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Original Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITIES OF SELECTED DISINFECTANTS SOLD IN OGIGE MARKET IN NSUKKA, NIGERIA, ON SOME CLINICAL BACTERIAL ISOLATES

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ABSTRACT

Disinfectants are chemicals that can kill or inhibit the growth of microorganisms and are widely used in hospitals and in households. This study was aimed at evaluating the antibacterial activity of six household disinfectants (D1, D2, D3, D4, D5 and D6) used in Nsukka, Enugu Nigeria on four clinical bacterial isolates including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The test organisms used were obtained from Medical Diagnostic Laboratory of University of Nigeria, Medical Centre, Nsukka. Dilutions of each disinfectant were prepared. The antibacterial activities of various dilutions of the disinfectants against the test organisms were determined using well diffusion technique. Phenol coefficient of each disinfectant was determined using Rideal-Walker method. Dilutions of phenol (1:80, 1:90 and 1:100) and disinfectants (1:400, 1:450 and 1:500) were prepared. Test organisms (0.1 ml suspension) were inoculated into different dilutions of the disinfectants and phenol. At intervals of 5, 10 and 15 minutes, 0.1 ml was taken from each dilution and inoculated into nutrient broth (2 ml) and incubated for 48 h at 37 ^{o}C. All experiments were carried out in duplicates. D3 with active ingredients (chlorhexidine gluconate (0.3%) and cetrimide (3%)) showed the highest inhibition against all the test organisms. D4 with active ingredient (Dichloro-meta-xylenol) showed the least inhibition against all the test organisms followed by D6 with Lysol as active ingredient. The phenol coefficient of the disinfectants ranged from 0-5.5 for the test organisms. All the disinfectants exhibited antibacterial activity and the most active disinfectant contains chlorhexidine gluconate and cetrimide.

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INTRODUCTION

Disinfectants are chemical agents used to inactivate or kill microorganisms on the surfaces of living or non-living objects to eliminate them from the environment. They are used for a variety of topical and hard surface applications in hospitals and other healthcare settings [1]. Disinfectants play a crucial role in infection control measures in helping to prevent transmission of infections in hospital and other healthcare environments. They are mostly used to sanitize fomites in outpatient department (OPD), wards and operation theatres. They are also used in households for cleaning surfaces and bathing. The primary purpose of disinfectants is to eliminate pathogenic organisms from inanimate objects [2].

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Disinfectants are an essential component of infection control procedures and help in the prevention of hospital acquired infections [3]. Growing concerns about the possibility of microbial contamination and the subsequent health risks in food and general consumer products has led to increase in public usage of antiseptics and disinfectants [3]. These products contain antimicrobial substances, which have been used for antisepsis, disinfection, and preservation for centuries [4].

Alcohols, quaternary ammonium compounds, hypo-chlorides, iodine, bromine, pine oils, peroxide, or phenolic compounds are examples of various types of disinfectants. However, their mechanism of action and range of organisms controlled varies among them [5]. Their mechanism of action varies from protein coagulation in bacteria, destruction of their lipids, nucleic acids, cytoplasmic membrane or by removal of a sulphonhydric group from the microbial cells [6, 7]. The British Standards Institution defined disinfection as not necessarily killing all the organisms but reducing them to a level which is neither harmful to health nor to the quality of perishable goods [7]. What is important then is the selection of disinfectants because microorganisms differ in their response to various disinfectants [8]. There is need for constant evaluation of consumer products to ascertain their quality and avoid risks posed by substandard products. There was no report on evaluation of disinfectants sold in Nsukka metropolis. Therefore, the present study was aimed at evaluating the antibacterial activities of the selected common disinfectants sold at Ogige Market at Nsukka, Enugu State, Nigeria and their phenol coefficients against some clinical bacterial isolates.

MATERIALS AND METHODS

Collection of Disinfectant Samples

Samples of six commonly used household disinfectants were randomly selected from Ogige Market at Nsukka, Enugu State, Nigeria. The disinfectants were designated as D1, D2, D3, D4, D5 and D6.

