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ORIGINAL ARTICLE



# Type 2 diabetes mellitus and oral *Candida* colonization: Analysis of risk factors in a Sri Lankan cohort

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**ABSTRACT**

**Aims:** Oral candidiasis is a major oral manifestation of uncontrolled diabetes mellitus, and a number of cofactors are associated with the pathogenesis of this infection. Here, we describe the prevalence of oral *Candida* in a Sri Lankan cohort of type 2 diabetes mellitus and risk factors that predispose them to this common fungal infection.

**Methods:** A case–control study was conducted in 250 diabetics with type 2 diabetes and 81 nondiabetic controls. Clinical and demographic data were collected using an interviewer administered questionnaire, and patient records. Oral rinse samples were collected to determine the candidal carriage, and the resultant yeast growth was quantified and speciated using multiplex-PCR and phenotypic analyses. Chi-square test ($\chi^2$ test) and Fisher exact test were used for the determination of the significant relationships between risk factors and oral candidiasis.

**Results:** The oral prevalence of *Candida* species among both groups was similar (81%) although a significantly higher proportion of diabetics (32.8%) yielded >2000 CFU/mL of yeasts compared with only 12.3% of the healthy controls ($p < .05$). Significant associations were noted between oral candidal carriage amongst diabetics, and (i) denture wearing, (ii) female gender and (iii) cigarette smoking (all, $p < .05$). Amongst both groups, *C.albicans* was the most common *Candida* species isolated followed by *C. parapsilosis*, *C. tropicalis* and *C. glabrata*.

**Conclusions:** The oral infestation of *Candida* in our Sri Lankan cohort of diabetics is significantly higher than their healthy counterparts, and co-carriage of multiple yeast species is a common finding in the study population. As there are no previous such reports of the latter phenomenon particularly from the Asian region it is noteworthy, mainly in view of the recent data on the emergence of drug-resistant yeast species the world over.

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Oral Candida; Diabetes; Sri Lanka; multiplex PCR

## Introduction

According to the International Diabetes Federation, diabetes mellitus (DM) is one of the commonest globally prevalent endocrine disorders, and one of the four priority noncommunicable diseases (NCDs) [1,2]. It is also the commonest NCD in South East Asia region. One in five individuals in Sri Lanka are either diabetic or exhibit impaired glucose tolerance [3]. Uncontrolled diabetes predisposes to a variety of superficial and systemic infections [4,5] and oral candidal infections are a common affliction in these individuals [6–8]. Additionally, heavy oral candidal colonization in diabetics with type 2 DM has been associated with oral thrush, periodontitis and dental carries [4,9,10]. Apart from diabetes, there are many other factors which predispose individuals to oral candidal colonization including denture wearing, smoking, xerostomia, use of steroids and broad spectrum antibiotics [11].

The predominant fungal species of the oral mycobiome is *C. albicans* both in health and disease followed by *Candida tropicalis*, *Candida parapsilosis* and other less common species [12–14]. Oral carriage of *C. albicans* in healthy individuals ranges from 1.9 to 62.3% with a mean of 50% [15] although in the Asian region the mean carriage rate is 12.5% [16].

To our knowledge, little if any data are available on the prevalence of either oral *Candida* infections or infestations in diabetics in Sri Lanka. Hence, we evaluated the oral carriage of candidal species in a Sri Lankan cohort of diabetics including their fungal spectrum. The opportunity was also taken to compare the associated risk factors such as their dental profile, the smoking habits and betel nut chewing on the oral prevalence and distribution of *Candida* species.

## Materials and methods

### Study design and study setting

A case control study was carried out over a period of 3 years (2014–2017) at the Endocrinology Clinic of the Colombo South Teaching Hospital (CSTH) and at the Department of

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Microbiology, University of Sri Jayewardenepura, Sri Lanka. Ethical approval for this study was obtained from the Ethics Review Committee of University of Sri Jayewardenepura, Sri Lanka (Ref. No: 764/13). Further, the patient consent was also sought and obtained prior to the sample, and data collection.

### Study population

A total of 331 individuals comprising of 250 patients (108 male and 142 female) and 81 age and sex matched nondiabetic healthy controls (47 male and 34 female), were enrolled in the study. Sample size was calculated based on prevalence data from current literature [8,17]. Male and female patients with DM (fasting blood sugar >126 mg/dL or 2-h post-prandial blood sugar >200 mg/dL or glycated hemoglobin A 1C >6.5%) (ADA guideline 2015) [18] attending the diabetes clinic at CSTH, over 18 years of age were included in the study.

Age and sex-matched controls were recruited after recruiting the patients. Upon receiving the consent to participate for the study, a fasting blood sugar test was performed. A cut off value of <100 mg/dL (ADA guideline 2015) [18] was considered as nondiabetes controls. Patients and individuals who were mentally unstable, less than 18 years of age and who did not consent to participate in the study were excluded.

