CHAPTER I

INTRODUCTION

Yeasts are fungi that grow as single cells reproduce by budding. They are distinguished from one another on the basis of the presence or absence of capsules, the size and shape of the yeast cells, the mechanism of daughter formation, the formation of true or pseudohyphae, and the presence or absence of sexual spores, along with physiologic data from biochemical testing [1]. *Candida* species are yeast like fungi that can form true hyphae and pseudohyphae. For the most part, *Candida* species are confined to the human and animal reservoirs; however, they frequently are recovered from the hospital environment, including on foods, counter tops, air-conditioning vents, floors, respirators, and medical personnel. They also are normal commensals of skin, mouth, throat, intestines, respiratory tracts and genitourinary tract. *Candida* is a normal part of the bowel flora. It has many functions inside our digestive tract, one of which is to recognize and destroy harmful bacteria.

Candida species are ubiquitous fungi and the most common fungal pathogens affecting humans. The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the pool of patients at risk and the increased opportunity that exists for *Candida* species to invade tissues normally resistant to invasion.

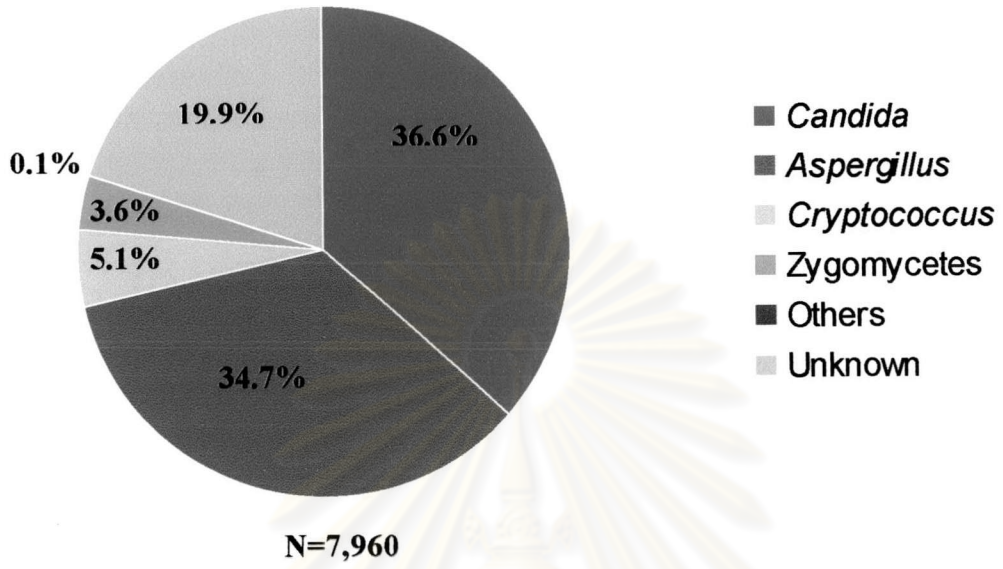
The increased prevalence of local and systemic diseases caused by *Candida* species has resulted in numerous new clinical syndromes, the expression of which primarily is dependent upon the immune status of the host. *Candida* species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to invasive illnesses, such as hepatosplenic candidiasis, *Candida* peritonitis, and systemic candidiasis.

Candida species are most frequently isolated from the oral cavity and are detected in approximately 31 to 55% of healthy individuals. Colonization rates increase with severity of illness and duration of hospitalization [2]. The data on visceral mycoses over the 6 years preceding 1994 showed that *Candida* was the most common causative agent, with 36.6% of the total of 7,960 cases, followed in order by *Aspergillus* (34.7%), *Cryptococcus* (5.1%), and Zygomycetes (3.6%) [3] (Fig.1). *Candida* species are true opportunistic pathogens that exploit recent technological advances to gain access to the circulation and deep tissues. It causes infection when antibiotics or other factors reduce our natural resistance to its overgrowth. Most *Candida* infections are superficial, limited to

mucous membranes. Disseminated infection by *Candida* of internal organs can occur in severely immunocompromised patients, such as those with cancer, serious burns, and acquired immunodeficiency syndrome (AIDS). This is a much more serious condition than superficial *Candida* infection. Disseminated *Candida* infection is usually determined by the presence of *Candida* in the blood, and is potentially life threatening. It should not be confused with superficial *Candida* infections, which are not life threatening. The two classes of infection are similar in that some of the same anti-fungal drugs may be used, and both can be difficult to eradicate [1, 4-7].

