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Laboratory investigation on candidastic potentials of bleach and toilet soaps

Adenike A.O. Ogunshe

Applied Microbiology and Infectious Diseases, Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Oyo State Nigeria

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ABSTRACT

Some medically-important Candia species can survive on inner clothing materials in close contact with human genital and extragenital areas. Since soaps and bleach are known to possess antimicrobial properties, this preliminary study was to investigate the candidastic effects of bleach products and commonly available industrial soaps on multi-drug resistant Candida species. Using a modification of agar well-diffusion method, 60 strains from oral rinses / swabs and vulvovaginal C. albicans, C. glabrata, C. pseudotropicalis and C. tropicalis isolated from endocervical / high vaginal swabs of patients presenting for sexually transmissible infections were screened to determine their in vitro susceptibilities to 18 industrial soaps and three brands of bleach samples. Undiluted bleach samples (RBL1, RBL2 and RBL3) were the most inhibitory concentrations, with 10.0-44.0%, 94.0-100% and 96.0-100% of the Candida strains inhibited in vitro by the respective bleach products. Only 4.0 - 10.0% (C. pseudotropicalis), 5.9 - 10.0% (C. albicans) and 20.0 - 30.0% (C. glabrata) strains were inhibited in vitro at the manufacturers' dilution specifications for the bleach products, while inhibitions at dilution 1:10 were 8.0-16.0% (C. glabrata) and 5.8-10.0% respectively. Only 4.0% and 5.8% of C. glabrata and C. albicans were inhibited in vitro at dilution 1:100; while none of the industrial soaps had in vitro candidastic potentials. In addition to whitening effect, topically-safe and minimally-diluted Clorox and JIK bleach can be used for personal hygiene by disinfection of contaminated inanimate objects, such as panties, undergarments and for toilet purposes to prevent infection / re-infection, in cases of vulvovaginal candidasis.

Keywords: bleach, candidasis, recurrent vulvovaginal candidasis, soaps, sodium hypochlorite.

INTRODUCTION

Bleach is sodium hypochlorite that has broad-spectrum disinfecting potentials, which mostly contains approximately 5% sodium hypochlorite (50,000 milligrams per milliliter chlorine [Cl_2]). It is known to serve as an effective disinfectant on many surfaces and is very commonly used as bleach in the home to disinfect drains, toilets and other surfaces. At concentrations of 0.05 - 0.5% (500-5,000 milligrams per millilitre of sodium hypochlorite), it contains free and available chlorine. It is generally considered to be intermediate-level disinfectant, and among the most convenient and least expensive germicides that is most effective against common microbial pathogens, including fungi [1]. Environmental Protection Agency (EPA) also registered sodium hypochlorite-based bleach as being effective in killing 99.9% of bacteria, viruses and some types of moulds. Additional reported advantages of bleach are that it kills the widest range of pathogens as an inexpensive disinfectant and is extremely powerful against viruses and bacteria at room temperature, Sodium hypochlorite, the active ingredient in household bleach, also helps to whiten, brighten and remove dirt and stains from surfaces and fabrics [2-4].

Bleach and soaps have been reported to exhibit certain antimicrobial properties, which led to evaluation of certain batches of soaps, germicides and disinfectants, as potential adjunct topical cleansing agents in cases of vulvovaginal discomforts, such as vaginal itching and burning sensations, due to candidiasis [5]. Vulvovaginal candidiasis (VVC) or *Candida* vaginitis (vulvovaginal candidosis) is a common, worldwide significant mucosal infection, and one of

the most common diseases that affect women's health, with over 50% of women experiencing at least one episode of vaginal infection in their lifetime. It accounts for 20-30% of gynaecological diseases observed in women, as well as also affecting all strata of the society worldwide [6-10]. Although VVC is one of the most common vaginal infections, it is a non-notifiable disease and not surprisingly, has received scant attention by public health authorities, funding agencies, as well as researchers. Epidemiologic data on risk factors and pathogenic mechanisms also remain inadequately studied [6].

Severe candidiasis sometimes gave rise to fissures and extensive erythema and oedema of the vulva [11], which most times would not respond well to short courses of antifungal treatments [12]. Furthermore, problems of human vulvovaginal candidasis and recurrent vulvovaginal candidasis (RVVC), associated topical / dermal discomforts, as well as re-infection had however, been attributed to contamination and recontamination of underwear and other inner clothing of females [13-17]. This study therefore, tried to determine the inhibitory potentials of three brands of bleach and 26 industrial laundry soaps on human pathogenic *Candida* species.