### Patient data collection

An interviewer administered, pretested validated questionnaire was used to obtain data related to host factors in both the test and the control group. These were also supplemented by referral to the patient clinical case notes. In particular, the diabetes patients (and the control group) were questioned verbally on whether they perceived the following five common oral symptoms that could be possibly related to DM: alterations in taste (dysgeusia), burning sensation of the mouth, dry mouth (xerostomia), gum abscess and halitosis.

### Specimen collection

The oral carriage of *Candida* was evaluated using the method of Samaranayake et al. [7]. Diabetics and the control group were given 10 mL of sterile phosphate-buffered saline (PBS) in universal containers and advised to rinse their mouth for 60 s, and then expectorate into the same container provided. The specimens were immediately transported to the laboratory, and then centrifuged at 6000 rpm (3300 g) for 10 min and the resultant pellet was resuspended in 1 mL PBS to obtain the concentrated oral rinse specimen which was subsequently used for culture, and DNA extraction.

### Phenotype determination

For phenotype determination, the concentrated oral rinse specimen (100 μL) was spread on Sabourauds dextrose agar (SDA) plates containing chloramphenicol (0.05 g/L) in triplicates and incubated at 37 °C for 48 h for semi quantitative analysis. *Candida* CFU was quantified by a single investigator (M.K.A.S), and sub-cultured to obtain a pure growth. The identity of the isolates was determined by colony morphology, germ tube test and carbohydrate fermentation and assimilation tests as described previously [19]. A colony count >2000 CFU/mL in the concentrated oral rinse samples was considered as the risk threshold for *Candida* infections in this study [20,21].

### DNA extraction

A 24-h old *Candida* colony on SDA was picked, and suspended in 100 μL STES buffer [200 mM Tris HCl (PH 7.6), 100 mM EDTA, 0.1% SDS] and the suspension was used for nucleic acid extraction using the bead beater extraction method. A 40 μL of TE buffer [10 mM Tris HCl (pH 8), 1 mM EDTA] and 120 μL Phenol: Chloroform mixture (1:1 V/V) was added to each suspension (culture isolate or concentrated oral rinse sample). This suspension was then decanted into a sterile bead beater tube containing 0.3 g sterile zirconium beads (0.1 mm diameter; BioSpec-Products). The sample was homogenized using a mini bead beater (model 3110BX; BioSpec Products) at 480 rpm for 5 min. The upper aqueous phase (100 μL) was transferred to a sterile micro centrifuge tube, and DNA was precipitated in the presence of 220 μL cold ethanol (100%) (VWR Chemicals, France) and 10 μL of 3 M sodium acetate (Sigma) at −20 °C for 18 h. The solution was then centrifuged at 13,000 rpm (15,493 g) for 12 min and the DNA pellet was air dried and dissolved in 30 μL TE buffer [14,22]. The extracted DNA samples were stored at −20 °C prior to analysis.

### Multiplex PCR

Extracted DNA from concentrated oral rinse samples were subjected to multiplex PCR to identify *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. glabrata*. Multiplex PCR was carried out in 25 μL volume with 2 μL template DNA, 1x green GoTaq Flexi buffer (pH 8.5), 3 mM $MgCl_2$, 0.2 μM of each primer (*ITS 4*, *CA*, *CT*, *CP*, *CGL*), 0.2 mM deoxy nucleotide triphosphate (dNTP) mix (Promega) and 1.25 unit of Taq DNA polymerase (Promega). ITS 1 and ITS 2 regions of the yeast genome was amplified as in our previous studies using *ITS 4*, *CA*, *CT*, *CP*, *CGL* primers [14,23].

PCR amplification was initiated at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min and a final elongation step at 72 °C for 5 min with a final hold at 4 °C. All PCR experiments included a negative (no template) control and a positive control. Resulting PCR products were separated by electrophoresis using 1× TAE (40 mM Tris HCl (pH 8), 20 mM acetic acid, 1 mM EDTA) on a 3% (w/v) agarose gel, stained with ethidium bromide

Table 1. Self perceived oral symptoms reported by the study population[a].

| | Diabetics | | Healthy controls | | |
|---|---|---|---|---|---|
| Perceived oral disorder | *N* | % | *N* | % | *p* Value† |
| Taste alterations (dysgeusia) | 30 | 12 | 0 | 0 | **.001** |
| Burning sensation | 6 | 2.4 | 1 | 1.2 | .526 |
| Dry mouth (xerostomia) | 43 | 17.2 | 4 | 4.9 | **.006** |
| Gum abscess | 28 | 11.2 | 5 | 6.2 | – |
| Halitosis | 14 | 5.6 | 1 | 1.2 | – |

[a]The results are based on the verbal data collected using the interviewer administered questionnaire, as perceived by the patient.
†Chi-square test ($\chi^2$ test) were carried out to assess possible association.
Bold values indicate *p* values that are lower than the 0.05 level of significance.

and viewed by UV trans-illuminator (VilberLourmat, QUANTUM ST5).

