



ANTIBACTERIAL EFFICACY OF SOME LOCALLY PRODUCED DISINFECTANTS ON SOME CLINICAL ISOLATES

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Abstract

The study aimed at evaluating the antibacterial efficacy of some locally produced disinfectants on some clinical isolates. The isolates obtained were identified through the morphology and biochemical tests. The organisms include *Staphylococcus aureus*, *Klebsiella* sp., *Salmonella* sp., and *Escherichia coli*. Antibacterial assay was carried out on test organisms using agar well diffusion method on Mueller Hinton agar and were incubated at 37°C for 24 hours. The results showed zone of inhibition in diameter. For sample A, the zones of inhibition were 33mm, 35mm, 38mm, 28mm on *Staphylococcus aureus*, *Klebsiella* sp., *Salmonella* sp., and *Escherichia coli* respectively. Sample B was 20mm, 18mm, 27mm, and 30mm on *Staphylococcus aureus*, *Klebsiella* sp., *Salmonella* sp., and *Escherichia coli* respectively. Sample C had a zone of inhibition of 36mm, 25mm, 20mm, and 23mm on *Staphylococcus aureus*, *Klebsiella* sp., *Salmonella* sp., and *Escherichia coli* respectively. Sample D showed a zone of inhibition of 35mm, 32mm, 31mm, and 35mm on *Staphylococcus aureus*, *Klebsiella* sp., *Salmonella* sp., and *Escherichia coli* respectively. This result showed that sample A and D had more antibacterial effect against the Gram positive and Gram negative organisms used while sample B and C were less effective on them.

Keywords: Antibacterial efficacy, locally produced disinfectants, clinical isolates, *Staphylococcus aureus*, *Klebsiella* spa.

INTRODUCTION

Disinfectants are chemical agent that eliminate undesirable microorganisms from inanimate environmental surfaces, because such surfaces are inanimate, they are considered contaminated rather than infected (Palezer *et al.*, 2006). Disinfectants are antimicrobial substances applied on inanimate objects such as instruments (medical or dental), plastics or working structural surfaces (kitchen surfaces, toilets, 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. Some studies confirmed that patients who stay in hospitals transmit these bacteria to facilities, which leads to the possibility of the transmission of these microbes to other patients later admitted to these rooms (Donskey, 2013; Eckstein *et al.*, 2007; Ontario Agency for Health protection and promotion, 2012). Proper disinfection techniques by broad spectrum biocidal compounds can prevent nosocomial

infections (Kundrapu *et al.*, 2012). Some studies have concluded that daily disinfection of medical equipment that is used by patients and their accommodation places by effective disinfectants limits the level of contamination by these organisms (Boyce, 2007; Tharaud *et al.*, 2004; John, 2016).

Disinfectants are chemical compounds commonly added to water for use during bath, laundry, mouth washing, wound dressing and other domestic activities such as toilet and general house cleaning (Akimitsu *et al.*, 2000). They are used to control or reduce the growth of pathogenic microbes found on human body (Fraise, 2002). The process can be affected by several factors such as temperature, acidity, concentration of the disinfectant and contact time during the disinfection process (Ferreira *et al.*, 2015). Pathogenic organisms vary in their degrees of response to different detergents, as they constantly acquire resistance to different formulas. The efficacy of disinfectants against targeted pathogens that are selected for the disinfection process must be studied and analyzed through a clinical test (Santos- Junior *et al.*, 2018).

