Oral Microbiology Immunology 2006: 21: 177–182
Printed in Singapore. All rights reserved

ORAL MICROBIOLOGY
AND IMMUNOLOGY

In vitro antifungal susceptibility to six antifungal agents of 229 *Candida* isolates from patients with diabetes mellitus

M. Manfredi[1,2], M. J. McCullough[1,3], L. Polonelli[4], S. Conti[4], Z. M. Al-Karaawi[1], P. Vescovi[2], S. R. Porter[1]

[1]Oral Medicine, Division of Infection and Immunity, Eastman Dental Institute, UCL, University of London, London, UK, [2]Sezione di Odontostomatologia, Dipartimento di Scienze Otorino-Odonto-Oftalmologiche e Cervico Facciali, Università di Parma, Italy, [3]School of Dental Science, University of Melbourne, Victoria, Australia, [4]Sezione di Microbiologia, Dipartimento di Patologia e Medicina di Laboratorio, Università di Parma, Italy

Manfredi M, McCullough MJ, Polonelli L, Conti S, Al-Karaawi ZM, Vescovi P, Porter SR. In vitro *antifungal susceptibility to six antifungal agents of 229* Candida *isolates from patients with diabetes mellitus.*
Oral Microbiol Immunol 2006: 21: 177–182. © Blackwell Munksgaard, 2006.

The most common antifungal drugs in current clinical use for the treatment of oral candidosis are polyenes and azoles, mainly used topically. Poor glycaemic control in association with other local factors, such as the presence of oral dental prostheses, salivary pH, salivary flow rate and tobacco habits, may lead to the development of oral candidosis. Topical antifungal agents are frequently used to prevent the development of candidal infections in patients with poor metabolic control, particularly in the elderly wearing dentures. The aim of this study was to assess the antifungal susceptibility of *Candida* isolates to six antifungal agents using a commercially available kit, Fungitest®. The isolated were collected from patients affected by diabetes mellitus from two different geographic localities (London, UK, and Parma, Italy) and from a group of healthy non-diabetic subjects. No differences in antifungal susceptibility to the six agents tested were observed between *Candida* isolates from diabetic and non-diabetic subjects. However, differences were observed between the two geographically different diabetes mellitus populations. Oral yeast isolates from diabetes mellitus patients in the UK more often displayed resistance or intermediate resistance to fluconazole ($P = 0.02$), miconazole ($P < 0.0001$), and ketoconazole ($P = 0.01$) than did isolates from diabetes mellitus patients in Italy. In addition, more *C. albicans* isolates were found in diabetic and non-diabetic subjects that were susceptible to fluconazole ($P = 0.0008$ and $P = 0.01$, respectively) than non-*albicans* isolates. The difference in the antifungal resistance of isolates from the two populations of diabetes mellitus patients may be related to differences in the therapeutic management of candidal infections between the two centres.

Key words: antifungal agents; oral candidosis; diabetes mellitus; oral *Candida* isolates

M. Manfredi, Sezione di Odontoestomatologia, Dipartimento di Scienze Otorino-Odonto-Oftalmologiche e Cervico Facciali, Università di Parma, Parma, Italy
E-mail: maddalena.manfredi@unipr.it
Accepted for publication October 21, 2005

Several antifungal agents (azoles and polyenes), with different modes of action, are available for the treatment of oral *Candida* infection (7, 9). The increasing availability and clinical application of these antifungal agents has led to a rise in the frequency of reports of *in vitro* and *in vivo* antifungal resistance of *Candida* spp. (11, 12, 24). Several yeast species (*Candida glabrata, Candida krusei, Candida guilliermondii*, *Candida lusitaniae*) have a higher prevalence of primary resistance to amphotericin B, and *C. glabrata* and *C. krusei* are intrinsically less susceptible to triazoles than *Candida albicans* itself. Furthermore, *Candida dubliniensis* has been shown, *in vitro*, to rapidly develop stable resistance to fluconazole (20, 31, 37). In addition to these intrinsically resistant species, there are intrinsically resistant strains of *C. albicans* which may be part of a commensal microflora or may be acquired from the environment or other individuals (37).