Collection of Test Organisms

The test organisms used for the antibacterial activity are: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Pure cultures of these organisms were obtained from Medical Diagnostic Laboratory of University of Nigeria, Medical Centre, Nsukka. The bacterial isolates were sub-cultured on appropriate media (Mannitol Salt agar for *S. aureus*, MacConkey agar for *E. coli*, Cetrimide agar for *P. aeruginosa* and Blood agar for *B. subtilis*). The isolates were sub-cultured on agar slants and stored at 4 ^{o}C in the refrigerator until needed for further studies.

Preparation of Test Inoculum

The test organisms were grown overnight at 37 ^{o}C on appropriate media in order to obtain fresh pure culture. Using the method described by Vandepitte *et al.* [9], each test inoculum was standardized. Saline solution was prepared by dissolving 8.5 g of sodium chloride (NaCl) into 100 ml of water and sterilized by autoclaving at 121°C for 15 minutes. Thereafter, 4 ml of the solution was dispensed into sterile test tubes. Using sterile wire-loop, each test organism from the overnight grown culture was transferred into a separate test tube containing the saline solution and properly labelled. The turbidity was adjusted by adding more of either test organism or normal saline solution until it got to McFarland standard of 0.5 which is approximately $1.0x10^6$ CFU/ml.

Preparation of Disinfectants

Increasing dilutions of the disinfectants were prepared employing serial dilution method. First, 5 ml of distilled water was dispensed into a set of test tubes for each disinfectant. Thereafter, 5 ml of disinfectant was transferred to the first tube and then mixed thoroughly to give a dilution of 1:1. From the first test tube, 5 ml was transferred to the second test tube to give a dilution of 1:2, and in this order the process was continued to get dilutions of 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512 and 1:1024. This procedure was repeated for D1, D2, D3, D4, D5 and D6. Distilled water was used as a control.

Antibacterial Activity using Agar Well Diffusion Method

The antibacterial activities of dilutions of the disinfectants against the test organisms were determined using well diffusion technique according to the Clinical and Laboratory Standard Institute [10] on Muller-Hinton agar medium. Mueller-Hinton agar (15-20 ml) was poured into sterile petri dishes and allowed to solidify and properly labelled. Each clinical bacterial isolate was then streaked on agar surface of plate using a sterile swab stick. Thereafter, a sterile cork-borer (6 mm) was used to make wells on the inoculated plate. A total of five wells were made on each plate, one for each dilution of a disinfectant and duplicate plates were made for each dilution of a disinfectant. Dilutions: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512 and 1:1024 were used for all the disinfectants. The disinfectant dilutions were delivered into the respective wells using sterile micropipettes. Thereafter the plates were allowed to standby for 30 minutes before incubation at 37 °C for 24 h. Zones of growth inhibition were measured in millimeters using transparent metric rule.

Determination of Phenol Coefficient (Pc) of the Disinfectants

Rideal-Walker Phenol Coefficient Test Method was used to determine the phenol coefficient (Pc) of the disinfectants [11]. Different dilutions of the phenol stock solution were made (1:80, 1:90 and 1:100) in sterile test tubes. Thereafter, 0.1 ml each of 24 h old suspension of each test organism which was adjusted to McFarland standard was delivered into each of the phenol dilutions and then thoroughly mixed. After time intervals of 5 minutes, 10 minutes and 15 minutes, an aliquot (0.1 ml) of each of the dilutions was taken and then

inoculated into tubes containing 2 ml of sterile nutrient broth. This procedure was carried out for each of the disinfectants using dilutions 1:400, 1:450 and 1:500. Thereafter the inoculated tubes were incubated at 37 ^{o}C for 48 h and then growth (turbidity) was measured. Phenol coefficient (Pc) for each of the disinfectants was determined as the ratio of the reciprocal of the highest dilution of disinfectant that prevented growth in 10 minutes to that of phenol that prevented growth at the same time.

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RESULTS

Table 1 shows the selected disinfectants designated D1-D6 and their active ingredients.

Figures 1-6 show the relative susceptibility of test organisms; *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis* to the disinfectants (D1, D2, D3, D4, D5 and D6). All the disinfectants showed inhibitory effects on all the test organisms but at varying dilutions.

