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Modified adherence of *Candida albicans* to human buccal epithelial cells in vitro following treatment with cationic, non-antibiotic antimicrobial agents

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Summary

The effects of the cationic, non-antibiotic antimicrobial agents chlorhexidine gluconate, dequalinium chloride, cetrimide and cetylpyridinium chloride on the adherence of *Candida albicans* blastospores (MEN and oral clinical isolates, exponential and stationary growth phases) to human buccal epithelial cells in vitro were examined using light microscopy. All agents expressed significant anti-adherence activities at concentrations both greater and less than the minimum inhibitory concentration. In all cases, the anti-adherence effects were observed following treatment (30 min, 37°C) of either buccal epithelial cells or blastospores. In addition, the majority of treatments significantly increased the number of buccal epithelial cells free from adherent *C. albicans*. These observations may be useful in the prophylaxis of oral candidosis in prone individuals.

Introduction

Candida albicans is a dimorphic fungus which is a normal resident of the oral cavity, gastrointestinal and female genital tracts of humans. However, under certain conditions, e.g., trauma, immunological compromisation, this organism may pathogenically invade a large number of different sites, including the oral cavity, skin, fingernails, genitals, respiratory, urinary and gastrointestinal tracts, central nervous and cardiovascular systems (Odds, 1988).

The choice of antifungal agent used in the treatment of candidosis is dependent upon the severity and nature of the infection and typically the polyenes (amphotericin B, nystatin), azoles (e.g., miconazole, ketoconazole, fluconazole) and flucytosine are preferred (Odds, 1988). In the treatment of oral candidosis topical preparations (mouthwashes and lozenges) frequently containing non-antibiotic, antimicrobial agents may be prescribed. An example in this category is chlorhexidine which, in addition to its use in oral candidosis (Langslet et al., 1974; Sharon et al., 1977), is employed in the treatment of other

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infective conditions of the oral cavity, e.g., denture stomatitis (Olsen, 1975a,b). Other antimicrobial agents prescribed as topical preparations include cetylpyridinium chloride (Cepacol™) and dequalinium chloride (Dequadin™).

In the pathogenesis of candidosis, it is accepted that the initial step involves adherence of *C. albicans* to the host epithelium (Douglas, 1987). This interaction will ensure that the flushing mechanism of bodily secretions is overcome and thus the organism will be retained on the epithelium. Amongst *Candida* spp., it has been reported that the ability to adhere to epithelia may be correlated with virulence potential (King et al., 1980; Douglas, 1987). The process of *C. albicans* adherence to host epithelia has been described to involve two stages (Douglas, 1987). Initially, as the yeast cell approaches the epithelial cell, it is subjected to both attractive and repulsive forces. If the attractive forces exceed the repulsive forces, then the organism may approach the epithelial cells at short distances (the so-called primary minimum). Consequently, whilst at this stage, the adherence interaction is rendered irreversible due to the interaction between adhesin molecules on the surface of *C. albicans* and the corresponding receptors on the surface of the epithelial cell (Douglas, 1987).

Therefore, in susceptible patients where prophylaxis against oral candidosis is important (e.g., AIDS), one approach may be to reduce or inhibit the interaction between *C. albicans* and the epithelia of the oral cavity. Several approaches have been investigated in vitro including the use of isolated adhesin or adhesin analogues (Lee and King, 1983; Collins-Lech et al., 1984), lectins (Sandin et al., 1982; Critchley and Douglas, 1987) and sublethal concentrations of antibiotics (Douglas and McCourtie, 1983). Recently, it has been reported that treatment with non-antibiotic, antimicrobial agents, e.g., taurolidine (Jones et al., 1986), polyvinylpyrrolidone-iodine and chlorhexidine (Gorman et al. 1987) significantly reduced the adherence of microorganisms (including *C. albicans*) to buccal epithelial cells in vitro.

