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Prevalence of oral *Candida* species in leprosy patients from Cambodia and Thailand

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BACKGROUND: Leprosy is a chronic bacterial infection which may lead to significant orofacial morbidity. However, reports on the oral mycotic flora of leprosy patients are rare. The aim of the current study was to explore the oral yeast carriage in two groups of leprosy patients.

METHODS: 40 Cambodian (seven men, 33 women) and 48 Thai (14 men, 34 women) leprosy patients from Leprosy Rehabilitation Centre Khien Kleang, Phnom Penh, Cambodia and McKean Rehabilitation Center, Chiangmai, Thailand were randomly selected and their demographic data and clinical history were recorded. Tongue and palatal swabs of each patient were collected using sterile Fungi-Quick swabs (Hain Diagnostika, Nehren, Germany) and they were cultured aerobically on Sabouraud's dextrose agar and CHROMAgar (CHROMagar, Paris, France). Yeast were identified by germ tube, chlamydospore production, and assimilation tests (API 20C AUX, Bio-Merieux, Marcy l'Etoile, France) and reconfirmed using APILAB Plus system (Bio-Merieux).

RESULTS: Two groups (Cambodian and Thai) had median age of 35 and 64 years. They had been with leprosy for median durations of 17.7 and 38.9 years ($P < 0.05$), respectively. Overall yeast carriage in two cohorts were 80% and 93.75%. *Candida albicans* had highest carriage rate in either group (65.6%, 44.4%). *Candida krusei* and *C. glabrata* existed as second-line colonizers after *C. albicans*. *Candida glabrata* carriage was significantly higher in Thai patients ($P < 0.05$). Multispecies carriage was seen in three Cambodian (9.4%) and five Thai (11.5%) patients.

CONCLUSIONS: This study indicates high oral yeast carriage in leprosy patients. *Candida albicans* remains predominant while *C. krusei* and *C. glabrata* are second-line oral colonizers. Co-inhabitation of multiple yeast species is also noted in these patients' oral mycotic flora.
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Introduction

Leprosy is a chronic disease caused by *Mycobacterium leprae*. Leprosy mainly affects the skin and nerves; progressive and permanent damage to skin, nerves, testicles, limbs, and eyes occur if left untreated. Over the past two decades, the global prevalence has fallen by almost 90% (1). Moreover, the global detection of new cases has fallen by about 35% since 2001. Presently, leprosy remains a public health problem in only nine countries in Africa, Asia, and Latin America (1). In Thailand and Cambodia, the prevalence rates per 10 000 are 0.3 and 0.36, respectively. At the beginning of 2004 the number of newly detected cases was 705 in Thailand and 509 in Cambodia. Since the introduction of multidrug therapy (MDT) in 1985, consisting of a combination of rifampicin, clofazimine, and dapsone the prognosis of leprosy has improved dramatically with about 4 million patients having been cured since 2000.

Leprosy may manifest as indeterminate, tuberculoid, borderline, and lepromatous variants. All of these may be associated with orofacial pathology (2). Most oral lesions are recorded in lepromatous leprosy patients (3). Due to the fact that most leprosy patients have received on time or are still receiving MDT, severe disability due to loss of limbs and destruction of orofacial structures like saddle nose and severe ocular pathology including blindness are less frequently seen (2). While formerly

patients were hospitalized or lived permanently in leprosy rehabilitation centers, in recent years most of leprosy patients have become outpatients.

Studies of the oral mycotic flora in leprosy patients are rare. In a pioneering study, Reichart et al. (4) studied the prevalence of oral *Candida* species in burnt-out leprosy cases of an institutionalized resident population at the McKean Rehabilitation Centre, Chiangmai, Thailand. This study revealed an unexpected high rate of oral carriage of *C. krusei* (36%) compared with a healthy control group. Among the *Candida*-positive patients 16 of 35 (46%) carried *C. krusei* while *C. albicans* was the second most common isolate with 34%. The corresponding figures for the control group were two of 13 (15%) and six of 13 (46%), respectively. Of further interest was that the antifungal resistance of the *C. krusei* isolates from patients indicated that all except one of the isolates were resistant to fluconazole. Further, two isolates were resistant to ketaconazole and all isolates were sensitive to amphotericin B. The high prevalence of *C. krusei* could not be explained.

