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EFFICACY OF SOME COMMONLY USED DISINFECTANTS IN SOME SELECTED HOSPITAL LABORATORIES IN SOKOTO

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Abstract: **This study was aimed at evaluating the efficacy of three (3) different commercial disinfectants commonly used in some selected Hospitals laboratories in Sokoto metropolis, the disinfectant preparations were investigated against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* by using tube dilution and agar diffusion methods respectively. Findings from agar diffusion method indicated that some disinfectants had wide efficacy against the test bacteria. The disinfectant effectiveness generally increased by increasing the disinfectant concentration. Also disinfectants were observed to be effective against the isolates by tube dilution method depending on exposure time. The result of the relative antimicrobial power from both the tube dilution and agar diffusion methods were in close agreement. However, some disinfectants were relatively ineffective even at their highest concentrations. Disinfectant C Jik ranked the highest in antimicrobial potency which could be attributable to the presence of mono-atomic chlorine as active agent. *Staphylococcus aureus* is the most sensitive bacteria to the disinfectants. *Salmonella typhi* and *Pseudomonas aeruginosa* were the most resistant organisms and these observations are attributable to the differences in the cell wall composition of the two groups of organisms. *Staphylococcus aureus* which is a gram-positive organism have more techoic acid and polysaccharides in their cell wall, whereas *Salmonella typhi* and *Pseudomonas aeruginosa* been gram-negative organisms have outer membrane which exhibits a typical phospholipid layer. In general, it could be said as observed from the research investigation that disinfectant C, (Jik) which is bleach is the most effective among the disinfectants and Methylated spirit ranked the lowest in antimicrobial potency.**

Keywords: **Efficacy, Disinfectants, Hospital, Laboratories, Sokoto.**

1. INTRODUCTION

As contamination with organic matter is common in the environment, the efficacy of cleaning agents in its presence is of greater practical significance (Vohra and Poxton, 2011). Appropriate disinfection and sterilization procedures are a must for control of hospital-acquired infection, as failure can result in many hospital-acquired infections thus leading to increased cost, morbidity and mortality (Singh *et al.,* 2012). Commonly used laboratory disinfectants include bleach (hypochlorite), ethanol (70%, vol/vol), and a variety of commercial preparations (Weir *et al.*, 2002). Useful disinfectants can be sprayed or poured onto laboratory surfaces and, after a brief period, cleaned up with paper towels for subsequent disposal. Laboratory disinfectants should kill target organisms in a short period of time (Weir *et al.*, 2002). Disinfectants may also be used to chemically treat infectious hospital waste, especially the disposable plastic and microbiological wastes. Different disinfectant formulations have different applications. The process of disinfection may be affected by many variables like temperature, contact period, pH and concentration of the disinfectant, bioburden, organic soil and hardness of water used for dilution (Singh *et al.,* 2012). Different disinfectant formulations have different applications. The process of disinfection may be affected by many variables like temperature, contact period, pH and concentration of

the disinfectant, bioburden, organic soil and hardness of water used for dilution. Therefore, the disinfectant ought to be tested in the field for the specified application to ensure its effectiveness. Because these standard tests cannot be performed by the laboratories belonging to small hospitals, one has to solely rely upon the literature provided by the manufacturer regarding the efficiency of the disinfectants. Almost all the manufacturers claim their disinfectant as a broad-spectrum antimicrobial agent suitable for diverse applications (Singh *et al.*, 2012).

Isolation and identification of bacteria

Culture media for the organisms *(* MacConkey Agar, Deoxychocolate citrate Agar& Blood Agar) were prepared, samples were aseptically inoculated on the media incubated at 37^{o}C for 24hours.

After 24hours of incubation, the colonies suggestive of the organisms were picked using a sterile wire loop and sub cultured on a nutrient agar in order to get pure colonies of the isolated organisms (*Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa*). Pure colonies of all the Isolates were identified using colony morphology on agar plates, Grams stain and Biochemical test.