From Figure 1, D1 showed inhibitory effect against all the test organisms at dilution of 1:16. Only *Pseudomonas aeruginosa* was inhibited at dilution of 1:32. This means that *Pseudomonas aeruginosa* was most susceptible to D1 and *Bacillus subtilis* being the least susceptible comparatively.

From Figure 2, D2 showed inhibitory effect to *Pseudomonas aeruginosa* at dilution of 1:128 and at dilution 1:64 *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* were inhibited except *E. coli. E. coli* was only inhibited at dilution of 1:32 being the most resistant.

From Figure 3, D3 showed inhibitory effect to *Pseudomonas aeruginosa* at dilution of 1:256 and at dilution of 1:128 all the test organisms except *E. coli* were inhibited. However, all the test organisms were inhibited at dilution of 1:64, *E. coli* being the most resistant.

From Figure 4, D4 showed inhibitory effect to *E. coli* and *P. aeruginosa* at dilution of 1:4 but all the test organisms were inhibited at dilution 1:2, *B. subtilis* being the least inhibited.

From Figure 5, D5 showed inhibitory effect against *P. aeruginosa* and *S. aureus* at dilution of 1:16. *B. subtilis* was inhibited at dilution of 1:8 and *E coli* could only be inhibited at dilution of 1:4, *E. coli* being the most resistant.

From Figure 6, D6 showed inhibitory effect against all the test organisms at dilution of 1:4 and *S. aureus* being the most resistant.

Table 1 shows the selected disinfectants designated D1-D6 and their active ingredients. Sample D1 and D2 have the same active ingredients as chloroxylenol but at different concentrations.

Table 2 shows the phenol coefficient (Pc) of the disinfectants against the test organisms. The phenol coefficients (Pc) of the six disinfectants against the test organisms showed that D3 has the highest Pc value for the four test organisms with a value of 5.5 against *E. coli*, *S. aureus* and *P. aeruginosa* and 5.0 against *B. subtilis*. D4 has 0 Pc value against all the test organisms while D6 has 0 Pc value against *S. aureus, P. aeruginosa* and *B. subtilis* but 4.4 Pc value against *E. coli*.

DISCUSSION

D1 and D2 have chloroxylenol as active ingredients, however, at different concentrations of 4.8% and 4.85% respectively. Both disinfectants had inhibitory effects on all the test organisms but at different concentrations (Figures 1 and 2). *P. aeruginosa* was the most susceptible to D1 at dilution 1:32 while for D2 it was at dilution 1:128. This susceptibility at different dilutions is explainable from the higher concentration of the active ingredient in D2 than in D1. *B. subtilis* was the least susceptible to D1 while *E. coli* was least susceptible to D2. Obi *et al.* [7] reported that *E. coli* and *S. aureus* were susceptible to D1 at dilution 1:16 which is of higher concentration than our finding. This difference could be due to differences in strains of test organisms. It could also be from differences in actual concentrations of the active ingredient in the disinfectant, considering that the dates of manufacture and batch are very wide apart (their report being in 2015).

D3 exhibited the highest activity against all the test organisms with *P. aeruginosa* being the most susceptible at dilution 1:256 while *E. coli* was the least at dilution 1:64. This high activity obviously came from the combined effect of chlorhexidine (0.3%) and cetrimide (3.0%) which were the active ingredients contained in D3.

Chlorhexidine is known to have broad-spectrum activity against Gram-positive and Gram-negative bacteria while cetrimide exhibits bactericidal effect against Gram-positive bacteria [12].

D4 was the least effective of all the disinfectants tested against the test organisms. Although it showed antibacterial activity against all the test organisms, this was at higher concentrations (Figure 4) when compared to the other disinfectants tested. Moreover, the concentration of the active ingredient was not stated on the product and, therefore, unknown and could be below expected standard. The active ingredient, Dichloro-meta-xylenol (DCMX) is known to have antibacterial activity against bacterial skin pathogens [13].

