

Full Length Research Paper

The antimicrobial activities of some commonly used disinfectants on *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*

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The disinfectants; Savlon^R, Jik^R, Methylated spirit and kerosene were observed for their inhibitory activities on *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*. This was done by measuring the zone of inhibition of the disinfectants on the tested organisms. The results showed that Savlon was very effective at 100% concentration, as it inhibits the growth of *P. aeruginosa* and *B. subtilis* to the level of 47 and 58 mm, respectively. However, there was no inhibitory activity on *C. albicans* at this concentration. At 6.25% concentration, it had remarkable antimicrobial activity on these organisms with 10 and 16 mm levels of inhibition respectively. Jik at 100% concentration inhibits the growth of *P. aeruginosa*, *B. subtilis* and *C. albicans* to a level of 17, 15 and 18 mm, respectively. After dilution to 50% concentration, its inhibitory activity on *P. aeruginosa* and *B. subtilis* reduced to a level of 15 and 4 mm, respectively, and there was no inhibition on *C. albicans*. Methylated spirit showed inhibitory activity on *P. aeruginosa*, *B. subtilis* and *C. albicans* to a level of 20, 22 and 21 mm, respectively. Dilution of methylated spirit had no inhibition on the organisms. Kerosene was only effective on *C. albicans* with 69 mm zone of inhibition at 100% concentration. The study showed that Methylated spirit and Jik have inhibitory activities on both fungi and bacteria, while kerosene had only but better antifungal activity and Savlon had only antibacterial activity.

Key words: Disinfectants, Organisms, Inhibition.

INTRODUCTION

Antiseptics and disinfectants are used extensively in the hospitals and other health care settings on a variety of topical and hard-surface applications. Over the years, rather than using antimicrobial drugs, antiseptics and disinfectants have played important roles in the control of infectious diseases, microbial food spoilage and unwanted microbes generally. In particular, they are an essential part of infectious control practices (Larson and Morton, 1996; Rutala, 1996). However, the antimicrobial activity of these agents may be influenced by their formulation effects, level of organic load, synergy, temperature, dilution rate and tests method (Denyer et al. 1985; Russell and Hugo, 1987; Russell and Russell, 1995).

Methylated spirit which is mainly alcohol is a generally acceptable disinfectant due to their ability to largely re-

duce skin flora, clean instruments or surfaces and have been widely used for skin preparation prior to injection or surgical preparation (Larson, 1995). JIK^R which is 3.5% sodium hypochlorite is used on a large scale for surface purification, bleaching, odor removal and water disinfection. Savlon^R is another disinfectant that composes of 2.8% n-propyl alcohol, 0.3 g chlorhexidine gluconate and 3.0 g cetrimide. They have been shown to have a cleansing property on wounds and burns. Moreover, kerosene which is a petroleum product has recently been known to have disinfectant properties mainly on cuts (Gardener et al., 2002) and it is now been used in barbing salon as after shave.

Although all the above-mentioned disinfectants are effective, recent researches have shown *Bacillus subtilis* to form a tough protective endospore allowing it to endure extreme environmental conditions thus, contaminate food and laboratory surfaces (Ryan and Ray 2004). More so, *Pseudomonas aeruginosa* have been know to be dange-

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

Organism	Zones of inhibition (mm)															
	Methylated spirit				Jik				Savlon				Kerosene			
<i>Bacillus subtilis</i>	20	19	21	20	15	16	14	15	58	57	59	58	-	-	-	-
<i>Pseudomonas aeruginosa</i>	22	21	23	22	17	16	18	17	47	45	49	47	-	-	-	-
<i>Candida albicans</i>	21	20	22	21	18	19	17	18	-	-	-	-	69	70	68	69

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

Organism	Zones of inhibition (mm)											
	Methylated spirit				Jik				Savlon			
<i>Bacillus subtilis</i>	-	-	-	-	4	4	4	4mm	24	23	25	24
<i>Pseudomonas aeruginosa</i>	-	-	-	-	15	16	14	15mm	21	22	20	21
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-

rous pathogen which is often called opportunistic pathogens and it can proliferate in pools, hot tubs, toilet, sinks and skin. In addition, the work of Gow (1994) has shown *Candida albicans* to have a typical pattern of proliferation and could extremely cause candidiasis in the gut, mouth, vagina and skin.

