after 10, 5, 20 and 20 min respectively.

Conclusion

In conclusion the results of this study showed that all the disinfectants tested showed strong bactericidal effect against the organisms used with exception to *Pseudomonas aeruginosa* to which only D5 and D3 were effective.

Acknowledgement

We wish to use this medium to appreciate the management and staff of the department of Microbiology and Biotechnology, NIPRD for their assistance during the course of this work. We also appreciate Dr. Yakubu Ngwai who took out time to review and edit this work. Our appreciation also goes out to Dr. Emeje Martins for His immense contributions towards the success of this work.

Reference

1. Onaolapo, J.A. (2001): Effects of sub- recommended dilutions of Savlon^{R} on the pathogenicity of *P. aeruginosa*. J.P.T 6 (2): 116.
2. Burdon, D.V and Whitby, J.L (1967): Contamination of hospital disinfectants with *Pseudomonas spp*. Brit. Med. J. 2: 153-157.
3. Segbetan, A,O., Alausa, K.O and Sobayo, E.L (1990): In – use quality control of hospital antiseptics. J. Med and Pharm. Marketing 4: 251-253.

4. French, G.L and Phillips, I (1996): Antimicrobial resistance in hospital flora and nosocomical infection. Hospital Epidemiology and infection control, Williams and Wilkins, Baltimore, MD (May hall,C.Ed), pg 980-990.
5. Hand washing liaison group. (1999): Hand washing a modest measure with big effects. Brit. Med. J. 318, 686.
6. National Committee for Clinical Laboratory Standard (2002). Performance standard for antimicrobial disc susceptibility testing. Twelfth International Supplement.
7. Bohdan Terleckyj and David A. Axler (1987): Quantitative neutralization assay of fungicidal activity of disinfectants. Antimicrobial agents and chemotherapy, 31(5):794-798.
8. Newman, K. A., Tenney, J. H., Oken, H. A. Moody, M. R. Wharton, R. and Schimpff S. C. (1984). Persistent isolation of an unusual Pseudomonas species from a phenolic disinfectant system. Infect. Control (Thorofare) 5:219-222.
9. Soliman, E. S.; Sobeih, M. A. A.; Ahmad, Z. H.; Hussein, M. M.; Moneim, H. A. A. (2009). Evaluation of commercial disinfectants against bacterial pathogens isolated from broiler farms. International Journal of Poultry Science 8(8): 728-732.