MATERIALS AND METHODS

Collection of Clinical Specimens:
Most of the *Candida* strains used in this study were clinical stock cultures [5, 18-20], originally isolated from endocervical swabs (ECS) and high vaginal swabs (HVS) of patients presenting at the sexually transmissible infections (STI) clinic of the Department of Medical Microbiology & Parasitology, University College Hospital (UCH), Ibadan, Nigeria.

Determination of anti-candidal activities of bleach samples / industrial and local soaps against *Candida* strains using a modified agar well-diffusion method:
Eighteen industrial soaps (Carat, Carex, Delta, Dettol, GIV, Halo antibacterial deodorant, London, Imperial Leather, Lux, Medisoft, Mekano, Meriko, Movate Savon, Premier antiseptic, Septol antiseptic & medicated, Tetmosol, Tura and Zee) bought from a major market in Dubai, United Arab Emirates (UAE) were assayed for *in vitro* inhibitory potentials.

Active ingredients of some of the industrial soaps were– GIV soap [*Cocos necitera* oil, *Elacsis guineensis* oil, sodium hydroxide, perfume, glycerin, rode oil, titariumdioxide, tetrasodium EDTA, water, Cl 15880, Cl 45100], Halo soap [sodium tallowate, sodium palm kernelate, water (Aqua), fragrance (Parfum), glycerin, sodium chloride, tetrasodium EDTA, tetrasodium etidronate, titanium dioxide Cl 77891, menthol, trichlocarbon 1% w/w, Cl 12490, limonene, butylphynyl, methylpropional, linalool, geraniol], Imperial Leather soap [soap base, water, glycerin, fragrance, stabiliser and colour], London soap [1.2% w/w mercuric iodide included as 3% potassium mercuric solution], Lux soap [sodium tallowate, sodium palmate, aqua, sodium palm kernelate, glycerin, paraffin, sodium sulphate, titanium dioxide, phosphoric acid, tetra sodium EDTA, etidronic acid, tocophenol acetate, disodium distyrylbiphenyl disulfonate, hexyl cinnamol, Geraniol, benzyl salicylate, butylphenyl, methylpropionol, coumarin, Limonene, Cl 74160, Min TFM = 68%], Meriko soap [sodium tallowate, sodium cocoate, aqua, perfume, Cl 74160, Cl 12490, Cl 77266], Tetmosol soap [5% monosulfiram B.P., sodium tallowate, *Citronella*], Tura soap [Triclosan, allantoin, vitamin E, sodium tallowate, sodium palm kernelate, aqua, perfume, Cl 12940 (Pigment Red 5), Cl 77266 (Carbon Black), Cl 74160 (Pigment Blue 15)] and Zee soap [native black soap base, palm kernel oil, Shea butter (rich in natural vitamins), cocoa pod and palm bunch ash solution, camwood extract (*osun*), native honey, aloe vera, aqua, fragrance].

JIK perfumed extra whitening stain removal bleach is a product of Reckitt Benkiser (Nigeria) Limited, a company that is part of the worldwide Reckitt family, founded in the United Kingdom over a century ago, and the active ingredient was 3.85% ml/v sodium hypochlorite. Clorox bleach is manufactured by Clorox Chemical Co. USA, which started its bleach factory in the early 1900s. The ingredients that made up 1% or more of the contents of Clorox bleach product by weight are listed in descending order of concentration as, water > sodium hypochlorite > sodium chloride > sodium carbonate > sodium hydroxide > sodium polyacrylate [3, 4].

A modification of Tagg *et al*. [21] method was used for the bioassay study to determine the *in vitro* inhibitory potentials of three brands of bleach and various batches of soaps. Sterile Mueller-Hinton agar was aseptically poured into sterile Petri dishes and allowed to set, after which holes that were 6.0 mm in diameter were aseptically punched out of the agar plates. The agar surfaces were then surface-sterilised with Bunsen flame and allowed to cool before separately seeding the agar by streaking with 500 µl (of inoculum size 10^3 cfu ml^{-1}) of each *Candida* strains [5]. One ml of each test agents (bleach samples and soap solutions incorporated into sterile semi-solid agar) was separately dispensed into the holes and incubated at 32-5^0C for 24-48 h. Demonstration of antagonism depends on the release of diffusible inhibitory materials from bleach or soap samples into the assay agar plates.

