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Soaps and Disinfectants / Germicides as Adjunct Antimycotic Cleansing-Agents in Cases of Vulvovaginal Candidasis

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Abstract: Vulvovaginal candidasis is a significant gynaecological infection, which causes extreme discomfort, such as vulvovaginal itching. Using modified agar well-diffusion method, this study therefore, investigates *in vitro* inhibitory / cleansing potentials of soaps, germicides and disinfectants on vulvovaginal *Candida* species, especially with regards to vulvovaginal itching. Crusader oil (100%), Meriko (95.0-100%), Tura (88.9%), Tetmosol (84.7%) and Aloe (68.4%) were the most-inhibitory soaps, while *in vitro* inhibitory activities of germicides and disinfectants at manufacturers' specified dilution concentrations were *C. albicans* (Dettol: 34.6%; Purit: 84.6%), *C. glabrata* (Roberts: 33.3%; Purit: 83.3%), *C. Pseudotropicalis* (Roberts: 21.4%; Purit: 92.9%) and *C. tropicalis* (Dettol: 35.0%; Purit: 87.5%), Inhibitory activities at first lower dilution concentrations were *C. albicans* (Dettol: 80.0%; Purit: 96.2%); *C. glabrata* (Roberts: 83.3%; Dettol, Purit, Septol, Izal: 100%); *C. pseudotropicalis* (Roberts: 85.7%; Dettol, Purit, Izal: 100%) and *C. tropicalis* (Purit, Septol: 87.5%; Roberts: 90.0%) but total (100%) inhibitions were recorded at second lower dilutions, except in Morigad. Vaginal *Lactobacillus* strains were not inhibited *in vitro* by the soaps, germicides and disinfectants. Current result findings indicated that certain soaps, germicides and disinfectants posses *in vitro* inhibitory potentials against vulvo-vaginal *Candida*; therefore, can safely serve as potential adjunct, topical cleansing-agents in cases of candidasis-associated vulvo-vaginal itching.

Key words: *Candida* · Mycotic infections · Topical cleansing agents · Women's health · Vaginal itching

INTRODUCTION

In spite of therapeutic advances in women's health, vulvovaginitis, *Candida* vaginitis (vulvovaginal candidosis), which accounts for 20-30% of gynaecological diseases observed in women remains a common worldwide problem that affects all strata of the society [1-3]. Vulvovaginal candidosis has been reported as one of the most frequent infections of the female genital tract with a high incidence, as well as increase in frequency of cervical-vaginal infection, a common mucosal infection caused by opportunistic yeasts of the *Candida* genus. The symptomatic infection by *Candida* spp. arises when there is an excessive proliferation of this microorganism among the vaginal flora; increase in its colonisation and outright adherence to the vaginal cells; thereby, causing the infection [4]. The patient presents thick, fetid vaginal secretions with a granular appearance and an itchy, erythematous vulva; with the vagina becoming hyperemic, while there may also be excoriation and dyspareunia [5].

Although sometimes dismissed as a minor infection, vaginal thrush caused by *Candida* species is currently on the increase [3, 6] and approximately 75% of sexually active women suffer at least one episode at some points in their lives, while 10% to about half of them have recurrent episodes [7, 8]. This vaginal infection constitutes one of the most common problems in clinical medicine, by being extremely common and resulting in millions of visits to obstetricians or gynaecologists, STD clinics and emergency rooms [9- 11], so women often seek medical care for vaginitis, which whether infectious

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or not, the complaint may be misdiagnosed by the woman and/or her health care provider. Many times, the cause of complaints may be related to infections, which when misdiagnosed or mistreated, can lead to more severe problems [12] and as a common complaint of adolescent and adult females can cause extreme distress for some patients, especially those with recurrent symptoms [13].

Most localised, cutaneous candidiasis may be treated with any number of topical antifungal agents (e.g., clotrimazole, econazole, ciclopirox, miconazole, ketoconazole, nystatin, clotrimazole, amphotericin B etc.) or systemic oral azoles (fluconazole, itraconazole etc.) and over the years, conventional use of vaginal antimycotics has been remarkably safe and free of untoward side effects. However, increasing resistance to many of the antifungal agents has been recorded [14]; while high-doses of some of the regimens, e.g., terconazole were associated with fever and flu-like symptoms in high concentration, which resulted in the withdrawal of this topical agent. Mild to moderate vulvovaginal burning is also an under-estimated but not infrequent side effect of topical antifungals like azoles; while traditional therapies, such as topical canesten are both prolonged and inconvenient; thereby, often leading to poor compliance and recurrence of symptoms. Although topical nystatin has been found to be useful in women whose thrush has not responded to imidazoles [15] but in addion to recorded resistance, it can stain clothes yellow, which may therefore, reduce its acceptability [16, 17].