### *Comparison of* candida *identification methods*

The two different yeast identification methods, i.e. multiplex PCR, and the culture based, phenotypic identification were compared. Efficacy of the identification methods was analyzed by calculating sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the tests, taking the PCR identification method as the bench mark.

### *Statistics*

Statistical analysis was carried out using 17.0 version of SPSS software. The chi-square test ($X^2$ test) and Fisher's exact test were carried out to assess possible association. All these tests were two-sided. The significance level was specified as $p < .05$.

## Results

### *Demographic data and risk factors for yeast colonization*

A total population of 331 subjects consisting of 250 patients with DM and 81 non-diabetes control group were included in this study. Patients with diabetes were age between 33 and 85 years with a mean age of 60 years. The age of the nondiabetes control group ranged from 34 to 88 years with a mean age of 54 years. Of the test cohort of 250 patients with diabetes, 102 (40.8%) were suffering from the disease over a period of 5–15 years, while 67 (26.8%) had the disease for more than 15 years, and approximately a third (32.4%) had less than a 5-year history of diabetes.

Fasting blood sugar (FBS) levels of diabetic patients ranged between 63 and 371 mg/dL with an average value of 139 mg/dL. Out of 250 patients, 121 (48.4%) had a FBS levels of more than 126 mg/dL. Of them 58 (47.9%) had a *Candida* colony count >600 CFU/mL while 42 (34.7%) had a colony count >2000 CFU/mL.

Mean post prandial blood sugar (PPBS) level was 166 mg/dL having a range of 70–432 mg/dL. Seventy seven patients had a PPBS levels >200 mg/dL. Of them 42 (54.5%) had a *Candida* colony count >600 CFU/mL while 34 (44.1%) had a colony count >2000 CFU/mL.

When considering risk factors for *Candida* colonization, 31/250 (12.4%) diabetics and 13/81 (16%) control subjects were smokers, while 39/250 (15.6%) diabetics and 19/81 (23.5%) controls were betel nut chewers; 6/250 (2.4%) diabetics claimed to have both habits. Overall, 72/250 (28.8%) diabetics wore either full or partial dentures compared to only 4/81 (4.9%) in the control group.

Further 71/250 (28.4%) diabetics did not brush their teeth twice a day compared with17/81 (21%) in the control group, while an equal proportion of diabetics 15/250 (6%) and controls 4/81 (6%) claimed they used a mouth wash for routine oral hygiene.

The five different oral symptoms of the diabetics and the control group as reported in the interviews and obtained from patient records are given in Table 1. A significantly higher number of diabetics complained of dysgeusia and xerostomia than the controls (both, $p < .05$). Collectively, a higher number of symptomatic oral conditions were reported by the diabetics compared with the control group (Table 1); 26/250 (10.4%) diabetics claimed to suffer from more than one oral complication.

### Prevalence of oral *Candida* species

The oral prevalence of *Candida* species amongst both groups was similar (~81%) although a significantly higher proportion of diabetics (32.8%) yielded >2000 CFU/mL of oral rinse compared with only 12.3% of the healthy controls ($p < .05$) (Table 2).

Furthermore, of the 250 diabetics, almost a third (32.8%) yielded >2000 CFU/mL and approximately one half (47.6%) yielded >600 CFU/mL when the oral rinses were cultured, in comparison to one-eighth (12.3%) and a quarter (27.2%) of the controls, respectively ($p < .05$) implying the significantly heavy oral yeast infestation extant in the diabetic cohort.

### Candida *speciation: phenotypic and molecular (PCR) analyses*

On qualitative analysis of the isolates by the phenotypic method, we noted that the predominant *Candida* species in both the diabetic and the control groups was *C albicans* with 66.7 and 53.4% prevalence, respectively (Figure 1). *C. parapsilosis* was the second most common (26.3%) species in both the diabetics (22.8%) and the controls (37%). Approximately one-third (33–35%) of the species isolated were neither *C. albicans* nor *C. parapsilosis* in both groups, and comprised *C. tropicalis, C. glabrata* and *C. krusei* with a few unidentifiable isolates.