Therefore, the purpose of current study was to re-examine institutionalized patients from McKean Rehabilitation Centre in Chiangmai, Thailand (Director: Dr Trevor Smith) and to compare these with leprosy patients from Cambodia at Phnom Penh (Leprosy Rehabilitation Centre, Khien Kleang, Director: Dr Khuon Nguon Heng). Of particular interest was whether the high prevalence of *C. krusei* could be confirmed in Thai leprosy patients and whether *C. krusei* was also prevalent in Cambodian leprosy patients.

Materials and methods

Patients at Leprosy Rehabilitation Centre, Khien Kleang were recruited at the outpatient department with only a few being residents for a short period of time (Group 1). Most of the patients at McKean Rehabilitation Centre, Chiangmai were long-term residents (Group 2). Generally, patients were randomly selected regardless their type of leprosy and whether the disease has been arrested by MDT earlier or whether MDT therapy was ongoing. At McKean Rehabilitation Centre, patients were examined by P. A. R., whereas at Leprosy Rehabilitation Centre Khien Kleang, patients were examined by Ch. B. The following parameters were recorded: age, gender, type and duration of leprosy, period of hospitalization, and type of antileprosy therapy. Period since a negative bacillary smear was also recorded. Ability to perform oral hygiene was recorded according to degree of mutilation of fingers and hands as possible, partly possible or impossible. In addition, smoking habits were also recorded. The oral cavities were examined for the presence of obvious clinical signs of oral candidiasis.

For evaluation of candidal carriage, the tongue and palate of each patient were sampled by rigorously swabbing their surfaces with a sterile Fungi-Quick swab (Hain Diagnostika). Afterwards, the swab was reinserted into alginate transport medium and kept at room temperature for immediate transport to the Oral Biosciences Laboratory at the Prince Philip Dental Hospital, Hong Kong. The swabs were then cultured aerobically on Sabouraud's dextrose agar at 37°C for 7 days. The cultures were inspected on a daily basis, discrete colonies selected, subcultured for purity, and stored in glycerol at −70°C until species identification. The yeast were identified by the germ tube test, growth at 45°C, chlamydospore production, and API 20C AUX (Bio-Merieux) assimilation tests; and the phenotype was further defined by using CHROMAgar *Candida* Plates (CHROMagar). Their identities were reconfirmed with the new improved APILAB Plus system (Bio-Merieux) to exclude *C. dubliniensis*. Carriage was defined as the presence of yeast on inoculated plates; attempts were not made to quantify the oral yeast load per individual.

All data were recorded in SPSS 11.0. Descriptive statistic analysis was performed by cross-table analysis and Fisher's exact test. In bivariate analyses, the Mann–Whitney *U*-test was used to determine significant differences between categorized variables. A *P*-value of < 0.05 was considered statistically significant.

Results

In Group 1, a total of 40 patients (seven men, 33 women) with a median age of 35 years were examined. Group 2 consisted of 48 patients (14 men, 34 women) with a median age of 64 years. Demographic data of Group 1 and Group 2 were significantly different ($P < 0.05$). Table 1 shows details of oral hygiene conditions and smoking habits of the experimental groups. Table 2 shows details on the type of leprosy and medication. Group 1 had a median duration of leprosy of 17.7 years, whereas Group 2 had a median duration of leprosy of 38.9 years ($P < 0.05$). Both groups except one patient in Group 1 had undergone former MDT.