Therefore, with this in mind, the aim of this study was to investigate whether other non-antibiotic, antimicrobial agents (cetrimide, cetylpyridinium chloride and dequalinium chloride) may similarly reduce the adherence of *C. albicans* to human buccal epithelial cells in vitro.

Materials and Methods

Chemicals

Chlorhexidine gluconate (Hibitane™) was a gift from ICI (NZ) Ltd.

Dequalinium chloride, as Dequadin™ throat lozenges, was purchased from Evans Medical (NZ) Ltd.

Cetylpyridinium chloride and cetrimide were purchased from Sigma Chemicals Ltd, St. Louis, U.S.A.

All other chemicals were obtained from BDH Chemicals Ltd, Poole, Dorset, U.K., and were of AnalaR or equivalent quality.

The appropriate concentrations of chlorhexidine gluconate were obtained following dilutions of the supplied solution using sterile deionised water. For cetylpyridinum chloride and cetrimide, the appropriate weights of pure substance were added to sterile deionised water.

Dequalinium chloride was extracted from Dequadin™ by dissolving one lozenge in sterile water. This was then diluted to the required concentration using sterile deionised water.

All solutions were prepared daily for use on the same day.

C. albicans isolates and growth conditions

Two isolates, MEN, from a diagnosed eye infection (Cannon, 1986) and one from a diagnosed oral infection of *C. albicans* were employed in this study. These were stored on Nutrient agar (Difco) slopes, at 4°C. Stationary phase *C. albicans* blastospores were harvested by transferring two loopfuls into prewarmed Nutrient broth (Difco) for incubation at 25°C for 18 h in an orbital incubator (150 oscillations/min).

Exponential phase blastospores were prepared by adding 10 ml of stationary phase *C. albicans* to fresh prewarmed Nutrient broth and incubation at 25°C for a further 6 h in an orbital incubator (150 oscillations/min).

Cultures were centrifuged at $2000 \times g$ for 15 min and the deposit washed once with and resuspended in phosphate-buffered saline (PBS, pH 7.3, 0.1 M) to the required cell density.

In each case the absence of hyphal forms was confirmed using light microscopy.

Epithelial cells

Buccal epithelial cells (BEC) were collected by scraping the buccal mucosa of healthy male and female volunteers using sterile ampoule files. These were subsequently placed in sterile PBS and the cells dislodged by shaking. After washing, the cells were resuspended in sterile PBS to the required cell count.

Minimum inhibitory concentrations (MIC)

The MIC of the antimicrobial agents under investigation were determined using a macrodilution method with doubling dilutions of chlorhexidine gluconate, cetrimide, cetylpyridinium chloride and dequalinium chloride in Nutrient broth (Gorman et al., 1987). A standardised suspension of washed stationary phase *C. albicans* (approx. 1×10^6 cfu/ml, 0.1 ml) was added to each tube containing broth and antimicrobial agent (9.9 ml). These were then incubated at 25°C for 24 h and the MIC read as the first concentration where no growth occurred.

Pretreatment procedures

Treatments were performed by incubating suspensions of *C. albicans* or BEC with either sterile water, chlorhexidine gluconate (0.05, 0.00005% v/v), dequalinium chloride (0.001, 0.0005% w/v), cetylpyridinium chloride (0.005, 0.00005% w/v) or cetrimide (0.001, 0.0001% w/v) for 30 min at 37°C in an orbital incubator (150 oscillations/min). The antimicrobial agents were removed by decanting the supernatant fluids after centrifugation ($2000 \times g$ for 15 min). The remaining pellet of either *C. albicans* blastospores or BEC was washed in sterile water and resuspended in PBS before inclusion in the adherence assay.

