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ORIGINAL ARTICLE

In vitro antifungal effect of amine fluoride-stannous fluoride combination on oral *Candida* species

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OBJECTIVE: The combination of amine fluoride and stannous fluoride (AmF/SnF_2) was, by chance, found to be antifungal in a clinical trial. This study investigated its effect on pathogenic *Candida* species with the hypothesis that the antifungal action on different species is variable.
MATERIALS AND METHODS: Growth inhibition effect of Meridol® mouth rinse which contains 250 ppm AmF/SnF_2 was evaluated on 43 reference and clinical strains of *Candida albicans*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*. Meridol® base solution without AmF/SnF_2 was used as a negative control.
RESULTS: Undiluted Meridol® mouth rinse killed most study strains within a few minutes. In ascending order, *C. parapsilosis*, *C. tropicalis*, *C. albicans*, *C. glabrata*, *C. krusei* and *C. dubliniensis* showed higher resistance against AmF/SnF_2 than *C. guilliermondii*.
CONCLUSION: AmF/SnF_2 could be used as a potent adjunct to antifungal therapy for oral yeasts. Although different *Candida* species demonstrated variable sensitivity the most prevalent oral yeast *C. albicans* appeared sensitive to the AmF/SnF_2 combination.
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Keywords: *Candida albicans*; *Candida glabrata*; *Candida krusei*; *Candida dubliniensis*; *Candida tropicalis*; *Candida guilliermondii*; *Candida parapsilosis*; amine fluoride; stannous fluoride; antifungal effect

Introduction

The emergence of antibacterial drug resistance is a growing global problem and there is also a reason for concern in general dental practice (Eliopoulos, 1998; Kilby and Dismukes, 1998; Austin *et al*, 1999; Sweeney *et al*, 2004). In relative terms, antifungal drug resistance is not very common and it has been mainly reported in special groups of critically ill patients, such as those undergoing treatment for HIV infection (Cartledge *et al*, 1997; Milan *et al*, 1998; Pelletier *et al*, 2000). Fluconazole resistance in particular has been observed among oral isolates of *Candida* sp. in HIV-infected patients (Hunter *et al*, 1998; Lopez-Ribot *et al*, 1999). In general, however, the number of resistant *Candida* strains seems to be increasing and elderly patients, in particular, are a risk group in this respect (Baran *et al*, 2000; Cowen *et al*, 2000). The elderly often harbour yeasts in the oral cavity and their concomitant use of several drugs, including antimicrobial agents, causes selection pressure for resident bacteria leading to yeast overgrowth. For example, in a group of 191 elderly referred to hospital because of general debility, yeast counts in saliva were noted in more than 80% (Meurman *et al*, 1997). Consequently, novel strategies are needed in order to combat the emergence of antimicrobial resistance in general.

In addition to antifungal agents, yeast infections of the oral cavity have been controlled by use of adjunctive treatment with antiseptic preparations, such as chlorhexidine (Lamfon *et al*, 2004). This chemical, however, is not recommended for long-term use because of its toxic and allergenic characteristics (Lockhart and Harle, 2001; Kudo *et al*, 2002).

We observed, in a 12-month open trial in elderly nursing home subjects who used a combination of amine fluoride and stannous fluoride (AmF/SnF_2) containing mouthwash and toothpaste twice daily that the number of patients with high salivary yeast counts decreased from 26% at baseline to 9% at follow-up (Meurman *et al*, 2001). This serendipitous finding led us to evaluate whether AmF/SnF_2 exerts antifungal effect against *Candida albicans*. Consequently, we hypothesized that AmF/SnF_2 has antifungal capacity against different oral *Candida* species. This paper reports a systematic study where the effect of the AmF/SnF_2 on seven human pathogenic *Candida* species was evaluated. We were especially interested in the non-*albicans* species where

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antifungal resistance against azole-group agents is becoming increasingly common (Samaranayake, 1997; Baran *et al*, 2000; Cowen *et al*, 2000).

Material and methods

Yeast strains

The tested *Candida* species were *C. albicans* (10 isolates), *C. dubliniensis* (5), *C. glabrata* (7), *C. guilliermondii* (2), *C. krusei* (7), *C. parapsilosis* (5) and *C. tropicalis* (7). Both reference strains and oral isolates were included in the study (Table 1). Because *C. albicans* is the predominant yeast in the mouth, it was tested more extensively than the other strains.