Table 3 shows the oral yeast carriage in Group 1 and Group 2. In Group 1, only 32 patients (80%) harbored yeast species compared with 45 (93.75%) in Group 2. The commonest yeast species in either group was *C. albicans* (Group 1: 65.6%; Group 2: 44.4%). Other common yeast species in Group 1 was *C. krusei* (15.6%) and *C. tropicalis* (9.4%) while in Group 2 the second-line colonizers were *C. glabrata* (24.4%) and *C. krusei* (17.8%).

There was no significant difference with regard to overall yeast carriage in two groups. There was,

Table 1 Demographic data of patients (Group 1 and Group 2)

Variable	*Group 1 (n = 40)*	*Group 2 (n = 48)*
Male	7 (17.5%)	14 (29.2%)
Female	33 (82.5%)	34 (70.8%)
Age	Median 35 (14–67)	Median 64 (20–80)
Inpatient since	Median 2 months (3 days–1 year)	Median 23 years (9 months–60 years)
Non-smoker	12 (30%)	4 (8.3%)
Smoker	28 (70%)	44 (91.7%)
Oral hygiene		
Possible	31 (77.5%)	29 (60.4%)
Partly possible	9 (22.5%)	4 (8.3%)
Impossible	0	15 (31.3%)

Table 2 Type of leprosy and medication

Variable	*Group 1 (n = 40)*	*Group 2 (n = 48)*
Duration of leprosy	Median 17.74 years (3–45 years)	Median 38.96 years (1–70 years)
Negative smears since	n.r.	Median 17.42 years (0–50 years)
Type of leprosy		
LL[a]	39 (97.5%)	25 (52.1%)
TT[b]	1 (2.5%)	21 (43.8%)
ENR[c]	12 (30.0%)	2 (4.2%)
Medication		
Formerly MDT	39 (97.5%)	48 (100%)
Formerly DDS	1 (2.5%)	43 (89.6%)
Presently MDT	9 (22.5%)	4 (8.3%)

[a]Lepromatous leprosy.
[b]Tuberculoid leprosy.
[c]Erythema nodosum reaction (LL and ENR may occur simultaneously).
n.r., not recorded.

Table 3 Yeast carriage rates in Group 1 and Group 2 (multiple yeast species carriage was observed in three and five subjects from the groups, respectively; percentages do not add up to 100 as there was multispecies carriage)

Yeast species	*Group 1 (n = 40; Cambodian group)*	*Group 2 (n = 48; Thai group)*
Yeast (all)	32/40 (80%)	45/48 (93.75%)
Candida albicans	21/32 (65.6%)	20/45 (44.4%)
C. glabrata	1/32 (3.1%)	11/45 (24.4%)
C. tropicalis	3/32 (9.4%)	2/45 (4.4%)
C. krusei	5/32 (15.6%)	8/45 (17.8%)
C. parapsilosis	0	1/45 (2.2%)
C. lusitaniae	2/32 (6.2%)	2/45 (4.4%)
C. famata	1/32 (3.1%)	2/45 (4.4%)
C. guilliermodii	1/32 (3.1%)	0
C. utilis	0	1/45 (2.2%)
Saccharomyces cerevisiae	0	1/45 (2.2%)
Hansenula polymorpha	1/32 (3.1%)	0
Pichia ohmeri	0	2/45 (4.4%)
Non-*albicans Candida*	12/32 (37.5%)	27/45 (60%)
Non-*Candida* species	1/32 (3.1%)	3/45 (6.7%)

however, a significant difference with regard to the isolation of *C. glabrata*, which was more common in Group 2 ($P < 0.05$). Moreover, there was no significant difference with regard to isolation of other yeast species including *C. albicans, C. tropicalis, C. krusei, C. parapsilosis, C. lusitaniae, C. utilis, C. famata, C. guilliermondii, Saccharomyces cerevisiae, Hansenula polymorpha*, and *Pichia ohmeri*.