Other side effects include itching and burning or inflamed vulva, fulminant hepatitis [18, 19], painful vulva during insertion of antifungal drugs [15] etc. It is therefore, important to assay for adjunct, topical cleansing agents that do not have side effects such as vulvovaginal burning and itching caused by the use of topical antimycotics and which can also relieve from vaginal itching, even during treatments with antimycotic agents.

MATERIALS AND METHODS

Microbial Isolates and Culture Conditions: A total of 98 *Candida* strains, which were stock culture collections of the Department of Medical Microbiology & Parasitology, University College Hospital, (UCH), Ibadan but originally isolated from high vaginal swabs (HVS) and endocervical swabs (ECS) of patients presenting at the Special Treatment Centre [3] were used in this study. The *Candida* species were reactivated in Sabouraud dextrose broth containing ofloxacin antibiotic and incubtated at 32°C for 24-48h, followed by streaking on sterile Sabouraud dextrose agar (SDA) to obtain pure cultures on modified Sabouraud dextrose agar (SDA) plates containing ofloxacin and then incubated aerobically at 30-32°C for 24-48 hours to obtain pure cultures. Representatives of each different pure *Candida* colony were identified based on characteristic colonial growth on CRHOM-agar and standard phenotypic taxonomic tools, including Gram's identity and sugar assimilation characteristics [20, 21]. In addition, fresh wet mount examinations (wet preparations) and germinal tube assay were also performed on the strains, after which they were kept in triplicates on SDA agar slants at 4°C as bench and stock cultures.

Forty five *Lactobacillus* strains collected from human vaginal specimens of healthy, pre-menopausal women who had not been on antimicrobial therapy in about six months prior to the collection of the swab specimens were also characterised in this study. The *Lactobacillus* species were grown in de Man, Rogosa and Sharpe (MRS) medium (Lab M, England) by cutting the swab sticks into modified MRS broth at pH 5.3 - 5.5 and incubating anaerobically in 5% CO_2 (Gas Pak Anaerobic System, Oxoid) at 32°- 35°C for 24-48 hours. The broth cultures were subsequently streaked on modified MRS agar and also incubated anaerobically at 32°- 35°C for 24-48 hours. The modified MRS agar (pH 5.5) was prepared by dissolving required MRS gram in specified volume of distilled water and after thorough mixing, the medium was allowed to setle and about half of the supernatant (broth) was dispensed into a conical flask and the pH adjusted to 5.5 with HCl. The remaining MRS agar was homogenised by heating, then seperately sterilised along with the modified MRS broth in an autoclave at 121°C for 15 minutes, after which both were mixed together before pouring into sterile Petri dishes at 45°C. This method of preparing pH-modified MRS agar was to prevent hydrolysis of the agar during autoclaving.

Representatives of each different colony type were randomly picked from the primary plates and sub-cultured by repeated streaking on sterile MRS agar plates to assure purity. The *Lactobacillus* species were characterised based on standard phenotypic taxonomic tools [22] and kept in triplicates on MRS agar slants as bench cultures, while the *Lactobacillus* stock cultures were stored in Hogness freezing medium (3.6mM K_2HPO_4; 1.3mM KH_2PO_4; 2.0mM Na-citrate; 1.0mM $MgSO_4$; 12% glycerol) and kept frozen.