This research is aimed at comparing the effectiveness of these unbranded disinfectants tested against the following clinical isolates: *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella sp.*

The possibility of more microorganism's particularly the nosocomial bacteria further developing resistance to more of these disinfectant formulations, just like they do with antibiotics, underscore the need to constantly evaluate the antimicrobial activities of various chemical disinfectant formulation against the nosocomial agents. Antiseptics are biocides or agents that destroy or inhibit the growth of microorganism in or on living tissue (e.g healthcare personnel hand wash and surgical scrubs); while disinfectants are similar but are used on inanimate objects or surfaces (Mckenry and Salerno, 2011). These agents such as alcohols and other health care settings for infections control and prevention of nosocomial infections (Mac Donnell and Rusell, 2019). Some disinfectants have a wide spectrum (Kill nearly all microorganisms), whilst others kill a smaller range of disease – causing organisms, but are preferred for other properties (they may be non – corrosive, non – toxic or expensive (Pelcza *et al.*, 2013). An ideal disinfectant to overcome the antimicrobial activity (Mandell and Pool, 2015) and the efficacy of these agents may be affected by organic matter, PH, ionic and type of the surfactants, temperature detergent base (Schorerand Eisele, 2017).

Due to the growing spread of bacteria infections, it is necessary to evaluate the effectiveness of disinfectants against pathogens before use and not rely on the information provided by the manufacturing companies to ensure the optimal efficacy (Kawamurasato *et al.*, 2008, Boyce and Pitted, 2002). Many health care workers are not properly informed on how to choose appropriate disinfectants, which have a generally low effectiveness in the disinfection process. This leads to an uncontrolled increase in infections among patients in hospitals (BSG, 2013; Alfa *et al.*, 2010; Manitoba, 2007; Shang *et al.*, 2015).

The aim of the study is to evaluate the antibacterial efficacy of some locally produced disinfectants on clinical isolates.

This work focuses on evaluation of the antibacterial efficacy of some locally produced disinfectants on clinical isolates.

MATERIALS AND METHOD

Sample Collection

The materials for the production of the sample were bought in Eke Oko market Anambra State. They were taken to the laboratory for preparation.

Sample Preparation

The disinfectants were prepared by gently pouring the texapon into a big empty bowl and phenol were dissolved in it. Pine oil was added and stirred properly. Chloroxylenol or Lysol or soda ash or vinegar was added and stirred also. I.P.A. (Isopropanol) or hydrogen peroxide or chlorine or carbolic acid was added. The castor oil was added and stirred properly. Water was poured into the content and stirred very well. The color was dissolved in water and was added to the content, it was mixed properly and left to settle for a while before packaging it.

Media and media preparation

The media used in this study are MacConkey agar, Mannitol salt, Eosine Methylene blue agar, Nutrient agar, Salmonella Shigella agar, Mueller Hinton agar, and Nutrient agar broth. All were prepared and sterilized according to the manufacturer's instructions at 121°C for 15 minutes.

Test organisms

The microorganisms used for this analysis were isolated from clinical samples. The samples were inoculated in Mannitol salt agar, Salmonella Shigella agar, MacConkey agar and Eosine methylene blue agar for 24 hours at 37°C. Distinct colonies were subcultured for pure culture and they were identified.

Identification of Test Organism

Gram's stain

The pure bacterial isolate was stained according to Gram's techniques. A thin smear was prepared on clean glass slide, air dried, and heat-fixed by placing the slide gently over the flame of the spirit lamp. The smear was stained with crystal violet for 1 minute; and then rinsed with tap water. The smear will then covered with Lugol's iodine for 60 seconds and washed off under gentle running tap water. The slide will then decolorized using 70% ethanol after which it washed under tap water and then counter-stained with safranin for 30 seconds. It was again rinsed with tap water and the slide blotted dry with a piece of filter paper. The stained cell was examined with the oil immersion objective lens of the light microscope. The gram positive organism was characterized by a purple colour while a gram negative organism takes on a pink colour.

Motility Test

The stabbing technique was used to carry out this test. Test-tubes containing sterilized motility medium were prepared. Sterilized inoculating needle was used to pick up isolates from their pure cultures. Each motility agar medium was stabbed with the inoculating needle carrying each isolate. A motile isolates usually grows away from the point where the medium was stabbed.