D5 (Figure 5) also showed inhibitory activity against the test organisms at relatively high concentration. With the combined effect of phenol (1.25 mg) and halogenated phenol (6.8 mg) *E. coli* was least inhibited. Our findings agreed with the reports of other workers [14, 15] who synthesized halogenated phenols and tested their antimicrobial activities.

D6 (Figure 6) containing lysol as the active ingredient also showed inhibitory activity against all the test organisms but at dilution 1:4 which is at relatively high concentration. Further dilutions did not show any inhibitory effect on all the test organisms. Okore *et al*. [6] reported that D6 (undiluted) had 17 mm (zone of inhibition) against *E. coli* but no inhibition at dilution 1:8 which is the same with our findings. Other workers reported the antimicrobial effects of disinfectants containing Lysol against *S. aureus*, *Salmonella choleraesuis*, *E. coli* O157:H7, *P. aeruginosa* and poliovirus [16], *S. aureus, E. coli, P. aeruginosa and B. subtilis.* [17, 18].

From Table 1, samples D1 and D2 have their active ingredients as chloroxylenol but at different concentrations. Chloroxylenol is one of the most common antimicrobial

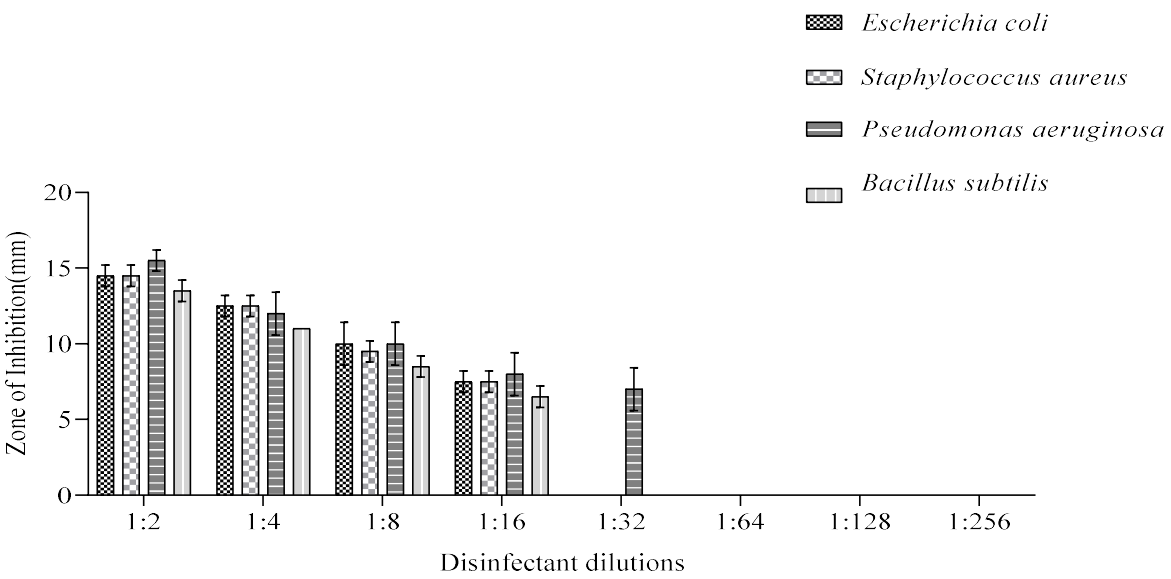

Figure 1: Zone of inhibition of D1 on test organisms at different dilutions

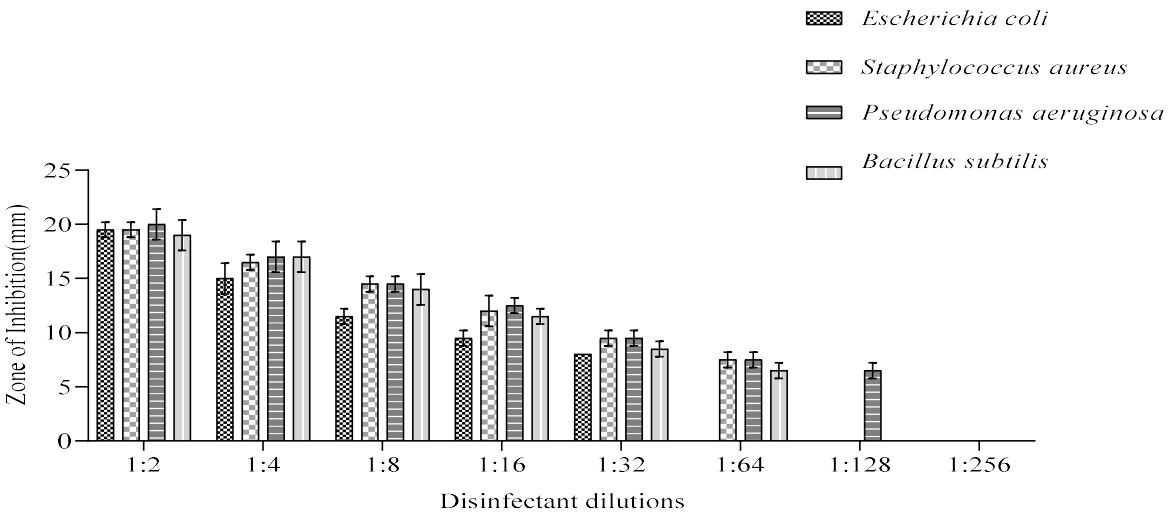

Figure 2: Zone of inhibition of D2 on test organisms at different dilutions

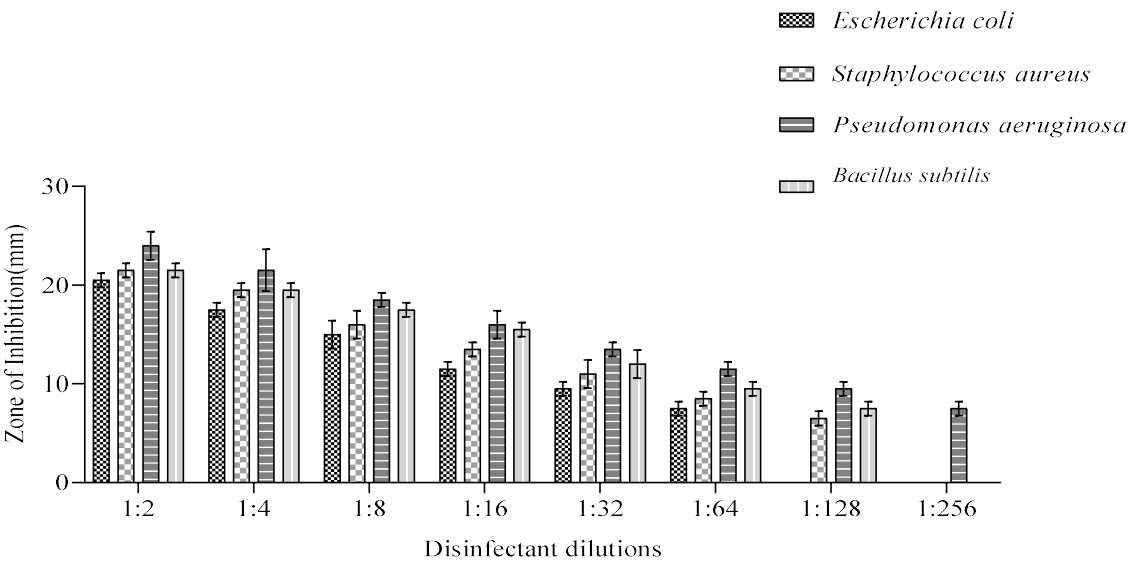

Figure 3: Zone of inhibition of D3 on test organisms at different dilutions

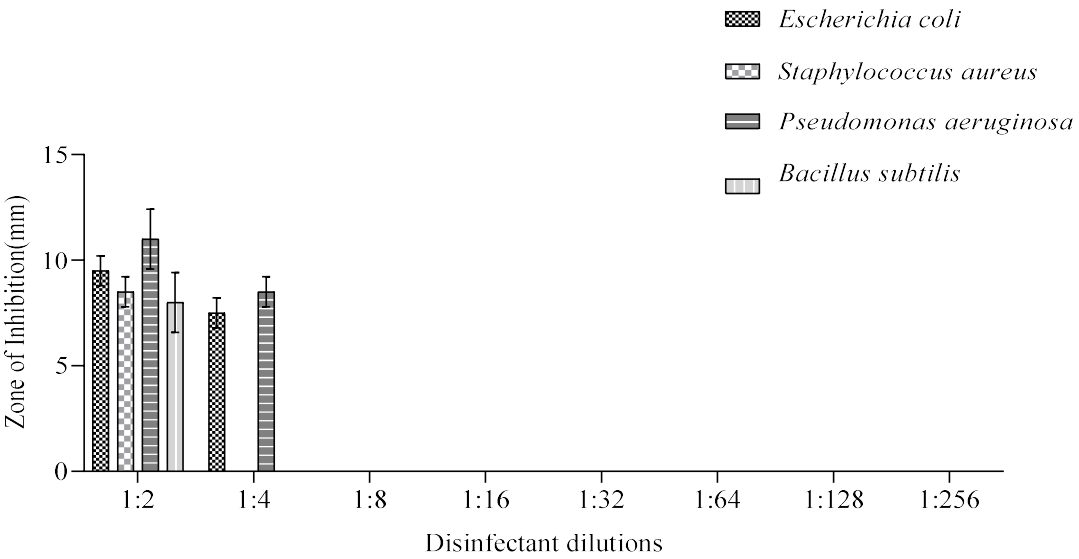

Figure 4: Zone of inhibition of D4 on test organisms at different dilutions

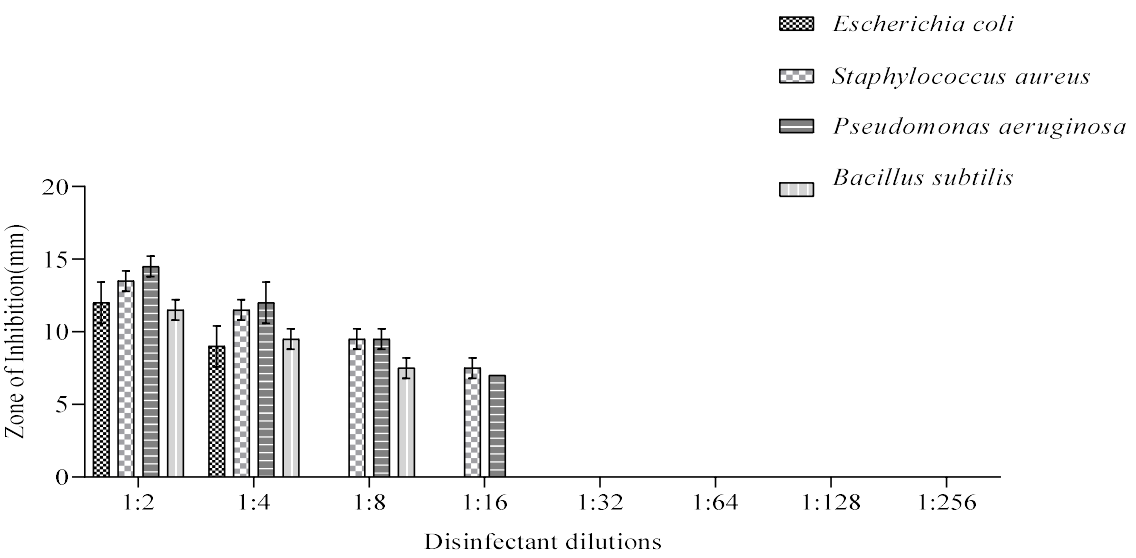

Figure 5: Zone of inhibition of D5 on test organisms at different dilutions