Antimicrobial Bioassay Using Agar Well-Diffusion Method: Test antimycotic agents used in this study and their active ingredients were mycoten tablets, mycoten

cream, canesten tablets, canesten cream (clotrimazole); fluconazole (flucamed tablets); tetradox tablet (doxycycline); interzol tablet (ketoconazole); mycostatine tablet, mycostatine cream (nystatin) and flagyl tablet (metronidazole); while the medicated / antiseptic and toilet soaps used were Imperial leather, Men Only, Joy, Lux, Zee, Valentine, Swan, Santex, Irish Spring, Movete, Dabur, Cussons, Skin Success, Zest, Medisoft, B29,Pears, Zarina, GIV, 21 Days, Mercury and Premier. Soaps used for bioassay were prepared as indicated for toilet purposes, with 10g of each soap or 10ml of each disifectants and germicides separately dissolved in 100 ml of sterile distilled water and the solutions used for the inhibitory studies. Active ingredients of the soaps were Tura (1.2% mercuric iodide); TCP (1.0% w/w total halogenated phenols, 0.254% w/v salicyclic acid); Dettol (0.5% w/v chloroxylenol); Tetmosol [ICC] (5% w/v monosulfiram B.P. [sulfiram]); trichlorocarbanilide); New Age (1.2% w/v trichlorocarbanilide); Meriko (1.2% w/v mercuric iodide); Carat (0.2% w/v triclosan) respectively but the active ingredients of *Dudu Osun*, Toto, Crusader oil and Aloe herbal soaps were not indicated by the manufacturers.

Dilution concentrations of the disinfectants and germicides used for bioassays were as specified by the manufacturers for toilet and midwifery purposes, and also at two lower dilution concentrations respectively: Dettol (1:40; 1:20; 1:10), Purit (1:60; 1:30; 1:20), Roberts (1:6; 1:3; 1:2), Septol (1:8; 1:4; 1:2); Morigad (1:400; 1:200; 1:50); Izal (1: 200; 1:100; 1:50). The active ingredients of the disinfectants and germicides were indicated as Dettol (4.8% chloroxylenol B.P.C.); Purit (0.3% w/v chlorhexidine gluconate B.P. and 3.0% w/v centrimide B.P.); Roberts (2% dichloroxylenol) and Septol (1.1% 6-chloro-hydroxy diphenyl methane).

Using a modification of agar well-diffusion method of Tagg *et al*. [23], the *Candida* strains were assayed for their *in vitro* antifungal susceptibility / resistance patterns against nine antimycotic agents commonly available in Nigeria for clinical cases, and commonly used soaps, disinfectants and germicides. Each test *Candida* strain was reactivated by being separately suspended in Sabouraud dextrose broth and incubated for 24 hours at 30°C. The modification was to avoid spreading of the soaps, disisnfectants and germicides' solutions on the agar surfaces. was separately dissolving one gram of each antimycotic tablet, capsule or cream in 10ml of sterile distilled water. 0.5ml of each test agent (soap solutions, germicides/ disinfectants incorporated into sterile semi-solid agar) was separately incorporated into sterile semi-solid SDA (45°C), which were separately dispensed into the agar wells (6.0 mm in diametre) bored into the SDA plates, already seeded with each *Candida* strain (500µl of inoculum size 10^3 cfu ml^{-1}). The plates were then incubated un-inverted for 24-48 hours at 30°C, while sterile, distilled water incorporated into sterile soft SDA served as control. Zones of inhibition surrounding the wells after incubation were measured and recorded in millimeters (mm) diameter and wells with no inhibition zones or zones less than 10.0 mm in diameter were recorded as resistant. The experiment was performed in duplicates.

Each *Lactobacillus* strain was suspended in MRS broth and incubated at 30°-35°C for 18-24 hours, while each soap solution (1000 µl) was screened against the test vaginal *Candida* and *Lactobacillus* strains and detection of antagonistic activities was determined by the modified (as stated above) agar well-diffusion method of Tagg *et al*. [23]. Sterile MRS agar was separately poured into sterile Petri plates and allowed to set at room temperature, after which holes 6.0 mm in diameter were bored into the set agar plates. The agar surfaces were separately seeded by streaking with 500 µl (of inoculum size 10^3 cfu ml^{-1}) of each *Candida* and *Lactobacillus* strains, while (1 ml) of each test agent (soap solution, germicides/ disinfectants incorporated into sterile semi-solid agar) was separately dispensed into the SDA and MRS agar wells. The plates were then incubated un-inverted for 24-48 hours at 32°C for the *Candida* and 35°C for the *Lactobacillus* strains, while sterile, distilled water incorporated into sterile soft agar served as control. The experiment was performed in duplicates and zones of inhibition surrounding the wells after incubation were measured and recorded in millimeters (mm) diameter, while wells with no inhibition zones or less than 10.0mm in diameter were recorded as resistant.