Adherence assay

Equal volumes of *C. albicans* (approx. 1×10^7 cfu/ml) and BEC (approx. 1×10^5/ml) were mixed and incubated at 37°C for 2 h in a shaking water bath. After this period, three loopfuls of the incubation mixture were removed, placed on a microscope slide and mixed with an equal vol-

TABLE 1

Effect of chlorhexidine gluconate (CHG) on the adherence of Candida albicans (an ocular clinical isolate, MEN, and an oral clinical isolate, exponential and stationary growth phases) to human buccal epithelial cells (BEC) in vitro

Concentration of CHG (% v/v)	Cell treatment	*C. albicans* isolate	Number [a] of adherent *C. albicans* ± SE/BEC		Percentage [b] of BEC free from adherent *C. albicans*	
			Exponential	Stationary	Exponential	Stationary
0 (water)	*C. albicans*	MEN	2.22 ± 0.22	1.40 ± 0.15	25	36
0.05			0.74 ± 0.10	0.48 ± 0.07	57	71
0.00005			1.69 ± 0.20	0.90 ± 0.09	41	49
0 (water)	*C. albicans*	oral	1.94 ± 0.18	N.D.	31	N.D.
0.05			0.57 ± 0.08	N.D.	64	N.D.
0.00005			0.43 ± 0.07	N.D.	71	N.D.
0 (water)	BEC	MEN	3.44 ± 0.30	4.05 ± 0.37	20	14
0.05			2.05 ± 0.17	2.18 ± 0.18	33	24
0.00005			2.19 ± 0.18	4.02 ± 0.32 [c]	29	16 [c]
0 (water)	BEC	oral	1.91 ± 0.18	4.43 ± 0.34	33	09
0.05			1.30 ± 0.15	2.03 ± 0.16	33 [c]	27
0.00005			1.92 ± 0.20 [c]	2.27 ± 0.17	29 [c]	23

Differences between water and antimicrobial agent treatments analysed using either a two-tailed unpaired *t*-test [a] ($p < 0.05$ significant), or χ^2 analysis [b] ($p < 0.05$ significant), [c] $p > 0.05$ (non-significant). N.D., not determined.

TABLE 2

Effect of cetylpyridinium chloride (CPC) on the adherence of Candida albicans (an ocular clinical isolate, MEN, and an oral clinical isolate, exponential and stationary growth phases) to human buccal epithelial cells (BEC) in vitro

Concentration of CPC (% w/v)	Cell treatment	*C. albicans* isolate	Number [a] of adherent *C. albicans* ± SE/BEC		Percentage [b] of BEC free from adherent *C. albicans*	
			Exponential	Stationary	Exponential	Stationary
0 (water)	*C. albicans*	MEN	2.22 ± 0.22	1.40 ± 0.15	25	36
0.005			0.99 ± 0.10	0.73 ± 0.01	44	59
0.00005			1.69 ± 0.23	0.88 ± 0.10	37	46
0 (water)	*C. albicans*	oral	1.94 ± 0.18	2.01 ± 0.20	31	27
0.005			1.42 ± 0.14	1.60 ± 0.18	39 [c]	39
0.00005			1.38 ± 0.13	1.62 ± 0.16	37 [c]	38
0 (water)	BEC	MEN	4.17 ± 0.47	N.D.	33	N.D.
0.005			1.64 ± 0.16	N.D.	33 [c]	N.D.
0.00005			2.15 ± 0.19	N.D.	23 [c]	N.D.
0 (water)	BEC	oral	2.40 ± 0.21	4.43 ± 0.34	33	09
0.005			1.57 ± 0.19	3.26 ± 0.39	47	32
0 (water)			1.91 ± 0.18	4.43 ± 0.34	33	09
0.00005			1.81 ± 0.22 [c]	1.54 ± 0.14	35 [c]	37

Differences between water and antimicrobial agent treatments analysed using either a two-tailed unpaired *t*-test [a] ($p < 0.05$ significant), or χ^2 analysis [b] ($p < 0.05$ significant), [c] $p > 0.05$ (non-significant). N.D., not determined.

ume of PBS. The cells were subsequently stained using crystal violet and the number of *C. albicans* adherent to at least 150 epithelial cells counted using light microscopy (Jones et al., 1986).

