CHAPTER I

INTRODUCTION

Oropharyngeal candidiasis (OPC) is the most common opportunistic infection in patients with HIV-infection, occurring in as many as 90% of HIV-positive patients at some point during the course of HIV disease (1). Increased retroviral replication and an associated decline in immune defenses render these patients particularly susceptible to OPC, to the extent that it is considered an early sign of HIV infection (2). The incidence of OPC has reportedly risen steadily overtime. A recent analysis of hospital discharge data from 1980 to 1989 by the National Center for Health Statistics, National Hospital Discharge Survey, and two commercially-generated hospital discharge data sources (PAS [Professional Activity Survey] and McAuto [McDonnell Douglas Automation Company Medical Records System]) revealed that the rate of OPC increased almost fivefold (from 0.34 cases per 1,000 admission per year to 1.6 per 1,000 admissions per year) from 1980 to 1989 (3). While most cases of candidiasis are still caused by *C. albicans*, there has been a striking increase in the frequency with which non-*albicans Candida* species (primarily *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. glabrata*) are isolated (4). For example, non-*albicans* strains of *Candida* accounted for only 3.4 % of OPC isolates from HIV-infected patients in the late 1980s, as compared with 16.8 % of isolates by the beginning of 1990-a fourfold increase in 3 years (5).

Treatment of OPC is generally effective and usually involves the use of topical or systemic antifungal therapy with drugs such as the polyenes (amphotericin B and nystatine) and the azoles (clotrimazole, ketoconazole, fluconazole and itraconazole). In recent years, oral fluconazole given its low toxicity, has become the most common form of treatment OPC in HIV-infected patients (6). The prolonged nature of AIDS predisposes these patients to recurrent episodes of OPC that can increase infrequency and severity with progressive HIV disease. Therefore, the prolonged management of OPC in this patient population causes the development of drug-resistant candidiasis (7). *Candida* resistance to the azoles has been frequently attributed to a selective

pressure caused by the use of these antifungal drugs as OPC prophylaxis or treatment (8). Many studies have estimated the incidence of clinical fluconazole resistance to be from 6 to 36%, depending on the patient group studies and the case definition used (9-11).

Several studies of DNA subtype variation and antifungal resistance among isolates of *Candida albicans* from AIDS patients with multiple episodes of oropharyngeal candidiasis have shown several results. Several authors have been shown that AIDS patients are frequently infected with the same DNA subtype over multiple infections (12-15). In some studies different DNA subtypes and fluconazole MICs were observed among isolates obtained from a single culture or emerged in subsequent cultures (16). The isolation of mixed fungal strains (*Candida albicans* with other species of *Candida*) in cultures of oral lesions has seldom been described (17).

In Southeast Asia, as of November 2002, an estimated six million adults and children live with HIV/AIDS. At the end of October 2001, 188,117 AIDS cases were reported from Thailand (18). In most countries of Southeast Asia the vast majority of HIV-infected patients have not received any antiviral therapy (ART) or highly active anti-retroviral therapy (HAART) until now. Consequently, the incidence of opportunistic infection by *Candida* remain a problem in this patient. In spite of oral candidiasis importance as an infections agents in HIV disease, little is known of the epidemiology of *Candida* spp. in Thailand.

To understand fully how and why *Candida* species cause disease, we must first be able to identify particular strains and track them in outbreaks of infection. With such knowledge, it should be possible to determine the source of individual infections. Since *Candida* infections can either be acquired endogenously from the host's normal flora or exogenously from the environment, other infected patients, or health care workers, this information can be of great help in monitoring outbreaks of infection and in assessing the quality of cross-infection control measures. Similarly, strain identification can establish whether relapses in infection are due to a novel infecting organism or to persistence/reinfection by the original strain, thus potentially affecting the choice of therapy. To define the infecting strain, effective typing systems capable of discriminating strains within the species are required. Previous typing methods, which were found to be more or less suitable for *C. albicans* strain delineation, have relied on biotyping, enzyme profiles, susceptibility to killer toxin,

streak morphology, and serological agglutination reactions (19). More recent literature indicates that DNA typing is the method that shows the greatest resolving power for *C. albicans* strain discrimination. Genetic typing systems such as electrophoretic karyotype by pulse-field gel electrophoresis (PFGE) (20, 21), restriction fragment length polymorphism (RFLP) of total DNA (22) and Southern hybridization pattern with the use of a DNA probe have been prevalent in epidemiological studies of *C. albicans* strains (22-24). Karyotyping has been reported to be more reproducible, more sensitive, and even simpler to interpret than RFLP for genotyping *C. albicans* strains; this is based on the much smaller number of DNA bands of karyotypes. On the other hand, the highly diversified Southern blot hybridization patterns produces by species-specific repetitive sequence probes have suggested that RFLP patterns are potentially more discriminative than karyotyping patterns. It has been suggested that use of combination of different systems may help distinguish certain isolates from each other (25).

A species-specific repetitive sequence is a useful probe for strain delineation by hybridization patterns of total *C. albicans* DNA digested by a restriction enzyme. Mid-repeat sequences Ca3 or 27A have been most frequently used as a probe to assess the genetic relatedness of commensal and infecting strains in *C. albicans*, and these sequences contain homologous regions to the *C. albicans*-specific repetitive sequence, RPS element (26, 27). RPS was found during research on the karyotype variation among the strains (28), and it was assumed to be involved in chromosome rearrangement (29, 30). RPSs were several different sizes ranging from 1.7 to 3.5 kbp, and contained several inner repeats of a short stretch (172 bp) called alt, whose repeating number gave rise to variation in the molecular sizes of RPSs (29). The distribution of the sizes of RPSs that were intrinsic to each respective strain allowed to use a RPS as a probe for delineation of the *C. albicans* strain (31).

In this study, we obtained *Candida* yeasts isolated from the oral cavities of HIV-infected patients with oral candidiasis by sampling two colonies from culture isolates. The purposes of this study were to examine the species distribution and genotype diversity of *C. albicans* in Thai HIV-infected patients with oral candidiasis and to analyze the in vitro susceptibility pattern of the isolates against amphotericin B, flucytosine, fluconazole, itraconazole, and ketoconazole by Epsilometer test (Etest). The DNA typing was performed by PFGE, RFLPs from *Sma*I digestion, and hybridization patterns with a *C. albicans* repetitive sequence RPS102.