Comparative Study of the Antimicrobial Activity of Chlorinated and Non-chlorinated Antiseptics against *C. albicans*

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Abstract: The efficacy of chlorinated and non- chlorinated antiseptics on *Candida albicans* is still not completely elucidated. Hence the general objective of this study is to determine the anti-candidal efficacy of nine commonly available antiseptics with chlorine (Purit, Savlon, Robert, Septol, Xylol and Dettol) and three without chlorine (Spring Mint, TCP and A.M.P.M.). The organism was challenged with diluted (according to the manufacturers instruction) of each of the antiseptics for a period between 30 seconds and 180 seconds and the microbial cell reduction rates were determined at every 30seconds contact by Time kill Test. The undiluted antiseptics with chlorine revealed 100% reduction in *C. albicans* cell count at 60secs contact time for Purit and Savlon while at 90secs undiluted Robert, Septol, Xylol and Dettol produced the same 100% lethal effect. Spring Mint, TCP and A.M.P.M. without chlorine did not produce significant cell reduction even at 180secs just like the control. Purit and Savlon, diluted according to the manufacturer's recommendations produced 100% cell reduction at 120 and 150secs respectively while Robert, Septol, Xylol and Dettol were able to produce 93.8% and 96.1% cell reduction at 180secs. Also, the pH of the antiseptics had significant association with their efficacy on *Candida albicans* (χ^2=3.54, $P < 0.05$). It is concluded that chlorination and pH of antiseptics has significant effect on the efficacy of antiseptics against *C. albicans.* [Academia Arena, 2010;2(9):35-40] (ISSN 1553-992X).

Keywords: *Candida albicans,* Antiseptics, Efficacy, Time Kill Test.

1. INTRODUCTION

Antiseptics and 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.,* 1999). They are used to control or reduce the growth of pathogenic microbes found on human body (Fraise, 2002). Many antiseptics in Nigeria markets today have varying degrees of effectiveness. These variations may be attributable to their active ingredients. Most of the antiseptics contain one of the following compounds: chlorhexidine, phenol, chloroxylenol and cetylpyridinium chloride (CPC) (Giuliana *et al.,* 1997). All, with the exception of the mouthwash, are applied externally to prevent proliferation of microbial population particularly during bath.

Most of the previously reported studies have been on the effects of these chemical agents on bacteria. There is paucity of reports on the effects of antiseptics on yeast- like organisms such as *Candida* species, which though are normal flora of human body but can become opportunistic pathogens when human immunity is compromised (Giuliana *et al.,* 1997). It is well known that bacterial and fungal cell-walls which are responsible for the cellular integrity of these microbes have different biochemical compositions (Henry *et al*., 2000). The cell- wall of bacteria is made up of peptidoglycan, techoic acid (in case of Gram positive bacteria and outer membrane (in case of Gram negative bacteria), while the cell wall of yeast is made up of mannan (Kobayashi *et al*., 1997). Also the cell-membrane of fungi is made up of ergosterols which is also found in mammalian cell-membrane but not found in bacterial cytoplasmic membrane (Henry *et al*., 2000). It is, therefore, expected that an antiseptic agent produced against bacteria may not be effective for yeast-like organisms such as *C. albicans*. However, an antiseptic which contains chemical compounds that have mode of action against the enzymes responsible for the biosynthesis of bacterial and fungal cell-walls and membranes will surely possess antimicrobial efficacy against these two groups of microbes.

Candida albicans is a normal flora of the human and animal digestive tract of the family

Saccharomycetaceae., It is the commonest fungus of medical importance. Candidiasis can be a superficial infection of skin, nails or mucous membrane with the yeast form of the fungus, causing mild inflammation (Larone, 2002). However, these tissues are rarely affected if they are entirely healthy (Brooks *et al*., 2004). *Candida albicans* is commonly found at low levels among the normal oral flora, but its overgrowth in immuno-compromised individuals or following broad-spectrum antibiotic therapy leads to oropharyngeal candidiasis (Sanglard**,** & Odds 2002). This is typically treated with fluconazole or related azole antifungals, which are inhibitors of ergosterol biosynthesis (Sanglard**,** & Odds 2002).