Epidemiological changes in the susceptibility of pathogenic fungi to antifungal drugs have led to the standardisation of antifungal resistance assays *in vitro* and resistance breakpoint definitions, first reported in 1992 and then in 1997 (21)

by the US National Committee for Clinical Laboratory Standards (NCCLS) as a reference method, named M27-A, for antifungal susceptibility testing. A second edition of the method, M27-A2, was released in 2002 (22). However, the NCCLS committee accepts that M27-A/M27-A2 have limitations in terms of their ability to distinguish between isolates susceptible and resistant to amphotericin B, newer azoles and echinocandins and the time-consuming nature of the methods. Many clinical laboratories therefore prefer to use commercially available products that have previously shown correlations with M27-A (31, 37). Three recent studies compared one or more of these commercial products with the NCCLS methodology (3, 19, 27). Fungitest® (Bio-Rad, Marnes-La-Coquette, France) was evaluated in the largest of these three studies (19). This multicentre evaluation of six commercial systems and the NCCLS microdilution method M27-A compared the susceptibility of 800 *Candida* isolates to fluconazole (19). The results of the study indicated that Fungitest® together with *E*-test (AB Biodisk, Slovna, Sweden) and Sensititre Yeast One can be considered useful for the *in vitro* evaluation of fluconazole susceptibility among *Candida* spp. isolates (19).

It has been reported that poor glycaemic control in association with other local factors, such as the presence of oral dental prostheses, salivary pH, salivary flow rate and tobacco habits, may lead to the development of oral candidosis (6). Topical antifungal agents are frequently used to prevent the development of candidal infections in patients with poor metabolic control, particularly in the elderly wearing dentures. Elderly people frequently suffer from an unpleasant sensation in the mouth that may give rise to intolerable distress (4). If the elderly are diabetic, typically type 2 DM, with poor glycaemic control, diabetologists and general practitioners tend to prescribe topical antifungal agents to prevent oral candidosis, even if signs of the disease are not present. The indiscriminate use of antifungal agents, even in diabetic populations, could change the prevalence of the *Candida* spp. causing the disease, resulting in increased antifungal resistance. Treatment of superficial oral *Candida* infections, both in diabetes and non-diabetic subjects, is mainly based on the use of topical antifungal agents, such as azoles (mainly miconazole and fluconazole, but also ketoconazole and itraconazole) or polyenes (amphotericin B and nystatin) (2, 7, 8, 29, 38). In this study, we evaluated the antifungal susceptibility of *Candida* isolates from diabetic patients to the most commonly used antifungal agents for the treatment of oral candidosis.

Material and methods

Oral *Candida* isolates

A total of 229 oral *Candida* yeasts 177 *C. albicans and* 52 non-*albicans* spp.) were isolated in Parma, Italy (DP group, 71 strains) and in the UK, London (DL group, 83 strains) from adult diabetes mellitus patients (both type 1 and type 2) and non-diabetic subjects (ND group, 75 strains), and identified by conventional and molecular methods (1, 15, 16). None of the diabetic patients or non-diabetic subjects evaluated in the present study was affected by oral candidosis at the time of the oral examination. Oral yeast load was expressed as colony forming units (cfu)/ml of mouth rinse. The medical history of each patient was recorded at the time of examination, as well as the characteristics of their disease (time since diagnosis, type of diabetic mellitus, haemoglobin glycosylation control and the most common long-term diabetes complications), as previously reported (15).

In vitro antifungal susceptibility test

Oral *Candida* yeasts isolated from diabetes mellitus patients and from the control population were evaluated *in vitro* for their antifungal susceptibility to six different antifungal agents using the commercial kit Fungitest® (Bio-Rad). The susceptibility of the individual isolates to commercially available antifungal agents was assessed by a broth microdilution assay using Alamar blue in an *in vitro* assay supplied by the manufacturer (Bio-Rad) and based on the NCCLS broth macrodilution assay. A single colony from a 48-h, 37°C SDA culture of each isolate was diluted into 3 ml of distilled water to give a turbidity reading of McFarland number 1 (equal to approximately 3×10^6 cells/ml). One hundred microlitres of this suspension was diluted into 1.9 ml of sterile distilled water and 20 µl were added to 3 ml of a suspension medium (supplied by the manufacturer, Bio-Rad). One hundred microlitres (approximately 1×10^3 cells/ml) of the yeast suspension were added to each of the 16 wells of a Fungitest® (Bio-Rad). This 16-well microplate contained two different concentrations of six different antifungal agents (fluconazole 8 and 64 µg/ml, itraconazole 0.5 and 4 µg/ml, miconazole 0.5 and 8 µg/ml, ketoconazole 0.5 and 4 µg/ml, amphotericin B 2 and 8 µg/ml and 5-FC 2 and 32 µg/ml), in modified RPMI 1640 buffered medium, with the presence of a redox indicator (Alamar blue). Two growth control wells and two negative control wells were also present in each microplate. After incubation at 37°C for 48 h, growth was assessed by colorimetric means as outlined by the manufacturer. Results were expressed in terms of resistance, intermediate resistance or susceptibility to each antifungal agent.

