Oral Colonization, Phenotypic, and Genotypic Profiles of *Candida* Species in Irradiated, Dentate, Xerostomic Nasopharyngeal Carcinoma Survivors

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The aim of this study was to investigate oral yeast colonization and oral yeast strain diversity in irradiated (head and neck), dentate, xerostomic individuals. Subjects were recruited from a nasopharyngeal carcinoma clinic and were segregated into group A (age, <60 years [n = 25; average age ± standard deviation (SD), 48 ± 6 years; average postirradiation time ± SD, 5 ± 5 years]) and group B (age, ≥60 years [n = 8; average age ± SD, 67 ± 4 years; average postirradiation time ± SD, 2 ± 2 years]) and were compared with age- and sex-matched healthy individuals in group C (age, <60 years [n = 20; average age ± SD, 44 ± 12 years] and group D (age, ≥60 years [n = 10; average age, 70 ± 3 years]). Selective culture of oral rinse samples was carried out to isolate, quantify, and speciate yeast recovery. All test subjects underwent a 3-month comprehensive oral and preventive care regimen plus topical antifungal therapy, if indicated. A total of 12 subjects from group A and 5 subjects from group B were recalled for reassessment of yeast colonization. Sequential (pre- and posttherapy) *Candida* isolate pairs from patients were phenotypically (all isolate pairs; biotyping and resistotyping profiles) and genotypically (*Candida albicans* isolate pairs only; electrophoretic karyotyping by pulsed-field gel electrophoresis, restriction fragment length polymorphism [RFLP], and randomly amplified polymorphic DNA [RAPD] assays) evaluated. All isolates were *Candida* species. Irradiated individuals were found to have a significantly increased yeast carriage compared with the controls. The isolation rate of *Candida* posttherapy remained unchanged. A total of 9 of the 12 subjects in group A and 3 of the 5 subjects in group B harbored the same *C. albicans* or *Candida tropicalis* phenotype at recall. Varying degrees of congruence in the molecular profiles were observed when these sequential isolate pairs of *C. albicans* were analyzed by RFLP and RAPD assays. Variations in the genotype were complementary to those in the phenotypic characteristics for some isolates. In conclusion, irradiation-induced xerostomia seems to favor intraoral colonization of *Candida* species, particularly *C. albicans*, which appeared to undergo temporal modifications in clonal profiles both phenotypically and genotypically following hygienic and preventive oral care which included topical antifungal therapy, if indicated. We postulate that the observed ability of *Candida* species to undergo genetic and phenotypic adaptation could strategically enhance its survival in the human oral cavity, particularly when salivary defenses are impaired.

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Nasopharyngeal carcinoma (NPC) is prevalent among people living in southern China. For instance, in Hong Kong, the age-standardized incidence rates of NPC are 23 and 9 per 100,000 for males and females, respectively (13). Radiotherapy is the treatment of choice for this condition, as surgical resection of the lesion is rarely possible. Such irradiation, however, inevitably involves oral and facial structures, including the major salivary glands, that cross the irradiation path. One major sequela of radiotherapy is prolonged xerostomia (7). Human saliva helps regulate oral health by its moisturizing, lubricating, buffering, and antimicrobial properties (43), and qualitative and quantitative changes in saliva inevitably affect oropharyngeal physiology, defense, and microbial ecology (5, 37).

Oral candidiasis is one of the most common fungal infections of man and is manifested in a variety of clinical presentations. *Candida albicans* is the main *Candida* species residing in the oral cavity and is responsible for the majority of such infections, although non-*C. albicans* species are sometimes implicated (30). The former opportunistic, dimorphic fungus is notable for causing local or systemic infections in an ever-increasing number of medically compromised individuals (2, 30). These include individuals undergoing chemotherapy, immunosuppressive treatment, or long-term broad-spectrum antibiotic therapy; patients with human immunodeficiency virus infection or advanced neoplasms; and organ transplant recipients. Being opportunistic pathogens, *Candida* species flourish and cause a spectrum of diseases in these individuals (2, 29), especially when immunological defenses are impeded (12).