Table 1 Yeast strains studied

Strain	*Source*
C. albicans CCUG32723	Culture Collection, University of Gothenburg, Sweden
C. albicans CCUG19915	Culture Collection, University of Gothenburg, Sweden
C. albicans F1206B	Helsinki, Finland
C. albicans C1374	Helsinki, Finland
C. albicans B1134	Helsinki, Finland
C. albicans F372	Helsinki, Finland
C. albicans F380	Helsinki, Finland
C. albicans F388	Helsinki, Finland
C. albicans F409	Helsinki, Finland
C. albicans F470	Helsinki, Finland
C. dublinensis Cd1	Hong Kong, China
C. dublinensis Cd2	Hong Kong, China
C. dublinensis Cd3	(ref. strain American Type Culture Collection, USA) MYA 646
C. dublinensis Cd4	(ref. strain American Type Culture Collection, USA) MYA 580
C. dublinensis Cd5	(ref. strain American Type Culture Collection, USA) MYA 577
C. glabrata CCUG32725	Culture Collection, University of Gothenburg, Sweden
C. glabrata G212	Helsinki, Finland
C. glabrata Cg1	Beijing, China
C. glabrata Cg2	Beijing, China
C. glabrata Cg3	Beijing, China
C. glabrata Cg4	Beijing, China
C. glabrata Cg5	Beijing, China
C. guilliermondii 6260	American Type Culture Collection, USA
C. guilliermondii B75B	Helsinki, Finland
C. krusei ATCC6258	American Type Culture Collection, USA
C. krusei D206B	Helsinki, Finland
C. krusei Ck1	Glasgow, UK
C. krusei Ck2	Glasgow, UK
C. krusei Ck3	Glasgow, UK
C. krusei Ck4	Glasgow, UK
C. krusei Ck5	Glasgow, UK
C. parapsilosis Cp1	Glasgow, UK
C. parapsilosis Cp2	Glasgow, UK
C. parapsilosis Cp3	Oslo, Norway
C. parapsilosis Cp4	Oslo, Norway
C. parapsilosis Cp5	Oslo, Norway
C. tropicalis ATCC750	American Type Culture Collection, USA
C. tropicalis D213	Helsinki, Finland
C. tropicalis Ct1	Beijing, China
C. tropicalis Ct2	Beijing, China
C. tropicalis Ct3	Beijing, China
C. tropicalis Ct4	Beijing, China
C. tropicalis Ct5	Beijing, China

Growth inhibition test

Growth inhibition effect of Meridol® mouth rinse (Gaba International, Inc., Basel, Switzerland) which contains 250 ppm amine fluoride/stannous fluoride combination (AmF/SnF_2) was evaluated.

In a pilot study, we noted that the commercial product Meridol® was inactive against yeasts at pH values lower than 4.3 and that the inhibition effect was stable at a pH range of 5–7. As the pH of Meridol® is approximately 4.0 we evaluated, using a panel of healthy volunteers ($n = 5$), the residual pH of expectorate after the volunteers rinsed the mouth with 10 ml of Meridol® for 30 s, according to recommendations of the manufacturer. After rinsing, the subjects expectorated the Meridol®-saliva mixture into a container for pH assessment. The pH of these solutions was found to vary from pH 4.7–5.2. Therefore, we decided to adjust the pH of the Meridol® preparation to a clinically relevant pH value of 5, throughout the experiments.

The inhibitory effect of Meridol® on yeasts was determined using the direct exposure of yeast cells to different concentrations of the preparation for varying periods of time followed by serial dilution and cultivation on Sabouraud agar (Lab M, Lancashire, UK). Four concentrations of 25–250 ppm of the active agent were used in these studies.

