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Effectiveness of Locally Formulated Unbranded Disinfectants on Three Clinical Bacterial Isolates

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ABSTRACT Antimicrobial effectiveness of seven unbranded locally formulated disinfectants sold in some major markets in Port Harcourt, were tested on three clinical bacterial isolates, which includes; *Streptococcus pneumoniae, Escherichia coli* and *Staphylococcus aureus*. These isolates were obtained from clinical samples collected from the Medical Microbiology and Parasitology Laboratory of the University of Port-Harcourt Teaching Hospital (UPTH). The antimicrobial activity was tested using the well diffusion and minimum inhibitory concentration (MIC) methods. The results indicates that *Streptococcus* sp was the most susceptible of the test organisms, having a zone of inhibition ranging from 0 to 38mm, *E. coli* ranging from 0 to 24mm while *Staphylococcus* sp ranges from 0 to 26mm. Disinfectant B (from Mile 3 market) produced the highest zone of inhibition (38mm) while sample D (from New market Borokiri) produced the least zone of inhibition of 18mm against *E. coli*, 21 mm against *Staphylococcus* sp and 22mm against *Streptococcus* sp, all at 100% concentration. The disinfectants exhibited inhibitory activity against the isolates at a minimum inhibitory concentration (MIC) of 20mg/ml. All the locally formulated disinfectants were effective against the test organisms used with varying degree of effectiveness. When the results of the seven unbranded disinfectants were compared with that of the branded control, only sample B had higher zone of inhibition than that of the branded control at 100% concentration.

Keywords: Antimicrobials, Disinfectant, pathogens, sensitivity, unbranded, zone of inhibition

Introduction

Disinfectants are antimicrobial substances applied on inanimate objects such as instruments (medical or dental), plastics or working structural surfaces (kitchen surfaces, toilets, washbasins, floors etc.), in hospitals, schools, hotels and homes etc. Most of the disinfectants are chemicals which are too toxic to use on human body as they are not selective in their antimicrobial action. Disinfectants play a very important role in infection control practices and aid in the prevention of nosocomial infections. For infection prevention, detergent-based cleaning alone is not sufficient to remove pathogens. The results of investigation conducted by Barker *et al*., 2001 revealed that cleaning with a detergent alone failed to decontaminate tested surfaces in all but one case. They demonstrated that when surfaces were treated with a solution containing 5000 ppm chlorine for 1 minute, noroviruses were only recovered from one surface. Therefore, there's need to disinfectant surfaces after cleaning. The effectiveness of a disinfectant depends on a number of factors, which includes: those inherent to the product, those inherent to the application, and those inherent to the microorganism. Product factors involve concentration formulation, water solubility and pH. For example, the concentration exponent, describing the relationship between dilution and activity of a disinfectant, must be considered, as well as the bioavailability of the substance and its stability. Application factors include the type of surface to be applied, the type of (organic) soil, the temperature and contact time as well as humidity and the method of application (with or without mechanical action) (Maillard, 2005).

The test used is based on the diffusion of the agent from the area of higher concentration (disc) to the area of lower concentration (media). If the organism is sensitive to the disinfectant, it will fail to grow up to the edge of the disc, creating a clear zone called the zone of inhibition around the disc. Microorganisms unaffected by the antiseptic or disinfectant will grow up to the disc. The larger the zone of inhibition does not mean the agent is more effective, only that it inhibits the microorganism. It also does not mean that the agent has a bactericidal effect. The diffusion rate depends on the concentration, and molecular weight of the agent being used.

In a bid to overcome poverty in the country, government and non governmental agencies have initiated different programmes and trainings, aimed at educating the people on the fundamentals of entrepreneurship skills. These programs seek to alleviate poverty and unemployment through the establishment of small and medium scale enterprises. In such programs, people are taught how to make different household washing, cleaning and disinfecting agents. When these products are produced by the individuals, they are usually put in unbranded containers (usually disposed plastic bottles). These un-

branded disinfectants are hawked from place to place and also sold in the local markets. This research is aimed at comparing the effectiveness of these unbranded disinfectants tested against the following clinical isolates: *Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae*.

Materials and Methods

Sample Collection

Clinical samples for the isolation of the test organisms were collected from the Medical Microbiology and Parasitology Laboratory of the University of Port-Harcourt Teaching Hospital (UPTH), Port Harcourt.