2.0 MATERIALS AND METHOD

2.1 Sample Collection of Antiseptics.

Market survey of various antiseptics available in Abeokuta markets, Ogun State, Nigeria was carried out. The antiseptics were purchased from markets and pharmacy shops in Abeokuta Ogun State, Nigeria. The compositions were obtained from the labels on the products.

2.2 Source of *Candida albicans.*

The Can*dida albicans* was isolated from the skin scrapings of a 12 year old primary school pupil attending St. Paul primary school I in Sagamu. The pupil was queried for cutaneous mycosis.

2.3 Purity Test

All the antiseptics used in this study were screened for sterility by aseptically streaking a loopful on MacConkey agar, Sabouraud dextrose agar and blood agar plates. The cultures were incubated at 37^0C for 48hours to isolate bacteria and yeasts, also at 27^0C for two weeks to isolate moulds.

2.4 Direct Microscopy and Culturing of Skin Scrapings

The skin scrapings were examined directly in 10% KOH with a cover glass and heated gently they were cultured on duplicate sets of Sabouraud dextrose agar with and without 0.5mg/ml of cycloheximide and incubated at both room temperature and 37^0C for 4 days.

2.5 Morphological and Biochemical Identification of *Candida albicans.*

Combination of morphological and biochemical tests were used to identify the *Candida albicans* in accordance with the guide to identification of medically important fungi (Davise, 2002). The identification test include : appearance and colour of colonies on Sabouraud dextrose agar, size and shape of cells, production of hyphae and/ or pseudohyphae, ability to produce germ tubes, ability to produce chlamydoconidia (chlamydospores), pellicle formation in broth , capsule stain, sugar assimilation, sugar
fermentation, nitrate assimilation, urease test and phenol oxidase test.

2.6 Time Kill Test

The *Candida albicans* isolate was challenged with each of the antiseptics between 30secs and 180secs using time kill test as described by Ogunledun *et al.,* (2008). An activated culture of *C. albicans* was obtained by inoculating a loopful of the stored organism into sterile nutrient broth and incubated at 37^0C for 18hrs. The suspension of the activated yeast- like fungus was adjusted to match the turbidity of 1.0 Mcfarland standard corresponding to 3.0×10^8 organisms per milliliter using phosphate buffer saline (Finegold & Martins, 1982). Then 0.1ml of the C. albicans suspension was separately inoculated into 10ml of phosphate buffer saline (PBS) to serve as control and into 10ml of each of the undiluted antiseptics to serve as test 1. Also, 0.1ml of the *C. albicans* suspension was inoculated into 10ml of each of the antiseptics diluted to the manufacturer's specification for use on humans to serve as test 2. Then 2.0ml of each of the inoculums (controls, test 1 and test 2) was transferred into 18.0ml molten sabouraud dextrose agar at 30, 60, 90, 120, 150 and 180secs intervals.
The contents were mixed by rotating the tubes between the palms of the hands and poured into a sterile disposable Petri dish. The media were allowed to set before incubating at 37^0C for 48hrs.

2.6.1. Interpretation of Time Kill Test.

At the end of incubation, the number of colony forming unit (CFU) of the controls and the tests were counted for each contact time from 30-180 seconds. The difference in colony counts between the control and test was divided by the colony count of the control before multiplying to obtain the percentage microbial cell reduction as shown below.

$$\text{Candida albicans cell reduction rate\%} = \frac{\text{CFU count at t sec of (control- test)}}{\text{CFU count at t sec of control}} \times \frac{100\%}{1}$$

2.7 pH Measurement.

An ATPH-6 waterproof digital pen pH meter (UNISCOPE surgifield medicals, LOT NO: 33-2006/20) was used for measuring the pH of the antiseptics used in this study. To take the pH, the key '*measure'* was pressed and letter '0' showed on the meter display. The electrode was rinsed in distilled

water and blot dried before dipping into the test solution, it was stirred once, then the key '*measure'* was pressed again and reading was recorded after 5seconds.

