

EFFICACY OF SODIUM METASILICATE IN REMOVING CANDIDA BIOFILMS IN RELATION TO THEIR ANTIFUNGAL SENSITIVITY

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Received: 05 November 2011, Revised and Accepted: 26 December 2011

ABSTRACT

Biofilm formation on medical devices, mucosal surfaces, implants etc. by *Candida* spp. is responsible for ever increasing problems throughout the globe because this increases mortality and morbidity, as antifungal agents often fail to destroy them. Thus it is our duty to find out a novel way to mitigate it. Although sodium metasilicate is utilized as a minor constituent in many formulations to remove biofilms but this nontoxic agent can be used singly in higher concentrations (8g/dL) more effectively which was proved in this study. At this concentration it will kill all *Candida* cells blocking any chance of any residual spread.

Key words: Biofilm, Sodium metasilicate, MIC, Antifungal susceptibility.

INTRODUCTION

Patient survival rates have greatly increased with the use of medical devices in all fields of medicine and it also has beneficial effect on quality of life. However, they are again associated with a variety of complications like the development of device-related infections. It normally begins with colonization of the microorganisms followed by a complex physiological metamorphosis by such organisms like bacteria or fungi resulting in biofilm formation ^{1,2,3,4}. In recent past, mycoses which threaten human life has been increasing in individuals with compromised immune system and there is a dramatic change in the incidence of different *Candida* spp. in candidiasis ⁵. *Candida* species are carried innocuously by a large proportion of humans, basically on the epithelial surfaces of the mouth, GI tract, vagina and skin. Predisposing factors to candidiasis include immuno-suppression, catheterization, premature birth, use of broad spectrum antibiotics⁶. *Candida* cells always try to overcome the immune response of human and they can cause a wide range of infections. Superficial *Candida* infections of the skin and the mucous membranes of the oral cavity and the vagina are mostly observed. *Candida* spp. also can infect a wide variety of organs including kidney, liver and brain when the cells penetrate through the epithelia with pseudohyphae and are disseminated throughout the body through blood ⁷. Over the past few decades, there is a significant increase of blood stream *Candida* infections ⁸.

Candida strains possess a number of virulence factors and due to these facts susceptible hosts are prone to be affected mainly with haematogenously disseminated infections. Among all virulent factors biofilm formation is the most important one. Slime or biofilm represents a structured community of fungal cells embedded in a self-produced polymorphic matrix adherent to the artificial surface ⁹. It is also a fact that antifungal treatment frequently fails to eradicate these infections despite the use of drugs with proven in vitro activity. Sometimes, pathogen from the implanted device could not be eliminated by host defense mechanism as they have the ability to adhere to plastic materials and to promote formation of a biofilm ^{10,11}.

The external layers of *Candida* cells actively take part in adhesion process to host surface, which ultimately leads to the biofilm growth and maturation, playing an important role in the pathophysiology of candidiasis ¹². *Candida* biofilm formation carries clinical manifestations because of their increased resistance to antifungal therapy and the cells within biofilms have this ability to withstand host immune defenses ¹³.

For clinical use, there are various types of antifungal agents available and these belong to different classes- polyenes, azoles, 5-flucytosine and echinocandins etc ¹⁴. Antifungal agents currently available for clinical use are inadequate against serious systemic fungal infections

and they also have side effects. In immunocompromised patients, extensive use of fluconazole results drug resistance to most of *Candida* spp. which are resistant to this drug. Amphotericin-B remains the preferred compound but problems associated with solubility in water, toxicity and ineffectiveness against mould diseases limit its therapeutic potential ¹⁵. In vitro, micafungin showed broad-spectrum fungicidal activity against clinically relevant *Candida* spp.; but it has also side effects ¹⁶. To overcome this situation, we have to concentrate on newer chemical compounds which possess antifungal activities against the causative agents with less side effects.

The purpose of our study is to evaluate the efficacy of chemical compounds like sodium metasilicate in removing biofilms which are mainly device associated caused by *Candida* spp.