Statistical analysis

Fisher's exact test and Chi-squared test were used for the statistical analysis of categorical data, numeric data were analysed by Student's *t*-test or ANOVA according to the postulates of each test, and differences among or between groups were considered significant when the probability (*P*) was less than or equal to 0.05. Although there are interesting variations in the number of isolates fully resistant to certain antifungal agents, it was not possible to undertake meaningful statistical analyses with all antifungal agents, as the number of resistant isolates in the other patient populations for the other antifungal agents was either too low or nonexistent. Furthermore, the intention of this analysis was to determine the changes in antifungal sensitivity over a large number of geographically dispersed isolates from specific patient populations utilising a rapid screening method. The intention of this study was not to report accurate and clinically significant antifungal susceptibility on specific strains to a limited number of antifungal agents. Therefore, isolates with any enhanced resistance to the antifungal agents tested were grouped together for statistical analysis.

Results

Antifungal susceptibility of all *Candida* isolates

The resistance rates of all *C. albicans* isolates (177/229) tested were 9% (fluconazole), 17% (itraconazole and miconazole), 15% (ketoconazole), 7% (amphotericin B) and 5%(5-FC). For non-*albicans* isolates (52/229), the resistance rates for these antifungals were 29% (fluconazole and itraconazole), 35% (miconazole), 23% (ketoconazole), 6% (amphotericin B) and 13% (5-FC).

Interestingly, *Candida* isolates that expressed resistance or intermediate resistance to fluconazole were also found to exhibit resistance to at least one other

Table 1. Analysis of the susceptibility of the *Candida* strains isolated from the oral cavity of all the *Candida* carriers to six different antifungal agents

Total patients	F S	F IR	*P*-value	M S	M IR	*P*-value	KS	K IR	*P*-value	I S	I IR	*P*-value	A S	A IR	*P*-value	5FC S	5FC IR	*P*-value
< 100 cfu	105	14	$P > 0.05$	98	21	$P > 0.05$	99	20	$P > 0.05$	95	24	$P > 0.05$	113	6	$P > 0.05$	113	6	$P > 0.05$
> 100 cfu	93	17		82	28		91	19		89	21		101	9		100	10	
C. albicans	161	16	$P = 0.0008$	147	30	$P = 0.01$	150	27	$P > 0.05$	147	30	$P > 0.05$	165	12	$P > 0.05$	168	9	$P > 0.05$
Non-*albicans*	37	15		34	18		40	12		37	15		49	3		45	7	
C. albicans A	130	15	$P > 0.05$	121	24	$P > 0.05$	122	23	$P > 0.05$	119	26	$P > 0.05$	135	10	$P > 0.05$	137	8	$P > 0.05$
C. albicans B	24	1		21	4		21	4		22	3		23	2		25	0	
C. albicans C	7	0		5	2		7	0		6	1		7	0		6	1	
Male	99	17	$P > 0.05$	95	21	$P > 0.05$	91	25	$P > 0.05$	89	27	$P > 0.05$	109	7	$P > 0.05$	108	8	$P > 0.05$
Female	99	14		85	28		97	16		95	18		105	8		105	8	
< 60 years old*	99	12	$P > 0.05$	89	22	$P > 0.05$	93	18	$P > 0.05$	92	19	$P > 0.05$	106	5	$P > 0.05$	108	3	$P > 0.05$
> 60 years old*	91	16		82	25		89	18		84	23		101	6		98	9	
Dentate	108	14	$P > 0.05$	103	19	$P = 0.02$	98	24	$P > 0.05$	99	23	$P > 0.05$	113	9	$P > 0.05$	118	4	$P = 0.02$
Dentures	90	17		77	30		92	15		85	22		101	6		95	12	
Tobacco users	43	5	$P > 0.05$	43	5	$P = 0.05$	41	7	$P > 0.05$	38	10	$P > 0.05$	48	0	$P = 0.05$	45	3	$P > 0.05$
Non-tobacco users	155	26		137	44		149	32		146	35		166	15		168	13	