The modification was that the bleach samples were incorporated into sterile semi-solid agar before dispensing into the agar wells, in order to avoid spreading of the bleach samples on the agar surface. Inhibition zones surrounding the *Candida* strains were noted and recorded in mm diameter, while zones that were less than 10.0 mm in diameter or absence of zones of inhibition were recorded as resistant (negative).

RESULTS

Demonstration of antagonism of *Candida* by bleach or soap samples was determined by zones of inhibition. Results of various concentrations of bleach samples indicated that raw (undiluted) bleach samples (RBL1, RBL2 and RBL3) were the most inhibitory concentrations with 10.0-44.0%, 94.0-100% and 96.0-100% of the *Candida* strains being inhibited *in vitro* by the respective undiluted bleach samples. Only 10.0%, 4.0%, 10.05 of *C. pseudotropicalis*; 5.9%, 10.0% of *C. albicans*; 30.0% and 20.0% of *C. glabrata* strains were inhibited *in vitro* at the manufacturers' dilution specifications, while inhibitions at dilution 1:10 were very negligible (5.8-16.0%). Just 4.0% and 5.8% of *C. glabrata* and *C. albicans* were inhibited *in vitro* at dilution 1:100 (Table 1). In the current study, none of the 18 tested soaps was inhibitory against the *Candida* species (Table 2).

DISCUSSION

Bleach is usually a laundry agent for whitening clothes and clothing materials but in some cases, it is used as a disinfectant. Results obtained in the present study indicated that undiluted JIK white (RBL3), Clorox white (RBL2) and Clorox bleach for coloured clothes (RBL1) bleach samples were mostly inhibitory *in vitro* against all the *Candida* strains, except RBL1, which was totally resisted by *C. tropicalis* strains. Precise mechanism through which bleach exerts its germicidal action has not been fully confirmed but it is believed to be mediated by ability of free chlorine to denature proteins, inactivate sulfhydryl-containing enzymes and damage nucleic acids (RNA and DNA) of targeted microorganisms or by oxidising cell membrane of such microorganisms, resulting in a loss of structure, and leading to cell lysis and death [22, 23]. These activities may thus, be responsible for the *in vitro* inhibitory potency of bleach samples recorded in this study.

It is commonly reported that full-strength bleach provides a more potent and convenient disinfectant [24-26] but once diluted, bleach breaks down quickly, mainly into salt and water [27]; meanwhile, household bleach samples are usually to be properly diluted to work best as whitening agent. However, bleach samples, which were diluted in this study according to the manufacturer's specifications and at lower dilutions, were far less inhibitory than the undiluted samples. Efficacy of a bleach solution to act as a disinfectant usually depends on the concentration of its free and available chlorine; thus, *chlorine demand* is a term used in describing the amount of chlorine that is expended in the course of reaction with inorganic and organic materials, such that any remaining chlorine after this demand is met is referred to as *available chlorine* [22]. The inability of the bleach samples at the manufacturers' and lower dilution specifications to serve as potent candidastic agents may therefore be due to lower available chlorine at higher diluted rates of the bleach samples.

It had been reported that bleach solutions prepared with tap water at pH $\geq$ 8 are stable for about a month when stored at room temperature in a closed, opaque container. But the third brand of bleach sample (white JIK bleach) lost potency faster with time (results not shown), probably due to defective covers of the containers, since most of the covers were broken as from the third / fourth time of opening and closing the container. Even same batch contents stored in well-covered transparent glass bottles were more potent over a longer period of time than the remaining contents in the manufacturer's containers with broken covers. Thus, old containers of partially used bleach may no longer have the labeled concentration [28].

Few data support that several factors like obesity, wearing of tight-fitting clothes and panty liners, use of underwear made from synthetic material etc. had been somehow implicated in VVC and urinary tract infections, as well as increase in colonisation of female genital and extragenital sites by *Candida* spp. Considering that extra-genital sites are often colonised by *Candida* spp. in women with a history of RVVC, and significantly more often, if the genital tract is also colonised, then, extra-genital sites may be reservoirs for re-colonisation of the genital tract in women, and few cases in men, which of course have serious implications in treatment failures [15, 16]. In a previous study [5], about 80.0% of test *Candida* strains were susceptible to 39 medicated / toilet soaps *in vitro*. However, apart from Meriko soap, *in vitro* inhibitory activities of the remaining soaps varied remarkably within two-year interval, e.g., Meriko (100% vs. 95.0%), Tura (88.9% vs. 8.2%) and Tetmosol (84.7% vs. 26.7%). None of the same brand of tested soaps was inhibitory *in vitro* against *Candida* species in the present study, and also as reported in a similar study for soaps bought in Nigeria [20].