The medicated / antiseptic and toilet soaps (Imperial leather, Men Only, Joy, Lux, Zee, Valentine, Swan, Santex, Irish Spring, Movete, Dabur, Cussons, Skin Success, Zest, Medisoft, B29, Pears, Zarina, GIV, 21 Days, Mercury and Premier) used for bioassay were prepared as indicated for toilet purposes. 10g of each soap was separately dissolved in 100 ml of sterile distilled water and the aliquant were used for the inhibitory studies. The active ingredients of the soaps were, Tura (1.2% mercuric iodide); TCP (1.0% w/w total halogenated phenols, 0.254% w/v salicyclic acid); Dettol (0.5% w/v chloroxylenol); Tetmosol [ICC] (5% w/v monosulfiram B.P. [sulfiram]); trichlorocarbanilide); New Age (1.2% w/v trichlorocarbanilide); Meriko (1.2% w/v mercuric iodide); Carat (0.2% w/v triclosan) respectively, while the active ingredients of *Dudu Osun*, *Toto*, Crusader oil and Aloe herbal soaps were not

indicated by the manufacturers. The pH of most (93.5%) of the soaps was 9.0, with only 6.5% having pH of 10 and 11.

The dilution concentrations of the disinfectants / germicides used for the anti-candidal assays were as specified by the manufacturers for toilet and midwifery purposes and also at two lower dilution concentrations respectively: Dettol (1:40; 1:20; 1:10), Purit (1:60; 1:30; 1:20), Roberts (1:6; 1:3; 1:2), Septol (1:8; 1:4; 1:2); Morigad (1:400; 1:200; 1:50); Izal (1: 200; 1:100; 1:50). The active ingredients of the disinfectants / germicides were indicated as Dettol (4.8% chloroxylenol B.P.C.); Purit (0.3% w/v chlorhexidine gluconate B.P. and 3.0% w/v centrimide B.P.); Roberts (2% dichloroxylenol) and Septol (1.1% 6-chloro-hydroxy diphenyl methane).

Nine-member control group (out of the initial 50 subjects), consisting of females having recurrent vaginal trush within one and ten years were the subjects in the study. Due to a protracted national industrial strike, the ethical approval could not be conclusively processed, so personal informed consents were obtained from nine subjects among the volunteers who still indicated interest in the study, in order to be able to use the test microbial cultures within expected time duration. Each of the subjects under investigation was trained on how to clean the pubic areas after bath in the morning with one of the selected toilet/medicated soaps (Meriko, Tura, Tetmosol, *Dudu-Osun* and Dettol) per week but without vaginal douching. The after-effect of soap cleansing was recorded after a weekly period of five weeks.

The subjects in the nine-member control study group were further subjected to cleansing with the disinfectants / germicides. Each of them using one of the disinfectants / germicides under investigation was also trained on how to clean the pubic areas with the disinfectants / germicides without vaginal douching, after bath in the morning and the after effect of the disinfectants / germicides' cleansing was recorded after a week period. The dilution concentrations of the disinfectants / germicides used for the cleansing assays were at two lower dilution concentrations to those specified by the manufacturers for toilet and midwifery purposes. After a week break from the studies, the subjects were allowed a *free week*, during which they were allowed to clean up as they wished, based on the after-effects of previous studies.

RESULTS

The isolated vaginal lactobacilli from healthy subjects were identified as *L. acidophilus*, *L. brevis*, *L. casei*, *L. fermentum*, *L. plantarum* and *L. reuteri*, while the isolated vaginal yeast strains from patients presenting for candidiosis in this study belong to the species, *Candida albicans* 26 (26.5%), *C. glabrata* 18 (18.4%), *C. pseudotropicalis* 14 (14.3%) and *C. tropicalis* 40 (40.8%). The *Candida* species assayed for in this study were mostly susceptible to the test antimycotic agents (but less susceptible after two years), with the overall susceptibility rates of 70.4 – 99.0%. Lower susceptibility rates were however, recorded among the *C. pseudotropicalis* strains towards mycoten / canesten tablets and creams (Table 1).