Collection of Disinfectant Samples

Disinfectants used for this study were purchased from the major local markets within Port Harcourt metropolis which includes: Mile 1, Mile 3, Creek road, New Market Borokiri, Choba weekly Market, Rumuokoro Market and Oil mill market.

Table 1: Sampling Locations

Sample code	Sampling Location
A	Mile 1
B	Mile 3
C	Creek road market
D	New Market Borokiri
E	Oil Mill market
F	Rumuokoro market
G	Choba market
H	Pharmacy shop(branded control)

Isolation and Identification of Test Organisms

All the test organisms were isolated from ear and wound swabs and urine samples obtained from the medical microbiology lab. Different differential media were used for the isolation of the organisms used. The isolates were confirmed using standard techniques in Biochemical testing of microorganisms and medical laboratory manual for tropical countries (Cheesbrough, 2005). After carrying out the various biochemical tests, the bacterial isolates

were identified according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). These isolates were subsequently maintained on nutrient agar slants at 4^0C in the refrigerator until required for use.

Standardization of Microbial Culture

Loop full of 24 hour old pure culture of the test organisms from nutrient agar plates, were put into sterile normal saline tubes and adjusted to 0.5 McFarland Turbidity Standard. Zero point five McFarland Nephelometer Standard is used in Microbiology as reference to adjust the turbidity of bacterial suspensions such that an approximate cell density of 1.5×10^8 cfu/ml is obtained (Cheesebrough, 2005).

Sensitivity Test

The following test methods were used to evaluate the effectiveness of the disinfectants against the test isolates:

Well-in-agar method

The sensitivity test was carried out using the well-in-agar diffusion method. This method involves the diffusion of antimicrobial agents into medium carefully seeded with the test organisms. Sterile swab sticks were dipped into the standardized inocula of each isolate on separate tubes and drained by pressing the swab stick above the fluid level. The swab sticks were used to streak the surface of already prepared Mueller Hinton agar medium and repeated twice rotating the plates about 60 degrees. Mueller Hinton media plates were allowed to dry for about five minutes. Thereafter, wells were bored in the medium using a sterile 6mm cork borer. The following concentrations of the disinfectants were used: 100%, 50%, 25%, 10% and 5%. The wells were filled with equal concentrations of the different disinfectants transferred into the holes using Pasteur pipette and plates were incubated at 37°C for 24hours in an upright position. Equal volume of sterile distilled water and the branded disinfectant served as negative and positive controls respectively. The procedure was carried out in triplicates. This procedure was repeated for all three test organisms. After incubation, plates were observed and the diameters of zones of inhibition that developed were measured in millimeters. This indicates the degree of susceptibility or resistance of the test organism to the disinfectant (Alekshun and Levi, 2007).

Agar Dilution Method

This method was used to determine the minimum inhibitory concentration of

the disinfectants which exerted the greatest inhibitory activity against the test organisms. Varying concentrations of the disinfectant were prepared (80, 40, 20, 10, and 5) mg/ml(Agwa *et al*., 2012). One milliliter of the standardized organisms and the various concentrations of the disinfectants were put into the sterile tubes of nutrient broth respectively. Tubes containing nutrient broth and organisms without the disinfectant served as negative control while the tube containing only the broth and disinfectant without organism served as positive control. These tubes were incubated at 37^0C for 18 to 24 hours. Thereafter, tubes were examined for visible growth or turbidity in the tubes and recorded. The MIC is the concentration at which no visible growth was observed when compared with the controls (Oke *et al*., 2013).

Results

The isolates used include *Staphylococcus aureus, Streptococcus pneumoniae* and *Escherichia coli*. Which were properly identified as stated above. The results of the antimicrobial activities are shown in Tables 2 to 4. The results indicate that *Streptococcus* sp was most sensitive with the highest zone of inhibition of 38mm at 100% concentration against sample B (from mile 3 market). While sample D (from Rumuokoro market) produced the least zones of inhibition of (*E. coli*, 18mm, *Staphylococcus* sp 21mm and *Streptococcus* sp 22mm respectively) against the isolates tested. Apart from *Streptococcus* sp, sample H (control) also produced higher zones of inhibition against the other test organisms (*E. coli* and *Staphylococcus* sp) at 100% concentration. The zones of inhibition for *E. coli* against the disinfectants ranged from 0 to 26mm, *Staphylococcus* sp has a range of 0 to 28mm while *Streptococcus* sp has a range of 0 to 38mm. Control sample H produced zone of inhibition in all concentrations and against all organisms tested, even when most of the unbranded disinfectants failed to produce zones at 5% concentration. Zones of inhibition produced by disinfectant H, at 5% concentration ranged between 7 to 11mm, while the sterile distilled water control failed to produce any zone of inhibition against all test organisms. For the unbranded disinfectants against *E. coli,* none produced a zone at 5% concentration. At 5% concentration of unbranded disinfectants against *Staphylococcus aureus,* only disinfectant B produced a zone of 7mm, while disinfectant B and E produced zones of 8 and 10mm against *Streptococcus* sp.