In view of the above facts, it may be necessary to determine the antimicrobial activities of these commonly used disinfectants on *B. subtilis*, *P. aeruginosa*, and *C. albicans*, as this will give better information on the efficacy of these agents on skin, toilet, instruments and laboratory surfaces contaminants.

MATERIAL AND METHODS

Chemicals

Methylated spirit, Jik, and Savlon were obtained from DOGAB Pharmacy, Iwaya Lagos, Nigeria. Kerosene was obtained from Mobil filling station, Fadeyi-Lagos, Nigeria.

Study design

The study was carried out using zone of inhibition method as described by WHO (2003). The standard strains of the organisms used, *B. subtilis*, *P. aeruginosa* and *C. albicans* were obtained from the Department of Molecular Biology and Genetics, Nigeria Instituted of Medical Research, Yaba-Lagos, Nigeria. Three replicated of each test was conducted.

Media preparation

The agar used was MacConkey agar, Sabroud dextrose agar and Nutrient agar. The media were prepared as described by LAB™ (Topley House, 52 Wash Lane, Bury, Lancashire, BL96AU, UK).

Inoculation

B. subtilis and *P. aeruginosa* were inoculated on MacConkey medium, and incubated at 37°C for 24 h. While *C. albicans* was inoculated on Sabroud dextrose medium and incubated at 30°C for 48 h.

Dilution of disinfectants

Serial dilution method was used to dilute the disinfectants into 50, 25, 12.5 and 6.25% concentrations. Kerosene was used at only the maximum 100% concentration.

Measurement of zone of inhibition

0.1 ml of each concentration of the disinfectant was introduced in triplicate on nutrient media plates that have been separately sub-cultured with each of the organism. They were incubated at 37°C for 24 h. The zone of inhibition was determined by measuring the diameter in millimeters of zone to which the disinfectant inhibited the growth of the organism.

RESULTS AND DISCUSSION

The results obtained showed that the antimicrobial activities of the tested disinfectants were concentration dependent. Table 1 showed Methylated spirit and JIK to be effective in inhibiting the three microbes at 100% concentration, while Savlon showed inhibitory activity on *B. subtilis* and *P. aeruginosa* only, but with higher zone of inhibition than Jik and methylated spirit. However, kerosene inhibited only *C. albicans* but with highest zones of inhibition. Table 2 illustrates that the inhibitory activity of JIK at 50% is 4 and 15 mm on *B. Substilis* and *P. aeruginosa*, respectively. While 50% Savlon showed 24 and 21 mm zone of inhibition on *B. Substilis* and *P. aeruginosa*, respectively. However, at 50% concentration, Methylated spirit has no effect on these organisms.

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

Organism	Zones of inhibition (mm)											
	Methylated spirit				Jik				Savlon			
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	-	20	21	19	20
<i>Pseudomonas aeruginosa</i>	-	-	-	-	15	15	15	15	19	18	17	18
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. Activities of disinfectant against organisms at 12.5% concentration.

Organism	Zones of inhibition (mm)											
	Methylated spirit				Jik				Savlon			
<i>Bacillus subtilis</i>	-	-	-	-	4	4	4	4	14	15	16	24
<i>Pseudomonas aeruginosa</i>	-	-	-	-	13	12	14	13	18	16	17	17
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-

Table 5. Activities of disinfectants against organisms at 6.25% concentration.

Organism	Zones of inhibition (mm)											
	Methylated spirit				Jik				Savlon			
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	-	10	11	9	10
<i>Pseudomonas aeruginosa</i>	-	-	-	-	12	13	11	12	16	17	15	16
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-

Table 3 demonstrates the activity of JIK at 25% concentration to be effective only on *P. aeruginosa* with 15 mm zone of inhibition. 25% Savlon showed activity against *B. subtilis* and *P. aeruginosa* with 20 mm and 18 mm zones of inhibition, respectively. Table 4 showed JIK at 12.5% concentration inhibits only *P. aeruginosa* with 13 mm zone of inhibition, while Savlon showed activity against *B. subtilis* and *P. aeruginosa* with 15 and 17 mm zones of inhibition, respectively. Table 5 shows JIK at 6.25% inhibits only *P. aeruginosa* with 12 mm zone of inhibition while Savlon inhibited *B. Substilis* and *P. aeruginosa* with 10 mm and 16 mm zones of inhibition, respectively.

