Research Letter

Antifungal Activity of Commercial Mouthrinses Against Candida Albicans

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ABSTRACT

Objectives
To investigate the effectiveness of a delmopinol, sodium chlorite, povidone iodine, and lactoferrin-lysozyme-lactoperoxidase mouthrinse against C. albicans planktonic cells and biofilms.

Methods
Two-fold dilutions of the mouthrinse solutions were prepared (1:2, 1:4, 1:8, 1:16, 1:32, 1:64), and dispensed into 96 well microtitre plates. Standardized inocula of wild type C. albicans SC5314 was added and the minimum inhibitory concentrations (MICs) were determined in accordance with Clinical and Laboratory Standards Institute (CLSI) Guidelines for the broth microdilution assay. Optically clear wells were cultured for determination of minimum fungicidal concentrations (MFCs). The effect of the mouthrinses against C. albicans mature biofilms was also evaluated. The metabolic activity of the biofilms was semi-quantitatively determined with a standard 2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay.

Results
The lowest MICs were exhibited by delmopinol (1:16), followed by sodium chlorite (1:8), povidone iodine (1:4), and lactoferrin-lysozyme-lactoperoxidase (1:4). Delmopinol exhibited fungicidal activity at 1:16 dilution, while MFCs were achieved by higher concentrations of povidone iodine (1:4) and sodium chlorite (1:2). Lactoferrin-lysozyme-lactoperoxidase did not exhibit fungicidal activity at all tested concentrations. Among the four agents at two-fold dilution, sodium chlorite and povidone iodine exhibited the greatest reductions (>90%) in C. albicans biofilms (p<0.005). Significantly greater reductions in metabolic activity were achieved with sodium chlorite compared to the other agents at dilutions of 1:4 through to 1:32 (p<0.0005).

Conclusions
Delmopinol exhibited the highest activity against planktonic C. albicans, while sodium chlorite exhibited greater effects against mature biofilms.

Clinical Significance
Sodium chlorite was shown in this study to exhibit potent antifungal activity against C. albicans biofilms, and products containing this active ingredient deserve consideration for inclusion in oral health promotion measures conducted amongst vulnerable patient groups.

Keywords
Yeast; Mouthrinse; Antifungal activity.

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**INTRODUCTION**

*Candida albicans* is an opportunistic pathogen commonly isolated from the oral cavity, and is a major etiological agent of oral mucosal infections. It is the fourth leading cause of nosocomial bloodstream infections, and the association of sepsis with preceding colonization has been demonstrated in AIDS patients, haematopoietic stem cell and bone marrow transplant patients, as well as low-weight neonates. *Candida albicans* has also been implicated as an etiological agent of pneumonia in certain medically compromised patient groups. While *C. albicans* is commonly isolated from various oral sites in healthy individuals, higher prevalence rates have been observed in hospitalized patients, and oral colonization has been associated with factors such as xerostomia, broad spectrum antibiotic therapy, immune status, nutritional deficiencies, and endocrine disorders.

Although conventional antifungal agents such as polyenes and azoles have been the mainstays of prophylaxis and treatment for systemic and oral candidiasis, resistance to such agents have been increasingly reported in medically compromised patients. There is thus an urgent need for the evaluation of the antifungal activity of existing commercial oral antiseptic agents, which is currently poorly understood.

The aim of this study was to investigate the efficacy of four existing commercial mouthrinses against *C. albicans* planktonic cells and biofilms. Tested products included: Betadine® mouthrinse (povidone-iodine 1% w/v, 8.7% ethanol, menthol, methyl salicylate, glycerol, saccharin sodium, purified water), Decapinol® mouthrinse (delmopinol hydrochloride 2mg/ml, aqua, alcohol, aroma, saccharin sodium, sodium hydroxide), TheraBreath® mouthrinse (sodium chlorite, water, PEG-40 hydrogenated castor oil, tetrasodium EDTA, sodium bicarbonate, sodium benzoate, mentha piperita, benzoic acid, saccharin sodium), and OralSeven® mouthrinse (lactoferrin, lysozyme, lactoperoxidase, potassium thiocyanate, calcium lactate, glucose oxidase, aqua, monopropylene glycol, xylitol, hydrogenated starch hydrolysate, poloxamer 407, hydroxyethyl cellulose, sodium benzoate, aloe barbadensis, mentha piperita, benzoic acid, zinc gluconate).