Gram's staining technique

Gram staining was done as described by Cheesebrough (2000) briefly, with a sterile wire loop; a colony of each of the test organisms was emulsified on a drop of sterile physiological saline placed on a clean glass slide. The emulsions were allowed to dry, and then fixed over the Bunsen flame briefly. The slides were placed on a staining rack, and then flooded with crystal violet. Stain allowed for 1 minute, after which the stain was washed off with water. The slides were again flooded with the mordant (Lugol's iodine) solution, and allowed to stain for 1 minute, then washed off with water. The slides were decolorized for 5 seconds with alcohol solution, and then washed off with water. The slides were counterstained with neutral red solution for 1 minutes and also washed off with water. These slides were air-dried and viewed microscopically using 100 x objective (oil immersion) and the Gram's reaction of the organisms were recorded.

Preliminary determination of antimicrobial activities of the test disinfectants

This was carried out using the agar diffusion techniques. Aliquots of 0.1ml of 24hours broth culture was spread plated on a 2ml solidified nutrient agar in a sterile petri dish and was allowed to dry, with the aid of a sterile cork borer of 10mm in diameter, wells were made on the agar. The test dilution (2.5 and 1.25 %v/v) of the disinfectant was made in sterile distilled water (SDW) and 0.1 ml introduced into each well, allowed to stand for complete diffusion to occur and incubated at 37°C for 24hours after which the diameter of the zone of incubation was measured to the nearest millimeters. The experiment was repeated three times and the mean diameter was calculated.

Determination of Minimum Inhibitory Concentration (MIC) and phenol coefficient (PC)

A serial dilution of the test disinfectants was carried out to obtain dilution of 0.5:5 (10% v/v), 0.5:10 (5.0% v/v), 0.5:20 (2.5% v/v), 0.5:40 (1.25 % v/v), 0.5:100 (0.5% v/v), 0.5:500 (0.1% v/v), 0.5:1000 (0.05% v/v), 0.5:2000 (0.025% v/v), 0.5:4000 (0.0125% v/v). Added to this chosen test dilution were two controls, one of which contained only the diluents (sterile distilled water) and the other contained the stock undiluted disinfectant.

0.5 ml of the standardized 24hours broth culture was subjected to the action of various dilution used, including the controls which were all maintained at a volume of 5ml. Subculture was made from this mixture into a sterile nutrient broth after 10 and 15mins to ascertain if the bacteria were still alive, using development of turbidity at 37°C for 24 hours.

The MIC and PC of each test disinfectant were determined as described by the Association of Official Chemists and U.S Food and Drug Administration (Pelczer *et al.*, 1993).

2. RESULTS

The result of mean diameter of the zone of inhibition is presented graphically in figure 1, 2 and 3. While the minimum inhibitory concentration (MIC) and Phenol Coefficient (PC) for the test disinfectants against *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa* are presented in tables 1, 2, 3 and 4.

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Dilutions of disinfectants used against Staphylococcus aureus

The highest activity against *Staphylococcus aureus* in 10mins was shown by both Dettol and Jik at the MIC of 0.5% v/v. while the MIC for Jik in 15mins was 0.1% v/v that of Dettol remains unchanged. The MIC for methylated spirit was maintained at 2.5% v/v with negligible change in time. The least antimicrobial activity against *Staphylococcus aureus* was shown by 10%phenol solution which gave MIC of 2.5% v/v and 1.25% v/v in 10 and 15mins respectively as presented in Table 1..

Dilutions of disinfectants used against Pseudomonas aeruginosa

The MIC for Jik against *Pseudomonas aeruginosa* was found to be 2.5% v/v in 10mins and 1.25% v/v in 15mins, Dettol maintained the same MIC of 5.0% v/v in 10 and 15mins respectively. The MIC for both Methylated spirit and 10%phenol solution against *Pseudomonas aeruginosa* was found to be the same 10.0% v/v in 10 and 15mins respectively as shown in Table 2..

Dilutions of disinfectants used against Salmonella typhi.

The MIC for Jik against *Salmonella typhi* was 1.25% v/v and 0.5% v/v in 10 and 15mins respectively. While the MIC for Dettol was 2.5% v/v and 1.25% v/v in 10 and 15mins respectively. Lowest antimicrobial activity against *Salmonella typhi* in 10 and 15mins was shown by both methylated spirit and 10%phenol solution at the MIC of 5.0% v/v respectively. The MIC for both disinfectants remains unchanged with time as presented in Table 3.

In general, the highest antimicrobial activity against the test organisms was shown by Jik. The result of the control shows turbidity for the tube containing only sterile distilled water that of the stock undiluted disinfectant shows no growth.