A loop full of an overnight growth of the tested yeast strain on Sabouraud agar was suspended in 5 ml of Sabouraud broth and incubated in a shaker at 35°C for 6 h. After this preincubation period, an aliquot of 0.2 ml was suspended in 30 ml Sabouraud broth and incubated similarly for 18 h. The yeast concentration in the suspension was adjusted to 5×10^7 CFU ml^{-1} using spectrophotometer (Multiscan RC, Labsystems, Helsinki, Finland). Optical density of 0.7–2.1 at 492 nm corresponded to the desired cell concentration depending on the cell size of the yeast strain. The suspension was divided into 2 ml aliquots and centrifuged at 3000 *g* for 3 min, and re-suspended in 400 μl Meridol® solution (pH 5) to yield a concentration of 2×10^8 CFU ml^{-1}. Samples of 20 μl were then withdrawn after each incubation interval (30 s, 1, 2, 3 and 5 min) and immediately diluted serially to 10^{-5} and plated on Sabouraud agar. Four different concentrations of Meridol® were studied; namely 250, 125, 50 and 25 ppm. The resultant yeast inoculates on Sabouraud agars were incubated at 35°C for up to 48 h, at which time the CFU's were counted. Meridol® base solution (Gaba International, Inc., Basel, Switzerland) without the active ingredient AmF/SnF_2 was used as the negative control. The CFU counts were compared with the negative controls and the percentage growth inhibition was calculated. All tests were done three times.

Statistics

Inter-species differences of growth inhibition effect were analysed with SPSS for Windows 12.0.1. Due to the non-parametric nature of the variable and small

frequencies the Mann–Whitney *U*-test was used for pair wise tests of differences.

Results

Inhibition of growth

The sensitivity of different *Candida* species to AmF/SnF_2 varied. It was observed that the undiluted commercial Meridol® (250 ppm AmF/SnF_2) preparation killed almost 90% of the *C. albicans* strains within 5 min and, 30% after 30 s. Of the tested species *C. guilliermondii* strains were the most sensitive to the 250 ppm concentration of AmF/SnF_2 as even the 125 ppm dilution killed all the cells within a 30 s exposure. Concentration of 25 ppm (10-fold dilution of Meridol® preparation) killed 90% of the *C. guilliermondii* cells in 5 min and, over 60% of the cells after 30 s exposure. In ascending order, *C. parapsilosis*, *C. tropicalis*, *C. albicans*, *C. glabrata*, *C. krusei* and *C. dubliniensis* showed higher resistance against Meridol® than *C. guilliermondii*. The growth inhibition curves for all tested *Candida* species are given in Figure 1. The variation of differences between species in the growth inhibition is shown as the minimum and maximum *P*-values of pair wise tests (Table 2).

Most *Candida* species exhibited some intra-species variation in growth inhibition. The degree of this variation varied between species. The variations among *C. dubliniensis* and *C. tropicalis* were most remarkable while *C. guilliermondii* showed almost no

