Salivary Candida Species Carriage Patterns and Their Relation to Caries Experience Among Yemeni Children

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Purpose: This study was carried out to assess the carriage rates, counts and species distribution of *Candida* in saliva of 6- to 12-year-old Yemeni children and relate that to their caries experience.

Materials and Methods: One hundred eighty children were recruited. Oral hygiene and caries were assessed using the simplified oral hygiene index and dft/DMFT index, respectively. Detection and quantification of 4 *Candida* species in unstimulated saliva were performed using CHROMagar Candida medium. Data were analysed using regression analysis.

Results: *Candida* was detected in 60% of the children with a mean count of 923 ± 1875 CFU/ml. *C. albicans* accounted for 60% of the isolates and was the only species to be detected with more than 1000 CFU/ml. Non-albicans candida and unidentified species represented 16.3% and 23.1% of the isolates, respectively. One novel finding was that a significant proportion (38%) of the carriers harboured two or more species, which for the first time allowed the identification of four age-dependent carriage patterns (clusters). Another somewhat new observation was that carriage at ≥ 1000 CFU/ml in particular significantly correlated with caries in primary and permanent dentitions ($r = 0.23$ and 0.18, respectively) as well as a caries-active status (OR = 6.9). Interestingly, the *C. glabrata* cluster had significantly lower primary caries scores than other clusters.

Conclusions: The findings substantiate claims of geographical variations in candida carriage and the relation between candida carriage and caries. The validity of carrier clusters and the use of 1000 CFU/ml as a risk marker should be further investigated.

Key words: candida species, dental caries, aetiology, risk factors, saliva

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Candida is a ubiquitous fungal genus belonging to the unranked mitosporic saccharomycetales, with more than 500 species members (Index Fungorum, 2008). About 20 of these are commonly found in humans, mostly as commensals and, under certain circumstances, as opportunistic pathogens. *Candida albicans* is the most commonly isolated species from both diseased cases and healthy carriers; however, the importance of non-albicans candida (NAC) species such as *C. parapsilosis, C. krusei, C. tropicalis, C. glabrata* and *C. dubliniensis* is being increasingly recognised (Sobel, 2006). Since *Candida* species differ in their production of virulence factors and susceptibility to antifungal agents, greater emphasis is being placed on differentiating them at the species level (Williams and Lewis, 2000).

In the oral cavity, the posterior part of the dorsum of the tongue is the main carriage site of *Candida* species, but they are also found on other mucosal surfaces and in saliva (Cannon et al, 1995). The asymptomatic carriage rates and intensities vary widely depending on the age, gender, salivary factors, smoking status and geographical location of the study population as well as the sampling and microbiological analysis method employed (Cannon

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et al, 1995; Kleinegger et al, 1996; Samaranayake, 2009). Geographical variations in particular are rather striking (Samaranayake, 2009; Xu and Mitchell, 2003). In children for example, carriage rates as low as 7.7% have been reported in Asian cohorts, while a prevalence of up to 70% has been found in Western populations (Samaranayake, 2009). While *C. albicans* accounts for the majority of the isolates from Europeans and Americans, NAC species seems to predominate among Chinese (Xu and Mitchell, 2003). It is prudent, therefore, to investigate oral carriage patterns of *Candida* species in other geographical areas, including developing countries, for which such information is nearly lacking.

It is well established that in susceptible hosts, *Candida* species can specifically infect the oral mucosa, resulting in a range of clinical pathologies collectively called oral candidiosis or candidiasis (Scully et al, 1994; Cannon et al, 1995). What remains controversial, however, is whether or not *Candida* has an aetiological role in caries. While it is not classically mentioned among the cariogenic microbiota, e.g. mutans streptococci and lactobacilli, there is growing evidence to support its potential cariogenic role. *Candida* has been detected in dentinal caries (Marchant et al, 2001; Shen et al, 2002) and its carriage has been repeatedly reported to correlate with caries experience (Coulter et al, 1993; Gabris et al, 1999; Raja et al, 2010) as well as caries risk (Raitio et al, 1996; Ollila and Larmas, 2008), particularly in primary teeth. *C. albicans* has been shown to possess a 20-fold greater ability to dissolve hydroxyapatite in vitro than *Streptococcus mutans* (Nikawa et al, 2003) and very recently to experimentally induce caries in rats (Klinke et al, 2011). Accordingly, the cariogenic potential of *Candida* should not be discounted.