Further of interest was multispecies *Candida* carriage/infection in the oral cavity. For instance, in Group 1 the following species combinations were identified in three patients each; *C. lusitaniae* and *H. polymorpha*, *C. krusei* and *C. glabrata* and *C. krusei* and *C. famata*. Whilst, in Group 2 co-infection was seen in five cases as follows: *C. albicans* and *C. krusei* ($n = 2$), *C. albicans* and *C. glabrata* ($n = 2$), *C. krusei* and *C. glabrata* ($n = 1$).

Discussion

Oral yeast carriage has often been attributed to the health and physiological conditions of individuals. For example, *C. dubliniensis* is frequently isolated from HIV-infected individuals (5) while *C. parapsilosis* (6) and *C. glabrata* (7) are common in infants and elderly, respectively. Leprosy causes orofacial manifestations such as leproma formation of oral soft tissues, and cutaneous adnexia involving the facial and trigeminal nerves that can affect patients' oral health and function. On the other hand, their oral health behaviors are affected by the general effects of leprosy that lead to permanent damage to nerves, eyes, and limbs. Moreover, these patients are treated with long-term antileprosy drug regimens and/or given institutionalized care. Thus, they constitute a noteworthy group of individuals where both the oral and systemic health may be compromised. However, there are only a few reports on the oral mycotic flora of leprosy-affected patients. In a previous study, we found a high prevalence rate of *C. krusei* in leprosy patients (46%) who were predominantly elderly institutionalized in McKean Rehabilitation Center (Chiangmai, Thailand) with reference to a healthy control group (15%; 4). In addition, those *C. krusei* isolates showed resistance to fluconazole with a complex genetic variety pointing to the intriguing possibility of species diversity in the oral cavity precipitated by chronic systemic disease and attendant environmental factors such as institutionalization. The present study was a natural extension of our previous investigation. For reasons of comparison we relied on the methodology used in previous candidal epidemiologic studies. Purpose was to identify all *Candida* species and not a single *C. albicans*. The use of multiple *Candida* markers and genetic analyses did not seem to be feasible for a field study. In addition, in routine clinical laboratories worldwide the API techniques are considered gold standard. Therefore, we consider the data to be reliable and comparable with international norms. Here, we report the oral mycotic flora of two subgroups of leprosy-affected patients from two Southeast Asian locations – Cambodia (Leprosy Rehabilitation Centre Khien Kleang, Phnom Penh) and Thailand (McKean Rehabilitation Center, Chiangmai).

Both groups showed high oral yeast carriage rates (80%, 93.75%) compared with the average carriage rate of 50% in healthy humans both from the east and the west (8). When the oral hygiene behaviors of the two groups are compared it is interesting to note that in Thai group 91.7% are smokers while 31.3% were unable to perform any oral hygiene procedures. Moreover, 70% of the Cambodian patients were smokers while 22.5% of them did not practice proper oral hygiene methods. Kanli et al. (9) in a study to examine the oral hygiene habits, denture cleanliness, and presence of yeast in elderly complete denture wearers found a significant positive correlation between poor oral/denture hygiene and candidal prevalence. Therefore, the higher yeast carriage particularly in Thai patients may be associated with their poor oral hygiene.

Furthermore, in both study groups, the most prevalent yeast species was *C. albicans* (65.6% and 44.4%, respectively) supporting the current consensus that the most frequently isolated oral *Candida* species is