Out of a total of 39 medicated / toilet soaps used in this study, the overall susceptibility results indicated that Crusader oil (SB9; 100%), Meriko (SB10; 95%/ SA1; 100%), Tura (SA7; 88.9%), Tetmosol (SB6; 84.7%) and Aloe (SB11; 68.4%) were the most inhibitory soaps against the *Candida* strains *in vitro*, with zones of inhibition between 10.0 and 35.0 mm in diameter; although, most of the soaps recorded moderate inhibition zones of between 10.0 and 22.0 mm in diameter but the *Candida* strains were not susceptible to 33.3% of the soaps *in vitro* (Table 2).

Using dilution preparations according to the manufacturer's specification, the inhibitory activities of the germicidal and disinfecting agents ranged between (Dettol; 34.6% and Purit; 84.6%) for *C. albicans*; (Roberts; 33.3% and Purit; 83.3%) for *C. glabrata*; (Roberts; 21.4% and Purit; 92.9%) for *C. pseudotropicalis*; (Dettol; 35.0% and Purit; 87.5%) for *C. tropicalis*. The zones of inhibition at the test dilutions were between 10.0 and 20.0 mm in diameter, while two of the disinfectants (Morigad and Izal), however did not inhibit any of the *Candida* strains at the specified dilutions by the manufacturers (Table 3).

Inhibitory activities at first lower dilution concentrations of the germicides and disinfectants also varied between (Dettol; 80.8% and Purit; 96.2%) for *C. albicans*; (Roberts; 83.3% and Dettol, Purit, Septol, Izal; 100%) for *C. glabrata*; (Roberts; 85.7% and Dettol, Purit, Izal; 100%) for *C. pseudotropicalis*; (Roberts; 90.0% and Purit, Septol; 87.5%) for *C. tropicalis* (Table 3). The zones of inhibition at these dilutions were between 10.0 and 22.0 mm in diameter. All the *Candida* strains were inhibited by the disinfectants and germicides at the second lower dilutions except Morigad (*C. albicans*; 69.2%), (*C. glabrata*; 72.2%), (*C. pseudotropicalis*; 92.9%) and (*C. tropicalis*; 82.5%). The zones of inhibition at these dilutions were between 10.0 and 34.0 mm in diameter (Table 3).

The nine-member study group reported relief from vulva itching and burning sensations after the first week of cleansing with the selected soaps. As at the time of the experiment, seven (77.8%) of the subjects preferred Meriko for fast relief from vaginal itching and burning

Table 1: *In vitro* susceptibility profiles of *Candida* species implicated in candidasis using antifungal agents

	% susceptibility profiles (g ml^{-1})									
Pathogens	Myct	Mycc	Cnstt	Cnstc	Flmd	Trdx	Intz	Mycs	Flgy	[2 yrs later]*
Overall susceptibility	75.0	76.5	70.3	70.3	95.3	89.0	98.4	98.4	75.0	
C. albicans [9]	88.9	88.9	88.9	88.9	100	88.9	100	100	88.9	[0.0-77.8]
C. glabrata [24]	70.8	62.5	62.5	66.7	87.5	91.7	91.7	91.7	70.8	[12.5-54.2]
C. pseudotropicalis [5]	60.0	60.0	40.0	60.0	100	100	100	100	75.0	[0.0-75.0]
C. tropicalis [26]	73.1	80.8	73.1	69.2	96.1	92.3	96.1	100	76.9	[7.6-57.7]

Keys: MYCT = mycoten tablet; MYCC = mycoten cream; CNSTT = canesten tablet; CNSTC = canesten cream;
FLMD = flucamed; TRDX = tetradox; INTZ = interzol; MYCS = mycostatine; FLGY = flagyl
* = Overall susceptibility values of the *Candida* species after 2 years

Table 2: *In vitro* susceptibility profiles of *Candida* species implicated in candidasis using medicated /antiseptic and toilet soaps