Based on the above, the objective of the current study was to examine the salivary carriage patterns of *Candida* species among Yemeni children and to assess their relation to caries experience.

MATERIALS AND METHODS

Study subjects

Sample size was calculated with the aim of detecting a difference in means of one decayed permament tooth or two decayed primary teeth between candida carriers and non-carriers, with a power of 90% and confidence level of 95%, assuming a carriers to non-carriers ratio of 1. Estimates for standard deviations were obtained from the study on oral health status among Yemeni children by Al-hadad et al (2009). The resultant sample size was around 150 and it was decided to increased it by 20%.

Consequently, a total of 180 healthy 6- to 12-year-old children, equally stratified by gender, were recruited from the paedodontic clinic at the University of Science and Technology's Dental Faculty, and from one private and one public primary school in Sana'a City. In the paedodontic clinic, children who fulfilled the inclusion criteria were sequentially recruited from among the visitors. In the schools, children were randomly selected from grades 1 to 6 to yield roughly equal numbers from each grade. To be included in the study, the children had to be free of symptomatic oral candidiosis, have no history of current use of antibiotics or antifungal agents and have not worn orthodontic or other intraoral appliances.

The study was approved by the Medical Research Committee at the faculty. Written consent was obtained from parents of children recruited at the clinic; for those at schools, consent was obtained from the schools' directors.

Clinical examination and sample collection

Each of the study subjects was examined for oral hygiene and caries. The former was recorded as poor, fair or good using the simplified oral hygiene index (Greene and Vermillion, 1964). The latter was assessed using the dft/DMFT index according to WHO's methodology for oral health surveys (World Health Organization, 1997). Children with a zero dft/DMFT score were considered caries free. Samples of unstimulated whole saliva were then collected in sterile containers and immediately cold-transferred to the laboratory for microbiological analysis. As a standard, all samples were collected between 9:00 and 11:00 a.m. to control for circadian changes in flow.

Microbiological analysis

The saliva samples were vortexed well for homogenisation before 100 µl of each sample were plated on CHROMagar Candida (CHROMagar; Paris, France) agar, a selective and differential medium for *Candida*, prepared according to manufacturer's directions. After 48–72 h of incubation at 37°C, plates

Table 1 Caries scores (mean ± SD) by gender and oral hygene index (OHI)

Component	Overall (N = 180)	Gender			OHI		
		Male (n = 90)	Female (n = 90)	*P**	Fair (n = 29)	Good (n = 151)	*P**
DT	1.02 ± 1.46	0.97 ± 1.60	1.08 ± 1.32	NS	1.03 ± 1.82	0.02 ± 1.39	NS
MT	0.00 ± 0.00	00.0 ± 0.00	0.00 ± 0.00	NS	0.00 ± 0.00	0.00 ± 0.00	NS
FT	0.02 ± 0.13	0.01 ± 0.11	0.02 ± 0.15	NS	0.00 ± 0.00	0.02 ± 0.14	NS
DMFT	1.04 ± 1.48	0.98 ± 1.63	1.10 ± 1.32	NS	1.03 ± 1.82	1.04 ± 1.41	NS
dt	2.95 ± 2.90	3.46 ± 2.92	2.44 ± 2.80	0.046	4.45 ± 3.71	2.66 ± 2.63	0.01
ft	0.12 ± 0.52	0.09 ± 0.32	0.14 ± 0.66	NS	0.00 ± 0.00	0.14 ± 0.57	NS
dft	3.07 ± 2.91	3.54 ± 2.92	2.59 ± 2.84	NS	4.45 ± 3.71	2.80 ± 2.67	0.02
dft/DMFT	4.11 ± 2.93	4.52 ± 2.92	3.69 ± 2.91	NS	5.48 ± 3.35	3.84 ± 2.78	0.01

* Stepwise multiple linear regression model adjusting for gender, OHI and age. NS: not significant.

were checked for growth (carriage) and, when present, colonies were counted and carriage levels recorded as the number of CFU per ml of unstimulated whole saliva. A count of 1000 CFU/ml was used as a cut-off to dichotomise subjects into non- or low-level carriers and high-level carriers.