Multiplex PCR was carried out from the DNA extracted from the concentrated oral rinse specimens of diabetes and the control groups. 223/250 (89.2%) diabetics were positive for *Candida* species by multiplex PCR. Of them, 137/250 (54.8%) diabetics had multiple *Candida* species when identified by multiplex PCR compared to the 88/250 (35.2%) as identified using phenotypic methods (Table 3). Both

**Table 2.** Oral *Candida* population density amongst diabetics and controls: comparison of culture and multiplex PCR.

| | Diabetics ($n = 250$) | Nondiabetic controls ($n = 81$) | $p$ Value[†] |
|---|---|---|---|
| *Candida* positive by culture | 204 (81.6%) | 66 (81.5%) | .981 |
| *Candida* levels > 600 CFU/mL | 119 (47.6) | 22 (27.2%) | **.001** |
| *Candida* levels > 2000 CFU/mL | 82 (32.8%) | 10 (12.3%) | **.000** |
| *Candida* positive by multiplex PCR | 229 (89.2%) | 66 (81.5%) | .070 |

[†]Chi-square test ($\chi^2$ test) were carried out to assess possible association.
Bold values indicate $p$ values that are lower than the 0.05 level of significance.

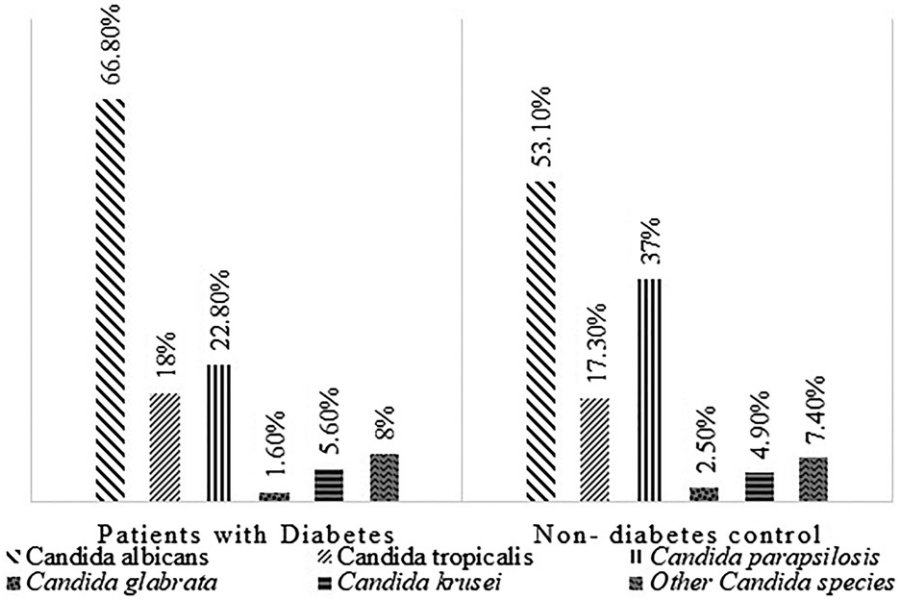


**Figure 1.** Oral *Candida* species isolated from the study population.

**Table 3.** Comparison of phenotypic and multiplex PCR identification of *Candida* species among the diabetics ($n = 250$).

| Results | Phenotypic method | Multiplex PCR |
|---|---|---|
| Positivity | 204 (81.6%) | 223 (89.2%) |
| Multiple species | 88 (35.2%) | 137 (54.8%) |
| *Candida albicans* | 167 (66.7%) | 198 (79.2%) |
| *Candida tropicalis* | 45 (18%) | 79 (31.6%) |
| *Candida parapsilosis* | 57 (22.8%) | 89 (35.6%) |
| *Candida glabrata* | 4 (1.6%) | 14 (5.6%) |
| *Candida krusei* | 14 (5.6%) | – |
| Other yeast species | 20 (8%) | – |

phenotypic and multiplex PCR methods reported the predominance of *C. albicans* followed by *C. parapsilosis*, *C.tropicalis* and *C. glabrata*, respectively, as depicted in Table 4.

Oral carriage of multiple species of *Candida* was common (Table 4) with 54.8% of the cohort carrying either two or three species simultaneously. The most common co-habitants were *C. albicans* and *C. parapsilosis*, and *C. albicans* and *C. glabrata*. A very small percentage of the cohort (5%) carried three *Candida* species simultaneously.

Further, using the multiplex PCR as the gold standard, the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of *Candida* species identification using phenotypic methods was determined (Table 5). Phenotypic identification method of *C. albicans* had a high sensitivity (>80%), specificity (>80%) and positive predictive value (>90%) albeit a low negative predictive value (<60%). In contrast, the sensitivity of the phenotypic identification methods of non-albicans *Candida* species was relatively low with a <40% positive predictive value; although specificity was high with <70% (Table 5).

### *Association of oral* Candida *species with risk factors*

The relationship between CFU/mL of oral *Candida* with the host risk factors including their age, gender, smoking habits, betel nut chewing, denture wearing and brushing frequency of teeth was assessed (Table 6).

In general, female diabetics were more likely to be positive for *Candida* compared to their male counterparts.