Candidiasis is an infection caused by one of the many species of *Candida*. They exist in several different forms during their life spans. One form, the oval "yeast" form, is the basis for the term "yeast infection" that is sometimes used to describe candidiasis. Nosocomial candidiasis is a major infection occurring mostly in patients undergoing prolonged hospitalization due to a variety of underlying conditions [8]. Bloodstream infections due to *Candida* are now regarded as the fourth most frequent cause of septicemia, with a mortality rate of about 50% [9]. Diagnosis of candidemia or hematogenous candidiasis has been problematic due to the low positivity of blood cultures. Even in patients with autopsy-proven systemic candidiasis, the rate of recovery from blood cultures ranged between 40 and 60% [10]. Mucocutaneous candidiasis often occurs in persons with human immunodeficiency virus (HIV) infection: oropharyngeal, esophageal, and vulvovaginal disease. Oropharyngeal candidiasis (OPC) is among the initial manifestations of HIV-induced immunodeficiency to be recognized [11, 12] and typically affects the majority of persons with advanced untreated HIV infection. Presenting months or years before more severe opportunistic disease, oropharyngeal candidiasis may be a sentinel event indicating the presence or progression of HIV diseases [13-15]. Although it is not usually associated with severe morbidity, OPC can be clinically significant. Severe OPC can interfere with the administration of medications and adequate nutritional intake, and may spread to the esophagus [16]. Esophageal candidiasis has been reported to be the most common opportunistic infection in developed countries, presumably due to the decreased incidence of other opportunistic infections since the introduction of highly active antiretroviral therapy (HAART) [17]. Vulvovaginal candidiasis is an important concern for women with HIV infection, although the relationship of vulvovaginal candidiasis to HIV infection remains unclear [18]. Despite the frequency of mucosal disease, disseminated or invasive infections with *Candida* and related yeasts are surprisingly uncommon.

Figure 1. Causative agents for visceral mycoses in autopsy cases [3]



During the past 50 years, the number of new species of *Candida* described increased significantly and currently total approximately 150 have been found [1]. *Candida albicans* (*C. albicans*) is the most common cause of candidiasis in humans. Less frequently, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and several other species may cause diseases. However, more recent epidemiological data reveal a mycological shift from *C. albicans* to the non-*albicans Candida* (NAC) species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii,* and *C. krusei* (Table 1 and 2) [2, 19-24]. Moreover, various reports of the recently described new yeast species *C. dubliniensis* indicate a world wide occurrence of this fungus, which is phenotypically very similar to *C. albicans*[25-29]. The vast majority of *C. dubliniensis* isolates have been recovered from the oral cavities of HIV infected individuals[30, 31]. However, *C. dubliniensis* has also been found as an oral carriage organism and has been implicated as an agent of oral [26, 30-32]. Some *C. dubliniensis* isolates showed higher levels of proteinase and were more adherent to buccal epithelial cells than isolates of *C. albicans* [33]. Current data from the SCOPE (Surveillance and Control of Pathogens of Epidemiological Importance) surveillance system confirm that *Candida* spp. was the fourth leading cause of bloodstream infection [34]. An increasing incidence of *Candida* species infection has been noted in

immunocompromised patients such as intensive care, cancer, post surgical, neutropenic patients and AIDS [2, 14, 19].