3. RESULTS

The results of the purity test of Savlon, Purit, Dettol, Septol, Xylol, Robert, T.C.P., Spring Mint and A.M.P.M. showed no microbial growth. Result from the Time kill test in table1 revealed ≥ 90% killing rate with diluted antiseptics with chlorine such as Savlon, Purit, Dettol, Robert, Xylol and Septol at 180s, while the undiluted antiseptics with chlorine such as Savlon, Purit, Dettol, Septol, Xylol, Robert, revealed 100% killing rate between 60s and 90s, (Table 2) while others (T.C.P., Spring Mint and A.M.P.M.) without chlorine could not. Table 3 displayed the pH values of antiseptics purchased from Abeokuta markets. Though, results from statistical analysis showed higher frequency of Candida killing rate at pH > 7.0 than pH < 7.0, and there was a significant difference ($\chi^2 = 3.54$, $P < 0.05$) (Table 4). Also, relationship between chlorine and 100% Candida killing rate was determined by Time kill test (Table 5). Antiseptics with chlorinated hydrocarbon were found with significantly higher Candida killing rate (100%) than in antiseptics without chlorinated hydrocarbon (0%) ($\chi^2 = 5.07$, $P < 0.05$).

Table 1. Killing Rate of *C. albicans* by the Diluted Antiseptics at Varying Time in seconds.

Antiseptics	Time Kill rate %					
	30s	**60s**	**90s**	**120s**	**150s**	**180s**
Savlon	53.9	68.2	94.1	97.8	100.0	100.0
Purit	55.4	71.2	93.1	100.0	100.0	100.0
Dettol	20.0	31.7	54.1	66.4	85.2	94.4
Robert	16.9	31.3	53.1	65.7	83.5	93.8
Xylol	21.1	35.1	59.5	70.3	88.6	96.1
Septol	25.2	40.2	63.1	67.1	84.2	94.1

Candida albicans reduction rate% = $\frac{\text{CFU count at t sec of (control- test)}}{\text{CFU count at t sec of control}} \times \frac{100\%}{1}$

Table 2. Killing Rate of *C. albicans* by the Undiluted Antiseptics at Varying Time in seconds.

	Time Kill rate %					
Antiseptics	30s	60s	90s	120s	150s	180s
Savlon	91.3	100.0	100.0	5.3	100.0	100.0
Purit	92.8	100.0	100.0	100.0	100.0	100.0
Dettol	75.4	92.2	100.0	100.0	100.0	100.0
Robert	74.3	91.4	100.0	100.0	100.0	100.0
Xylol	76.9	92.9	100.0	100.0	100.0	100.0
Septol	76.6	92.9	100.0	100.0	100.0	100.0
TCP	0.0	11.0	13.2	15.1	17.1	19.6
Spring- Mint	0.0	2.5	5.3	6.3	9.2	11.9
A.M.P.M.	0.0	2.2	5.3	6.3	8.9	11.9

Candida albicans reduction rate% = $\frac{\text{CFU count at t sec of (control- test)}}{\text{CFU count at t sec of control}} \times \frac{100\%}{1}$

Table 3. pH Values of the Antiseptics Purchased from Abeokuta Markets.

Antiseptics	pH value
Savlon	6.9
Purit	6.9
Dettol	10.6
Robert	9.4
Xylol	9.6
Septol	10.0
TCP	4.0
Spring mint	5.4
A.M.P.M.	5.3

Table 4. Relationship between pH and 100% Killing Rate at 180s

pH of antiseptics	100% killing rate at 180s		Total
	Yes (%)	No(%)	(%)
<7.0		5	(100)
>7.0		4	(100)
		4	(100)

$\chi^2 = 3.54, P < 0.05$

Table 5. Relationship between Composition of Antiseptics and 100% Killing Rate at 180s

100% cell reduction in 180s	Antiseptics		
	chlorinated n (%)	un-chlorinated n (%)	N
Positive	6 (100.0)	0(0)	6
Negative	0 (0)	3 (100.0)	3
Total	6 (100.0)	3 (100.0)	9