There was a significant correlation between denture wearing and culture positivity with *Candida* counts ranging from >600 to >2000 CFU/mL ($p < .001$; Table 6).

Cigarette smoking was significantly associated with oral *Candida* carriage ($p = .009$). Out of 31 smokers, 20 were positive for *Candida* species by culture and 25 were positive by multiplex PCR (Table 5). Meanwhile, 12 out of 13 smoking nondiabetes subjects were positive for oral *Candida* species by culture.

A total of 33 of 39 betel nut chewing, diabetics were positive for *Candida* on oral rinse culture,(of which 12 had a colony count >2000 CFU/mL) as opposed to 17 of 19 betel nutnut chewing non *diabetic* controls ($p < .05$).

A total of 56 diabetics (78.9%) claimed to brush their teeth only once a day and were positive for oral *Candida* species by culture. Of them, 28 had a *Candida* colony counts more than 600 CFU/mL (Table 5).

**Table 4.** Single and multiple species oral carriage of *Candida* in the diabetic and the control population (analyzed by both the phenotypic and multiplex PCR methods).

| | Diabetic (*n*) | | Control (*n*) | |
|---|---|---|---|---|
| *Candida* species | Phenotypic analysis | Multiplex PCR analysis | Phenotypic analysis | Genotype (multiplex PCR) |
| Single species | | | | |
| *C. albicans* | 93 | 64 | 25 | 18 |
| *C. parapsilosis* | 14 | 11 | 10 | 3 |
| *C. tropicalis* | 6 | 10 | 2 | 1 |
| *C. glabrata* | 1 | 1 | 0 | 0 |
| *C. krusei* | 0 | – | 1 | – |
| Other | 2 | – | 1 | – |
| Sub total | 116 | 86 | 39 | 22 |
| Multiple species | | | | |
| *C. albicans* + *C. parapsilosis* | 30 | 61 | 10 | 28 |
| *C. albicans* + *C. tropicalis* | 20 | 49 | 4 | 6 |
| *C. albicans* + *C. glabrata* | 3 | 4 | 0 | 0 |
| *C. tropicalis* + *C. parapsilosis* | 6 | 3 | 3 | 0 |
| *C. tropicalis* + *C. glabrata* | 0 | 1 | 1 | 0 |
| *C. albicans* + other | 5 | – | 1 | – |
| *C. albicans* + *C. krusei* | 4 | – | 0 | – |
| *C. parapsilosis* + other | 2 | – | 2 | – |
| *C. tropicalis* + other | 3 | – | 0 | – |
| *C. krusei* + other | 1 | – | 0 | |
| *C. albicans* + *C. tropicalis* + *C. parapsilosis* | 4 | 10 | 1 | 7 |
| *C. albicans* + *C. parapsilosis* + *C. glabrata* | 0 | 3 | 1 | 2 |
| *C. albicans* + *C. tropicalis* + *C. krusei* | 2 | – | 1 | – |
| *C. albicans* + *C. tropicalis* + *C. glabrata* | – | 6 | – | 1 |
| *C. albicans* + *C. tropicalis* + Other | 1 | – | 0 | – |
| *C. albicans* + *C. parapsilosis* + *C. krusei* | 1 | – | 0 | – |
| *C. albicans* + *C. krusei* + other | 3 | – | 0 | – |
| *C. parapsilosis* + *C. krusei* + other | 0 | – | 1 | – |
| *C. tropicalis* + *C. parapsilosis* + *C. krusei* | 0 | – | 1 | – |
| *C. tropicalis* + *C. parapsilosis* + other | 0 | – | 1 | – |
| *C. tropicalis* + *C. krusei* + other | 2 | – | 0 | – |
| *C. albicans* + *C. tropicalis* + *C. krusei* + other | 1 | – | 0 | – |
| Sub total | 88 | 137(54.8%) | 27 | 44(54.3%) |

**Table 5.** Comparison of phenotypic and multiplex PCR identification of *Candida* species (from concentrated oral rinse specimens)[a].

| | Culture identification | | | |
|---|---|---|---|---|
| | Sensitivity | Specificity | PPV | NPV |
| *C. albicans* identification | 79.79% (158/198) | 82.69% (43/52) | 94.61% (158/167) | 51.80% (43/83) |
| *C. tropicalis* identification | 37.97% (30/79) | 91.22% (156/171) | 66.66% (30/45) | 76.09% (156/205) |
| *C. parapsilosis* identification | 37.07% (33/89) | 85.09% (137/161) | 57.89% (33/57) | 70.98% (137/193) |
| *C. glabrata* identification | 14.28% (2/14) | 99.15% (234/236) | 50.0% (2/4) | 95.12% (234/246) |

[a]Multiplex PCR was considered as the bench mark standard (NPV, negative predictive value; PPV, positive predictive value).