Data was subdivided according to patient *Candida* colonisation (cfu/ml), species and *C. albicans* genotype subgroups.
Statistical analysis was performed using Fisher's exact and Chi-squared parametric tests.
FS, fluconazole susceptible; F IR, fluconazole intermediately resistant/resistant; MS, miconazole susceptible; M IR, miconazole intermediately resistant/resistant; KS, ketoconazole susceptible; K IR, ketoconazole intermediately resistant/resistant; I S, itraconazole susceptible; I IR, itraconazole intermediately resistant/resistant; A S: amphotericin B susceptible; A IR, amphotericin B intermediately resistant/resistant; 5-FC S, 5-FC susceptible; 5FC IR, 5-FC intermediately resistant/resistant.
*****Of the 12 non-DM control subjects who did not give their date of birth (special needs patients), 10 harboured oral yeasts. Of these 10, nine yielded one *Candida* strain each and one additional non-diabetic subject yielded two *Candida* strains.

antifungal agent. This finding of fluconazole cross-resistance with other antifungals was not encountered with any other combination of antifungals.

No relationship was evident between oral candidal load and the rates of antifungal resistance for the drugs tested ($P > 0.05$) (Table 1). It was, however, found that the non-*albicans* isolates exhibited higher or intermediate resistance rates to fluconazole ($P = 0.0008$) and miconazole ($P = 0.01$) than did *C. albicans* isolates (Table 1). There was no equivalent relationship to susceptibility with any other antifungal agents tested (Table 1).

There was no association between patient age or gender and the antifungal susceptibility of *Candida* spp. ($P > 0.05$). Fewer of the oral yeasts isolated from patients without dentures were resistant or intermediately resistant to miconazole ($P = 0.02$) and 5-FC ($P = 0.02$) (Table 1). Furthermore, isolates from non-tobacco users had a higher resistance or intermediate resistance to miconazole ($P = 0.05$) or amphotericin B ($P = 0.05$) (Table 1).

Table 2. In vitro susceptibility to six different antifungal agents of oral *Candida* isolates from patients with and without diabetes mellitus

Antifungal agents	Diabetic patients	Non-Diabetic subjects	*P*-value
Fluconazole			
S	132/154(86%)	66/75(88%)	$P = 0.57$
I	16/154(10%)	8/75(11%)	
R	6/154(4%)	1/75(1%)	
Miconazole			
S	122/154(79%)	59/75(79%)	
I	32/154(21%)	16/75(21%)	
R	0/154	0/75	$P = 1.00$
Ketoconazole			
S	123/154(80%)	67/75(89%)	
I	20/154(13%)	5/75(7%)	
R	11/154(7%)	3/75(4%)	$P = 0.20$
Itraconazole			
S	120/154(78%)	64/75(85%)	
I	20/154(13%)	7/75(9%)	
R	14/154(9%)	4/75(5%)	$P = 0.40$
Amphotericin B			
S	147/154(95%)	67/75(89%)	
I	7/154(5%)	8/75(11%)	
R	0/154	0/75	$P = 0.09$
5-FC			
S	146/154(95%)	67/75(89%)	
I	6/154(4%)	8/75(11%)	
R	2/154(1%)	0/154	$P = 0.08$

Statistical analysis was performed using Fisher's exact and Chi square parametric tests.
S, susceptible; I, intermediately resistant; R, resistant.

Comparison of antifungal susceptibility between *Candida* from diabetic and non-diabetic subjects

No difference in the antifungal susceptibility to the six agents tested were observed between *Candida* isolates from diabetic and non-diabetic subjects (Table 2). However, significant differences were observed in the antifungal susceptibility when the two diabetic populations from London and Parma were compared (Table 3).

Oral yeasts from London diabetic patients had a higher (or intermediate) resistance to fluconazole ($P = 0.02$), miconazole ($P < 0.0001$), and ketoconazole ($P = 0.01$) than the isolates from Parma diabetic patients. There was also a trend in London diabetic patients of an increased resistance of isolates to itraconazole and 5-FC ($P = 0.08$) (Table 3).

No statistically relevant associations could be found when antifungal susceptibility was compared against type of dia-

Table 3. Analysis of the antifungal susceptibility of the *Candida* species isolated from the oral cavities of London and Parma patients with diabetes mellitus

Antifungal agents	Diabetic London patients	Diabetic Parma patients	*P*-value
Fluconazole			
S	66/83(79.5%)	66/71(93%)	
IR	17/83(20.5%)	5/71(7%)	$P = 0.02$
Miconazole			
S	55/83(66.3%)	67/71(94.4%)	
IR	28/83(33.7%)	4/71(5.6%)	$P < 0.0001$
Ketoconazole			
S	60/83(72.3%)	63/71(88.7%)	
IR	23/83(27.7%)	8/71(11.3%)	$P = 0.01$
Itraconazole			
S	60/83(72.3%)	60/71(84.5%)	
IR	23/83(27.7%)	11/71(15.5%)	$P = 0.08$
Amphotericin B			
S	77/83(92.8%)	70/71(98.6%)	
IR	6/83(7.2%)	1/71(1.4%)	$P = 0.08$
5-FC			
S	78/83(94%)	68/71(95.8%)	
IR	5/83(6%)	3/71(4.2%)	$P = 0.72$