Oral candidiasis is common in individuals with head, neck, and other malignancies, especially when radiotherapy is used as the mainstay of treatment (8, 28, 37). It is thought that irradiation-induced histologic changes leading to oral mucositis, together with quantitative and qualitative changes in saliva and salivary flow, facilitate yeast infection (9). In a very recent study which monitored the weekly oral yeast carriage in 30 patients with head and neck cancers undergoing irradiation therapy, Redding and coworkers (28) noted oral *Candida* carriage in 73% of patients on at least one visit and when those positive for *Candida* were recalled, the researchers noted oral *Candida* carriage at 51% of the recall visits (28). Further, they reported that almost identical *Candida* strains consistently col-
ozended the oral cavity despite the use of antifungals by 27% of the study population. However, there are others who were unable to note any discernible differences in oral yeast colonization in control and test subjects after radiation therapy (38). Possible reasons for such discrepant results may be the intrinsic differences in the study populations and the irradiation protocols used (8, 9, 28, 38).

The aims of the present study, therefore, were to investigate the oral colonization profile of yeasts (i) in a homogenous cohort with a history of NPC managed using a similar irradiation protocol, at least 6 months following irradiation therapy, and (ii) before and after professional oral hygiene care with or without antifungal therapy. In addition, the phenotypic and genotypic characteristics of sequential yeast isolates from a subgroup of studied individuals were monitored to explore the degree of similarity between isolates.

MATERIALS AND METHODS

Study group. A total of 33 patients who survived NPC (confirmed by two consecutive negative biopsies 10 weeks apart starting at week 8 postirradiation) or more than 6 months posttreatment were recruited from the Department of Clinical Oncology, Queen Mary Hospital, the University of Hong Kong (34). All subjects underwent almost identical radiotherapy protocols, and the total irradiation dose received was similar (34). The irradiated subjects were divided into two cohorts according to age (group A [<60 years] and group B [≥60 years]), with reference to a previous study (14, 16). Age- and sex-matched nonirradiated individuals were selected for the control groups, as follows: 20 subjects younger than 60 years old were randomly selected from the Outpatient Dental Clinic, Faculty of Dentistry, the University of Hong Kong (group C) and 10 healthy males 60 years of age or older were randomly selected from more than 100 attendees at a local senior citizen center (group D).

Clinical examination, treatment, and recall. At baseline, a comprehensive clinical examination was carried out for all subjects. The detailed data from this examination, including the plaque index (35) and gingival index (17), which are indicators of personal oral hygiene, have been reported elsewhere (34). All subjects in groups A and B were given comprehensive oral health care which included oral hygiene education, daily home fluoride gel application, scaling and polishing of the teeth, restoration of carious lesions, and topical antifungal therapy for those with clinically evident oral candidiasis; jaw muscle exercises were prescribed for subjects with trismus. Three months after completion of this therapy for those with clinically evident oral candidiasis; jaw muscle exercises were prescribed for subjects with trismus. Three months after completion of this study population. However, there are others who were unable to note any discernible differences in oral yeast colonization in control and test subjects after radiation therapy (38). Possible reasons for such discrepant results may be the intrinsic differences in the study populations and the irradiation protocols used (8, 9, 28, 38).

Preparation of Candida DNA for molecular analysis. C. albicans isolates obtained from subjects harboring yeasts were identified by species-specific primers (1) and RAPD or restriction fragment length polymorphism (RFLP) analysis, the yeast pellet was resuspended in 1 ml of SE buffer (1.2 M sorbitol–0.1 M EDTA, pH 8.0) containing 3 μl of β-mercaptoethanol (Sigma, St. Louis, Mo.) and 0.5 μg of yeast lytic enzyme (lyticase; Sigma) and incubated at 37°C for up to 1 h until spheroplasts were noted. The spheroplasts were harvested by centrifugation at 4,000 × g for 5 min, washed twice in SE buffer, and resuspended in 1.5 ml of 0.53 M NaCl–0.1 M EDTA, 8.0. They were then lysed by the addition of proteinase K (final concentration, 500 μg/ml) and sodium dodecyl sulfate (final concentration, 1% [wt/vol]), along with RNase (final concentration, 500 μg/ml), at 55°C for 1 h. The lysate was boiled for 10 min and then incubated first at 50°C for 24 h and then at 50°C for 24 h in 100 mg of lyticase (Sigma) per ml of solution (900 U of lyticase/ml). The resulting agarose plugs were washed thrice with 50 mM EDTA, pH 8.0, and loaded onto 0.8% (wt/vol) agarose grade Ultra (Beverl

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denaturation at 94°C, 2 min of annealing at 32°C (primer NA) or at 57°C (primers JWF and JWFF), and 2 min of primer extension at 72°C. The reaction was held at 72°C for 15 min. Control tubes without template DNA were included in each run, and reproducibility was checked for each reaction (39). The PCR products were electrophoresed in a 0.8% agarose gel in TBE buffer, stained with ethidium bromide, and visualized under UV transillumination. The RAPD analysis was repeated on two further separate occasions with strains recovered from the stock kept at –70°C.