High oral *Candida* prevalence was observed among patients who complained of oral symptoms including dry mouth, dysguesia, gum abscesses and halitosis. Out of the total 250 patients, 43 (17.2%) claimed to have dry mouth followed by 30 (12%) dysguesia, 28 (11.2%) gum abscesses and 14 (5.2%) halitosis. Of the 43 diabetics claimed to have dry mouth, 34 (79.2%) were culture positive and 13 (38.2%) had *Candida* colony count of >2000 CFU/mL. Further majority (27/30) of diabetics who complained of dysguesia were positive for *Candida* species. Of them, 14 had *Candida* colony count of >2000 CFU/mL. Out of 28 diabetics with a history of one or more gum abscesses, 24 were positive for *Candida* species. Among them 13/24 (46.4%) had a colony count >2000 CFU/mL. Among the patients (06) who claimed to have burning sensation, 5 (83.3%) were positive for *Candida* species while 3 (50%) had a *Candida* colony count >2000 CFU/mL (Table 7).

Among the non-diabetes control group who claimed to have oral complications *Candida* species were isolated from those having dry mouth 4/4 (100%), gum abscess 3/5 (60%), halitosis 1/1 (100%) and burning sensation 1/1 (100%). Of these 1of 4 controls had dry mouth and 1 of 3 with gum abscess were positive for *Candida* colony counts of >2000 CFU/mL (Table 7).

## Discussion

*Candida* species have a predilection for colonising the human oral cavity, particularly in patients with DM. A recent review spanning the last half century notes that the mean cumulative oral carriage rate of *Candida* in diabetics ranges from 60 to 80% [8], although in the Asian region the mean carriage rate is 12.5% [16]. On the other hand, in healthy individuals the carriage was lower and ranged from 40 to 70% [7].

Interestingly we noted a remarkably high oral carriage rate of *Candida* not only in our test population of diabetics but also in the controls (82–89%), irrespective of the phenotypic or the molecular identification tools used. However, the population density of the yeasts in diabetics (>2000 CFU/mL of the rinse) was significantly higher than that of the controls implying that the oral mucosa in diabetics offer a less hostile ecosystem for oral candidal colonization. The reasons for this could be local factors such as xerostomia, wearing of

**Table 6.** The association between oral *Candida* levels and the studied host risk factors[a].

| | Culture positive | | | Colony count > 600 CFU/mL | | | Colony count > 2000 CFU/mL | | | Multiplex PCR positive | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diabetics | Nondiabetics (controls) | p Value[†] | Diabetics | p Value[†] | Nondiabetics (controls) | p Value[†] | Diabetics | p Value[†] | Nondiabetics (controls) | p Value[†] | Diabetics | p Value[†] | Nondiabetics (controls) | p Value[†] |
| Age | | | | | | | | | | | | | | | |
| <55 years | 63/78 (80.8%) | 38/47 (80.9%) | .819 | 31/78 (39.7%) | .094 | 15/47 (31.9%) | .258 | 24/78 (30.8%) | .645 | 05/47 (10.6%) | .583 | 68/78 (87.2%) | .488 | 36/47 (76.6%) | .183 |
| >55 years | 141/172 (82.0%) | 28/34 (82.4%) | | 88/172 (51.2%) | | 7/34 (20.6%) | | 58/172 (33.7%) | | 05/34 (14.7%) | | 155/172 (90.1%) | | 30/34 (88.2%) | |
| Gender | | | | | | | | | | | | | | | |
| Male | 79/108 (73.1%) | 36/47 (76.6%) | **.003** | 45/108 (41.7%) | .101 | 11/47 (23.4%) | .372 | 35/108 (32.4%) | .908 | 03/47 (6.4%) | .055 | 91/108 (84.3%) | **.028** | 34/47 (72.3%) | **.013** |
| Female | 125/142 (88.0%) | 30/34 (88.2%) | | 74/142 (52.1%) | | 11/34 (32.4%) | | 47/142 (33.1%) | | 07/34 (20.6%) | | 132/142 (93%) | | 32/34 (94.1%) | |
| Denture wearer | 67/72 (94.4%) | 3/4 (75%) | **.003** | 52/72 (72.2%) | **.000** | 2/4 (50%) | – | 42/72 (58.3%) | **.000** | 2/4 (50%) | .019 | 69/72 (95.8%) | **.032** | 3/4 (75%) | .732 |
| Smoking | 20/31 (64.5%) | 12/13 (92.3%) | **.009** | 14/31 (45.2%) | .771 | 5/13 (38.5%) | .317 | 10/31 (32.3%) | .945 | 0/13 (0%) | – | 25/31 (80.6%) | .101 | 9/13 (69.2%) | .215 |
| Betel nut chewing | 33/39 (84.6%) | 17/19 (89.5%) | .597 | 23/39 (58.9%) | .122 | 5/19 (26.3%) | .925 | 12/39 (30.7%) | .769 | 2/19 (10.5%) | – | 38/39 (97.4%) | – | 15/19 (78.9%) | – |
| Brushing < 2/day | 56/71 (78.9%) | 10/17 (58.8%) | .483 | 28/71 (39.4%) | .104 | 1/17 (5.9%) | – | 20/71 (28.2%) | .326 | 0/17 (0%) | – | 62/71 (87.3%) | .547 | 11/17 (64.7%) | .045 |