Study of Fuls *et al*. [29] indicated that non-antimicrobial soaps were less active, so it can be deduced from the result findings of this study that it is likely that the test *Candida* strains were intrinsically resistant to the test soaps or that the levels of chemical compositions (active ingredients) with inhibitory properties in such soaps were less than the effective concentrations. However, there were reductions in *Candida* colony counts (cfu log^{-1}) of panty washings (pers. comm.), which means that soaps can be effective in washing off pathogenic *Candida* species from contaminated panties and under-wears.

Table 1: *In vitro* inhibitory activities of bleach on *Candida* species

Candida species	% inhibitory activities at various concentrations of bleach samples (v/v)											
	RBL1	**MSP1**	**1:10**	**1:100**	**RBL2**	**MSP2**	**1:10**	**1:100**	**RBL3**	**MSP3**	**1:10**	**1:100**
C. albicans [17]	17.6	0.0	0.0	0.0	94.1	5.9	5.8	0.0	100	10.0	10.0	5.8
C. glabrata [25]	44.0	0.0	8.0	0.0	96.0	30.0	16.0	4.0	96.0	20.0	12.0	0.0
C. pseudotropicalis [10]	10.0	10.0	0.0	0.0	100	4.0	0.0	0.0	100	10.0	0.0	0.0
C. tropicalis [8]	0.0	0.0	0.0	0.0	100	0.0	0.0	0.0	100	0.0	0.0	0.0

Keys: *RBL1 = raw Clorox coloured bleach sample 1; RBL2; = raw Clorox white bleach sample 2; RBL3 = raw JIK bleach sample 3; MSP1 = manufacturer's dilution specification for bleach sample 1; MSP2 = manufacturer's dilution specification for bleach sample 2; MSP3 = manufacturer's dilution specification for bleach sample 3.*

Table 2: *In vitro* inhibitory activities of toilet soaps on *Candida* species

	SP1	**SP2**	**SP3**	**SP4**	**SP5**	**SP6**	**SP7**	**SP8**	**SP9**	**SP10**	**SP11**	**SP12-SP18**
C. albicans [43]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Candida glabrata [52]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. pseudotropicalis [19]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. tropicalis [15]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Keys: *SP1 – SP11 = soap samples bought in Nigeria; SP12-S18 = soap samples bought in Dubai.*

CONCLUSION

Most cases of vaginitis are the result of infection that accounts for millions of physician office visits and most importantly, standards of care, including diagnosis and therapy remain undefined. [30]. Meanwhile, some medically-important *Candia* species can survive on fabrics [31, 32], as aetiological agents of VVC or RVVC, a significant gynaecological infection that causes extreme discomfort in infected women. Even non-infectious *Candia* strains may become opportunistic pathogens when there is colonisation of human genital and extragenital sites, as well as inanimate personal effects like panties and inner clothing. There is dearth of scientific reports on candidastic effects of bleach but the most effective means of getting rid of pathogenic *Candida* from clothing materials that are in close contact to the genital and extragenital areas is either not known or poorly studied. But based on findings of this first study in the country on candidastic potential of bleach, combination of soapy water and well-diluted bleach for safe topical application can be used to wash off and disinfect significant pathogenic *Candida* loads from inanimate objects like contaminated pants, under-wears, inner clothing, household linens, toilet seats and bathtubs, as well as cleansing of human extragenital sites or washing of extragenital sites for personal hygiene, to prevent contamination /recontamination, which can ultimately cause infection/re-infection in cases of VVC. Moreover, reduction of infection/re-infection through this cleansing / disinfecting procedure can also serve as a peculiar control therapeutic measure for VVC and RVVC. Considering the various implications of antimicrobial therapy, including adverse drug effects due to adulterated and substandard medications, as well as antifungal resistance in developing countries like Nigeria [33], there would be less need for treatments of VVC and RVVC when there is reduction in infectious cases; therefore, there would be less therapeutic hazards.

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