Statistical comparisons of the mean number of adherent *C. albicans* to BEC following treatments with the water control and cationic antimicrobial agents were performed using a two-tailed

TABLE 3

Effect of dequalinium chloride (DQC)on the adherence of Candida albicans (an ocular clinical isolate, MEN, and an oral clinical isolate, exponential and stationary growth phases) to human buccal epithetial cells (BEC) in vitro

Concentration of DQC (% w/v)	Cell treatment	*C. albicans* isolate	Number [a] of adherent *C. albicans* ± SE/BEC		Percentage [b] of BEC free from adherent *C. albicans*	
			Exponential	Stationary	Exponential	Stationary
0 (water)	*C. albicans*	MEN	2.22 ± 0.22	N.D.	25	N.D.
0.001			1.62 ± 0.17	N.D.	36	N.D.
0.0005			1.39 ± 0.13	N.D.	33 [c]	N.D.
0 (water)	*C. albicans*	oral	1.94 ± 0.18	2.01 ± 0.20	31	27
0.001			2.00 ± 0.19 [c]	0.82 ± 0.09	28 [c]	49
0.0005			1.40 ± 0.15	1.93 ± 0.23 [c]	47	34 [c]
0 (water)	BEC	MEN	4.27 ± 0.47	4.30 ± 0.31	33	14
0.001			1.60 ± 0.18	1.38 ± 0.19	41 [c]	41
0.0005			1.98 ± 0.19	2.64 ± 0.19	32 [c]	24 [c]
0 (water)	BEC	oral	2.34 ± 0.21	3.23 ± 0.33	25	18
0.001			0.82 ± 0.09	1.32 ± 0.16	52	38
0.0005			1.67 ± 0.19	1.46 ± 0.18	36	47

Differences between water and antimicrobial agent treatments analysed using either a two-tailed unpaired *t*-test [a] ($p < 0.05$ significant), or χ^2 analysis [b] ($p < 0.05$ significant), [c] $p > 0.05$ (non-significant). N.D., not determined.

TABLE 4

Effect of cetrimide (C) on the adherence of Candida albicans (an ocular clinical isolate, MEN, and an oral clinical isolate, exponential and stationary growth phases) to human buccal epithelial cells (BEC) in vitro

Concentration of C (% w/v)	Cell treatment	*C. albicans* isolate	Number [a] of adherent *C. albicans* ± SE/BEC		Percentage [b] of BEC free from adherent *C. albicans*	
			Exponential	Stationary	Exponential	Stationary
0 (water)	*C. albicans*	MEN	2.22 ± 0.22	1.40 ± 0.15	25	36
0.001			1.75 ± 0.22	0.65 ± 0.08	25 [c]	58
0.0001			2.27 ± 0.22 [c]	0.63 ± 0.09	41	64
0 (water)	*C. albicans*	Oral	1.94 ± 0.18	2.01 ± 0.20	31	27
0.001			1.21 ± 0.15	1.13 ± 0.13	50	49
0.0001			0.95 ± 0.13	1.33 ± 0.20	57	45
0 (water)	BEC	MEN	3.44 ± 0.30	4.31 ± 0.32	20	14
0.001			2.77 ± 0.21	2.72 ± 0.21	22 [c]	25
0.0001			N.D.	N.D.	N.D.	N.D.
0 (water)	BEC	oral	2.40 ± 0.21	4.43 ± 0.34	25	14
0.001			1.23 ± 0.15	1.31 ± 0.12	51	37
0 (water)			1.91 ± 0.18	4.43 ± 0.34	33	14
0.0001			1.16 ± 0.18	2.00 ± 0.20	43	32

Differences between water and antimicrobial agent treatments analysed using either a two-tailed unpaired *t*-test [a] ($p < 0.05$ significant), or χ^2 analysis [b] ($p < 0.05$ significant), [c] $p > 0.05$ (non-significant). N.D., not determined.

unpaired *t*-test, $p < 0.05$ indicating significance (Jones et al., 1986; Woolfson et al., 1987). Chi-squared (χ^2) analysis was used to statistically compare the number of BEC free from adherent *C. albicans* following treatment with water or antimicrobial agents ($p < 0.05$ indicating significance).

