CHAPTER VI

DISCUSSION

Oral candidiasis has increased markedly during the past two decades parallely with the increasing numbers of immunocompromised patients. Along with this rising incidence, molecular typing techniques have become fundamental for studying the epidemiology of *Candida* isolates and for developing rational therapeutic strategies (86, 134-137). Longitudinal studies on the oral colonization and infection patterns of the human fungal pathogen *C. albicans* have mostly been performed with a single isolate from each patient per visit (138), and there have been calls for studies using multiple strains from a single visit due to the polyclonality of *C. albicans* in the oral niche (22). Hence, we employed karyotyping and genotyping methods to characterize 2-3 randomly selected isolates per visit to investigate the population of multiple clones of *C. albicans* among a group of HIV-infected individuals over a period of 1 year.

C. albicans is the most pathogenic species of the genus *Candida* and is consistently the most frequently causative agent of *Candida* infection in human. In AIDS patients with OPC, *C. albicans* is followed in frequency by *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis* and other non-*albicans* species (139, 140). In this study we found a high prevalence of *C. albicans* recovered from HIV-infected patients with oral candidiasis (95%). Non-*albicans* species were isolated from 8 patients either as the sole isolate (n = 1) or in addition to *C. albicans* (n = 7), these being considered cases of co-infection. *C. glabrata* was the most frequent non-*albicans* species recovered in this series, followed by *C. tropicalis*, *C. krusei* and *C. rugosa*. Seven cases of co-infection (7%) were detected. The 95% recovery of *C. albicans* from the oral cavities in the present studies is in agreement with the recovery rates reported by others (range from 88-95%) (141). It is clear that there is a significant correlation between the recovery of *C. albicans* and the presence of oral lesions in HIV-infected patients. Only very few studies reported including this report that *C. rugosa* was isolated from oral cavity in HIV-infected patients and in diabetes

patients (142). Up to 15% of *Candida* spp. mixed cultures from oral cavities of HIV-infected patients has been described by other authors and the isolated species in addition to *C. albicans* did not seem to play an important role in the pathogenesis (8, 143). However, the presence of two or more species in the same patient may predispose to recurrent candidiasis, mainly in the presence of species for which azole MICs are intrinsically high, like *C. glabrata* or *C. krusei* (13). Of note, episodes of co-infection were observed in our study among patients with none exposure or exposure, both, to antifungal drugs, as well as a CD4+ count lower than 200/mm^3. These data suggest that co-infections may reflect not only previous exposure to antifungal drugs, but also the severity of the immunosuppression condition of patients and contact with patients harboring different species (8).

The 187 isolates of *C. albicans* showed highly homogeneous patterns in biochemical analysis. To enhance the accuracy of analysing the strains characteristics, both the karyotypic and genotypic variability were evaluated. The DNA typing illustrates a number of important features of oral colonization by *C. albicans*. In evaluating electrophoretic karyotype diversity of oral candidiasis infections in HIV-infected patients, the result of this study showed that the majority (59%) of patients were harboring more than one karyotypes of *C. albicans*, that the same karyotype was rarely shared among two or more individuals, indicating a high level of karyotype variability. This heterogeneity within oral isolates agrees with that reported for oral *Candida* colonization/infection (59, 135-137, 144, 145). There are numerous reports of karyotypic diversity in *C. albicans* with chromosome size ranging from 4 to 0.5 Mb (103, 146, 147). Variability in the number of DNA bands has been widely reported for *C. albicans* karyotyes by several investigators who used different systems (74, 147). In the present investigation, the numbers of DNA bands obtained with a CHEF does not represent the numbers of chromosomes of *C. albicans* because *C. albicans* is diploid, most likely aneuploid, organism (87) with a haploid chromosome number of eight, including the R chromosome, as determined by karyotype and hybridization experiments (93).