Based on their colour and as described previously (Odds and Bernaerts, 1994; Pfaller et al, 1996), colonies were identified as *Candida albicans* (light green), *Candida tropicalis* (metallic blue with or without a purple halo), *Candida krusei* (pink, rough, spreading colonies with pale edges), *Candida glabrata* (dark pink colonies with pale egdes) or other species (white or gray). Colony counts for each species were then obtained.

Statistical analysis

Demographic and clinical data were described as means ± SD or proportions as appropriate. Microbiological data were described in terms of carriage status (carriers and non-carriers), species distribution, total and species-specific salivary counts (CFU/ml) and dichotomous carriage level status (non- or low-level carriers and high-level carriers). Association of the microbiological variables with caries status and, separately, caries scores was assessed using multiple logistic regression and multiple linear regression, respectively. Age, gender and oral hygiene status were included in each model as covariates. Odds ratios and/or correlation coefficients (r) were obtained as appropriate. Two-step clustering was used to cluster carriers based on carriage patterns. Differences with a *P*-value of ≤ 0.05 were considered significant. Statistical analyses were performed using SPSS and LogXact software (Cytel Corporation; San Diego, CA, USA).

RESULTS

Oral hygiene and caries experience

The mean age of the girls and boys was 9.83 ± 1.48 and 9.70 ± 1.53 years, respectively; the difference was statistically not significant. The oral hygiene status was good in 83.9%, fair in 16.1% and poor in 0% of the study population. The overall mean oral hygiene score was 2.84 ± 0.37. Differences in oral hygiene by age and gender were not statistically significant.

Twenty-three (12.8%) of the children were caries-free; this did not vary significantly by age, gender or oral hygiene status. The caries scores are presented in Table 1. The D and d components accounted for 98% and 96% of the DMFT and dft scores, respectively, while the F and f components represented only 2% and 4%, respectively. There were no missing permanent teeth in any of the examined subjects. As expected, age was inversely associated with dft, while proportionally associated with DMFT scores ($r = -0.28$ and 0.35, respectively; $P < 0.0001$). Being a female and having good oral hygiene were significantly associated with lower d scores (r = - 0.17 and -0.20, respectively; Table 1).