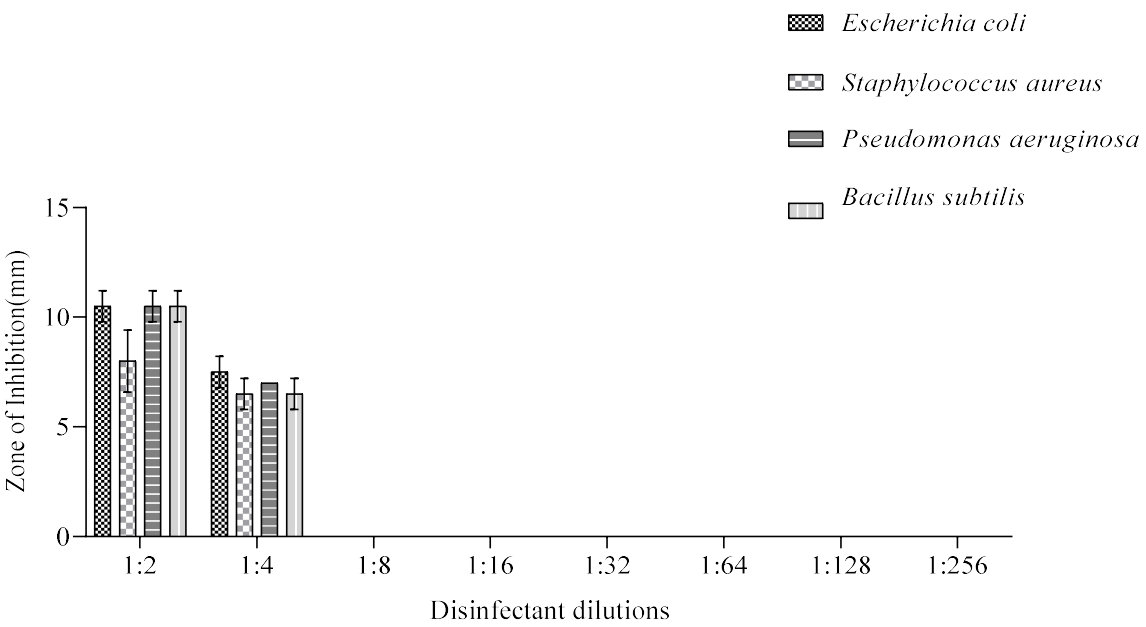

Figure 6: Zone of inhibition of D6 on test organisms at different dilutions

Table 1: Selected disinfectants' active ingredients and their shelf life

Disinfectants	Active ingredient(s)	Concentration	MFD	EXPD
D1	Chloroxylenol	4.8 %	04/2021	04/2025
D2	Chloroxylenol	4.85 %	10/2020	10/2024
D3	Chlorhexidine gluconate	0.3 %	08/2021	07/2024
	Cetrimide	3.0 %		
D4	Dichloro-meta-xylenol	Not stated	08/2021	09/2025
D5	Phenol,	1.75 mg	03/2021	03/2026
	Halogenated phenol	6.8 mg		
D6	Lysol	Not stated	03/2020	04/2023

Legend: MFD = Manufactured date; EXPD = Expiring date

Table 2: The Phenol coefficients (Pc) of disinfectants against the test organisms

Test organism	Disinfectants	Phenol coefficient comparison with phenol
Escherichia coli	D1	4.4
	D2	5
	D3	5.5
	D4	0 (R)
	D5	5
	D6	4.4
Staphylococcus aureus	D1	5
	D2	5.5
	D3	5.5
	D4	0 (R)
	D5	4.4
	D6	0 (R)
Pseudomonas aeruginosa	D1	4.4
	D2	4.4
	D3	5.5
	D4	0 (R)
	D5	5
	D6	0(R)
Bacillus subtilis	D1	5
	D2	5
	D3	5
	D4	0 (R)
	D5	4.4
	D6	0 (R)

Note: R = Resistance

agents widely used in the medical field. Its mechanism of action is by damaging the bacterial cell wall, hence reducing the activity and number of bacteria [12]**.** It is used in hospitals and households for disinfection and sanitation.

Chlorhexidine (introduced as an antiseptic in the mid twentieth century) is a broad-spectrum antibacterial agent and also active against yeasts [19]. Its application to wound is considered generally safe but recommended to be used at the lowest bactericidal concentration of 0.05 % [20].

Cetrimide has bactericidal activity against Gram-positive bacteria but at higher concentrations [21]. It is used in the treatment of minor burns and to prevent infection in cuts, small bruises, chapped hands and nappy rash.