**Statistical Analysis.** The demographic and microbiological data of the subjects were analyzed by Statview 4.5 for Macintosh computer. Differences between individual groups were tested by Bonferroni's multiple comparison for nonparametric data, analysis of variance, or Fisher exact test, as appropriate. Groups were regarded as significantly different from each other if P < 0.05.

**RESULTS**

The demographic data of the subjects recruited in the first part of the study, including the length of the postirradiation period, are shown in Table 1. On initial examination, 42% (group A [n = 10], 40%; group B [n = 6], 50%) of the irradiated individuals were diagnosed as having oral candidiasis.

All isolates belonged to *Cryptococcoideae* and were *Candida* species. The species isolated were *C. albicans* (group A [n = 19], 76%; group B [n = 5], 62.5%; group C [n = 2], 10%; group D [n = 4], 40%), *C. tropicalis* (group A [n = 5], 20%; group B [n = 4], 50%; groups C and D [n = 0], 0%), *Candida parapsilosis* (group A [n = 1], 5%; group C [n = 1], 5%; groups B and D, [n = 0], 0%), *Candida famata* (group A [n = 1], 4%; groups B, C, and D [n = 0], 0%). Two species of *Candida* were isolated from each of two rinse samples from group A and one rinse sample from group B. Significantly higher prevalences of *C. albicans*, *C. tropicalis*, and total *Candida* species were noted in the irradiated individuals (groups A and B) than in the healthy individuals (groups C and D) (Fisher exact test, P < 0.05). The total counts (in CFU per milliliter of oral rinse) of yeast species isolated are summarized in Table 2. The oral rinse samples of irradiated individuals, (i.e., groups A and B), yielded a mean of at least one yeast species as opposed to less than 0.5 yeast species recovered from groups C and D (Table 2). The quantities (in CFU per milliliter) of total *Cryptococcoideae* recovered from Group B subjects were significantly elevated compared with those recovered from the other three cohorts (Table 2).

Altogether, 12 (6 males; average age ± SD, 45.9 ± 5.8 years; average postirradiation time ± SD, 5.0 ± 3.1) and 5 (all males; average age ± SD, 67.1 ± 3.4 years; average postirradiation time ± SD, 2.0 ± 1.1 years) subjects from groups A and B, respectively, participated in the recall reassessment for yeast colonization. The remainder either missed the recall, attended their own general dentist, or succumbed to illness. Clinically, most of the subjects exhibited a fair standard of oral hygiene, i.e., ≥81% of the studied sites had a plaque index score of ≤1 during both examination events, with the number of plaque-free sites increasing from 12% to 33% at the second examination. As for periodontal health, ≥69% of the studied sites exhibited clinically healthy or mild marginal gingival swelling without bleeding on probing; 95% of sites were free of calculus at the initial examination. On first presentation 35.3% (group A, n = 4; group B, n = 2), i.e., 6 of the 17 individuals were clinically diagnosed with candidiasis and received topical antifungal therapy (100,000 U of nystatin per gram of ointment, three times a day [20]), whereas at the recall session, 23.5% (group A, n = 3; group B, n = 1), i.e., 4 of the original 6 subjects remained affected by candidiasis, albeit with markedly smaller lesions. Antifungal therapy was helpful in eradicating the yeast or reducing the yeast numbers to below detectable levels in only 1 of the 6 individuals. In four cases, the colonization pattern remained unchanged while in one case *C. albicans* was replaced by *C. tropicalis* on the second sampling visit. None of the affected individuals complained of discomfort related to the residual lesions at the recall session.

Eight of the 11 asymptomatic individuals harbored the same biotype of *Candida* in the sequential oral rinse samples (*C. albicans*, n = 7; *C. tropicalis*, n = 1), while the remainder yielded different *Candida* species on the second visit (one yielded *C. albicans* and then *C. tropicalis*, one yielded *C. tropicalis* and then *C. albicans*, and one yielded *C. albicans* and then *C. glabrata*).

The prevalences of yeast species in oral rinse samples at baseline and recall sessions is summarized in Table 3. The quantity of yeast species isolated from both groups (total or individual counts), from the first or second oral rinse samples, irrespective of antifungal therapy, fell within a similar range. The range and median values (in CFU per milliliter of oral rinse) were determined for *C. albicans* before (range, 0 to 4.8 × 10³; median, 132) and after (range, 0 to 1.2 × 10⁴; median, 711) treatment and for *C. tropicalis* before (range, 0 to 2.3 × 10³; median, 0) and after (range, 0 to 4.5 × 10³; median, 0) treatment. There was no significant difference in either the yeast recovery patterns or the yeast harvests between the two visits.