This present study, we used RFLP in combination with Southern hybridization and the repetitive sequence RPS102 probe to characterize a group of oral *C. albicans* isolates recovers from HIV-infected patients that each individual was contained identical karyotype of two colonies. Almost two isolates with identical karotype from individual were also shown the same hybridization profile. Minor

differences between two isolates in each individual with identical karyotypes revealed by RFLP and hybridization with RPS102 in a few cases (6%). By using Southern hybridization method, this study demonstrates that isolates of *C. albicans* in different individuals were showed either similar or different hybridization patterns, although their electrophoretic karyotypes were similar or different to each other (Table 10). According to Doi et al. (31), found that recurrent isolates from one patient and his mother showed four patterns in karyotype analysis that were common in both individuals, but only a single pattern in RFLP by *Sma* I digestion and Southern hybridization with RPS1. This finding indicates that the yeasts cells are closely related or had a common origin. The appearance of different karyotypes in the same individual was assumed to be due to a minor chromosomal rearrangement that did not influence the *Sma* I digestion pattern. This rearrangement may have occurred by the action of certain agents or may have developed spontaneously in cells colonized in the patients. The possibility remains that the karyotypic variants represent strains that have ungergone profound genomic changes affecting both karyotype and genotype. However, initial work with the 27A sequence showed that the fingerprint pattern of strains is very stable despite in vitro passaging and changes in drug resistance (80). Magee et al. (25) suggested that the relatedness between different strains depends on the rate at which certain parameters, such as restriction sites, chromosome length, and chromosomal configuration of repeat sequences, change as a chromosomal configuration of repeat sequences, change as a function of cell growth. The stability of DNA electrophoretic patterns of *C. albicans* strains was tested by Bart-delabesse et al. (12) in patients with AIDS who had developed resistance to fluconazole. They reported that after approximately 500 generations, progeny karyotypes were not always identical to the parental type, while *Eco*RI and *Hin*fI patterns were identical.

As HIV-infected patients become more immunosuppressed, more occurrences of candidiasis occur, often leading to more frequent or prolonged exposure to azole antifungal agents. Extended use of azoles can lead to increased resistance and thereby reduce the efficacy of these drugs (148). Several studies have reported that the extent of prior fluconazole exposure correlates with reduction in the susceptibility of *C. albicans* (12). Likewise, itraconazole resistance has occurred following long-term exposure (149). Long-term exposure to the antifungal agents can select for mutations in the original strain, which may lead to reduced susceptibility in those isolates. In patients who are colonized or infected with multiple strains or species, antifungal

prophylaxis can select for the few organism that are able to develop resistance, allowing them to flourish (150). Suzuki et al. (92) and Rystchenko-Bulgac et al. (75) proposed that there was a correlation between the diversity of karyotype and diversity of phenotype. Perepnikhaka et al. (88) found an association between the loss of one of the homologs of chromosome 4 and resistance to fluconazole.

In our studies, it was found that susceptibility of *Candida* spp. to antifungal drugs varies among the different species and among different isolates within the same species. *C. krusei*, *C. glabrata*, *C.parapsilosis* and *C. tropicalis* are less susceptible to antifungal drugs than most *C. albicans* isolates. In the last few year, the increased use of fluconazole may have contributed to a shift in the incidence of fungal infections due to yeasts other than *C. albicans* which may be less responsive to fluconazole. All non-*albicans* species mentioned are usually less susceptible or resistant to azoles (151). No difference variations in antifungal susceptibility patterns were noted among two different karyotypes in individual patients and no relationship between a particular level of susceptibility to antifungal agents and a specific DNA type of *C. albicans* isolates. The changed in karyotype patterns illustrated no association with the variations in susceptibility to antifungal agents, according to previous study by Mori et al. (152) that found no correlation between the diversity of karyotype and resistance to the antifungal drug fluconazole. Millon et al. (13) found that susceptible and resistant strains from each patient had the same genotype as defined by electrophoretic karyotype and restriction fragment length polymorphism. Despite a low overall incidence of antifungal-resistant *C. albicans* strains in this study of HIV-positive patients, examples of antifungal resistance were delineated. In patient no. 35, 44 and 71, in vitro azoles resistant were found. This findings well correlated with the clinical presentation of these patients, as all of them had received azole therapy. It is known that the emergence of resistance strains of *C. albicans* frequently occurs in patients previously exposed to azoles (70).

In summary, high polymorphism in genotype within oral *C. albicans* isolates recovered from HIV-positive individuals is shown. There is no correlation between antifungal susceptibility patterns and genotypic patterns. We found a co-infection of the same species that showed similar antifungal susceptibility profile but was genetically distinct. Co-infection of difference species of *Candida* were also found. It is interesting that the non-*abicans* species demonstrated the less susceptible against

antifungal agents than *C. albicans*. To perform the phenotypic and genotypic study, the method to select the colony is the very important factor.