Table 2: Effect of the different concentrations of the disinfectants against E. coli

Test organ-ism	Disin-fectants	5%	10%	25%	50%	100%
Esche-richia coli	A(mm)	R	9	14	20	23
	B(mm)	R	7	13	18	26
	C(mm)	R	10	16	19	20
	D(mm)	R	8	12	15	18
	E(mm)	R	10	15	18	22
	F(mm)	R	7	14	19	22
	G(mm)	R	8	13	17	21
	H (control mm)	7	10	15	20	33

Results for each concentration represent mean value of triplicate readings, while R is resistance.

Table 3: Effects of the different concentration disinfectants against Staphylo-coccus aureus

Test or-ganism	Disin-fectant	5 %	10 %	25 %	50%	100%
Staphylococcus aure-us	A(mm)	R	9	13	17	23
	B(mm)	7	12	17	21	28
	C(mm)	R	8	14	18	25
	D(mm)	R	10	15	20	21
	E(mm)	R	8	13	19	24
	F(mm)	R	10	15	18	22
	G(mm)	R	11	15	20	22
	H(mm)	8	15	21	27	34

Results for each concentration represent mean value of triplicate readings, while R is resistance.

Table 4: Activities of the disinfectants against Streptococcus pneumoniae

Test organism	Disinfectants	5%	10%	25%	50%	100 %
Streptococcus pneumonia	A(mm)	R	10	16	20	25
	B(mm)	10	12	17	29	38
	C(mm)	R	11	16	23	28
	D(mm)	R	10	15	20	22
	E(mm)	8	12	18	20	23
	F(mm)	R	8	12	18	27
	G(mm)	R	9	13	20	27
	H(control mm)	11	20	25	29	35

Results for each concentration represent mean value of triplicate readings, while R is resistance.

The MIC test results showed that all the test organisms were sensitive to the different disinfectants but the rate of sensitivity varies with each organism and each disinfecting agents. The results of the MIC are displayed in Table 6, samples B and G exerted the highest activity of 40mg/ml against *Staphylococcus* sp and the lowest of 80mg/ml by samples A, C, D, E and F. Samples B, E, F and G had the highest activity against *Streptococcus* sp at 20mg/ml, Sample A at 40mg/ml and the lowest activity of 80mg/ml for C and D. the highest activity of 20mg/ml of samples B, E, F were observed against *E. coli* while sample G produced highest activity at 40mg/ml and C and D 80mg/ml against *E. coli.*

Table 6: Minimum inhibitory concentration of the disinfectants on the isolates

Organ-isms	Disin-fectants	5mg/ml	10mg/ml	20mg/ml	40mg/ml	80mg/ml
Staphy-lococcus aureus	A	-ve	-ve	-ve	-ve	+ve
	B	-ve	-ve	-ve	+ve	+ve
	C	-ve	-ve	-ve	-ve	+ve
	D	-ve	-ve	-ve	-ve	+ve
	E	-ve	-ve	-ve	-ve	+ve
	F	-ve	-ve	-ve	-ve	+ve
	G	-ve	-ve	-ve	+ve	+ve
Strepto-coccus pneu-moniae	A	-ve	-ve	-ve	+ve	+ve
	B	-ve	-ve	+ve	+ve	+ve
	C	-ve	-ve	-ve	-ve	+ve
	D	-ve	-ve	-ve	-ve	+ve
	E	-ve	-ve	+ve	+ve	+ve
	F	-ve	-ve	+ve	+ve	+ve
	G	-ve	-ve	+ve	+ve	+ve
E. coli	A	-ve	-ve	-ve	-ve	+ve
	B	-ve	-ve	+ve	+ve	+ve
	C	-ve	-ve	-ve	-ve	+ve
	D	-ve	-ve	-ve	-ve	+ve
	E	-ve	-ve	+ve	+ve	+ve
	F	-ve	-ve	+ve	+ve	+ve
	G	-ve	-ve	-ve	+ve	+ve

Key: -ve which means no growth, +ve, indicates growth.