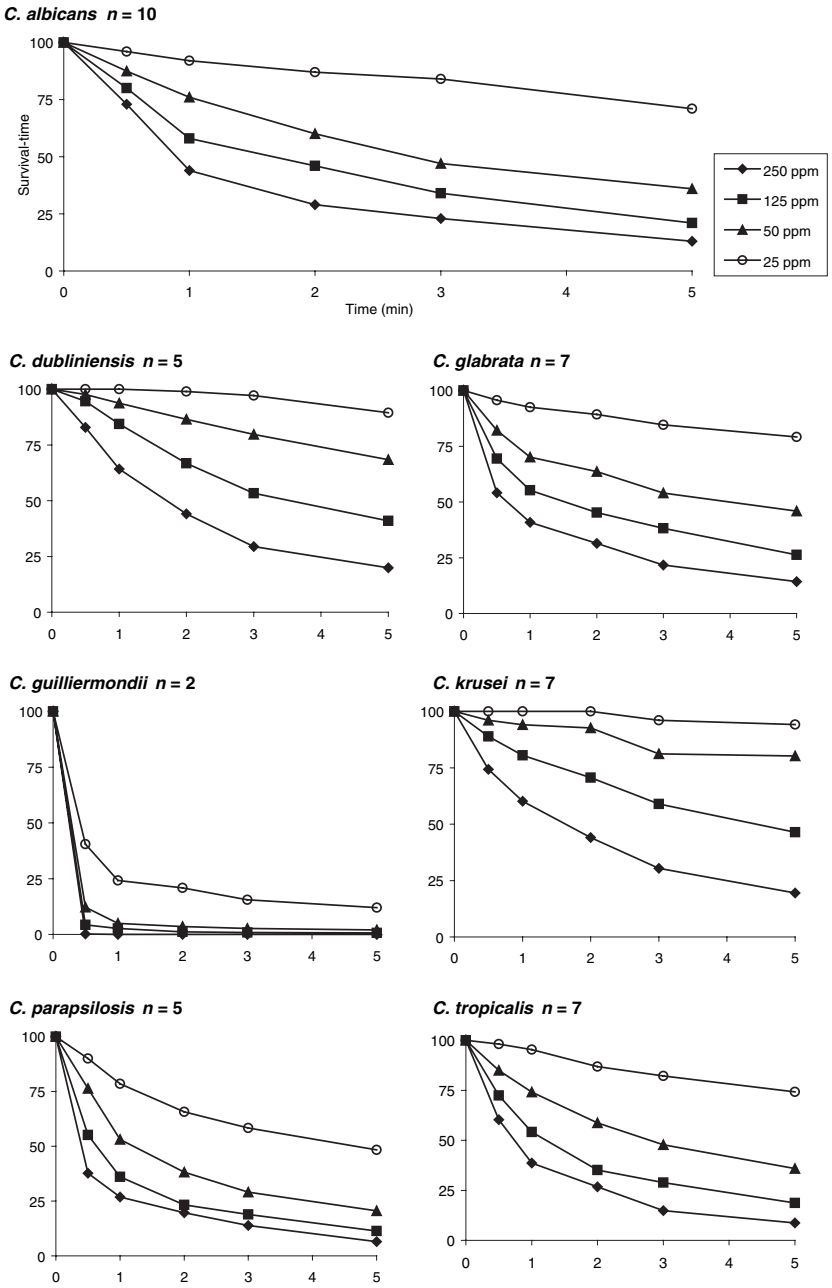

Figure 1 Means of survival percentages as a function of time (min)

Table 2 The minimum and maximum *P*-values for pair wise tests of differences between the growth inhibitions of *Candida* species

	C. albicans	*C. dubliniensis*	*C. glabrata*	*C. guilliermondii*	*C. krusei*	*C. parapsilosis*	*C. tropicalis*
C. albicans		0.000–0.655	0.001–0.988	**0.000–0.000**	0.000–0.676	0.000–0.261	0.034–0.884
C. dubliniensis	0.000–0.655		0.000–0.806	**0.000–0.002**	0.059–1.000	0.000–0.072	0.000–0.203
C. glabrata	0.001–0.988	0.000–0.806		**0.000–0.001**	0.000–0.392	0.000–0.769	0.020–0.741
C. guilliermondii	**0.000–0.000**	**0.000–0.002**	**0.000–0.001**		**0.000–0.001**	**0.000–0.001**	**0.000–0.001**
C. krusei	0.000–0.676	0.059–1.000	0.000–0.392	**0.000–0.001**		**0.000–0.028**	0.000–0.774
C. parapsilosis	0.000–0.261	0.000–0.072	0.000–0.769	**0.000–0.001**	**0.000–0.028**		0.002–0.943
C. tropicalis	0.034–0.884	0.000–0.203	0.000–0.203	**0.000–0.001**	0.000–0.774	0.002–0.943	

The most differing species are highlighted. The Mann–Whitney test was used for each five time points, and four dilutions investigated, altogether in 20 occasions.

Table 3 The intra-species variability of growth inhibition in seven *Candida* species. The values show the minimum and maximum values of the survival percentages

Meridol® *conc. time (min)*	*C. albicans* ($n = 10$)	*C. dubliniensis* ($n = 5$)	*C. glabrata* ($n = 7$)	*C. guilliermondii* ($n = 2$)	*C. krusei* ($n = 7$)	*C. parapsilosis* ($n = 5$)	*C. tropicalis* ($n = 7$)
0.2%							
0.5′	53–100	49–100	26–89	0–1	49–100	8–73	20–100
1′	13–73	12–100	15–78	0–0	29–85	0–51	6–100
2′	5–56	0–100	11–75	0–0	14–63	0–43	4–83
3′	5–49	1–80	7–59	0–0	3–59	0–34	1–53
5′	1–36	0–80	3–51	0–0	0–55	0–17	0–36
0.1%							
0.5′	48–100	75–100	36–100	0–11	62–100	23–80	34–100
1′	17–85	20–100	25–100	0–8	59–100	16–65	18–100
2′	9–74	4–100	19–93	0–4	38–89	7–59	8–100
3′	8–73	1–100	10–82	0–3	37–82	6–53	0–100
5′	4–48	1–79	2–77	0–2	18–75	2–36	0–83
0.05%							
0.5′	46–100	87–100	55–100	4–28	72–100	40–100	46–100
1′	26–100	73–100	40–100	0–13	65–100	19–93	42–100
2′	21–100	54–100	45–85	0–10	67–100	15–69	29–100
3′	10–75	44–100	36–75	0–9	10–100	11–51	18–100
5′	7–76	31–100	22–72	0–6	46–100	9–30	12–100
0.02%							
0.5′	72–100	100–100	78–100	29–64	100–100	62–100	86–100
1′	58–100	100–100	76–100	10–34	100–100	48–100	59–100
2′	54–100	90–100	59–100	4–28	100–100	40–100	39–100
3′	53–100	88–100	46–100	5–23	72–100	32–100	54–100
5′	42–100	60–100	14–100	2–20	76–100	22–100	41–100