The 10 sequential isolate-pairs of *C. albicans* were biotyped using the API 20C AUX and API ZYM kits. All were found to belong to the primary biotype type J of the classification system of Williamson et al. (46) (Table 4), indicating that they all possessed alkaline phosphatase, acid phosphatase, esterase, and acid maltase activity. As for periodontal health, ≥69% of the studied sites exhibited clinically healthy or mild marginal gingival swelling without bleeding on probing; 95% of sites were free of calculus at the initial examination. On first presentation 35.3% (group A, n = 4; group B, n = 2), i.e., 6 of the 17 individuals were clinically diagnosed with candidiasis and received topical antifungal therapy (100,000 U of nystatin per gram of ointment, three times a day [20]), whereas at the recall session, 23.5% (group A, n = 3; group B, n = 1), i.e., 4 of the original 6 subjects remained affected by candidiasis, albeit with markedly smaller lesions. Antifungal therapy was helpful in eradicating the yeast or reducing the yeast numbers to below detectable levels in only 1 of the 6 individuals. In four cases, the colonization pattern remained unchanged while in one case *C. albicans* was replaced by *C. tropicalis* on the second sampling visit. None of the affected individuals complained of discomfort related to the residual lesions at the recall session.

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lipase esterase, leucine arylamidase, valine arylamidase, phosphomonoesterase, N-acetyl-β-glucosaminidase activity (data not shown). Eight sequential isolate pairs belonged to the secondary biotype 1 (per API 20C AUX), while the remainder were of biotypes 11 and 24 (21) (Table 4).

The resistotype profiles of the 12 sequential isolate pairs of *C. albicans* and *C. tropicalis* are also shown in Table 4. The susceptibilities of the isolate pairs to various chemicals differed to varying extents. Five of the 12 isolate pairs tested were of resisototypes that were considerably different (>3 of 9 tests) from those of their counterparts. It was found that among individuals receiving only hygienic therapy *C. albicans* isolate pairs with fewer phenotypic differences were significantly more prevalent than they were among subjects that received antifungal therapy (Table 4).

The 10 sequential *C. albicans* isolate pairs from the irradiated patients were subjected to three different molecular typing methods, namely, PFGE, RFLP, and RAPD analyses. Identical banding patterns were observed in specimens from the same stock culture (data not shown). Electrophoretic karyotyping using PFGE revealed six to eight chromosomes per *C. albicans* isolate tested, with chromosome sizes ranging from 1 to 3.2 Mb. All but two isolate-pairs (L1-L1’ and L2-L2’) revealed stringent conservation of karyotypes (Fig. 1). When comparing PFGE patterns of isolates from different subjects, only isolates from subjects L8 and L10 showed identical PFGE profiles (Fig. 1B). The restriction enzyme *Hinf*I was employed to further profile the isolates by genotype (3, 6, 36). Enzymatic digestion of sequential *C. albicans* isolate pair DNA specimens by *Hinf*I revealed that 7 of 10 patients carried genetically different strains after the antifungal and/or hygienic therapy as illustrated by the polymorphism of restriction fragments at the higher-molecular-weight region, i.e., 2 to 10 kb (6). The remaining 30% of the DNA profiles exhibited almost identical restriction fragment length patterns (Table 5; Fig. 2).

When the 10 sequential *C. albicans* isolate-pairs were analyzed by RAPD, remarkable genetic variation was observed (Table 5). The three different primers yielded isolate-specific arrays of 10 to 15 prominent fragments under the PCR conditions employed (Fig. 3). Only one isolate pair was found to possess identical DNA profiles when the primers NA and JWF were used (Fig. 3). However, with the primer JWFR, four *C. albicans* isolate pairs were found to be identical, although none of these was the pair which was deemed identical with NA and JWFR (Table 5).