[a]The results are based on the data collected using the interviewer administered questionnaire.
[†]Chi-square test ($\chi^2$ test) were carried out to assess possible association.
Bold values indicate p values that are lower than the 0.05 level of significance.

dentures and systemic factors including the level of glycaemic control, and the therapeutic regimens used. Some have opined that a sugar rich oral environment in uncontrolled diabetics due to the high salivary glucose levels may also contribute to the persistence of aciduric yeasts in the oral cavity [24]. It is also known that dietary carbohydrates promote, adhesion, biofilm formation and colonization of yeasts in the oral milieu and this may be another contributory factor [25]. As the significantly high level of xerostomia was one of the self-perceived oral symptoms reported by the diabetic population, this indeed may have been a co-factor for the high yeast density in the latter group.

Another crucial reason for the wide disparity in the oral yeast prevalence observed in previous studies [26] is the mycological sampling technique. Over the years, researchers have attempted to assess the density of oral yeast population using a number of sampling methods ranging from imprint culture, swab samples, mixed saliva, mouth wash and oral rinse samples [7]. However, there is consensus that the oral rinse sampling method used in the current study, is the most appropriate and sensitive technique for evaluating overall oral yeast carriage. Future workers should therefore pay heed to the sampling as well as laboratory culture methods, as well as appropriate patient (and control) selection and standardization in order to obtain globally comparable data.

An incidental finding in our diabetic cohort was the significantly high prevalence of dysgeusia or altered taste sensation compared to the control group (Table 1). Dysgeusia is known to be associated with diabetes [27] and is unlikely to be a co-factor impacting on oral yeast carriage.

We determined the identity of isolated *Candida* species using traditional phenotypic methods of carbohydrate fermentation and assimilation as well as a molecular identification method of multiplex PCR [14]. Both methods revealed the predominance of *C. albicans* followed by *C. parapsilosis, C. tropicalis, C. glabrata* and *C. krusei* in both the diabetic and the control groups confirming previous work [13,28–30]. We have previously reported similar findings of oral yeast carriage in a Sri Lankan cohort with angular cheilitis where *C. albicans* was the predominant species followed *by C. tropicalis* and *C. glabrata* [31]. One caveat in the current study, however, was the predominance of *C. parapsilosis* as the second commonest isolated species among the diabetics. However studies by Lynne et al. and Usha et al. showed a high prevalence of *C. parapsilosis* among diabetes patients supporting this finding [28,32].

A noteworthy finding of our study is the relatively common oral carriage of multiple *Candida* species among diabetic (54.8%) as well as healthy controls (54.2%) as depicted in Table 4. The two most common co-habitus were *C. albicans, C. parapsilosis* and *C. albicans, C. glabrata*. A very small percentage of the cohort (5%) carried three *Candida* species simultaneously. Multiple oral carriage of *Candida* species in not uncommon and the largest study thus far form a dental school in Scottish patients with candidiasis has reported a 15% multi-species carriage rate [33]. Studies by Coco et al. and Silva et al. suggest pathogenic synergy of *C. albicans*

Table 7. Association of *Candida* levels in oral cavity with different oral complication[a].

| | Culture positive | | | | Colony count > 600 CFU/mL | | | | Colony count > 2000 CFU/mL | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Condition of mouth | Diabetes | *p* Value[†] | Nondiabetes | *p* Value[†] | Diabetes | *p* Value[†] | Nondiabetes | *p* Value[†] | Diabetes | *p* Value[†] | Nondiabetes | *p* Value[†] |
| Dry mouth | 34 (79.1%) | .638 | 4 (100%) | – | 20 (46.5%) | .875 | 1 (25%) | – | 13 (30.2%) | .694 | 1 (25%) | – |
| Taste alteration | 27 (80.5%) | .206 | – | – | 17 (56.7%) | .289 | – | – | 14 (46.7%) | .085 | – | – |
| Gum abscess | 24 (85.7%) | .551 | 3 (60%) | – | 16 (57.1%) | .283 | 1 (20%) | – | 13 (46.4%) | .103 | 1 (20%) | – |
| Halitosis | 12 (85.7%) | – | 1 (100%) | – | 7 (50%) | .853 | 1 (100%) | – | 4 (28.6%) | – | 0 | – |
| Burning sensation | 5 (83.3%) | – | 1 (100%) | – | 4 (66.7%) | – | 0 | – | 3 (50%) | – | 0 | – |