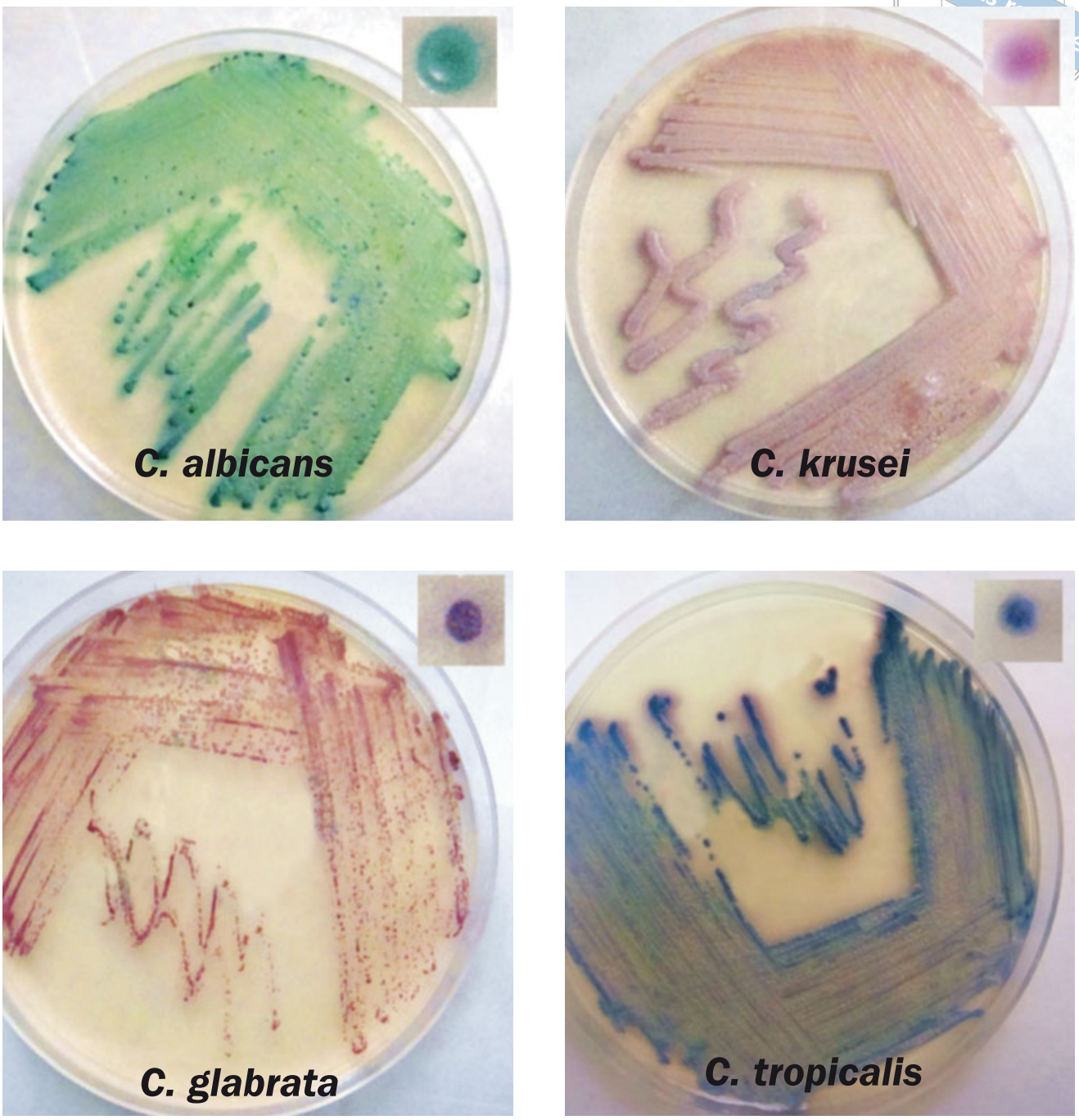

Fig 1 Clinical isolates of four *Candida* species in pure form on CHROMagar Candida.

Candida species carriage patterns

Candida was detected in 108 children (60%) with 160 species isolates recovered. The mean carriage count was 923 ± 1875 CFU/ml unstimulated saliva. Dichotomising by the 1000 CFU/ml carriage count cut-off, 156 subjects (86.7%) were non- or low-level carriers and 24 (13.3%) were high-level carriers. All sought species were detected based on the expected colony morphologies (Fig 1). Among the carriers, 67 subjects (62%) harboured only one *Candida* species, 30 subjects (27.8%) carried two species, 10 subjects (9.3%) had three species and one subject (0.9%) harboured four species (Fig 2).

The detection rates, distribution by dominance and mean counts of each species are presented in Table 2. *C. albicans* was the most frequently detected species (in 89.8% of the carriers), accounting

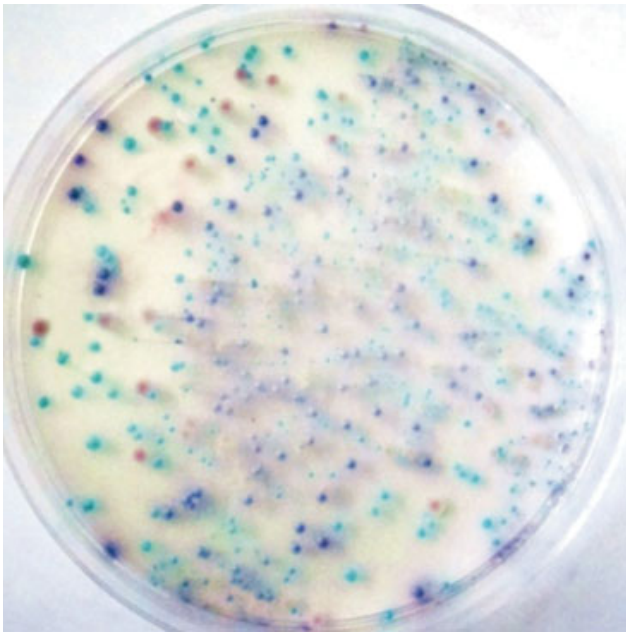

Fig 2 Four *Candida* species harboured simultaneously by one of the study subjects.