Methyl Red Test

This test were used to detect which of the isolates could produce and maintain sufficiently a stable acid product from glucose fermentation. The test is usually used as an aid in the identification and differentiation of the *Enterobacteriaceae*. This test was performed according

to Monica (CheeseBrough, 2005). The suspected organism was incubated at 37°C for 24 hours. After 24 hours 5 drops of methyl red indicator was added and the mixture was shaken and observe. A bright red colour shown shows a positive result.

Catalase Test

This test was used to demonstrate which of the isolates could produce the enzyme catalase that release oxygen from, hydrogen peroxide. This test is usually used as an aid to differentiate *Staphylococci* from *Streptococci* and to differentiate other catalase positive organism from catalase negative (Edward, 2005).

A loopful of the pure colony was transferred into a plane, clean glass slide. The test sample were then mixed with a drop of 3% v/v hydrogen peroxide. The reaction was observed immediately. Gas production indicated by the production of gas bubbles confirmed the presence of catalase.

Citrate Utilization Test

This test was done according to (Monica CheeseBrough, 2005), the test was used to identify which isolates can utilize citrate as the sole source of carbon for metabolism. The test is usually used as an aid in the differentiation of organism in the *Enterobacteriaceae* group. Simons citrate medium was inoculated in sterile test tube with a loopful of culture. The test tubes will then incubated at 37°C for about 24hours. Change in the colour indicates a negative reaction.

Indole Test

Escherichia coli is indole positive and only some Shigella are indole positive. The test organism was incubated in a test tube containing 3ml of sterile Tyrone water. Incubation was done at 37°C for 24 hours, the test for indole was done by adding 0.5ml of koracs reagent and shake gently. Examination for a red color in the surface of the layer within 10 minutes means positive, while no color changes means negative.

Voges-proskauer Test

This test is used to detect which of the isolates was able to produce a neutral red end point. Acetyl methyl carbinol (aceton) from glucose fermentation or its reductive product butylenes glycerol. The test is usually used to differentiate between gram negative organisms especially members of *Enterobacteriaceae*. Inoculate the suspected organism into a test tube containing buffered glucose peptone water and incubate at 37°C fir 24hours into the incubated medium, add 0.6% w/v solution of A and 0.2 of solution B shake the mixture and live to stand. A red color is a positive result while the development of a yellow color indicates a negative reaction. Solution A contains 5g of naphol 100ml absolute ethyl alcohol, solution B contains 100ml distilled water 40g potassium hydroxide. The alkalis oxides, the acetyl methyl carbonyl (acetone) to diacetyl which gives the pink color.

Urease Test

The test was used to demonstrate the ability of the isolate to produce the enzymes urease which splits the urea forming ammonia. The test is usually used to differentiate organism like proteus from other non urease positive organisms. A loopful of the isolates was used to inoculate the tube of urea-agar. The tube was inoculated at 37°C. A change in color from yellow to red confirm the presence of urease.

Oxidase Test

Place a piece of filter in paper in Petri dish and add 3 drops of freshly prepared oxidase reagent using a sterile glass rod. Remove a colony of test organisms from a culture plate and smear it on the filter paper. Oxidase reagent is specially prepared as log/1 or 1% solution of tetramethyl-p-phenylenediaminedihydrochloride-oxidase. Positive organism gives blue color within 5-10 seconds and in oxidase negative organisms, colour does not change.

Antibacterial Activity of the Disinfectants using Agar Well Diffusion Method.

The Agar well diffusion method was adopted to evaluate the antibacterial efficacy of some locally produced disinfectants on clinical isolates. Muller Hinton agar was prepared and distributed into different sterile petri dish. The bacterial strains were grown in a nutrient agar broth which was used for the bacterial susceptibility test. The broth culture was grown for 24 hours and serially diluted in the same broth (sterilized at 121°C for 15 minutes).