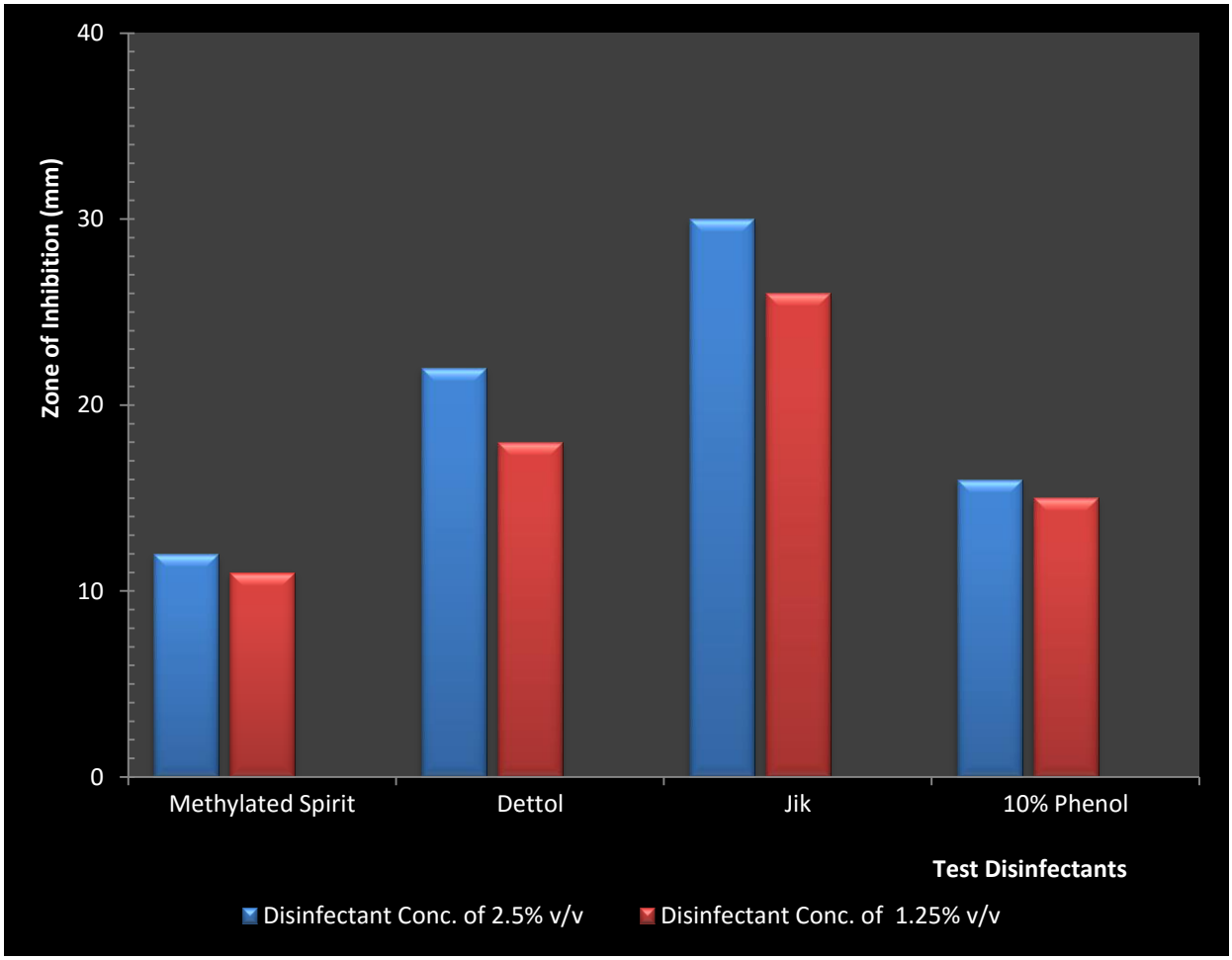

FIG 1: The Antimicrobial Activity of the test Disinfectants against *Staphylococcus aureus*