variation. Table 3 gives the intra-species variations as minimum and maximum values of the survival percentages.

Discussion

As stated, we observed serendipitously, in a previous clinical trial in elderly nursing home residents who used AmF/SnF_2 containing mouthwash and toothpaste twice daily, a significant decrease in salivary yeast counts from 26% at baseline to 9% at follow-up over a 12-month observation period (Meurman *et al*, 2001). The current laboratory data, we believe, tend to confirm our hypothesis that AmF/SnF_2 may indeed have antifungal potential. In the concentration range investigated, the AmF/SnF_2 was found to exert antifungal effect on all seven *Candida* species studied. The fact that a number of strains, both reference and clinical isolates, belonging to each species, were killed by the AmF/SnF_2 combination indicates that the chemicals are likely to have a generalized antifungal effect on the vast majority of pathogenic *Candida* species.

The duration of an antibacterial effect of AmF/SnF_2 has been shown to last up to 5 h after a single 30 s rinse with the preparation (Netuschil *et al*, 1997). This prolonged antimicrobial effect of AmF/SnF_2 combination has been alluded to its chemical structure where the amine moiety is likely to favour its attachment to the epithelial cells thus forming an 'in situ' reservoir of the chemical. Our laboratory data taken together with previous clinical observations indicate that AmF/SnF_2 rinse may indeed exert a fairly durable antifungal activity on the oral mucosa. Other workers have also shown the surface activity of AmF/SnF_2. For instance, Banoczy *et al* (1989) demonstrated in a 12-week double-blind study of school children that such a combination reduces gingival bleeding. Attin and Hellwig (1996) have shown that saliva concentrations of amine fluoride are higher after tooth brushing than compared with sodium fluoride toothpaste.

Whilst the retention of the AmF/SnF_2 combination on the mucosa is likely to be because of the amine moiety, its antifungal effect may be ascribed to the stannous fluoride component which is known to have both antibacterial and anti-plaque activity (Tinanoff, 1990). It may also interact with the plasma membrane of the yeasts as in the case of chlorhexidine (Hiom *et al*, 1996; McDonnell and Russell, 1999). Whether the actual target site is the plasma membrane or other cellular components of the yeast cell remains to be investigated by further ultrastructural and biochemical studies.

We also noted that *C. albicans*, the most prevalent causative agent of oral yeast infections, was highly sensitive to the agent while the non-*albicans* strains *C. dubliniensis*, *C. glabrata*, and *C. krusei* were the least sensitive. There are reports from different regions of the world that non-*albicans* species are increasingly becoming common in both the hospital-acquired and community-acquired infections. Thus Foongladda *et al* (2004) recently reported from Thailand that the prevalence of non-*albicans* yeasts is increasing among hospitalized patients while Reichart *et al* (2002) have reported similar findings in leprosy patients, also in Thailand. *C. glabrata* is another emerging pathogen that has been shown to colonize the oral cavities of elderly in particular (Lockhart *et al*, 1999). It is also a significant nosocomial pathogen, second only to *C. albicans* (Samaranayake *et al*, 2002). Furthermore, it is known that *C. glabrata* and *C. krusei* are intrinsically resistant to the azole-group agents (Fortun *et al*, 1997; Pfaller *et al*, 2004). Consequently, the alarming worldwide emergence of candidal resistance to commonly used antifungal drugs (Ellepola and Samaranayake, 2000) calls for new approaches to management of fungal infections, such as adjunct therapy with AmF/SnF_2 reported here. However, the present results need to be clinically confirmed in properly controlled randomized trials prior to clinical recommendations and interventions.