Sterile swab sticks was used to inoculate the test microorganisms by dipping it in the diluted culture and streaking on all the surface of the agar plates. The agar plate was punched with a sterile cork borer of 8mm size and 0.1ml of each sample was poured in the holes bored. The plates was then incubated for 24 hours at 37°C. After incubation, the diameter zones of the inhibition was measured using millimeter rule.

RESULTS

The result of the cultural characteristics and biochemical reactions of the test organisms as shown in the Table 1 below.

Table 1: Cultural characteristics and Biochemical Reactions of the Test Organisms

Cultural characteristics	Citrate Test	Coagulase Test	Indole Test	Methy/Red (MR)	Vogesproskauer (VP)	Urease Test	Probable organ
Greenish metallic sheen on Eosine Methylene blue agar	+	-	-	+	+	-	<i>Escherichia coli</i>
Yellow colonies within yellow zone on mannitol salt agar	+	+	+	+	-	+	<i>Staphylococcus aureus</i>
Colourless colonies usually with black center on <i>Salmonella</i> Shigella agar	-	+	-	+	-	-	<i>Salmonella spp</i>
Large, mucoid glistening pink colonies on MacConkey agar plate	+	-	-	+	-	-	<i>Klebsiella spp.</i>

+ = Positive

- = Negative

The results of the antimicrobial activities of the locally produced disinfectants on the clinical isolates.

Table 2: Antimicrobial Activities of the Locally Produced Disinfectants on the Clinical Isolates

Zone of inhibition in millimeter (mm)				
Test organism	Sample A	Sample B	Sample C	Sample D
<i>Staphylococcus aureus</i>	33	20	36	35
<i>Klebsiella sp.</i>	35	18	25	32
<i>Salmonella sp.</i>	38	27	20	31
<i>Escherichia coli</i>	28	30	23	35

Discussion

In this study, the antibacterial efficacy of some locally produced disinfectant that are used for washing and cleaning purposes and in other to remove dust and microbes present on the surfaces were evaluated on some clinical isolates.

Some of the clinical isolates gotten were *Staphylococcus aureus*, *Klebsiella sp.*, *Salmonella sp.*, and *Escherichia coli*. They were culturally and morphologically identified, and also they were identified using biochemical tests as seen in table 1 above.

Result obtained from the experimental data revealed that most of the studied locally produced disinfectant showed antibacterial activity, though with varying degree of zone of inhibition as indicated by the antibacterial sensitivity pattern of the isolates.

Sample A and sample D were most effective in all the test organisms with the following zone of inhibition 33mm, 35mm, 38mm, and 28mm; 35mm, 32mm, 31mm and 35mm on *Staphylococcus aureus*, *Klebsiella sp.*, *Salmonella sp.*, and *Escherichia coli* respectively on sample A and sample D respectively though with varying zone of inhibition and its correlates with the work of Obi and Onyekaozuru (2015). It also, correlate with the work of Okore *et al.*, (2014).

Sample B and C were also effective on *Staphylococcus aureus*, *Klebsiella sp.*, *Salmonella sp.*, and *Escherichia coli* with the following zone of inhibition 20mm, 18mm, 27mm, and 30mm; 36mm, 25mm, 20mm, and 23mm respectively though with varying zone of inhibition and this correlates with the work of Obi and Onyekaozuru (2015).

Conclusion

In conclusion it showed that sample A and D were most effective in all the test organisms though with varying zone of inhibition. Also, it showed that they were active in both gram positive and gram negative test organisms used. Also, it showed that sample B and C were also active in both gram positive and gram negative test organisms used. Hence, sample A and D has a high long term spectrum though with varying zone of inhibition than sample B and C.

Recommendation

In view of the result obtained, antibacterial efficacy of some locally produced disinfectant on some clinical isolates are recommended for the cleaning and disinfection of surfaces such as door handles, floors, walls, desk, test tubes, restroom fixtures.

- Regular surface cleaning and disinfection is also highly recommended to reduce chances of transmission of these potentially pathogens.

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