MATERIALS AND METHODS

A total of 30 blood samples were collected from ICU patients. Blood cultures of these patients were done in automated blood culture system (Becton Dickinson, BD, BACTEC 9050) in MYCO/F-Lytic bottles. The positive samples were then subcultured on Sabouraud dextrose agar at 37^o C ¹⁷. A total of 6 *Candida* species isolates were recovered and these were tested for antifungal susceptibility and biofilm formation. To identify the species, several tests like germ-tube test, colonial study on corn meal agar (CMA) with 1% Tween80 / trypan blue, growth pattern on candida chromogenic media, carbohydrate assimilation and fermentation tests were done ¹⁸. The isolates included *Candida tropicalis*, *C. albicans* and *C. glabrata*. One international standard strain of *C. albicans* (ATCC-10231) was also used for the comparative study.

The antifungal susceptibility testing

The antifungal susceptibility of fluconazole, amphotericin-B and micafungin against isolated *Candida* strains was done by MIC strip method. The inoculum suspensions were prepared as instructed in M27-A2, NCCLS, USA (Approved Standard Second Edition). The MIC strips were applied to the Mueller Hinton agar surface of the plate with the MIC scale facing upwards. Then it had been placed properly on the surface of the agar media. The plates were incubated at 35-37 °C and examined after 24-48 hours ¹⁹.

BIOFILM FORMATION

Test tube method

Using a modified tube adherence test proposed by Brachini *et al.*, the slime or biofilm production was determined ²⁰. A loopful of organisms from the surface of Sabouraud's -dextrose agar plates was inoculated into the tubes (tubes were taken in pair for each

Candida strain) containing 6mL of Sabouraud's broth supplemented with 8% glucose in the final concentration and a control tube with broth but without cell suspension was taken for comparison ²¹. The tubes were incubated at 37° C for 48 hours, after that 6mL of distilled water or 4% / 8% sodium metasilicate solutions were added in each tube. These were kept for 30 minutes and then the cell suspension in the tubes were poured or aspirated out and the walls of the tubes were stained with 1% safranin, the adhesive layer produced on the walls of the tubes were interpreted as biofilm or slime production . Biofilm production was scored as negative (-), weak positive (1+), moderate positive (2+) or strong positive (3+) ²⁰.

RESULTS

Six *Candida* species were isolated from 30 collected blood samples. Amongst the six isolates, 3 were *C. tropicalis*, 2 were *C. albicans* and one was *C. glabrata*. At first, the antifungal susceptibility test was done and afterwards efficacy of sodium metasilicate on removing biofilm was tested for those species. The zone of inhibitions was in the form of an ellipse. MIC values were the value at which the zone convenes the comb like projections of the strip. The antifungal susceptibility test results were shown in Table 1.