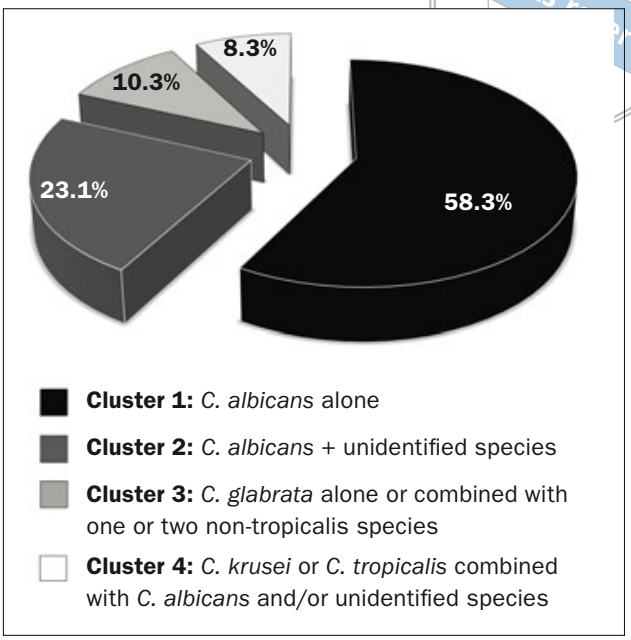

Fig 3 Pie chart showing the four carrier clusters based on species co-carriage patterns.

Table 2 Species detection frequency, distribution by dominance and mean counts among carriers (n = 108)

Species	Frequency No. (%)	Distribution by dominance No. (%)				CFU/ml (mean ± SD)
		First	Second	Third	Fourth	
C. albicans	97 (89.8)	92 (85.2)	03 (02.8)	2 (1.9)	0 (0.0)	951 ± 1927
C. glabrata	10 (9.3)	04 (03.7)	03 (02.8)	3 (2.8)	0 (0.0)	67 ± 68
C. tropicalis	05 (04.6)	02 (01.8)	03 (02.8)	0 (0.0)	0 (0.0)	22 ± 16
C. krusei	11 (10.2)	04 (03.7)	05 (04.6)	2 (1.9)	0 (0.0)	103 ± 138
Others	37 (34.2)	06 (05.6)	26 (24.1)	4 (3.7)	1 (0.9)	143 ± 201

for the majority (60.6 %) of the isolates; it was also the only species to be detected above 1000 CFU/ml, representing the predominant species in about 85% of the carriers. *C. krusei, C. tropicalis* and *C. glabrata* were detected in 10.2%, 4.5% and 9.3% of the carriers and accounted for 6.9%, 3.1% and 6.3% of the isolates, respectively. They were present at much lower counts, but still represented the predominant species in about 9% of the carriers. Unidentified species (others) were recovered from 34.2% of the carriers, accounting for 23.1% of the isolates and were present as the most predominant species in 5.6% of the carriers and the second most dominant ones in 24%.

Gender and oral hygiene status did not correlate with carriage patterns. Age, on the other hand, appeared as a determinant of *C. tropicalis* and *C. glabrata* carriage; the former was exclusively detected in subjects ≥ 11 years old, while the latter appeared only in subjects ≤ 10 years old.

Carrier clustering by carriage patterns

Two-step clustering by carriage data revealed the presence of four age-dependent clusters of carriers (Fig 3). The largest cluster (58.3%) with a mean age of 9.9 years included carriers of *C. albicans*

Table 3 Caries scores (mean ± standard deviation) by carriage status				
	Non-carriers (n = 72)	Carriers (n = 108)	r*	*P**
DT	0.88 ± 1.24	1.12 ± 1.59	-	NS
MT	0.00 ± 0.00	0.00 ± 0.00	-	NS
FT	0.00 ± 0.00	0.03 ± 0.17	-	NS
DMFT	0.88 ± 1.24	1.15 ± 1.62	-	NS
dt	2.24 ± 2.24	3.43 ± 3.18	0.17	0.016
ft	0.70 ± 0.26	0.15 ± 0.64	-	NS
dft	2.31 ± 2.30	3.57 ± 3.16	0.20	0.007
DMFT/dft	3.18 ± 2.46	4.72 ± 3.07	0.24	0.001

* Stepwise multiple linear regression model adjusting for gender, OHI and age. r: correlation coefficient; NS: not significant.