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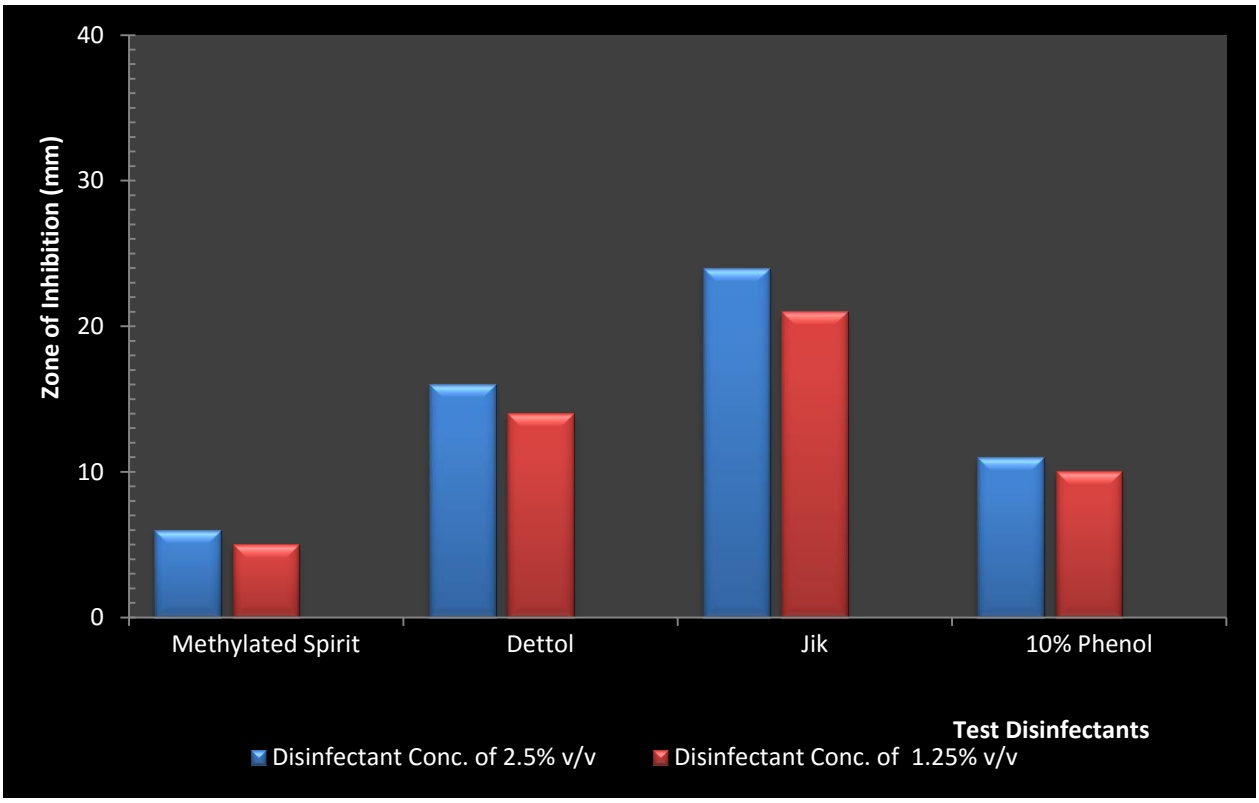

FIG 2: The Antimicrobial Activity of the test Disinfectants against *Pseudomonas aeruginosa*.