Statistical analysis was performed using Fisher's exact and Chi-squared parametric tests.
S, susceptible; IR, intermediately resistant/resistant.

betes mellitus, the duration of the diabetes and haemoglobin glycosylation levels. However, it was apparent that *Candida* isolated from diabetic patients with neuropathy and retinopathy were more susceptible to miconazole ($P = 0.05$) than those strains from patients who had nephropathy.

No statistically significant ($P > 0.05$) association was found between antifungal susceptibility and patient age, gender, tobacco usage or denture wearing between the two diabetes populations.

No significant associations were found between resistance to any tested antifungal agent and the level of oral yeast colonisation (cfu/ml) or *C. albicans* genotype in diabetic patients from London and Parma.

Discussion

The objective of this study was to establish whether increased *in vitro* antifungal resistance occurs in isolates from diabetes mellitus patients that might lead to problems in the management of patients prone to oral candidal infections. As reported in the Results section, no differences in the antifungal susceptibility were observed between *Candida* isolates from diabetic and non-diabetic subjects. However, differences were observed between the two geographically different diabetic populations.

The higher incidence of intermediate resistance to certain antifungals in the UK diabetes mellitus isolates was probably not related to recent exposure to antifungal treatment, as none of these patients had received antifungal drugs in the preceding 6 months. However, their exposure to antifungal agents prior to this time was unknown. Therefore, there may well be a significant variation in lifetime antifungal exposure between the two diabetes mellitus populations, explaining the elevated antifungal resistance rates observed in the UK diabetes mellitus population.

Interestingly, one of the most common antifungal agents used to treat patients with dentures suffering from oral candidosis is miconazole (5). Although patients in the present study had not taken any antifungal agents in the preceding 6 months, those patients with dentures likely had increased exposure to this antifungal agent during their lifetime, which may well be more significant than antifungal exposure in the preceding 6-month period.

The difference in the antifungal resistance of isolates from the two populations of diabetes mellitus patients may be related to differences in the therapeutic management of candidal infections between the two centres in Italy and the UK since the introduction of these antifungal agents in the two countries. However, no available long-term data were available to support this. Although no association could be observed in the diabetes mellitus status of the patients in the UK and Italy, it could be assumed that as the UK group had more long-standing diabetes mellitus with more complications (data not shown), they would be more likely to have received or taken the antifungal agents themselves. As has been reported in literature (29, 31), it is more likely that intermittent rather than long-term antifungal therapy leads to the development of azole resistance, as may be the case with the present group of diabetes mellitus patients in the UK. The *Candida* isolated from the oral cavities of the diabetes mellitus patients resident in the UK may have acquired resistance as a consequence of the selective pressure of azole treatment (31). The most important clinical consequence of antifungal resistance is usually a failure to successfully treat patients affected by candidosis as well as changes in the prevalence of the *Candida* spp. causing the disease (31). Interestingly, none of the patients with resistant *Candida* species had signs or symptoms of oral *Candida* infections, indicating that a patient's own immune system can control the yeast pathogenicity.

Although some of the yeasts isolated from the diabetes mellitus population from the UK (6/83, 7.2%) and from the control group (8/75, 10.6%) showed an *in vitro* intermediate resistance to amphotericin B, none of the *Candida* isolates from the three groups of patients enrolled in the study was completely resistant to amphotericin B. This antifungal drug (10, 14) is the most widely used in the UK as a topical agent (lozenges, suspensions) for the treatment of oral candidosis, and as some of these topical formulations are not available in Italy, it is possible that this geographical variation might explain the trends for increased amphotericin resistance in UK isolates. In any case, secondary resistance to amphotericin B generally seems to be an infrequent development (37). However, the methodologies and results of the present study do not clarify whether the antifungal resistance of examined isolates is intrinsic or acquired. In addition, because diabetes mellitus had little effect on the presence of antifungal resistance of oral yeast isolates, there remains the possibility that any resistance shown has been acquired from family members (or indeed other hospital patients) (30–32). It is well known that wearing acrylic dentures is an important predisposing factor for the development of oral candidosis, as these prostheses, when not kept clean, act as a reservoir for infection (8, 9). Furthermore, increased oral candidal load is much more common in full-denture wearers than dentate individuals (15, 23). *In vitro* biofilms, likely to show denture-associated stomatitis, have increased resistance to azoles (13). In this study, isolates from denture wearers were less susceptible *in vitro* to miconazole and 5-FC than isolates collected from dentate