The results obtained from this study demonstrates the antimicrobial activities of some commonly used disinfectants; JIK, methylated spirit, Savlon and kerosene on *B. Substilis*, *P. aeruginosa* and *C. albicans* that are known to be contaminants of skin, laboratory surfaces, toilets and pools. The results obtained from this study showed methylated spirit to be most effective against all the tested organisms at 100% concentration, but lost its activity when diluted by half. This result is consistent with the report of Larson (1995) that showed alcohol to be effective disinfectant on the skin. This work has demonstrated JIK to have inhibitory activities against all the tested organisms at 100% concentration. However, a serially diluted JIK produced no inhibitory activity against *C. albicans*,

but maintained its activity against *B. Substilis* and *P. aeruginosa* with lower zones of inhibition.

The result obtained from this study is in accordance with the previous report of Boyce and Pilet (2002) showed chlorhexidine, which is a component of Savlon to have excellent activity against gram positive organisms and good activity against Gram negative organisms. The result demonstrates Savlon to be highly effective against *B. substillis* and *P. aeruginora* at 100% concentration and further showed this agent to be relatively as effective as 100% concentration of methylated spirit and JIK on these organisms after dilution. However, Savlon has no activity against *C. albicans*.

The result obtained with kerosene indicated that it has good activity against *C. albicans* at 100% concentration which is in order with the work of Gerdner et al. (2002) that showed kerosene to have good disinfectant activity against skin cuts. Further dilution could not be made because of immiscibility of kerosene and water. The study of Logan (1994) that showed bacteria to survive in kerosene agreed with this work because kerosene at 100% concentration had no inhibiting effects on *B. substillis* and *P. aeruginora*. These findings may underscore the use of kerosene by barbers for aftershave as inappropriate especially for bacteria infections. Thus, the barbers should be enlightened about this effect.

In conclusion, this study revealed Savlon to be very effective against *B. substillis* and *P. aeruginosa*, while

kerosene was effective against *C. albicans*. However, JIK and Methylated spirit have moderate activities against *B. subtilis*, *P. aeruginas* and *C. albicans*, with Methylated spirit having better activity than JIK. Therefore, the study showed Methylated spirit and JIK to have good activity against both bacteria and fungi, while Savlon and kerosene to excellent activity against bacteria and fungi, respectively.

REFERENCES

- Boyce JM, Pittet D (2002). Guidelines for hand hygiene in healthcare settings: Recommendation of the healthcare infection control practices Advisory committee and HICPAC/SHSA/APIC/IDSA Hand Hygiene Task Force. *Infect control Hosp Epidemiol* 23(Suppl): S3-S40. Available at: <http://www.cdc.gov/handhygiene>.
- Denyer SP, Hugo WB, Harding VD (1985). Synergy in preservative combination. *Int. J. Pharm.* 25: 245-253.
- Gow NAR (1994). Growth and guidance of fungal hypha. *Microbiology* 140: 3139-3205.
- Larson EL (1995). APIC guideline for handwashing and hand antisepsis in health care settings. *Amer J infect cont.* 23(4): 251-269.
- Larson, EL, Morton HE (1996). Alcohol's. In *Disinfection, sterilization and preservation*, 4th ed, S.S. Block (ed), pp. 191-203. Lea and Febiger Philadelphia, Pa.
- Logan NA (1994). *Bacteria mysematies*. Oxford Blackwell Science. pp. 335-336.
- Russel AD, Russel NJ (1995). Biocides: activity, action and Resistance. *Symp. Soc. Gen. Microbial.* 53: 327-365.
- Russell AD, Hugo WB (1987). Chemical disinfectants, . In AH Linton, WB Hugo, AD Russell (ed.), *Disinfection in veterinary and farm animal practice*. Blackwell Scientific Publications, Oxford pp. 20-23.
- Rutala WA. (1996). APIC guideline for selection and use of disinfectants. *Amer J infect Control* 24(4): 313-342.
- Ryan KJ, Ray CG (2004). *Bacteria*. In: *Sherris Medical microbiology*, 4th ed, Mc Gram Hill ISBN 0838585299.
- World Health Organization (WHO) (2003). *Manual for the laboratory identification and antimicrobial susceptibility testing of Bacterial Pathogens of public health importance in the developing world*. Boyce and Pillet