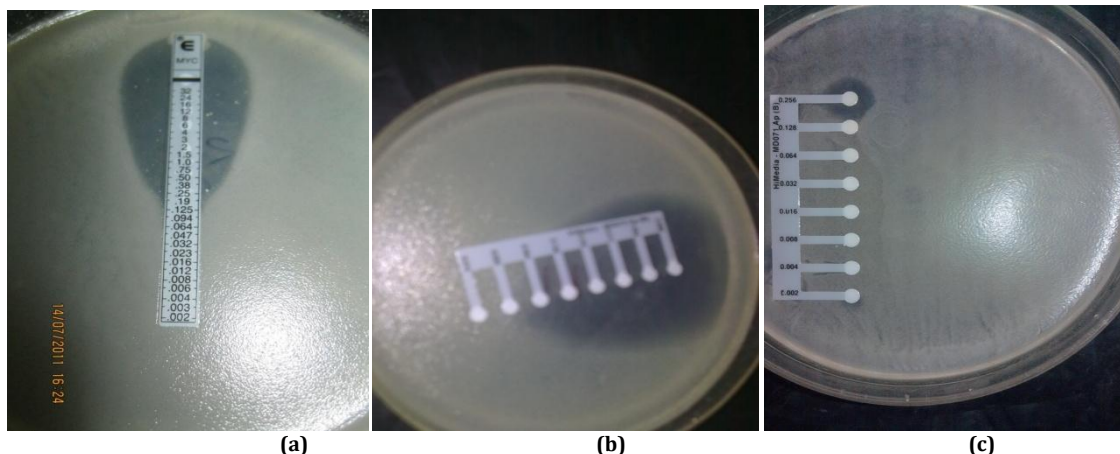


Fig 1: (a) MIC of Micafungin, (b) MIC of Fluconazole, (c) MIC of Amphotericin-B

Table 1: The MIC values of Fluconazole, Amphotericin-B and Micafungin

Strain No.	Species	MIC values		
		FLUCONAZOLE Range:0.016-256 µg/mL	AMPHOTERICIN-B Range:0.0002-32 µg/mL	MICAFUNGIN Range:0.002-32 µg/mL
1	<i>C. glabrata</i>	0.064	0.064	0.25
2	<i>C. tropicalis</i>	0.032	0.128	0.012
3	<i>C. tropicalis</i>	0.016	0.256	0.012
4	<i>C. tropicalis</i>	0.016	0.128	0.006
5	<i>C. albicans</i>	Resistant	0.128	0.006
6	<i>C. albicans</i>	0.064	0.064	0.016
7	<i>C. albicans</i>	0.064	0.064	0.004
	ATCC-10231			

The MIC values indicated that out of seven *Candida* species including the ATCC strain six were susceptible to fluconazole, amphotericin-B and micafungin. Micafungin had showed very good result against these isolates and one *C. albicans* isolate was resistant to fluconazole.

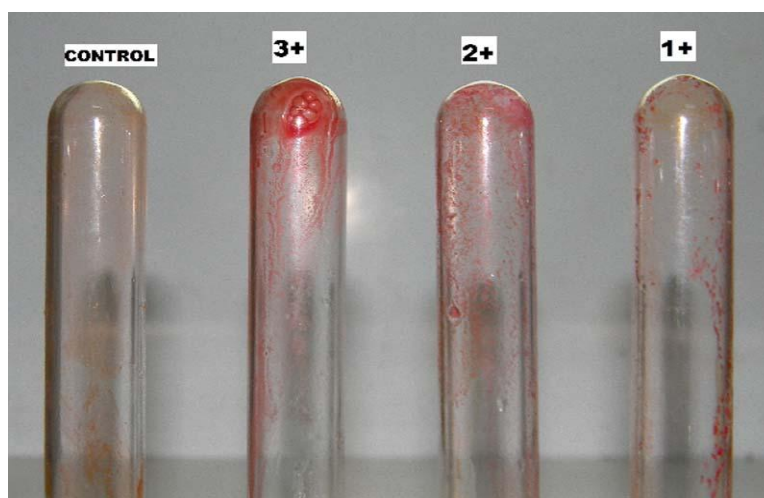


Fig 1: Biofilm formation by *Candida* strains

Table 2: Biofilm formation and action of sodium metasilicate.

Species	Without adding D/W(vol-6 mL)	Add 6 mL D/W(vol-12 mL)	Add 6 mL 4% metasilicate(vol-12 mL)	Add 6 mL 8% metasilicate(vol-12 mL)
<i>C. glabrata</i>	1+	1+	-	-
<i>C. tropicalis</i>	3+	1+	-	-
<i>C. tropicalis</i>	2+	2+	2+	1+
<i>C. tropicalis</i>	3+	2+	1+	1+
<i>C. albicans</i>	2+	2+	2+	1+
<i>C. albicans</i>	3+	1+	2+	1+
ATCC 10231	3+	2+	1+	-

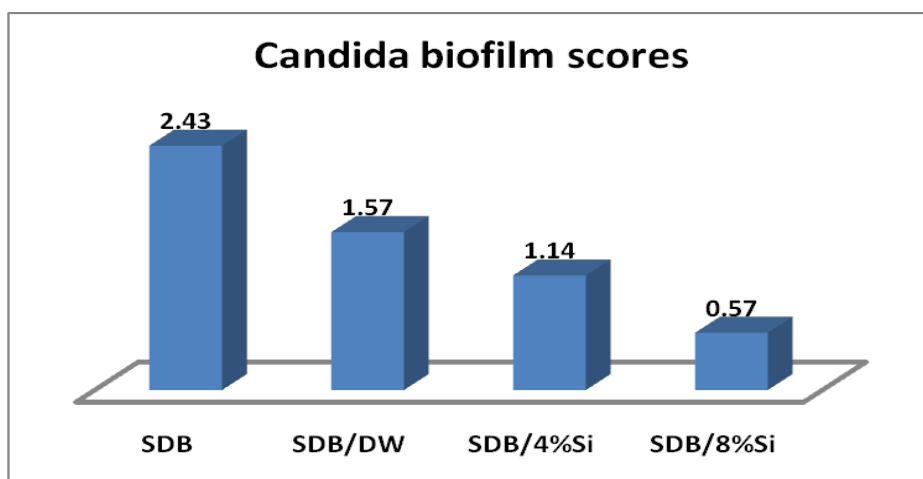


Fig 2: The graph shows scores of Candida biofilm treating with sodium metasilicate and distilled water.