patients. It could be hypothesised that the *in vitro* resistance of these *Candida* isolates is a consequence of the past widespread topical use of miconazole for the treatment of *Candida*-induced denture stomatitis. Alternatively, it may be that some of the resistance of *Candida* within biofilms of denture-associated stomatitis reflects inherent change within the fungi (rather than simply reflecting the inhibition of diffusion of antifungals within biofilms) (13). The analysis of the susceptibility of all the oral *Candida* isolates (i.e. from the three groups of patients) to the different antifungal agents has shown that *C. albicans* strains were more susceptible to fluconazole and miconazole than non-*albicans* strains. It has been demonstrated that *C. albicans* is usually susceptible to all major antifungal agents (29), and other *Candida* spp., such as *C. glabrata* and *C. krusei*, are intrinsically less susceptible to triazole and amphotericin B (31). However, *C. albicans* resistance to different antifungal agents, such as triazoles and particularly fluconazole, has recently been reported among different groups of immunocompromised patients in whom this agent is frequently used in prophylaxis and the treatment of fungal infections (28, 29, 34). Furthermore, although the susceptibility of *Candida* spp. to the available antifungal agents can be predicted if the species of the infecting isolate is known, individual isolates do not necessarily follow the general pattern (25, 26, 29).

The findings in this study confirm the results of others indicating that the previously reported emergence of *C. albicans* fluconazole and, more generally, triazole resistance, was probably mainly due to the non-standardised susceptibility methods rather than a real increase in the resistance of *C. albicans* species (30). However, the issue is still controversial. The commercial method (Fungitest®) used in the present study is considered a useful kit for the *in vitro* evaluation of fluconazole sensitivity among *Candida* spp. isolates in clinical laboratories, with a positive agreement among laboratories regarding its reproducibility. Although this method provides a limited number of drug concentrations and has some limitations as a commercial kit compared to the NCCLS reference methods, it is regarded as one of the easiest and most rapid commercial kits available (19). Furthermore, the NCCLS M27-A antifungal concentration breakpoints are only available for three of the most common drugs used (21).

The results obtained in this study would appear to confirm that the rates of triazole resistance in *Candida* spp. are low and possibly overestimated (31), perhaps suggesting that the associated clinical problem could be less serious than hypothesised. However, because the present non-*albicans Candida* spp. isolates were less susceptible to fluconazole and miconazole (Table 1) than the *C. albicans* isolates, the increased use of 'over the counter' azole formulations could result in increased oral carriage of non-*albicans Candida* and thus the possibility of acquisition by, and infection of, immunocompromised groups (31, 37).

Finally, it has been reported (18) that *C. albicans* genotype B (which harbours a 379-nucleotide (Group I intron) in the 25S rDNA) and *C. albicans* genotype C (a mixture of intron-containing and intronless 25S rDNA) both showed a greater susceptibility to 5-FC than the intronless strain *C. albicans* genotype A. This observation was further confirmed by other studies (17): the different levels of susceptibility to this agent by these subgroups of *C. albicans* seemed to be due to the inhibitory effect of biosynthetic incorporation of this base analogue into the group I intron ribozymes (18). However, in this study no differences were observed in the susceptibility to 5-FC of the *C. albicans* subgroup B isolates from all the patients evaluated in the study, and only one isolate from the *C. albicans* subgroup C expressed intermediate resistance to the same agent. This finding is in agreement with others (33) and suggests that the contribution of the group I intron to the susceptibility of *Candida* spp. to 5-FC may only be one factor, and that other factors (35, 36) may play more important roles in the 5-FC susceptibility of *Candida* spp.

In conclusion, diabetes mellitus does not appear to influence the frequency or nature of antifungal resistance of *Candida* isolates of the mouth. Antifungal resistance of oral isolates may indeed be more influenced by the geographical locale of patients and perhaps local practices for the prescribing of antifungal agents. Denture wearing may likewise increase the possibility of antifungal resistance in patients with or without diabetes mellitus. It would seem that the emergence of antifungal resistance within many oral *Candida* isolates has an iatrogenic basis.