$\chi^2 = 5.07, P < 0.05$

4. DISCUSSION

The present study tested and compared the antifungal activities of Savlon, Purit, Xylol, Septol, Robert, Dettol, TCP, A.M.P.M., and Spring mint in direct contact with *Candida albicans* using time kill test

In a bid to unravel some the factors that may be responsible for the exhibition of excellent and preferential killing rate by some of the studied antiseptics, analysed data revealed a significant relationship between killing rate at 180s and pH (χ^2 =3.54, P< 0.05), indicating that pH of the studied antiseptics may play a role in the killing of *Candida albicans* (Table 4.9). Also the investigation on the relationship between the killing rate of *Candida albicans* at 180s and the composition of the antiseptics was able to establish the fact that antiseptics with chlorinated hydrocarbon were more efficacious than those without (χ^2 =5.07, P< 0.05) as shown in Table 4.10. This finding suggests that chlorination of disinfectants play a vital role in the efficacy of antiseptics against *Candida albicans.* This result is in agreement with the observation of Russell & Furr (1996) who also reported that chlorinated hydrocarbon antiseptics were more efficacious than non-chlorinated types. The Spring Mint, A.M.P.M. and TCP were found not efficacious in this study as an anticandidal antiseptics probably because they are unchlorinated (Table 4.1). Chlorinated antiseptics including Savlon and Purit produced 100% candida cell reduction in 60s and Roberts, Dettol, Septol and Xylol produced similar effects in 90s. Chlorine has also been reported as active against even vegetative forms of microbes and can remain on human skin following evaporation of alcohol Russell & Furr (1996).

Candida albicans is the most common cause of candidiasis, which can be acute, sub-acute or chronic infection involving any part of the body. This organism is found as part of the normal flora in the skin, mouth, vaginal tract and gastrointestinal tract (Larone, 2002).The single most important procedure that can aid in the prevention of hospital- associated diseases is the practice of hand washing. Physicians, nurses and other hospital personnel are frequently in contact with patients and thus serve as vectors in the transmission of infectious agents from one patient to another. Hand washing with antiseptics such as Savlon, Purit, Dettol, Robert, Xylol and Septol can be candicidal and, therefore, should be used before such procedures as surgery or the manipulation of invasive device such as catheter.

Comparison between the time-kill rate of *Candida albicans* among the tested antiseptics in this study has shown that Purit and Savlon when diluted to the manufacturers specification for human bathing were effective for 100% cell reduction rate of *C. albicans* at *120secs* and 150secs respectively (Table 4.11) while Dettol, Robert, Xylol and Septol were unable at the same time limit. However, in the undiluted antiseptics, Savlon and Purit were able to achieve100% cell reduction rate of *Candida albicans* in 60s of contact time while it took Dettol, Robert, Xylol and Septol 90s. The other undiluted antiseptics (Spring Mint, A.M.P.M. and TCP) could only produce less than 20.0% cell reduction even at 180s.

The extent of killing of the *C. albicans* as observed from the results of this study may be governed by two principal factors such as concentration of the antiseptics and their chemical composition.

Scientific facts from published articles suggested that antiseptics bind readily to microbial cell; with the amount absorbed increasing with concentration of the antiseptic solution and that most site of absorption is the cytoplasmic membrane (Barett-Bee *et al.,* 1994). The attribute of an antiseptic is to reduce microbial population or eliminate it, but there is often a low and rather narrow concentration range in which their effect is bacteriostatic (Ogunledun *et al.,* 2008). At these concentrations, certain biochemical functions associated with bacterial membrane may be inhibited. However, in the presence of higher concentrations of antiseptics and after prolonged treatment, the compound usually penetrates the cell and brings about extensive ill-define disruption of normal cellular functions (Ogunledun *et al.,* 2008).

The results obtained in this study showed that Savlon and Purit are very efficacious as anticandida antiseptics followed by Robert, Dettol, Septol and Xylol. The presence of chlorine in these anti-Candida antiseptics is probably responsible for their efficacy. Based on the findings, it is recommended that producers of antiseptics against pathogenic microbes such as *Candida albicans* must include chlorine in their formulations.

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