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Impact of Disinfectants on Antimicrobial Potentials of Some Microorganisms

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Abstract: *In hospitals and other health care sceneries, disinfectants are used expansively for a diversity of topical and hard-surface applications. They are vital parts of infection regulator exercises that also aid in the prevention of nosocomial infections. In order to avoid related diseases causedby pathogenic organisms, disinfectants are necessary for external use. This study investigated the effectiveness of varying commercial disinfectants for the control of pathogenic organisms. The clinical isolates used for the test were Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Epidermophyton floccosum while the commercial disinfectants used were liquid dettol, JIK, methylated spirit and kerosene. The method employed in assessing the efficacies of the disinfectants in this study was agar well plate diffusion and broth dilution methods. The antimicrobial activities of the disinfectants presented dettol as most effective against the experimental microorganisms as the other disinfectants. This was done by evaluating the zones of inhibition of the disinfectants on the experimental microorganisms.*

Keywords: *Disinfectants, antimicrobial, effectiveness, experimental microorganisms.*

I. Introduction

An antimicrobial is an agent that eradicates or hinders thedevelopment of microorganisms.Their classification is dependent on the roles they perform. Agents that eradicate microbes are known as microbicidalwhile those that merely hinder their growth are called microbiostatics (U.S.CDC, 2016).Since microorganisms have been recognized as the causative agents of communicable diseases, there has been the implementation of different ways for the reduction as well as prevalence of the microorganisms. Chemotherapy, immunization, sterilization and disinfection are some of the methods used (Omoruyi*et al.*, 2011).The European Committee for the Standardization of disinfectants defined disinfectionas the selective eradication of certain undesirable organisms so as to inhibit their transmission which is realized by action on their structure or metabolism irrespective of their functional state (Block,1991).The conception of the disinfectants and antiseptics is to curb or minimize the presence of microorganisms (Van, *et al.,* 2002). In order to prevent infections as it regards injury, the most vital measure is to kill or inhibit the growth of microorganisms on the skin, wounds and in human body cavity (Awodele, *et al.,* 2007). The antimicrobial potentials of these disinfectants could be influenced by their formulation properties, concentration of organic components, temperature,synergy, rate of dilution and experimental procedures (Denyer*et al.,* 1985).Antimicrobial agents like antibiotics (that destroy microorganisms within the body) and antiseptics (that attack microorganisms associated with the living body (external) and when used on microorganisms, render them inactive by inhibiting their multiplication) are quite different from disinfectants.

A wide range of different substances are used as disinfectants including alcohols, aldehydes, bleaches etc. Bacteria consist of protein known as heat shock protein33 (Hsp33) which protect proteins from reactions that could damage them. The role of heat shock protein33 is predominantly significant when cells are under stress e.g. temperature rise due to infection. At high temperatures, proteins begin to lose their three-dimensional molecular structure and start to clump together and form large, insoluble aggregates. Hypochlorite, the chemical in chlorine bleach has a similar effect on proteins as heat stress resulting in clumping together of proteins. Hypochlorite inactivates the proteins essential for bacterial growth,thereby, kills the bacteria. Though bleach will certainly kill the germs on kitchen counters or in tubs, it is also hazardous to the environment after being washed down the drain, as well as to the health if work area is not properly ventilated (Thompson,2008) Methylated spirit is principally alcohol though generally acknowledged as a disinfectant due to its capability to essentially decrease skin flora, sterilize equipment or surfaces and has been extensively used for skin preparation before injection or surgery (El-Mahmood*et al.,* 2009). Methylated spirit acts by dissolving cell membranes, proteins denaturation and dehydration of the cells due to evaporation. It is a bacteriostatic antiseptic, mild rubifacient and counter irritant when used on excerated skin and mucosa (Iroha*et al.,* 2011). Dettol is a disinfectant that is composed of chloroxylenol B.pc.4.8% w/v, OleumpiniAromtieum 8.38%w/w, Isopropyl Alcohol 9.43% w/w, Sapovegetalis 5.60% w/w, and sacchariumustumqs. Aqua 100vols.It has been established to have a cleansing property on lacerations and scalds. JIK is 3.5% sodium hypochlorite and it is

used on a huge scale for decontamination of surface, removal of odor, bleachingand disinfection of water (Olowe*et al.,* 2004). Recently, kerosene,a petroleum product was discovered to have a cleansing property (Ryan *et al.,* 2004). Even though all the above mentioned disinfectants are effective, some bacteria form tough endospores that enable them tolerate harshecological situations, hence, pollute food and workroom surfaces (Ray *et al.,* 2004). It has also been known that *Pseudomonas aeruginosa* is a dangerous organism which is often called an opportunistic pathogen and can multiply in swimming pools, toilets, skin and sinks. *Candida albicans* has a characteristic pattern of multiplying and could tremendously result in candidiasis in the vagina, mouth, skin and gut(Gow, 1994). The aim of this study was to determine the impact of disinfectants on the antimicrobial potentials of *Pseudomonas aeruginosa, Staphylococcus aureus, Epidermophytonfloccosum and Candida albicans*.