C. albicans. Nonetheless, a range of non-*albicans Candida* species were also isolated from these patients. Moreover, the profiles of non-*albicans Candida* prevalence rates were different in two groups. Patients from Cambodia carried *C. krusei* (15.6%) and *C. tropicalis* (9.4%), whereas Thai patients harbored *C. glabrata* (24.4%) and *C. krusei* (17.8%) after the major colonizer *C. albicans*. These data reconfirm our previous observation that *C. krusei* has a particular predilection for colonizing the oral cavities of leprosy-affected patients (4). In a comprehensive review of the literature on *C. krusei* carriage in humans, Samaranayake and Samaranayake (10) noted differential carriage rates of *C. krusei* in various body niches (6.1% in the oral cavity, 10.3% in the gastrointestinal tract and 12.5% in the vagina) either in health or disease. Furthermore, it has been reported that oral inoculation of *C. krusei* into immunosuppressed rats produced oral candidiasis similar to *C. albicans* infection (11). Thus, present data which shows a propensity of *C. krusei* to colonize the oral cavities in two groups of leprosy patients from disparate geographic locations warrants further investigations with larger cohorts possibly in a multicenter study due to latter's importance as an opportunistic pathogen in this potentially compromised group of patients.

Strikingly, the carriage of non-*albicans Candida* species in Thai patients (60%) was relatively high with reference to Cambodians (37.5%). In a survey to assess the oral yeast flora in patients with advanced cancer, Bagg et al. (12) noted an increased prevalence of non-*albicans Candida* species as high as 51% in a total of 194 yeast isolates. Furthermore, it was revealed that many non-*albicans Candida* isolates have developed resistance to fluconazole and itraconazole. Therefore, high carriage rate of non-*albicans Candida* in Thai patients with reference to Cambodians elaborate the importance of improved oral care regimes and continuous monitoring of oral microflora of this potentially compromised community. Moreover, the most frequently isolated non-*albicans Candida* species in Thai patients was *C. glabrata* and its carriage rate (24.4%) was significantly higher ($P < 0.05$) than that of Cambodians (3.1%). At least two studies have related high incidence of *C. glabrata* to poor oral hygiene, particularly in elderly institutionalized patients (7, 13). In this study, Thai patients were in hospital care for a considerably longer period than the Cambodian group. There was a predominance of smokers in the Thai group and significant number (39.6%) did not follow good oral hygiene procedures. Our data on the age distribution also show that the Thai cohort is much older than the Cambodian cohort (Table 1). Thus, high *C. glabrata* prevalence in the Thai leprosy group could be attributed to the older age as well as the poor oral hygiene status of this population and this adds credence to the previous observations of Lockhart et al. (7) and Grimoud et al. (13).

In total, we found 12 different fungal species from the leprosy patients denoting a considerable diversity in their mycotic flora. This included *Candida* species as well as other yeast such as *H. polymorpha*, *Pichia ohmeri*, and *S. cerevisiae*. Our data are comparable with that of Xu and Mitchell (14) who identified great heterogeneity of oral *Candida* species in Chinese and North American populations. These investigators speculated that such variations of the human yeast flora are due to sociodemographic and geographical differences. Additionally, we also noted many instances of multispecies yeast carriage in both our cohorts. Thus, multispecies carriage was seen in three Cambodian (9.4%) and eight Thai (17.8%) patients. Commonest species combinations in either group were *C. albicans* + *C. krusei* and *C. albicans* + *C. glabrata*. This finding of multiple *Candida* species carriage in leprosy patients is higher than a similar finding in diabetic patients (6.1%) from the UK (15). The phenomenon of multiple species carriage of *Candida* in the oral cavity was first reported by Samaranayake et al. (16) and subsequently by other groups. Cohabitation of several *Candida* species within a single niche could modify the pathogenic mechanisms such as adhesion to host tissues, production of extracellular enzymes, and biofilm formation whilst the clinical relevance of these findings remains to be determined.

In conclusion, the results of the present study confirm the phenomenon of high prevalence of oral colonization by yeast in leprosy patients. It was found that elderly institutionalized and/or compromised oral hygiene increase the risk of yeast colonization in the mouth. Our data indicate that *C. krusei* and *C. glabrata* are possibly the second commonest oral yeast species in leprosy patients although *C. albicans* is the commonest in the general population. Oral microbiological prevalence studies with frequently monitored, bigger population groups are required to clarify and extend the present findings.

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