Results and Discussion

As may be observed (Tables 1–4), all cationic antimicrobial agents examined significantly decreased, but did not totally inhibit the adherence of *C. albicans* (both isolates) to human BEC in vitro following pretreatments of either blastospores or BEC. These effects were consistently observed whenever the larger concentrations were employed, although, in a small number (i.e., a minority of cases), pretreatments using the lower concentration of antimicrobial agents failed to significantly reduce adherence. However, in many instances, pretreatments with the lower concentrations produced statistically similar reductions in adherence to treatments with the higher concentrations ($p > 0.05$).

The minimum inhibitory concentrations (MIC) of the antimicrobial agents are shown in Table 5. When these concentrations are considered in the light of the findings described in Tables 1–4, it may be observed that all antimicrobial agents examined exhibited an anti-adherence effect at sublethal (sub-MIC) concentrations.

TABLE 5

Minimum inhibitory concentrations [a] of chlorhexidine gluconate, cetylpyridinium chloride, cetrimide and dequalinium chloride against C. albicans (isolates from an eye infection, MEN, and an oral infection)

Antimicrobial agent	Minimum inhibitory concentration (% w/v or v/v)	
	MEN isolate	Oral isolate
Chlorhexidine gluconate (% v/v)	0.0001	0.0001
Cetylpyridinium chloride (% w/v)	0.0001	0.0005
Cetrimide (% w/v)	0.0005	0.0005
Dequalinium chloride (% w/v)	0.001	0.001

[a] Estimated using a macrodilution method as described in Materials and Methods.

The percentage of BEC free from adherent *C. albicans* is also shown in Tables 1–4. In the majority of cases, pretreatments of either *C. albicans* or BEC with the antimicrobial agents significantly increased the number of BEC free of adherent blastospores ($p < 0.05$). Generally, treatments with the antimicrobial agents were associated with significant increases in the percentage of BEC free from adherent *C. albicans* and with significant decreases in the number of adherent *C. albicans* per BEC.

The agents investigated in this study are clinically employed in the treatment of infection of epithelial surfaces (Hugo and Russell, 1982), and consequently, one application is in the treatment of oral infections, e.g., oral candidosis. As adherence of microorganisms is accepted to be the initial step in the process of infection, a clinical interest has emerged in compounds which possess the ability to reduce or inhibit this microorganism/epithelial cell interaction. The ability of chlorhexidine to interfere with the adherence of *C. albicans* to epithelial cells has been observed previously (Gorman et al., 1987) and therefore the observed anti-adherence property of chlorhexidine was of no suprise. Gorman et al. (1987) reported that treatment of *C. albicans* (NCYC 1467) for 30 min at 37°C with chlorhexidine acetate (0.0002–0.05% w/v) significantly reduced the subsequent adherence to BEC. In the present study, under similar experimental conditions, an anti-adherence effect was observed at a lower concentration (0.00005% v/v). However, it should be borne in mind that different isolates of *C. albicans* and a different pool of BEC donors were used, factors which have previously been reported to influence the degree of adherence (King et al., 1980; Sandin et al., 1987). Interestingly, in both studies, anti-adherence effects, associated with chlorhexidine treatments, were observed at sublethal concentrations.

Other non-antibiotic, antimicrobial agents have been reported to reduce the adherence of *C. albicans* to BEC in vitro, including noxythiolin (Gorman et al., 1986), povidone-iodine (Gorman et al, 1987), polynoxylin (Gorman et al., 1988) and taurolidine (Jones et al., 1986). However, to our knowledge, this is the first study to report that cetrimide, cetylpyridinium chloride and dequalinium chloride treatments of either blastospores or BEC reduce the adherence of *C. albicans* to human BEC in vitro. As the binding sites on the surface of the microbial cell are thought to be similar for chlorhexidine and the quarternary ammonium compounds (Bonesvoll and Gjermo, 1978), it is likely that the mechanisms by which these compounds interfere with the adherence of *C. albicans* are similar. This aspect is currently under further investigation.