II. Materials And Methods

Sample collection

Soil samples were collectedaseptically at the farm land around Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt. Mouth wash and skin scrape were obtained from students.The microbiological analyses of the samples were carried out according to the methods of Oyeleke and Manga (2008a). The fungal isolates were identified according to Oyeleke and Okusanmi (2008b).The study was carried out using standard zone of inhibition method as described by WHO(2003). The microorganisms isolated from the samples above were*Pseudomonas aeruginosa, Staphylococcus aureus, Epidermophytonfloccosum*and *Candida albicans.*

Chemicals (disinfectants/antiseptics)

Jik, Nigeriandettol, methylated spirit was obtained from B.M LINE supermarket, EmekukuStreet in Port Harcourt, Nigeria. Kerosene was obtained from Total filling station, Port Harcourt, Nigeria.Serial dilution method was used to dilute the disinfectants into 50% and 25% concentrations respectively. The kerosene was used at only the 100% concentration.

Bacteriological Analyses

The total heterotrophic bacterial count was performed in duplicates on dried nutrient agar plates and incubated 30◦c for 24hrs. At the end of the incubation period, isolation for pure culture was done.

Acidified potato dextrose agar plates containingstreptomycin (1mg/100ml) were used to obtain fungal isolates. The plates were incubated at 30◦c and observed after 48 hours for yeasts and 96 hours for mould, after this, isolation of pure isolates was done.

Identification and Characterization of isolates

The methods described in Cheesebrough (2000) were adopted in characterization of isolates. Isolates were identified by standard methods (Williams and Odokuma, 2014).

Statistical Analysis

Results were subjected to statistical analysisemploying the student t-test at 95% probability levels using SPSS (VERSION 14.0) statistical package.

Determination of minimum inhibitory concentration (MIC) of the various Disinfectants/antiseptics on the test organisms using the agar well diffusion assay

0.1ml of broth culture of the various test microorganisms (*Pseudomonasaeruginosa, Staphylococcus aureus, Candida albicans, Epidermophytonfloccosum)* was placed on the nutrient and sabouraud dextrose agar plates respectively. It was evenly spread on the entire surface of the agar plates with the aid of a sterilized bent glass rod. The organisms were allowed to pre- diffuse. Using a sterile borer two wells were bored on each of the plates. The different concentrations of the disinfectants were dispensed into the wells in each plate using one disinfectant per plate. Sterile distilled water was used in each of the other well as control. The plates were allowed to stand for 30minutes to allow proper diffusion of the disinfectants into the culture plates. The plates were incubated at 37˚C for 24 hours.

The plates were observed for zone of inhibition after the 24 hours. The zones of inhibition were measured and recorded. This was performed as described by (Sahu, 2013).

Determination of minimum inhibitory concentration (MIC) of the various Disinfectants/antiseptics on the test organisms using the tube dilution method

0.1ml of 24hours peptone water cultures of various test organisms were inoculated into the tubes and was properly mixed then incubated at 37˚C for 24hours.

Positive control contained 9ml of peptone water with organisms without disinfectants while negative control had 9ml of peptone water and antiseptic without organism. The dilution tube without visible growth as seen by turbidity was taken as the minimum inhibitory concentration (French *et al*., 2006).

III. Results And Discussion

Results achieved revealed that the antimicrobial activities of the tested disinfectants were centered on concentration. The effectiveness of dettol, JIK and methylated spirit in inhibiting *Pseudomonas aeroginosa, Staphylococcus aureus, Candida albicans*and *Epidermophytonfloccosum*is shown on table 1 though with different zones of inhibition at 100% concentration. Dettol showed 40mm (4.0), 49mm(4.9cm), 20mm (2.0cm) ,29mm(2.9cm) which were the highest zones of inhibition compared to that of JIK and, methylated spirit. However, kerosene inhibited 20mm and 10mm on *Candida albicans and Epidermophytonfloccosum* respectively.

Table 1: Activities of disinfectants against organisms at 100% concentration.

Test organism	**Zones of inhibition (mm).**			
	Dettol	JIK	Methylated spirit	Kerosene
Pseudomonas aeruginosa	40mm	10mm	10mm	Nil
Staphylococcus aureus	49mm	20mm	20mm	Nil
Candida albicans	20mm	5mm	10mm	20mm
Epidermophytonfloccosum	29mm	2mm	5mm	10mm

Table 2 illustrates that the inhibitory activity of dettol at 50% was 30mm, 40mm, 5mm, and 26mm on *Pseudomonas aeruginosa, Staphylococcus aureus Candida albicans,* and *Epidermophytonfloccosum* respectively. At 50% concentration, JIK had 5mm as zone of inhibition for both *Pseudomonasaeruginosa and Staphylococcus but no antimicrobial activity on Candida albicans and Epidermophytonfloccosum*. At 50%concentration, methylated spirit had the following zones of inhibition: 3mm, 12mm, 5mm and 1mm on *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Epidermophytonfloccosum* respectively. This indicates that at 50% concentration, methylated spirit had less effect on the tested microorganisms.