Finally, in clinical terms, the present results in combination with the previously reported inhibitory effect of AmF/SnF_2 against dental plaque bacteria (Meurman *et al*, 1989) imply that the compound may have potential to prevent other oral infections in addition to gingivitis and dental caries, which are the main indications for its use. We have tested that if a subject rinses the mouth with 10 ml of Meridol® preparation for 30 s, the volume of the expectorate is approximately 12 ml (the preparation mixed with saliva), and the resulting concentration of AmF/SnF_2 is reduced from the original 250–200 ppm. Thus, the diluting effect of saliva is clinically irrelevant and the preparation is anticipated to remain effective also in clinical use. Clearly, although, the topical treatment modes such as the use of AmF/SnF_2 solutions can only be an adjunct therapy in treating and controlling oral yeast infections, which needs to be emphasized.

If the present findings are compared with reported antifungal effect of chlorhexidine the main difference in the eventual clinical use of these two topical agents would be that chlorhexidine may not be highly desirable for daily use because of its side-effects, while AmF/SnF_2 preparations may have the potential for daily use. The reported adverse effects of AmF/SnF_2 on continuous use are mainly staining of the teeth (Tinanoff, 1990; Paraskevas *et al*, 2004). To conclude, our data clearly indicate that an additional significant advantage of AmF/SnF_2 mouth rinse would be its antifungal effect which would synergise its well known anti-plaque activity.

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References

Attin T, Hellwig E (1996). Salivary fluoride content after toothbrushing with a sodium fluoride and an amine fluoride dentifrice followed by different mouthrinsing procedures. *J Clin Dent* **7:** 6–8.

Austin DJ, Kristinsson KG, Anderson RM (1999). The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proc Natl Acad Sci* **96:** 1152–1156.

Banoczy J, Szöke J, Kertesz P, Toth Z, Zimmermann P, Gintner Z (1989). Effect of amine fluoride/stannous fluoride-containing toothpaste and mouthrinsings on dental plaque, gingivitis, plaque and enamel F^- accumulation. *Caries Res* **23:** 284–288.

Baran J Jr, Klauber E, Barczak J, Riederer K, Khatib R (2000). Trends in antifungal susceptibility among Candida sp. Urinary isolates from 1994 and 1998. *J Clin Microbiol* **38:** 870–871.

Cartledge JD, Midgley J, Gazzard BG (1997). Clinically significant azole cross-resistance in *Candida* isolates from HIV-positive patients with oral candidosis. *AIDS* **11:** 1839–1844.

Cowen LE, Sanglard D, Calabrese D, Sirjusingh C, Anderson JB, Kohn LM (2000). Evolution of drug resistance in experimental populations of *Candida albicans*. *J Bacteriol* **182:** 1515–1522.

Eliopoulos GM (1998). Mechanisms of bacterial resistance to antimicrobial drugs. In: Gorbach SL, Bartlett JG, Blacklow NR, eds. *Infectious diseases*. Saunders: Philadelphia, PA, pp. 319–330.

Ellepola AN, Samaranayake LP (2000). Oral candidal infections and antimycotics. *Crit Rev Oral Biol Med* **11:** 172–198.

Foongladda S, Sakulmaiwatana P, Petlum P, Vanprapar N (2004). *Candida* species, genotypes and antifungal susceptibility of *Candida* isolates from blood samples of patients at the largest tertiary care hospital in Thailand during 1999–2002. *J Med Assoc Thai* **87:** 92–99.

Fortun J, Lopez-San Roman A, Velasco JJ *et al*. (1997). Selection of *Candida glabrata* strains with reduced susceptibility to azoles in four liver transplant patients with invasive candidiasis. *Eur J Clin Microbiol Infect Dis* **16:** 314–318.

Hiom SJ, Furr JR, Russell AD, Hann AC (1996). The possible role of yeast cell walls in modifying cellular response to chlorhexidine diacetate. *Cytobios* **86:** 123–135.

Hunter KD, Gibson J, Lockhart P, Pithie A, Bagg J (1998). Fluconazole-resistant *Candida* species in the oral flora of fluconazole-exposed HIV-positive patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontics* **85:** 558–564.