### TABLE 3. Frequencies of yeast species isolated from oral rinse samples of irradiated individuals before and after antifungal and/or hygienic therapy

<table>
<thead>
<tr>
<th>Species</th>
<th>Before therapy</th>
<th>After therapy</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>75.0</td>
<td>80.0</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Candida lipolytica</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>25.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Total <em>Cryptococcus</em></td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Group A, n = 12; Group B, n = 5. Data from subjects who participated both before and after antifungal and/or hygienic therapy were considered. Some samples contained more than a single species, so the total is not equal to the sum of the frequencies for individual species.*

### DISCUSSION

The prevalence and quantity of cultivable oral yeasts and their colonization patterns in a homogenous group of NPC survivors subjected to therapeutic head and neck irradiation were investigated by using a cross-sectional study design. To our knowledge this is the first study to characterize sequential oral *Candida* isolates from irradiated individuals suffering from solid tumors similar in nature and subjected to the same therapeutic irradiation protocol. The baseline data obtained were compared with data for age- and sex-matched nonirradiated healthy individuals. The oral yeast colonization pattern of the irradiated subjects after antifungal and/or hygienic therapy was reassessed, and the phenotypic and genotypic characteristics of biochemically identical, sequential oral yeast isolate pairs were studied.

The oral yeast colonization profile, including the candidal prevalence and the predominant *Candida* species isolated, from our test cohort was similar to most previous reports from irradiated subjects with various head and neck tumors (20, 27). According to some reports, early oral candidal colonization in these patients coincides with the commencement of irradiation therapy (28). In our study topical antifungal therapy effectively reduced lesion sizes and resolved clinical symptoms in approximately one-third of the recalled patients with candidiasis, although the oral yeast prevalence was persistently high (Table 3). Similarly, Ramirez-Amador et al. (27) have shown that systemic antifungal therapy eradicated clinical oral infection in only five of eight irradiated patients. In another recent study Redding et al. (28) reported that progressive, incremental systemic antifungal dosage could eliminate only 27% of clinical *Candida* infections, and oral yeast carriage cannot be totally eradicated. Based on these observations, and mindful of the selection and emergence of drug-resistant strains, both groups expressed caution in using prophylactic antifungals during irradiation therapy (27, 28).

It is well established that *Candida* species are oral commensals in diseased individuals such as the irradiated subjects in the current study. Hence, one premise of our study was that the *Candida* strains isolated on the recall visit were persistent oral strains derived from the original parent stock but subjected to exogenous insult such as postirradiation xerostomia. Particular attention was paid in studying these sequential yeast isolates in an attempt to trace their phenotypic and genotypic lineage. Thus, we found that 59% of the recalled subjects harbored *C. albicans* and 12% harbored *C. tropicalis*, respectively, at both time points studied. Although the introduction of exogenous *Candida* strains into the oral cavity between the sampling intervals cannot be totally ruled out, this would be minimal, as all subjects suffered from the same type of tumor (NPC), successfully treated with a single course of irradiation therapy without resorting to surgery, immunosuppressants, or long-term steroïd therapy. Second, the subjects were all from southern China, having comparable dietary habits. All had undergone similar, professionally delivered oral hygiene therapy.

Although, it would have been desirable to follow up both the control and the test group to decipher the lineage of sequential oral *Candida* isolates, we did not, due to the low prevalence of oral yeasts in control group C (age, ≤60 years). Furthermore, the fact that the denture-wearing habit, which fosters yeast colonization, was more prevalent in control group D (age, ≥60 years) reinforced our decision to abort such a parallel study. Resistotyping allows differentiation of *C. albicans* isolates from clinical samples and has been used in clinical epidemiology studies (24). This method is based on the susceptibility of the test strains to a select group of organic and inorganic
The phenotypic characteristics of sequential isolate pairs of Candida spp. derived from oral rinse samples are shown in Table 4. Distinctly different resistotypes were obtained with C. albicans isolate pairs L4-L4, L8-L8, L9-L9, and L10-L10; the last three being from subjects who had received topical antifungal therapy. The rest of the isolate pairs were found to be moderately or minimally different from their partner strain. The C. tropicalis isolate pair, L11-L11', was found to differ in resistotype after hygienic therapy, while the isolate pair L12-L12' was minimally different despite antifungal therapy (Table 4).

Due to the potential inconsistencies of the phenotyping methods, several molecular typing methods have been widely used in epidemiologic studies of C. albicans. These include RAPD (22), RFLP analysis of total genomic DNA (40), Southern hybridization analysis using a number of different probes (11, 33), and electrophoretic karyotyping using PFGE (25). However, the resolution, specificity, and discriminatory power of each of these methods differ greatly, thus affecting their utility. For instance, electrophoretic karyotyping of chromosome-size DNA elements of medically important yeasts is of relatively less technically demanding approach for strain differentiation of C. albicans (15).
Karyotyping was carried out on an 0.8% agarose gel. Lane M, *H. wingei* chromosome molecular size standard (Bio-Rad).