Chlorhexidine gluconate is mainly used in combination with cetrimide for topical antisepsis. The combination can be applied in preoperative skin antisepsis and for disinfection of materials [19].

Dichloro-meta-xylenol (DCMX) is known to have antimicrobial activity against bacteria, fungi and algae [21]. It is utilized as a major constituent of some disinfectant formulations like hand cleaners, surgical cleaners, pre-operative skin sanitizing composition [22]. DCMX is used as an ingredient in pin–type disinfectants and in medicated soaps and hand scrubs [23].

Phenols, originally derived from coal tar, are among the oldest established active disinfectant substances. They have a wide spectrum of activity against bacteria, fungi and mycobacteria [24]. Miklasinska-Majdanik [25] reported that

phenol attacked the cytoplasmic membrane of microorganisms causing the release of intracellular constituents. However, from the time of Lister the use of phenol as active ingredient in compounding antiseptics and disinfectants has steadily declined, most probably due to its low antimicrobial activity and high toxicity. Many phenol derivatives and other more effective antimicrobial compounds have replaced phenol. Nowadays phenol is primarily used to compare the effectiveness of other compounds with antimicrobial activities [26]. Halogenation increases the antibacterial activity of phenols [23]. Halogenated phenols have broad spectrum antibacterial activity but are less effective in the presence of soiling matter. They have very pungent odour and, therefore, they are not used in food items [26]. D5 can be used as a mouth wash when diluted apart from its general use as a disinfectant.

Lysol as a household and industrial cleaning agent was invented in the late 19th century and it has antimicrobial effect against a wide variety of fungi and bacteria [27]. Lysol is a commercial preparation consisting of a 50% mixture of cresol (a member of the phenol group) with linseed oil soap. As an antiseptic it is about 3 times as efficient as phenol [28]. It has been in use since as a household cleaning agent.

Obi *et al.* [7] reported that disinfectants with the same composition as D1 and D6 had 6.25 and 4.0 Pc values respectively against *E. coli,* and against *S. aureus* the same products as D1 and D6 also had "6.25" and 0 Pc values respectively. Our findings only agreed with the Pc value of their D6 equivalent against *S. aureus*. Raut *et al*. [29] also reported that a product with the same composition as D1 had Pc value of 9 against *E. coli* and 8 against *S. aureus* which are higher than our findings. This could be because of the incubation period in determining their phenol coefficient which was 24 h before observing for growth (turbidity) [29]. If the Pc is less than 1 then test disinfectant is less effective than phenol but if the Pc is greater than 1 then test disinfectant is more effective than phenol. Therefore, D1, D2, D3, D5 are all more effective than phenol against all the test organisms while D6 is more effective than phenol against *E. coli* only but ineffective against other organisms. D4 is less effective than phenol against all the test organisms. From the results it is evident that different microorganisms differ in their responses to different types of disinfectants.

CONCLUSION

This study evaluated the antibacterial activities of disinfectants designated D1, D2, D3, D4, D5 and D6 on clinical isolates; *E. coli, P. aeruginosa, B. subtilis and S. aureus* and concluded that all the six common household disinfectants used exhibited antibacterial activity against the test organisms though at different concentrations. D3 with active ingredients**;** - chlorhexidine gluconate (0.3 %) and cetrimide (3 %) showed the highest antibacterial effect against all the test organisms. D4 with active ingredient; - dichloro-meta-xylenol showed the least inhibitory effect on the test organisms followed by D6 with Lysol as active ingredient. The phenol coefficient of the disinfectants ranged from 0-5.5 for the test organisms.

RECOMMENDATION

Among the six disinfectants, D4 and D6 need a regular monitoring because of their low antibacterial activity. Especially, as the concentration of the active ingredient was not stated on D4. It is important that the manufacturers of D4 and D6 should upgrade the constituents of the products to improve their antimicrobial potentials for use as effective disinfectants.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTION

SCE designed and supervised the research and also edited the manuscript; BAE was a student under the first author and carried out the experiments and prepared the manuscript; CKE contributed to editing the manuscript.

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