	% susceptibility profiles (g ml^{-1})													
Pathogens	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	SB12		
[Batch B]														
C. albicans	7.7	19.2	7.7	46.2	38.5	73.1	0.0	0.0	100	100	84.6	0.0		
C. glabrata	22.2	22.2	0.0	61.1	64.3	94.4	0.0	0.0	100	100	44.4	0.0		
C. tropicalis	2.5	17.5	5.0	30.0	50.0	85.0	2.5	2.5	100	95.0	68.4	0.0		
C. pseudotropicalis 7.1	21.4	0.0	42.9	42.9	92.9	0.0	0.0	100	92.9	64.3	0.0			
Overall susceptibility	8.2	19.4	4.1	41.8	45.9	84.7	1.0	1.0	100	95.0	68.4	0.0		
[Batch A]														
	SA1	SA2	SA3	SA5	SA7	SA10	SA12	SA13	SA14	SA15	SA16	SA18	SA19	SA23
Overall susceptibility	100	8.9	4.4	2.2	88.9	20.0	4.4	28.9	4.4	11.1	6.7	2.2	26.7	2.2

Keys: Batch B [SB1 = Tura; SB2 = TCP; SB3 = Dettol; SB4 = *Dudu osun*; SB5 = Toto; SB6 = Tetmosol; SB7 = Delta; SB8 = New Age; SB9 = Crusader Oil; SB10 = Meriko; SB11 = Aloe; SB12 = Carat]
Batch A [SA1= Meriko; SA2 = Toto; SA3 = Asepso; SA5 = Men Only; SA7 = Tura; SA10 = Dudu-Osun; SA12 = Valentine; SA13 = Swan; SA14 = Santex; SA15 = Irish Spring; SA16 = Movete; SA18 = Cussons; SA19 = Tetmosol; SA23 = Crusader Ultra]

Table 3: *In vitro* susceptibility profiles of *Candida* species implicated in candidasis using germicidal agents

	% susceptibility profiles (ml l^{-1}) (Manufacturer's specified dilution concentrations)					
Pathogens	G1 (1:40)	G2 (1:60)	G3 (1:6)	G4 (1:8)	G5 (1:400)	G6 (1:200)
Overall susceptibility	38.8	86.7	36.7	69.4	0.0	0.0
Candida albicans	34.6	84.6	38.5	69.2	0.0	0.0
Candida glabrata	50.0	83.3	33.3	72.2	0.0	0.0
Candida tropicalis	35.0	87.5	42.5	72.5	0.0	0.0
Candida pseudotropicalis	42.9	92.9	21.4	57.1	0.0	0.0
	% susceptibility profiles (ml l^{-1}) (First lower dilution concentrations)					
	G1 (1:20)	G2 (1:30)	G3 (1:3)	G4 (1:4)	G5 (1:100)	G6 (1:100)
Overall susceptibility	91.8	99.0	86.7	96.9	0.0	92.9
C. albicans	80.8	96.2	84.6	92.3	0.0	84.6
C. glabrata	100	100	83.3	100	0.0	100
C. tropicalis	92.5	100	90.0	100	0.0	92.5
C. pseudotropicalis	100.0	100	85.7	92.6	0.0	100
	% susceptibility profiles ml l^{-1} (Second lower dilution concentrations)					
	G1 (1:10)	G2 (1:20)	G3 (1:2)	G4 (1:2)	G5 (1:50)	G6 (1:50)
Overall susceptibility	100	100	100	100	78.6	99.0
C. albicans	100	100	100	100	69.0	96.1
C. glabrata	100	100	100	1007	2.2	100
C. tropicalis	100	100	100	100	82.5	100
C. pseudotropicalis	100	100	100	100	92.9	100

Keys: GMC1 = Detttol; GMC2 = Purit; GMC3 = Roberts; GMC4 = Septol; GMC5 = Morigad; GMC6 = Izal

sensations, while Tetmosol, *Dudu osun* and Tura were rated as second best by five (55.6%) of the subjects. *Dudu osun* was preferred best (77.8%) based on effectiveness and fine honey aroma; while, Meriko was indicated as being slightly harsh on the skin and as regards the smell (66.7%). Overall, Meriko, *Dudu osun*, Tura and Tetmosol soaps were preferred by the subjects but subject 9 was eliminated for further examination and treatment after the first week because of continued vaginal discomfort and laboratory examination confirmed combination of trichomatis with candidasis in the subject.

The study group also indicated relief from vulva itching and burning sensations after the first week of cleansing with the germicides / disinfectants and all the subjects preferred Purit and Septol for fast relief from vaginal itching and burning sensations, while Izal was discontinued after the second day due to complaint of harshness and pungent smell. Overall, Septol, Purit and Dettol were preferred by the subjects, while the results obtained from the *free-week* indicated that most (77.8%) of the subjects preferred to clean with combination of preferred soap and disinfectant twice (morning and evening) per day. Five of the subjects also informed of slight douching during the study due to more relief than the normal cleaning of the vaginal area.