Table 4 Caries scores (mean ± standard deviation) by dichotomous carriage level categories (cut-off at 1000 CFU/ml)				
	Non- or low-level carriers (n = 156)	High-level carriers (n = 24)	r*	*P**
DT	0.94 ± 1.42	1.54 ± 1.62	0.153	0.029
MT	0.00 ± 0.00	0.00 ± 0.00	-	NS
FT	0.00 ± 0.00	0.13 ± 0.34	0.33	0.001
DMFT	0.94 ± 1.42	1.67 ± 1.71	0.18	00.01
dt	2.72 ± 2.46	4.46 ± 4.67	0.22	0.002
ft	0.10 ± 0.45	0.25 ± 0.85	-	NS
dft	2.81 ± 2.49	4.71 ± 4.54	0.23	0.001
DMFT/dft	3.76 ± 2.61	6.38 ± 3.87	0.31	0.0001

* Stepwise multiple linear regression model adjusting for gender, OHI and age. r: correlation coefficient; NS: not significant.

alone. Carriers of both *C. albicans* and unidentified species (others), with a mean age of 9.2 years, formed the second cluster (23.1%). The third cluster (10.2%) consisted of a group of younger carriers (mean age of 8.6 years) who harboured *C. glabrata* alone or combined with one or two of the non-tropicalis species. A group of older children (mean age 11 years) who carried *C. tropicalis* or *C. krusei* along with *C. albicans* and/or unidentified species constituted the fourth cluster (8.3%).

Dental caries in relation to candida carriage

The carriage status (carriers vs non-carriers) significantly correlated with the d scores (Table 3), while the carriage counts showed significant association with both the d ($r = 0.15$; $P = 0.042$) and f ($r = 0.17$; $P = 0.023$) scores as well as the F component scores ($r = 0.2$; $P = 0.009$). Adjusted for other factors, neither the presence nor the mean counts of salivary *Candida* correlated with caries status (caries-free/caries-active). However, carriage at counts ≥ 1000 CFU/ml of unstimulated saliva was significantly associated with being caries-active (odds ratio = 6.9; $P = 0.03$). It also strongly correlated with both primary and permanent dentition caries scores (Table 4).

Interestingly, the carriers in cluster three (the glabrata cluster) showed significantly much lower d and dft scores than those of the carriers in the other clusters even after adjustment for carriage

levels. The mean d and dft scores (1.91 ± 3.60 and 1.91 ± 3.76, respectively) were even lower than those of the non-carriers, although the differences were not significant. This cluster also tended to have more caries-free subjects (28%) than other carrier clusters (7%) and non-carriers (18%), but the differences were marginally significant after adjustment for other factors.

DISCUSSION

Information about oral *Candida* carriage from different geographical locations is required to improve our understanding of the global distribution of this genus among humans (Xu and Mitchell, 2003). This seems particularly important, as the interest in *Candida* as a cariogenic micro-organism is reviving (Raja et al, 2010; Klinke et al, 2011). To the best of our knowledge, this is probably the first report about salivary carriage patterns of *Candida* species and their relation to caries experience among children from Arabia.

Various sampling techniques for assessment of oral *Candida* carriage or infection are described in the literature including smears, swabs, imprints, paperpoints, concentrated oral rinse (COR) and whole saliva (Williams and Lewis, 2000). The latter two are more sensitive and allow accurate quantification of the fungus for correlation and comparison purposes; however, saliva rather than COR was chosen for the current study because it is probably more relevant to caries risk assessment. *Candida* species were detected, quantified and differentiated using CHROMagar Candida. The medium has high specificity (95%–100%) and is recommended for rapid screening of clinical samples (Pfaller et al, 1996; Willinger and Manafi, 1999). However, some species have been shown to be not easily differentiated from the 4 sought species, particularly *C. dubliniensis*, which appears similar to *C. albicans* on the medium (Hospenthal et al, 2006). Since these species are very rare in healthy carriers, it is doubtful that any mistyping could have influenced the results of the current study. Surprisingly, CHROMagar Candida medium has hardly been used in assessment of oral *Candida* carriage.