Chlorhexidine, cetylpyridinium chloride and hexadecyltrimethylammonium bromide (a component of cetrimide) have been reported to be well retained within the oral cavity, and when instilled two to four times daily as a mouth rinse, to provide a plaque-inhibiting effect (Bonnesvoll and Gjermo, 1978). This retention is postulated to be due to electrostatic interactions between the cationic antimicrobial agents and the surfaces of the oral cavity. The ability of cationic antimicrobial agents to interact rapidly with surfaces of the oral cavity is interesting as their anti-adherence effects are most likely surface phenomena. In this study, anti-adherence effects were observed at sublethal concentrations of the antimicrobial agents. Therefore, several rinses with saliva may be required to reduce the concentrations on the oral surfaces to a level not associated with an anti-adherence effect. This may be important in the in vivo situation where the epithelial cells are continuously bathed with saliva. In addition, several hours after rinsing chlorhexidine, cetylpyridinium chloride and cetrimide have been reported to be present in saliva at sublethal concentrations (Bonesvoll and Gjermo, 1978). This may also influence the in vivo anti-adherence properties of these compounds.

There have been few in vivo investigations of the anti-adherence properties of antimicrobial agents. In one study, chlorhexidine (0.2% v/v), following a rinsing period of 1 min, was shown to significantly decrease the adherence of *C. albicans* to buccal epithelial cells for periods 30 min post-rinsing (Tobgi et al., 1987). The strong interaction of quarternary ammonium compounds (cetylpyridinium chloride, cetrimide) with oral surfaces, in combination with the observed in

vitro anti-adherence abilities, may suggest similar in vivo anti-adherence properties to chlorhexidine. These aspects are currently under investigation.

An interesting characteristic of the observed anti-adherence properties of these cationic antimicrobial agents, at super- and often sublethal concentrations, is the ability to significantly increase the number of epithelial cells devoid of adherent *C. albicans*. This property has been previously associated with treatments of both *C. albicans* and BEC with chlorhexidine, povidone-iodine and taurolidine (Gorman et al., 1987) and may further illustrate the potential benefits of non-antibiotic, antimicrobial agents in the prophylaxis of oral candidosis.

Therefore, this study has shown that treatment (37°C, 30 min) of either *C. albicans* or BEC in vitro with the cationic, non-antibiotic antimicrobial agents, chlorhexidine gluconate, cetrimide, dequalinium chloride and cetylpyridinium chloride, significantly reduced the subsequent adherence to BEC, and the number of BEC free from adherent *C. albicans*, in vitro. As adherence of *C. albicans* is accepted to be the initial step in the infective process, these findings may be of clinical benefit in the prophylaxis of candidosis in prone individuals, e.g., AIDS patients or patients receiving cancer chemotherapy.

References

Bonesvoll, P. and Gjermo, P., A comparison between chlorhexidine and some quaternary ammonium compounds with regard to retention, salivary concentration and plaque-inhibiting effect in the human mouth after mouth rinses. *Arch. Oral Biol.*, 23 (1978) 289–294.

Cannon, R.D., Isolation of a mycelial mutant of *Candida albicans*. *J. Gen. Microbiol.*, 132 (1986) 2405–2407.

Collins-Lech, C., Kalbfleisch, J.H., Franson, T.R. and Sohnle, P.G., Inhibition by sugars of *Candida albicans* adherence to human buccal mucosal cells and corneocytes in vitro. *Infect. Immun.*, 46 (1984) 831–834.

Critchley, I.A. and Douglas, L.J., Isolation and partial characterisation of an adhesin from *Candida albicans*. *J. Gen. Microbiol.*, 133 (1987) 629–636.