DISCUSSION

Several years ago, *Candida* species were commonly regarded as little more than culture contaminants; however, in less than two decades, these microorganisms have become major human pathogens, even as the most common cause of vulvovaginitis [2, 9, 11, 24-27] and just as disconcerting is the increasing rate at which drug-resistant *Candida* spp. are regularly reported [17-19, 27- 29]. As also studied by Adad *et al*. [11], there was an increase in the frequency of *Candida* spp. over four decades (1960's, 1970's, 1980's and 1990's) and similarly, according to Ogunshe *et al*. [3], out of 4047 patients presenting at the Special Treatment Clinic (STC) of the University College Hospital (UCH) Ibadan, Nigeria, between May 13 1997 and November 29 2005, the most recovered pathogens from the patients were *Candida* species (55.6%).

Performing aetiologic diagnosis of vulvovaginitis is necessary in order to take appropriate therapeutic and preventive measures, especially in patients with recurrent infection [1] but as earlier reported by Riordan *et al*. [30], no single specimen was found ideal for all pathogens in the female reproductive tract; therefore, endocervical and vaginal swabs were cultured for the recovery of *Candida* spp. in this study, while the isolated vaginal yeast strains were found to belong to *Candida albicans* 26 (26.5%), *C. glabrata* 18 (18.4%), *C. pseudotropicalis* 14 (14.3%) and *C. tropicalis* 40 (40.8%) species. These species of *Candida* have similarly been previously reported in various studies as the most frequent causes of acute and chronic vulvovaginitis [1, 6, 26, 30-32].

As earlier suggested by Sweeney *et al*. [33], information obtained from *in vitro* antifungal sensitivity testing can be used to direct *in vivo* antifungal therapy, therefore, widespread application of standardised *in vitro* antifungal sensitivity testing is needed in cases of candidasis. Currently, there are no surveillance on the epidemiology and therapy of candidal infections in Nigeria; and in addition, *in vitro* antifungal sensitivity testing is not as common as *in vitro* antibiotic sensitivity testing in bacterial species; therefore, prescriptions are basically made on most-readily available antifungal agents in the market, since the variety of newer, highly effective topical azole agents [34], which are presently available in a variety of formulations in most developed countries are not common in Nigeria; thus, the commonly available products in the market are the ones that can be administered in cases of candidasis.

Increased resistance to common antifungal agents have however, been generally noted [34- 36] and there is also the notion that antifungal agents used to treat vaginitis may be contributing to the drug resistance problem by promoting cross-resistance to a range of clinically used antifungals [14]; meanwhile, in the current study, the *Candida* strains were initially mostly susceptible to the test antimycotics, except among the *C. pseudotropicalis* strains, in which lower susceptibility to mycoten (40.0%) were recorded. However two years after, lower susceptibility rates were recorded among the *Candida* species; which thus supported the earlier findings of Bhutani *et al*. [37], Yamazumi *et al*. [38] and Verghese *et al*. [32]. Meanwhile, it is important to note that inconsistencies in production batches of drugs produced or imported into developing countries like Nigeria can lead to different susceptibility rates of the *Candida* species and ultimately have varying effects on the therepeutic efficacy of the antimycotic agents due to the varying drug quality [14].

At least half of women suffer a recurrence of candidasis once the treatment drugs are stopped [18]. As an example, topical antifungal products may be used during pregnancy but oral treatments are not normally given to either pregnant women or to nursing mothers; therefore, decrease in long-term mycological cure might

result in re-growth of *Candida* as part of the vaginal flora. Similarly, women with chronic or recurrent vaginal thrush seldom have recognisable precipitating factors and the condition can lead to psychosexual problems and depression [15, 18]. In addition, vulvovaginal candidiasis is a non-notifiable disease and was excluded from the ranks of sexually transmitted diseases; not surprisingly, vulvovaginal candidiasis has received scant attention by public health authorities, funding agencies and researchers, most especially in developing countries such as Nigeria, while epidemiological data on risk factors and pathogenic mechanisms also remain inadequately studied. Most importantly, standards of care, including diagnosis and therapy therefore, remained undefined [39].