The antifungal activity of mouthrinses was tested against wild type *C. albicans* strain SC5314, which was cultured as previously described. Two-fold dilutions of the mouthrinse solutions were prepared (1:2, 1:4, 1:8, 1:16, 1:32, 1:64) in RPMI, and minimum inhibitory concentrations (MICs) were determined in accordance with Clinical and Laboratory Standards Institute (CLSI) Guidelines for the broth microdilution assay. Optically clear wells were cultured on Sabouraud’s agar for determination of minimum fungicidal concentrations (MFCs). The effect of the mouthrinses against *C. albicans* mature biofilms was also evaluated using a previously established protocol (Figure 1). In brief, standard *C. albicans inouela* inocula (2-3x10^6 cells/ml) were added to 96-well polystyrene microtitre plates (Iwaki) and incubated at 37 °C with agitation. Following the adhesion phase, supernatant was aspirated, and wells were washed twice with PBS. Plates were incubated for a further 24 hours at 37 °C. Following aspiration of liquid and washing with PBS (2x), previously prepared serial dilutions of mouthrinses (1:2, 1:4, 1:8, 1:16) in YNB were added. Plates were incubated for an additional 24 hours, and YNB without mouthrinses were included in positive control wells. The metabolic activity of the biofilms was semi-quantitatively determined with a standard 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay. All assays were done in triplicate.

The lowest MICs were exhibited by delmopinol (1:16), followed by sodium chloride (1:8), povidone iodine (1:4), and lactoferrin-lysozyme-lactoperoxidase (1:4). Fungicidal activity was achieved at 1:16 dilution for delmopinol, and at higher concentrations for povidone iodine (1:4 dilution), and sodium chloride (1:2 dilution). Lactoferrin-lysozyme-lactoperoxidase did not exhibit fungicidal activity at all tested concentrations. Among the four agents at two-fold dilution, sodium chloride and povidone iodine exhibited the greatest reductions (>90%) in *C. albicans* biofilms (p<0.005) (Figure 2). Significantly greater reductions in metabolic activity were achieved with sodium chloride compared to the other agents at dilutions of 1:4 through to 1:16 (p<0.005). Lactoferrin-lysozyme-lactoperoxidase consistently exhibited the weakest antifungal activity, with only a 40% reduction in biofilm metabolic activity at 1:2 dilution.

Prior studies have investigated the effectiveness of a wide range of commercial mouthrinses (eg. chlorhexidine, benzylamine hydrochloride, lawsonine methyl ether, povidone iodine, lactoperoxidase-hypothiocyanate, amine fluoride, melaleuca) against oral yeasts in hospitalized and medically compromised patients, as well as their acceptability among both patients and nursing staff in hospital wards. Nevertheless, it has been clear that *in vitro* activity against *Candida* often does not equate with clinical effectiveness, and there currently remains a lack of evidence supporting the clinical efficacy of chemical agents contained in mouthrinses. In this study, sodium chloride demonstrated potent antifungal activity against *C. albicans* biofilms, and products containing this active ingredient deserve consideration for inclusion in oral health protocols.
Figure 2. Effect of Four Commercial Mouthrinses on Candida Albicans Biofilms Metabolism Following Incubation with Mouthrinses At A) 1:2 Dilution: Kruskal-Wallis Test (P<0.001). Pairwise Mann-Whitney U Tests (A<B<C; P<0.005). B) 1:4 Dilution. Kruskal-Wallis Test (P<0.001). Pairwise Mann-Whitney U Tests (A<B<C; P<0.001). C) 1:8 Dilution: Kruskal-Wallis Test (P<0.001). Pairwise Mann-Whitney U Tests (A<B<C; P<0.001). D) 1:16 Dilution: Kruskal-Wallis Test (P<0.001). Pairwise Mann-Whitney U Tests (A<B; P<0.001).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.
REFERENCES