The fragments of interest (in the region of 4 to 9 kb) generated by *Hin*II digestion of total genomic DNA are derived from the spacer region of the DNA repeat sequences of *C. albicans* (19). Smith et al. (36) postulated that the above-mentioned spacer region was not genetically conserved, hence generating the diversity observed. Among them, RAPD is the most favored, probably due to its relatively simple and quick protocol. One limitation, however, is that no universally established guidelines are available for the selection of primers and the subsequent interpretation of the data generated. Whereas some have used combined profiles derived from multiple primers (45), others suggest the use of a single primer in combination with different molecular typing methods for profiling *Candida* genotypes (41). Bart- Delabesse and coworkers (3) appreciated these difficulties and suggested that minor variations of RAPD profiles of isolates derived from the same individual should be disregarded. We followed these guidelines in the current study, together with recommendations of Sullivan and coworkers (41), who suggested the selection of at least three different molecular typing methods for adequate characterization of *C. albicans*.

At the karyotype level, only *C. albicans* isolate pairs L1-L1' and L2-L2' appeared to yield disparate profiles, in contrast to all other isolate pair profiles, which were identical (Fig. 1). A total of 11 unique *C. albicans* karyotypes were observed in this study, including four isolates (isolate pairs L8-L8' and L10-L10') with the same karyotype profile (Fig. 1; Table 5). Interestingly, isolate pairs L8-L8' and L10-L10' were dissimilar with regard to their (API 20C AUX) biotype.

The banding profiles of *C. albicans* we obtained using PFGE were similar in size range to those observed by Bostock et al. (3). RAPD profiles were generated with three different primers: NA (5'-GGCA TCCCCA3') (1), JWF (5'-GGTCCGTTTCAAGCAG3'), and JWFF (5'-G CATATCAATAGCGGAGGAAAA3') (10). Observable band staining intensity differences and variation of one band were disregarded (3).

As opposed to PFGE and RFLP, PCR-based protocols have become popular in recent years for genotyping *Candida* (3). Among them, RAPD is the most favored, probably due to its relatively simple and quick protocol. One limitation, however, is that no universally established guidelines are available for the selection of primers and the subsequent interpretation of the data generated. Whereas some have used combined profiles derived from multiple primers (45), others suggest the use of a single primer in combination with different molecular typing methods for profiling *Candida* genotypes (41). Bart-Delabesse and coworkers (3) appreciated these difficulties and suggested that minor variations of RAPD profiles of isolates derived from the same individual should be disregarded. We followed these guidelines in the current study, together with recommendations of Sullivan and coworkers (41), who suggested the selection of at least three different molecular typing methods for adequate characterization of *C. albicans*.

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The rationale for using primers JWF and JWF was their previous utility in the analyses of the V3 variable region of the large ribosomal subunit genes of C. albicans (10, 42). In the current study, JWF and JWF were used individually rather than in tandem as previously described (10, 42) in an attempt to generate an extra set of RAPD profiles. NA has also been previously employed for RAPD fingerprinting of C. albicans (1). All primers employed appeared to well suit the present analyses, as they yielded effectively similar annealing frequencies in most of the C. albicans isolates tested.

Drawing together the phenotypic and genotypic characteristics of the C. albicans isolate pairs studied, we could make the following conclusions. The chromosomal attributes of the sequential C. albicans isolate pairs appear relatively subject specific (except for the two pairs L8-L8 and L10-L10). There was no significant disparity in the chromosome-size bands derived from PFGE, except for the possibility of chromosomal translocations observed in two isolate pairs. As for RAPD analyses, identical genotypic characteristics could be detected only in six isolate pairs with all three primers. Further, the utility of the Hinfl RFLP regimen for profiling C. albicans genotype appears questionable, as there was no correlation between the latter and the Hinfl RFLP profile for any of the isolate pairs tested (Tables 4 and 5). This may be due to the characteristics of the highly variable rDNA spacer region, the use of which may confer minimal effects on the C. albicans phenotype.

In conclusion, we postulate that the irradiation-induced changes of the intraoral environment, such as xerostomia, lead to increased intraoral colonization by Candida species. The question of whether clonal selection and propagation of Candida occurs in these patients either due to irradiation and/or to concomitant antifungal therapy is still unresolved. A comprehensive study of a large cohort using precise analytical tools appears to be necessary to resolve this issue.

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