The fact that severe candidiasis sometimes gave rise to fissures and extensive erythema and oedema of the vulva [31] and did not respond well to short courses of treatment [40]; led to the evaluation of soaps and germicides / disinfectants' for their potency as adjunct topical agents in cases of vulvo-vaginal discomforts due to candidiasis. Though varied susceptibility patterns were exhibited by the *Candida* species but the results obtained in this study indicated strong *in vitro* inhibitory effects of the soaps and germicides / disinfectants on the *Candida* strains, irrespective of the species. The qualitative and semi-quantitative changes in the aerobic microbial flora of normal skin with the prolonged use of a chlorhexidine scrub (6 months) have earlier been found to produce a reduction in the total aerobic counts in the axilla, groin and between the toes and fingers [41]. Also, in a double-blinded, randomised clinical trial of hands of primary caretakers in 238 inner city households, a single handwash with antimicrobial (containing 0.2% triclosan) handwashing soap was found to have minimal effect on quantity of bacterial counts of the hand flora [42]. This study similarly reported the *in vitro* anti-candidal effects of soaps in cases vulvo-vaginal candidiasis.

Delta, *Dudu-Osun*, Meriko, TCP, Tetmosol, *Toto* and Tura soaps were assayed for during two sets of the study; however, apart from Meriko soap, the *in vitro* inhibitory activities of the remaining soaps varied. The active ingredients of the soaps were probably not adequate in the soap batches with lower inhibitory activities, which is usually the case with most products in the country, including toiletries. It can thus, be inferred that production batches can also have effect on the inhibitory activities of the soaps, especially since there are no regulating bodies in the country directly responsible for surveillance of industrial cosmetic products. This can therefore, pose as a limitation in this study. However, since treatment regimen chosen for successful treatment of vulvovaginal candidiasis should fit the patient's daily lifestyle [43].

There is a classic view that the vagina of healthy women is colonised by a homogeneous population of lactobacilli [44], while the predominance of *Lactobacillus* species present in the vagina has been found to confer protective activities against urinary tract infections. For example, adherence of *Lactobacillus acidophilus* to the vaginal cell wall has been known to block the attachment of uropathogenic bacteria to the surface of the uroepithelial cells, while the prevention and control of infections by *Lactobacillus* species has been known to be due to the production of some metabolites such as aminoglycosides, bacteriocins, hydrogen peroxide, diacetyl and lactic acid, which act as inhibitory barriers against some pathogenic microorganisms [45, 46]. None of the vaginal lactobacilli under the current study was inhibited by any of the test soaps and germicidal agents, indicating the safety in usage of toilet or medicated soaps and disinfecting / germicidal agents at the appropriate concentrations, as adjunct, topical cleansing agents.

Even when candidiasis has been coreectly diagnosed, majority of the infected women cannot afford to combine oral and topical medications in cases of vulvovaginal candidosis due to high poverty level in the country and in addition, although the pathogenic *Candida* strains were mostly susceptible to the test antifungal agents but they could not prevent burning sensations and accompanying itching usually experienced during vulvovaginal candidosis; thus, soaps and germicides can serve as adjunct, topical cleansing agents in cases of vulvovaginal candidosis, even when combined oral and topical treatments are affordable. However, one of the limitations of the study is that consistency of the product quality of the soaps and germicides / disinfectants cannot be ascertained because the regulating bodies for such consumer products are not quite effective; therefore, in most cases, there are variations in the products based on differences in production batches. *Candida* strains used in this study were multi-drug susceptible; therefore on-going studies are looking into the susceptibility patterns of multi-drug resistant *Candida* strains towards soaps and germicides / disinfectants.

CONCLUSION

Adjunct effect of certain Nigerian toilet /medicated/ antiseptic soaps and disinfectants/germicides as topical cleansing agents in cases of vaginal itching

and burning sensations has been provided by the result findings of this study. Though most *Candida* species that are responsible for vulvo-vaginal candidasis may be depedent on geographical locations, diet and other factors but there is the possibility that similar findings can be identified in various countries. The study concluded that the current findings also add to the list of advantages and safety of of soaps, germicides and disinfectants in human hygiene with regards to their potentials in cases of vulvovaginal itching and burning sensations associated with vaginal candidasis.

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