SDB: Sabouraud's dextrose broth ,SDB/DW: Equal volume of distilled water added to SDB *Candida* growth after 48 hours; SDB/4%Si: Equal volume of 4% Na metasilicate added to SDB *Candida* growth after 48 hours; SDB/8%Si: Equal volume of 8% Na metasilicate added to SDB *Candida* growth after 48 hours.

Table 3: Biofilm score in different sets of experiments along with standard deviation and standard error of mean*

	Mean	Standard deviation	Standard error of mean	t-value between (a)and (b)
Original growth in Sabouraud dextrose broth (a)	2.43	0.78	0.20	4.94 (P value significant at 0.001)
With equal volume distilled water (b)	1.57	0.53	0.141	t-value between (a) and (c)
With equal volume 4% sodium metasilicate solution (c)	1.14	0.90	0.24	6.45 (P value significant at 0.0001)
With equal volume of 8% sodium metasilicate solution (d)	0.57	0.53	0.141	t-value between (a) and (d)
				10.94 (P value significant at 0.0001)
				t-value between (b) and (c)
				2.53 (P value significant at 0.05)
				t-value between (b) and (d)
				7.14 (P value significant at 0.0001)
				t-value between (c) and (d)
				3.35 (P value significant at 0.01)

*In b, c and d equal volumes were added after growth as described in a, kept for 30 minutes.

Decrease of biofilm in (b) was 35.4%, Decrease of biofilm in (c) was 53.1%, Decrease of biofilm in (d) was 76.6%.

DISCUSSION

Various model systems have been used to investigate the properties of *Candida* biofilms *in vitro*. These ranged from simple assays with catheter disks to more complex flow systems, such as the perfused biofilm fermentor²². The cells are surrounded by a matrix of extracellular polymeric material, the synthesis of which markedly increases when developing biofilms are exposed to a liquid flow²³. Results from several studies have shown that *Candida* biofilms are resistant to clinically important antifungal agents, including amphotericin B, fluconazole and micafungin and less effective against biofilms²⁴. The mechanisms of biofilm resistance to antimicrobial agents are not fully understood. One long-standing hypothesis for the resistance of fungal biofilms is that the matrix material restricts drug penetration by forming a reaction-diffusion barrier²⁵ and that only the surface layers of a biofilm are

exposed to a lethal dose of antifungal agent. The extent to which the matrix acts as a barrier to drug diffusion would depend on the chemical nature of both the antimicrobial agent and the matrix material.

The mean score of the original growth of biofilms in Sabouraud dextrose broth of the different strains of *Candida* spp was found to be 2.43 with a standard deviation of 0.78 and 0.20 standard error of mean. The mean value of the growth of biofilms in Sabouraud dextrose broth with equal volume of water was found to be 1.57 with a standard deviation of 0.53 and 0.141 standard error of mean. The mean score of the growth of biofilms in Sabouraud dextrose broth with equal volume of 4% sodium metasilicate solution was found to be 1.14 with a standard deviation of 0.90 and 0.24 standard error of mean. The mean score of the growth of biofilms in

Sabouraud dextrose broth with equal volume of 8% sodium metasilicate solution was found to be 0.57 with a standard deviation of 0.53 and 0.141 standard error of mean. A considerable decrease of biofilm was observed with 8% sodium metasilicate solution (76.6%).

In this study we observed a definite *Candida* biofilm lowering activity of sodium metasilicate even up to 76% which is very significant. It is probably due to the detergent action of its aqueous solution depending on wetting power, emulsifying ability, deflocculating power and dissolving power. Sodium metasilicate can be removed easily by washing and it is nontoxic, thus simple washing with this chemical is sufficient to prevent *Candida* biofilm.

ACKNOWLEDGMENT

We are most grateful to the Managing Director and Medical Director of Peerless Hospital and B. K. Roy Research Centre for giving us clinical data.

FUNDING

All funds were provided by Peerless Hospital and B.K.Roy Research Centre

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