NAC species cause 35-65% of all candidaemias in the general patient population. They occur more frequently in cancer patients, mainly in those with haematological malignancies and bone marrow transplant (BMT) recipients (40-70%), but are less common among intensive care unit (ITU) and surgical patients (35-55%), children (1-35%) or HIV-positive patients (0-33%). The proportion of NAC species among *Candida* species is increasing: over the two decades to 1990, NAC represented 10-40 % of all candidaemias. In contrast, in 1991-1998, they represented 35-65% of all candidaemias. The most common NAC species are *C. parapsilosis* (20-40% of all *Candida* species), *C. tropicalis* (10-30%), *C. krusei* (10-35%) and *C. glabrata* (5-40%). Although these four are the most common, at least two other species are emerging: *C. lusitaniae* causing 2-8% of infections, and *C. guilliermondii* causing 1-5%. Other NAC species, such as *C. rugosa, C. kefyr* and *C. famata* are rare, accounting for less than 1% of fungaemias in man [35]. Moreover, Coleman *et al.* [30] and Willis *et al.* [36], reported that *C. dubliniensis* has been recovered from 31.7%, 26% and 14 % of AIDS , HIV-positive and HIV-negative patients, respectively.

Table 1. Distribution of *Candida* species recovered from blood culture in Europe, Canada, Latin America, and the United State, report by SENTRY anti-microbial surveillance program, 1997 [22]

Species	Distribution of *Candida* spp. (% of all isolates)			
	United States (n = 203)	Europe (n = 170)	Canada (n = 61)	Latin America (n = 42)
C. albicans	56	53	53	41
C. parapsilosis	9	21	23	38
C. glabrata	19	12	11	2
C. tropicalis	7	6	8	12
C. guilliermondii	1	4	-	2
C. krusei	2	1	2	-
Candida spp.	6	3	3	5

Table 2. Species distribution of *Candida* bloodstream isolate in the EIEIO program, 1998 to 2001 [23]

Species	N	%
C. albicans	148	58
C. glabrata	51	20
C. tropicalis	28	11
C. parapsilosis	17	7
C. krusei	5	2
Candida spp.	5	2

Antifungal susceptibility varies significantly in contrast to *C. albicans*: some NAC species are inherently or secondarily resistant to azole and polyene. A recent study in Canada reported 53%, 47% and 47% of *C. krusei* isolates were resistant to fluconazole, itraconazole and amphotericin B, respectively [28]. In the same study, 27% and 32% of *C. tropicalis* isolates were resistant to fluconazole and itraconazole. In 2002 [23], 51 *C. glabrata* bloodstream isolates tested, 10% were fluconazole resistant (MIC $\geq$ 64 μg/ml) and 53% were itraconazole resistant (MIC $\geq$ 1 μg/ml). Moran *et al.* [37], 16 isolates were susceptible to fluconazole (MIC range, 0.125-1 μg/ml), while four were more fluconazole resistant (MIC $\geq$ 64 μg/ml). In the same report, stable fluconazole resistant was induced by exposing fluconazole susceptible strains of *C. dubliniensis* to increasing concentrations of fluconazole *in vitro*.

Various laboratory tests based on detection of *Candida*-specific antibodies, antigens, or metabolites have been developed; they all suffer from lack of specificity and/or sensitivity, besides being time-consuming [38]. Moreover, these tests fail to clearly discriminate between the infecting *Candida* species, information that is crucial for initiating specific anti-fungal therapy since several non-*albicans Candida* (NAC) species are known to be inherently less susceptible to commonly used antifungal drugs [39, 40]. Traditional methods used for the identification and typing of clinical isolates of *Candida* include morphological and biochemical analysis, colony morphotyping, resistogram typing, and serotyping [41-45]. These techniques are time-consuming, and their reliance on phenotypic expression makes them potentially unreliable. In order to overcome the limitations of conventional diagnostic tests, DNA-based methods have been developed for the detection of *Candida* species and offer a potentially more sensitive means of diagnosis systemic candidiasis [46]. This method would be one based on genotypic properties. Genotypic methods have been used extensively for the detection and typing of *Candida* strains [47-51]. Recently, a combination of molecular techniques was used to analyze single isolates of eight species of *Candida*, and this allowed the identification of four species [51]. In addition, identification of *C. albicans*, *C. stellatoidea*, *C. tropicalis*, *C. guilliermondii*, *C. glabrata*, *C. parapsilosis* and *C. krusei* has also been achieved by restriction fragment length polymorphism (RFLP) analysis of the ribosomal DNA (rDNA) (genes encoding rRNA) repeat of *Candida* species [52-56]. Therefore, we have developed a PCR-RFLP assay for the identification of seven *Candida* species on the basis of size and variation of the rDNA internal spacer regions.