The prevalence of carriage in this study (60%) is almost identical to carriage rates reported for children of the same age from the UK (Raja et al, 2010), North America (Jabra-Rizk et al, 2007), Brazil (Cortelli et al, 2006) and Israel (Berdicevsky et al, 1984). The highest rate reported so far is 71% for Scottish children (Martin and Wilkinson, 1983). Lower prevalences (27% to 50%) have been found in children from Poland (Rozkiewicz et al, 2006) and Turkey (Kadir et al, 2005), while a rate as low as 7.7% has been found among Chinese children (Sedgley et al, 1997). While methodological variations probably account for some of these differences, geographical variation is also thought to be an important factor (Xu and Mitchell, 2003). The current study shows that children can asymptomatically harbour high counts of *Candida* (in thousands of CFU/ml), which is consistent with findings from some previous reports (Coulter et al, 1993; Signoretto et al, 2009; Raja et al, 2010) but contradicts another study (Epstein et al, 1980) which showed that a cut-off of 400 CFU per ml unstimulated saliva can be used to differentiate carriers from infected patients.

C. albicans accounted for 60.6% of all the isolates in this study, which is lower than the proportions reported from Brazil (Moreira et al, 2001), Turkey (Kadir et al, 2005) and North America (Jabra-Rizk et al, 2007), while it was considerably higher than those found among the Chinese, for whom *C. albicans* was shown to represent only 9.4% of all isolates (Xu and Mitchell, 2003). In the current study, *C. albicans* was also found to account for 100% of carriage at > 1000 CFU/ml. Kleinegger et al (1996) found comparable findings using a cut-off of 500 CFU in mucosal swabs. This is the first study to report mean counts for NAC species. The lowest counts were found for *C. tropicalis* followed by *C. glabrata* and *C. krusei*. Nevertheless, the NAC species were still detected as the most predominant species in some of the carriers. Distribution of NAC species varies considerably among the studies. Notably, however, while *C. glabrata* has not been detected in several previous reports (Kleinegger et al, 1996; Moalic et al, 2001; Kadir et al, 2005; Raja et al, 2010), it was detected in 9.3% of the carriers in this study.

Thirty-eight percent of the carriers harboured two or more species, which is far higher than the range (2%–5%) reported earlier (Kleinegger et al, 1996; Moreira et al, 2001). In fact, this is also the first time co-carriage of more than 2 species has been reported (about 10%). Because of these high co-carriage rates, it was possible to perform cluster analysis, which identified 4 clusters of carriers according to carriage patterns, another novel finding in this study. One limitation here, however, is that it was not possible to identify a considerable fraction (23%) of the isolates with CHROMagar,

which may have compromised the accuracy of clustering. Nevertheless, since most of the unidentified species isolates were co-carried with *C. albicans* (carrier cluster 2), it seems appropriate to assume that they represented one species.

The current study substantiates previous evidence of the association between *Candida* carriage and caries. Being a cross-sectional study, it actually provides a lower level of evidence compared to previous case-control (Signoretto et al, 2009; Raja et al, 2010) or longitudinal risk-assessment studies (Russell et al, 1991; Raitio et al, 1996; Ollila and Larmas, 2008); however, it does present new information with potential clinical and research implications. First, it shows that carriage of *Candida* at counts >1000 CFU/ml of unstimulated saliva was associated with higher caries scores in both dentitions as well as with caries status (caries-active vs caries-free). In line with this, Raja et al (2010) and Signoretto et al (2009) reported mean counts of > 1000 CFU/ml in their caries-active groups. This cut-off could therefore be used for defining caries risk, but it needs to be assessed in further studies. Second, it demonstrates that the carrier cluster carrying *C. glabrata* had much lower caries scores and tended to include more caries-free subjects. This is an interesting finding that also needs to be explored further.

CONCLUSION

To sum up, the current study substantiates evidence on the association between *Candida* carriage and caries, particularly in primary dentition. The clinical implication of this is that salivary *Candida* levels may be used to assess caries risk in children and that controlling them, for example by use of antifungal agents or perhaps vaccination, may represent a strategy for prevention of caries. Thus, these are strongly recommended as lines of future research.

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