Kilby JM, Dismukes WE (1998). Antifungal drugs. In: Gorbach SL, Bartlett JG, Blacklow NR, eds. *Infectious diseases*. Saunders: Philadelphia, PA, pp. 350–359.

Kudo K, Ikeda N, Kiyoshima A, Hino Y, Nishida N, Inoue N (2002). Toxicological analysis of chlorhexidine in human serum using HPLC on a polymer-coated ODS column. *J Anal Toxicol* **26:** 119–122.

Lamfon H, Porter SR, McCullough M, Pratten J (2004). Susceptibility of *Candida albicans* biofilms grown in a constant depth film fermentor to chlorhexidine, fluconazole and miconazole: a longitudinal study. *J Antimicrob Chemother* **53:** 383–385.

Lockhart AS, Harle CC (2001). Anaphylactic reactions due to chlorhexidine allergy. *Br J Anaesth* **87:** 940–941.

Lockhart SR, Joly S, Vargas K, Swails-Wenger J, Enger L, Soll DR (1999). Natural defenses against *Candida* colonization breakdown in the oral cavities of the elderly. *J Dent Res* **78:** 857–868.

Lopez-Ribot JL, McAtee RK, Kirkpatrick WR, La Valle R, Patterson TF (1999). Low levels of antigenic variability in fluconazole-susceptible and -resistant *Candida albicans* isolates from human immunodeficiency virus-infected patients with oropharyngeal candidiasis. *Clin Diagnostic Lab Immunol* **6:** 665–670.

McDonnell G, Russell AD (1999). Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* **12:** 147–149.

Meurman JH, Jousimies-Somer H, Suomala P, Alaluusua S, Torkko H, Asikainen S (1989). Activity of amine-stannous fluoride combination and chlorhexidine against some aerobic and anaerobic oral bacteria. *Oral Microbiol Immunol* **4:** 117–119.

Meurman JH, Pajukoski H, Snellman S, Zeiler S, Sulkava R (1997). Oral infections in home-living elderly patients admitted to an acute geriatric ward. *J Dent Res* **76:** 1271–1276.

Meurman JH, Kari K, Äikäs A, Kallio P (2001). One-year compliance and effects of amine and stannous fluoride on some salivary biochemical constituents and oral microbes in institutionalized elderly. *Spec Care Dentist* **21:** 32–36.

Milan EP, Burattini MN, Kallas EG, Fischmann O, Costa PR, Colombo AL (1998). Azole resistance among oral *Candida* species isolates from AIDS patients under ketokonazole exposure. *Diagnostic Microbiol Infect Dis* **32:** 211–216.

Netuschil L, Rauh T, Riethe P (1997). Substantivität und antibakterielle Wirkung von Aminfluorid/Zinfluorid in situ. *Parodontologie* **1:** 7–16.

Paraskevas S, Danser MM, Timmerman MF, Van der Velden U, Van der Weijden GA (2004). Effect of a combination of amine/stannous fluoride dentifrice and mouthrinse in periodontal maintenance patients. *J Clin Periodontol* **31:** 177–183.

Pelletier R, Peter J, Antin C, Gonzalez C, Wood L, Walsh TJ (2000). Emergence of resistance of *Candida albicans* to clotrimazole in human immunodeficiency virus-infected children: in vitro and clinical correlations. *J Clin Microbiol* **38:** 1563–1568.

Pfaller MA, Diekema DJ, International Fungal Surveillance Participant Group (2004). Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of Candida. *Clin Microbiol Infect* **10** (Suppl. 1)**:** 11–23.

Reichart P, Samaranayake LP, Grote M, Pow E, Cheung B (2002). High oral prevalence of *Candida krusei* in leprosy patients in Northern Thailand. *J Clin Microbiol* **40:** 4479–4485.

Samaranayake LP (1997). *C. krusei* infections and fluconazole therapy. *Hong Kong Med J* **3:** 312–314.

Samaranayake LP, Cheung LK, Samaranayake YH (2002). Candidiasis and other fungal diseases of the mouth. *Dermatol Ther* **15:** 252–270.

Sweeney LC, Dave J, Chambers PA, Heritage J (2004). Antibiotic resistance in general dental practice – a cause for concern? *J Antimicob Chemother* **53:** 567–576.

Tinanoff N (1990). Review of the antimicrobial action of stannous fluoride. *J Clin Dent* **2:** 22–27.