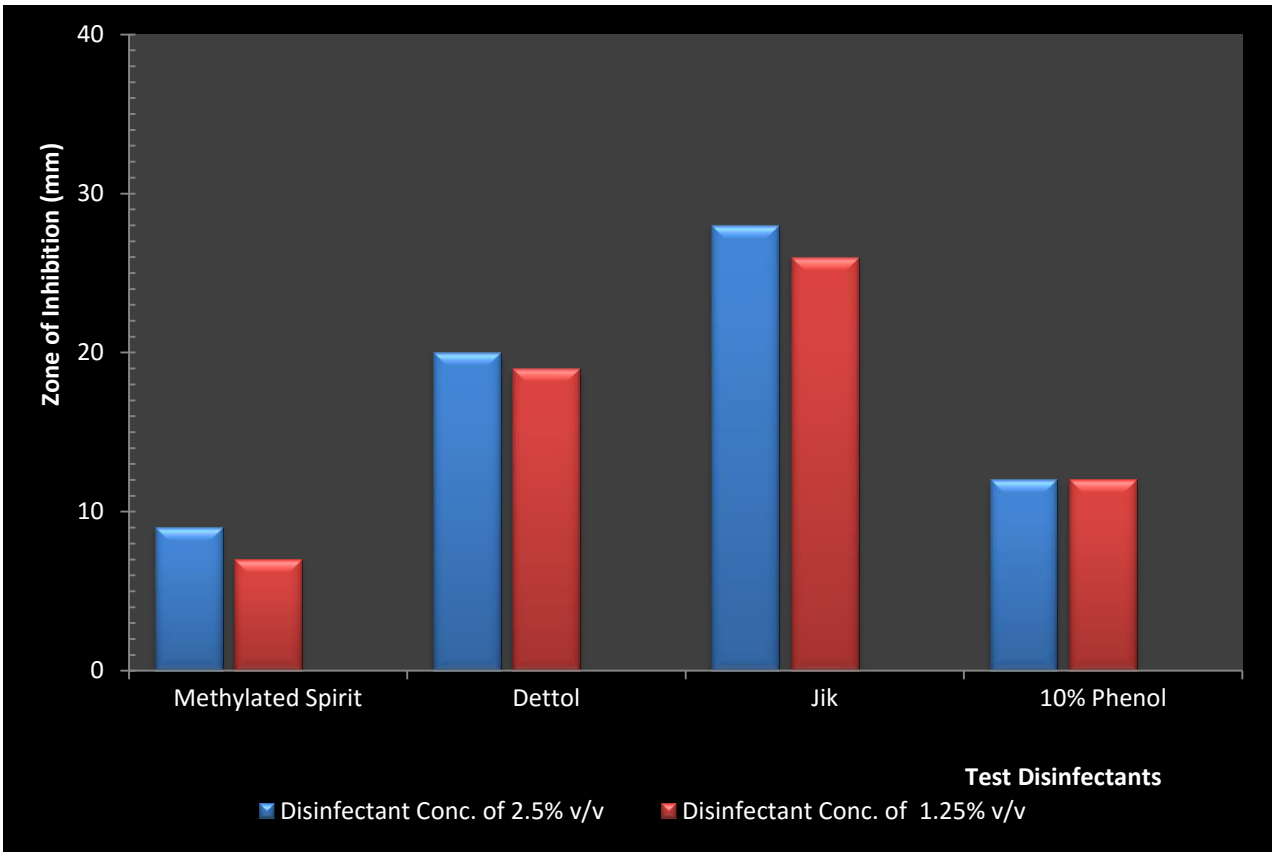

FIG 3:The Antimicrobial Activity of the test Disinfectants against *Salmonella typhi*

3. DISCUSSION

Result obtained in this study showed that chemical disinfectants are more active against gram positive organisms than they are to gram negative organisms. It also shows that gram negative organisms are more resistant and show relatively less changes with time of exposure compared to those of gram positive organisms. These observations are attributable to the differences in the cell wall composition of the two groups of organisms. Gram positive organisms have more in layers of techoic acids and polysaccharides in their cell walls. While gram negative organisms have outer membrane which exhibits a typical phospholipid layer and hence the relative resistance of the gram negative bacteria to a number of antibacterial agents (Jawetz *et al.*,1998).

The result of the relative antimicrobial power from both the tube dilution and agar diffusion method were in agreement, except for the Jik which ranked among the highest with Dettol in anti microbial potency in the tube dilution method, and shared the lowest potency with 10% phenol solution in the agar diffusion technique. The reason for this observation is the diffusion potential of Jik and Dettol through agar gel.

Bleach is a better disinfectant since it contains mono-atomic chlorine and literally blows holes in bacteria, unlikely places where *Pseudomonas aeruginosa* have been found includes antiseptics such as Quatenary ammonium compounds such as Dettol. Their cell wall contains porins which gives them the ability to thrive in harsh conditions. It possesses a wide range of secretion systems which export numerous proteins relevant to the pathogenesis of chemical strains.

Barindra *et al.*, 2006, reported that oxidation reactions will occur when bleach is dissolved in water, which can destroy organisms fold structure leading to sterilization. Another study also found similar result that bleach is rapidly bactericidal achieving a 5log10 kill of *Pseudomonas aeruginosa* and other vegetative organisms in one minute (Fraise, 1999).

Based on that fact, the more resistant organism would provide a more stringent test for any disinfectant (Rawlins, 1977). Jik could be termed the most potent followed by Dettol based on their antimicrobial efficacy against *Salmonella typhi* and *Pseudomonas aeruginosa* in 15minutes.

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