[a]The results are based on the data collected using the interviewer administered questionnaire. Oral examinations were not performed.
[†]Chi-square test ($\chi^2$ test) were carried out to assess possible association.

and *C. glabrata* [34–36]. The tissue destruction caused by secretion of proteolytic and lipolytic enzymes contribute to invasiveness of hyphae of *C. albicans* which acts as a structural scaffold for the invasion of *C. glabrata* contributing to increased pathogenesis of both species. A feature of mixed species biofilms is the higher recalcitrance to antimicrobial treatment. It is suggested that microorganisms from mixed communities may benefit each other resulting in perpetuation of infection which is difficult to eradicate. Synergistic associations have been reported between *Candida albicans* and bacterial cohabitants such as *Staphylocccus* species and *Streptococus* species which are also common inhabitants of the oral cavity [37,38]. These organisms have been reported to have both positive and negative effects on each other through various quorum sensing signaling systems which can have an impact on the clinical outcome of oral diseases.

In clinical terms, the findings of this study are noteworthy as they may impact on the the protocols guiding the management of oral candidiasis. Clearly, therefore, vigilance by clinical microbiologists for the presence of multi-species candidal infections is necessary, in view of the global emergence of drug resistant *Candida*.

A number of investigators have estimated that the mean *Candida* counts in diabetics with overt oral candidal infections to be between 2–3 $\times$ $10^3$ CFU/mL [20,21]. Hence, in this study, we determined the diabetic sub-set with more than 2000 CFU/mL of oral rinse, who could be at risk for oral candidal infection. Whilst a significant proportion of the diabetics compared with the controls crossed this threshold limit of oral yeasts, none complained of oral yeast infections. Clinical examination for the presence or absence of overt signs of candidal infection, which we were unable to perform, in our cohort should have clarified this.

We evaluated the association between age, gender, denture wearing, smoking, betel nut chewing and tooth brushing in diabetics and the healthy controls using both PCR and culture techniques (Table 7). Of these the gender, denture wearing and smoking appeared to have impacted significantly on oral yeast carriage amongst diabetics compared with the controls. The association between gender and oral *Candida* colonization is rather ill defined with contradictory findings [39,40]. Some have noted that oral mucosal inflammation in females associated with periodontitis may lead to such yeast infestation in females than in males [29]. This is in agreement with the observation of higher risk of *Candida* colonization of females (88%) compared to males (73.1%) in this study. The high prevalence of oral *Candida* carriage associated with denture wearing is consistent with many previous findings. *Candida* species avidly bind and adhere to acrylic surfaces [29], and therefore dentures may act as a reservoir for these organisms. Many have shown that the tissue fitting surface of the denture is colonized by a relatively thick layer of bacterial–fungal biofilm that cannot be easily eradicated [41]. Wearing dentures promotes a microenvironment conducive to the growth of *Candida* under low levels of oxygen, low pH and an anaerobic environment. This may be a result of the enhanced adherence of *Candida* species to the acrylic substrates, in dentures, reduced saliva flow under the surfaces of the denture fittings, improperly fitted dentures or poor oral hygiene [39]. Hence, the current study together with others, imply that diabetics wearing dentures have a higher risk of oral candidal infestation by virtue of their acrylic dentures. The higher number of denture wearers among the diabetic population may contribute to the higher colonization of *Candida* species observed. Clinically, therefore, meticulous denture hygiene should be observed by diabetics wearing dentures to obviate oral candidal infections.

It is generally accepted that tobacco smoking is associated with high oral candidal colonization, as was observed in the current study [11,42]. Arendorf and Walker [43] were the first to report this phenomenon. As cigarette smoke induced mucosal permeability and consequent pathogen adherence are thought to contribute to this phenomenon [44].

Betel nut quid chewing is a popular habit in Southeast Asia. However, no significant association has been reported between oral candidal colonization and betel nut chewing [45–48], as confirmed in the current study. Our data and those of others indicate that the chemical constituents of the betel nut quid may have no overall effect on the oral carriage of *Candida* species.

In conclusion, we have demonstrated that oral infestation of *Candida* in our Sri Lankan cohort of diabetics is significantly higher than their healthy counterparts, possibly increasing their susceptibility to recurrent candidal infections, and in addition, multiple yeast carriage is a common finding in the study population. As there are no previous such reports of the latter phenomenon either from Sri Lanka or

the Asian region it is worthy of further inquiry, particularly due to its clinical importance.

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## Disclosure statement

The authors report no conflict of interest.

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