References

1. Al-Karaawi ZM, Manfredi M, Waugh AC, McCullough MJ, Jorge J, Scully C, et al. Molecular characterization of *Candida* spp. isolated from the oral cavities of patients from diverse clinical settings. Oral Microbiol Immunol 2002: **17**: 44–49.
2. Budtz-Jorgensen E, Lombardi T. Antifungal therapy in the oral cavity. Periodontol 2000 2000: **10**: 89–106.
3. Chryssanthou E. Trends in antifungal susceptibility among Swedish *Candida* species bloodstream isolates from 1994 to 1998: comparison of the E-test and the Sensititre YeastOne Colorimetric Antifungal Panel with the NCCLS M27 – A reference method. J Clin Microbiol 2001: **39**: 4181–4183.
4. Collin HL, Niskanen L, Uusitupa M, Toyry J, Collin P, Koivisto AM, et al. Oral symptoms and signs in elderly patients with type 2 diabetes mellitus. A focus on diabetic neuropathy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000: **90**: 299–305.
5. Dias AP, Samaranayake LP, Lee MT. Miconazole lacquer in the treatment of denture stomatitis: clinical and microbiological findings in Chinese patients. Clin Oral Invest 1997: **1**: 47–52.
6. Dorocka-Bobkowska B, Budtz-Jorgensen E, Wloch S. Non-insulin-dependent diabetes mellitus as a risk factor for denture stomatitis. J Oral Pathol Med 1996: **25**: 411–415.
7. Ellepola AN, Samaranayake LP. Antimycotic agents in oral candidosis: an overview: 2. Treatment of oral candidosis. Dent Update 2000: **27**: 165–170, 172–164.
8. Ellepola AN, Samaranayake LP. Oral candidal infections and antimycotics. Crit Rev Oral Biol Med 2000: **11**: 172–198.
9. Ellepola AN, Samaranayake LP, Martin MV. Antifungal agents. In: Samaranayake LP, Macfarlane TW, eds. Oral candidosis. London: Wright-Butterworth, 1990: 238–255.
10. Epstein JB, Polsky B. Oropharyngeal candidiasis: a review of its clinical spectrum and current therapies. Clin Ther 1998: **20**: 40–57.
11. Espinel-Ingroff A, Pfaller M, Messer SA, Knapp CC, Killian S, Norris HA, et al. Multicenter comparison of the sensititre YeastOne Colorimetric Antifungal Panel with the National Committee for Clinical Laboratory standards M27 – A reference method for testing clinical isolates of common and emerging *Candida* spp., *Cryptococcus* spp. and other yeasts and yeast-like organisms. J Clin Microbiol 1999: **37**: 591–595.
12. Espinel-Ingroff A, Warnock DW, Vazquez JA, Arthington-Skaggs BA. *In vitro* antifungal susceptibility methods and clinical implications of antifungal resistance. Med Mycol 2000: **38** (Suppl. 1): 293–304.
13. Lamfon H, Al-Karaawi Z, McCullough M, Porter SR, Pratten J. Composition of *in vitro* denture plaque biofilms and susceptibility to antifungals. FEMS Microbiol Lett 2005: **242**: 345–351.
14. Lefebvre JL, Domenge C. A comparative study of the efficacy and safety of fluconazole oral suspension and amphotericin B oral suspension in cancer patients with mucositis. Oral Oncol 2002: **38**: 337–342.
15. Manfredi M, McCullough MJ, Al-Karaawi ZM, Hurel SJ, Porter SR. The isolation,