Table 2: Activities of disinfectants against organisms at 50% concentration.

Test organism	Zones of inhibitions(mm)			
	Dettol	JIK	Methylated spirit	Kerosene
Pseudomonas aeruginosa	30mm	5mm	3mm	Nil
Staphylococcus aureus	40mm	5mm	12mm	Nil
Candida albicans	5mm	Nil	5mm	20mm
Epidermophytonfloccosum	26mm	Nil	10mm	10mm

On table 3,a good level of antimicrobial activity of dettol disinfectant at 25% concentration was shown against the four microbes. At 25% concentration, JIK had less effect on *Pseudomonasaeruginosa and Staphylococcus aureus* with 1mm and 2mm zone of inhibition respectively. No zone of inhibition was seen on *Candida albicans and Epidermophytonfloccosum*. 25% concentration of methylated spirit showed less activity against *Staphylococcus aureus* and *Candida albicans* and no zone of inhibition on *Pseudomonas aeruginosa* and *Epidermophytonfloccosum*. The results obtained from this study proves that some commonly used disinfectants such asdettol (composed chloroxylenol B.pc.4.8% w/v, OleumpiniAromtieum 8,38%w/w, Isopropyl Alchohol 9.43% w/w, Sapovegetalis 5.60% w/w, and sacchariumusteumqs. Aqua 100vols.), JIK(composed of sodium hypochlorite) methylated spirit(Ethanol, absolute 99/100%) and kerosene have antimicrobial activities on *Pseudomonas aeruginosa, Staphylococcus aureus, Candida and Epidermophytonfloccosum* that can be found on the skin, swimming pool, toilets and lab surfaces.

Table 3: Activities of disinfectants against organisms at 25% concentration.

Test organism	Zones of inhibitions(mm)			
	Dettol	JIK	Methylated spirit	Kerosene
Pseudomonas aeruginosa	27mm	1mm	1mm	Nil
Staphylococcus aureus	34mm	2mm	5mm	Nil
Candida albicans	2mm	Nil	2mm	20mm
Epidermophytonfloccosum	20mm	Nil	Nil	10mm

The result obtained from this study showed Dettol to be most effective against all the tested microorganisms at 100% concentration even at 50% and 25% concentration though with less zones of inhibition as a result of reduction in concentration. Dettol showed excellent result against gram positive andnegative organisms. This work has demonstrated JIK to have inhibitory activities against all the tested microorganisms at 100% concentration. However, at 50% and 25%, JIK produced no inhibitory activities against *Candida albicans and Epidermopytonfloccosum* due to reduced concentration but maintained its activity against *Pseudomonasaeruginosa* and *Staphylococcus aureus*with zones of inhibition of 5mm at 50% concentration, 1mm and 2mm at 25% concentration respectively.

Table 4.5 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on test organisms produced by disinfectants.

Disinfectant	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	*Epidermophytonfloccosum*
Dettol	-	-	-	-
JIK	-	-	-	-
Methylated spirit.	-	-	-	-
Kerosene	+	+	-	-

KEYS;
+ = visible growth by turbidity
- = No visible growth

The result obtained with kerosene indicated that it has a good antimicrobial activity against *Candida albicans* and Epidermophyton*floccosum* at 100% concentration which concurs with the findings of Gerdner*et al*.,(1991). Kerosene was not diluted because of its immiscibility with water. The study of Logan (1994) that showed bacteria to survive in kerosene agreed with this work because at 100% concentration, kerosene had no inhibitory effect on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This study has shown that use of kerosene by some barbers as after-shave is inappropriate especially because of bacterial infection. From this study, it was observed that methylated spirit was active against all the tested organisms at 100% concentration though became less effective with reduced concentrations of 50% and 25%. Larson (1995) observed that alcohol is an effective disinfectant against microbes on the skin.

IV. Conclusion

The results acquired from this research work showed that disinfectants have broad activities against pathogenic microorganisms like *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Epidermophytonfloccosum*. The study revealed dettol to be more effective against *Pseudomonas aeruginosa and Staphylococcus aureus.* Kerosene was effective against *Candida albicans and Epidermophytonfloccosum.* Butmethylated spirit and JIK had moderate effect against *Pseudomonas aeruginosa and Staphylococcus aureus.* However, the study proved that dettol has good action against both bacteria and fungi.With the use of disinfection, a great reduction in nosocomial infections as well as other infective diseases could be achieved. This could be attained when the disinfectants are effectively diluted and in clean environments. When disinfectants are over diluted their efficacy becomes reduced hence, pathogenic organisms develop resistance to such disinfectants.

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