Discussion

The results of the investigation indicated that the different organisms used vary in their response to the different disinfectant samples. Using the disinfectants without diluting produced the highest zone of inhibition, and as the dilution increases the zone of inhibition decreases. From the results obtained, it was observed that all the disinfectants used for this study exerted greater inhibitory activity against *Streptococcus pneumoniae*. Sample B produced the highest zone of inhibition against the test organisms at all concentrations tested. The inhibition of the growth pattern of the isolates indicates the varying ability of the organism to resist the antimicrobial effects of the disinfectants. However, the variations could be due to the differences in the nature and structures of the microbial cell wall since it is the ultimate target of any antimicrobial agent or disinfectants. The disinfectant compounds function by denaturing or disrupting cell activity and interfering with microbial metabolism. All these depend on a number of factors such as the inherent properties of the organisms, the composition of the disinfectants (active ingredient), contact time, concentration of individual formulation and microbial sensitivity (Ikpoh *et al.,* 2012). It was also observed that both the branded and branded disinfectants had a broad spectrum of activity as they all inhibited the growth of Gram positive organisms (*Staphylococcus aureus* and *Streptococcus pneumoniae)* and Gram negative (*E. coli*). The *Streptococcus pneumoniae* is the most susceptible to the disinfectants. Okore *et al*., 2014, also observed in their work that *Streptococcus* sp was the most sensitive to the popular disinfectants in the market tested. The MIC result showed higher activity against the *Streptococcus* sp and *E. coli* while *Staphylococcus* sp had low activity.

Bacterial resistance to the antimicrobial agent can either be inherent to the bacterium or to the bacterial population. In addition, some of the resistance mechanisms may be intrinsic to the microorganism while others have been acquired through forced mutations or through the acquisition of mobile genetic elements (Poole, 2002). The development of bacterial resistance through acquired mechanisms such as mutation and the acquisition of resistant determinants are of concern since a bacterium that was previously susceptible can become resistant to a group of compounds (Russell, 2002). The acquisition of resistance might confer cross-resistance or co-resistance on occasions (Poole, 2004). However, there is little information on the effects of biocides on the transfer of genetic determinants. According to Pearce *et al.,* (1999), some antimicrobial sub inhibitory concentration could inhibit genetic transfer, while others bring about increased genetic efficiency. The disinfectants used showed great antimicrobial activity against these organisms that are associated with human infections. For instance, *E. coli* is a nor-

mal inhabitant of the intestinal tract of humans and other warm-blooded animals, their presence in water is an indication of faecal contamination of the water, which could result in potential health risk because feacal-borne pathogens might also be present, to cause food and water borne diseases. Presence of *Staphylococcus* sp is the leading cause of wound infections (and other community acquired infections), both surgical and accidental; the two most important species are *Staphylococcus aureus* and *Staphylococcus epidermidis*, which survive well in the environment, making their transmission from person to person easy. While *Streptococcus* sp is involved in the cause of throat infection (sour throat). The antibacterial effectiveness of the disinfectants was concentration dependent, with disinfectant B being highly effective against all the organisms tested, while sample D was least effective. Others, A, C, E, F and G showed moderate antibacterial activity. However, right use of disinfectants can help to reduce greatly Nosocomial infections and other infectious diseases, which could be achieved when the disinfectants are properly diluted and after proper cleaning to remove organic matter from surfaces and materials. Over dilution of disinfectant results in decreased effectiveness and also make the pathogenic organisms build resistance to such disinfectants. The major challenge in the use of these unbranded disinfectants is that, since they are not branded getting a very effective one becomes difficult. What could be done is using a very high concentration of the disinfectant during application to ensure the different microbial groups present on the surfaces are destroyed, which is not very economical though. All the disinfectants used in this study showed different level of antimicrobial activity on the different microorganisms. With the level of antimicrobial activity possessed by these disinfectants they can be used to control infections, with disinfectant B the most promising for use in the control and prevention of *Streptococcus pneumoniae* infections.

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