identification and molecular analysis of *Candida* spp. isolated from the oral cavities of patients with diabetes mellitus. Oral Microbiol Immunol 2002: **17**: 181–185.
16. Manfredi M, McCullough MJ, Conti S, Polonelli L, Vescovi P, Al-Karaawi ZM, et al. *In vitro* activity of a monoclonal killer anti-idiotypic antibody and a synthetic killer peptide against oral isolates of *Candida* spp. differently susceptible to conventional antifungals. Oral Microbiol Immunol 2005: **20**: 226–232.
17. McCullough MJ, Clemons KV, Stevens DA. Molecular and phenotypic characterization of genotypic *Candida albicans* subgroups and comparison with *Candida dubliniensis* and *Candida stellatoidea*. J Clin Microbiol 1999: **37**: 417–421.
18. Mercure S, Montplaisir S, Lemay G. Correlation between the presence of a self-splicing intron in the 25S rDNA of *C. albicans* and strains susceptibility to 5-fluorocytosine. Nucl Acids Res 1993: **21**: 6020–6027.
19. Morace G, Amato G, Bistoni F, Fadda G, Marone P, Montagna MT, et al. Multicenter comparative evaluation of six commercial systems and the national committee for clinical laboratory standards M27-A broth microdilution method for fluconazole susceptibility testing of *Candida* species. J Clin Microbiol 2002: **40**: 2953–2958.
20. Moran GP, Sanglard D, Donnelly SM, Shanley DB, Sullivan DJ, Coleman DC. Identification and expression of multidrug transporters responsible for fluconazole resistance in *Candida dubliniensis*. Antimicrob Agents Chemother 1998: **42**: 1819–1830.
21. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing for yeasts. Approved standard. Wayne, PA: NCCLS, 1997.
22. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard, 2nd edn. Wayne, PA: NCCLS, 2002.
23. Parvinen T, Kokko J, Yli-Urpo A. Miconazole lacquer compared with gel in treatment of denture stomatitis. Scand J Dent Res 1994: **102**: 361–366.
24. Pfaller MA, Bale M, Buschelman B, Lancaster M, Espinel-Ingroff A, Rex JH, et al. Quality control guidelines for National Committee for Clinical Laboratory Standards recommended broth macrodilution testing of amphotericin B, fluconazole, and flucytosine. J Clin Microbiol 1995: **33**: 1104–1107.
25. Pfaller MA, Messer SA, Bolmstrom A. Evaluation of Etest for determining *in vitro* susceptibility of yeast isolates to amphotericin B. Diagn Microbiol Infect Dis 1998: **32**: 223–227.
26. Pfaller MA, Messer SA, Hollis RJ. Strain delineation and antifungal susceptibilities of epidemiologically related and unrelated isolates of *Candida lusitaniae*. Diagn Microbiol Infect Dis 1994: **20**: 127–133.
27. Posteraro B, Romano L, Sanguinetti M, Masucci L, Morace G, Fadda G. Commercial systems for fluconazole susceptibility testing of yeasts: comparison with the broth microdilution method. Diagn Microbiol Infect Dis 2000: **38**: 29–36.
28. Rex JH, Pfaller MA, Walsh TJ, Chaturvedi V, Espinel-Ingroff A, Ghannoum MA, et al. Antifungal susceptibility testing: practical aspects and current challenges. Clin Microbiol Rev 2001: **14**: 643–658.
29. Rex JH, Walsh TJ, Sobel JD, Filler SG, Pappas PG, Dismukes WE, et al. Practice guidelines for the treatment of candidiasis. Infectious Diseases Society of America. Clin Infect Dis 2000: **30**: 662–678.
30. Sanglard D, Bille J. Current understanding of the modes of action and resistance mechanisms to conventional and emerging antifungal agents for treatment of *Candida* infections. In: Calderone RA, ed. Candida and candidiasis. Washington DC: American Society of Microbiology Association (ASM) Press, 2002: 349–383.
31. Sanglard D, Odds FC. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. Lancet Infect Dis 2002: **2**: 73–85.
32. Sullivan DJ, Coleman DC. Molecular approaches to identification and typing of *Candida* species. In: Calderone RA, ed. Candida and candidiasis. Washington DC: American Society of Microbiology Association (ASM) Press, 2002: 427–441.
33. Tamura M, Watanabe K, Mikami Y, Yazawa K, Nishimura K. Molecular characterization of new clinical isolates of *Candida albicans* and *C. dubliniensis* in Japan: analysis reveals a new genotype of *C. albicans* with group I intron. J Clin Microbiol 2001: **39**: 4309–4315.
34. Vazquez JA, Peng G, Sobel JD, Steele-Moore L, Schuman P, Holloway W, et al. Evolution of antifungal susceptibility among *Candida* species isolates recovered from human immunodeficiency virus-infected women receiving fluconazole prophylaxis. Clin Infect Dis 2001: **33**: 1069–1075.
35. von Ahsen U, Davies J, Schroeder R. Antibiotic inhibition of group I ribozyme function. Nature 1991: **353**: 368–370.
36. von Ahsen U, Schroeder R. Streptomycin inhibits splicing of group I introns by competition with the guanosine substrate. Nucl Acids Res 1991: **19**: 2261–2265.
37. White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev 1998: **11**: 382–402.
38. Willis AM, Coulter WA, Fulton CR, Hayes JR, Bell PM, Lamey PJ. The influence of antifungal drugs on virulence properties of *Candida albicans* in patients with diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001: **91**: 317–321.