Douglas, L.J., Adhesion of *Candida* species to epithelial surfaces. *CRC Crit. Rev. Microbiol.*, 15 (1987) 27–43.

Douglas, L.J. and McCourtie, J., Effect of tunicamycin treatment on the adherence of *Candida albicans* to human buccal epithelial cells. *FEMS Microbiol. Lett.*, 16 (1983) 199–202.

Gorman, S.P., McCafferty, D.F., Woolfson, A.D. and Anderson, L., Decrease in adherence of bacteria and yeasts to human mucosal epithelial cells by noxythiolin in vitro. *J. Appl. Bacteriol.*, 60 (1986) 311–317.

Gorman, S.P., McCafferty, D.F., Woolfson, A.D. and Jones, D.S., A comparative study of the microbial anti-adherence capacities of three antimicrobial agents. *J. Clin. Pharm. Ther.*, 12 (1987) 393–399.

Gorman, S.P., McCafferty, D.F., Woolfson, A.D. and Kennedy, G.E., Reduced adherence and inhibition of hyphal development of *Candida albicans* by the antimicrobial agent polynoxylin. *Lett. Appl. Microbiol.*, 6 (1988) 145–148.

Hugo, W.B. and Russell, A.D., Types of antimicrobial agents. In Russell, A.D, Hugo, W.B. and Ayliffe, G.A.J. (Eds), *Principles and Practice of Disinfection, Preservation and Sterilisation*, Blackwell, Oxford, 1982, pp. 8–107.

Jones, D.S., Gorman, S.P., McCafferty, D.F. and Woolfson, A.D., Microbial anti-adherence properties of taurolidine. *J. Pharm. Pharmacol.*, 38 (1986) 39P.

King, R.D., Lee, J.C. and Morris, A.L., Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. *Infect. Immun.*, 27 (1980) 667–674.

Langslet, A., Olsen, I., Lie, S.O. and Lokken, P., Chlorhexidine treatment of oral candidiasis in seriously diseased children. *Acta Pediatr. Scand.*, 63 (1974) 809–811.

Lee, J.C. and King, R.D., Characterisation of *Candida albicans* adherence to human vaginal epithelial cells in vitro. *Infect. Immun.*, 41 (1983) 1024–1030.

Odds, F.C., *Candida and Candidosis: A Review and Bibliography*, Bailliere Tindall, London, 1988, pp. 115–230.

Olsen, I., Denture stomatitis. Effects of chlorhexidine and amphotericin B on the mycotic flora. *Acta Odontol. Scand.*, 33 (1975a) 41–46.

Olsen, I., Denture stomatitis. The clinical effects of chlorhexidine and amphotericin B. *Acta Odontol. Scand.*, 33 (1975b) 47–52.

Sandin, R.L., Rogers, A.L., Patterson, R.J. and Beneke, E.S., Evidence for mannose-mediated adherence of *Candida albicans* to human buccal epithelial cells in vitro. *Infect. Immun.*, 35 (1982) 79–85.

Sandin, R.L., Rogers, A.L., Patterson, R.J. and Beneke, E.S., Variations in affinity to *Candida albicans* in vitro among human buccal epithelial cells. *J. Med. Microbiol.*, 24 (1987) 151–155.

Sharon, A., Berdicevsky, I., Ben-Aryeh, H. and Gutman, D., The effect of chlorhexidine mouth rinses on oral *Candida* in a group of leukemic patients. *Oral Surg.*, 44 (1977) 201–205.

Tobgi, R.S., Samaranyake, L.P. and MacFarlane, T.W., Adhesion of *Candida albicans* to buccal epithelial cells exposed to chlorhexidine gluconate. *J. Med. Vet. Mycol.*, 25 (1987) 335–338.

Woolfson, A.D., Gorman, S.P., McCafferty, D.F. and Jones, D.S., On the statistical evaluation of adherence assays. *J. Appl. Bacteriol.*, 63 (1987) 147–151.