CHAPTER I

INTRODUCTION

In this recent, the number of HIV infected patients were increase in a past decade year , the rate of *Candida* infection were increasing in the parallel way (1). And now there are many report of *Candida* infection in other patients such as in organtransplant patients and cancer pateints (2). Among the disease caysed by *Candida* infection , the oropharyngeal candidiasis (OPC) was the most frequent opportunistic infection in these patients, almost 90 percent of HIV-infected patients whom deveolped it during the course of the disease (3).

C. albicans is an important opportunistic fungal pathogen of human. It is a normal flora that present in cutaneous , mucocutaneous surface and the intestine. *Candida* species are most frequently isolated from the oral cavity and are detected in approximately 31 to 50 percent of healthy individuals. But it can developed the disease and increase the colonization rate with severity of illness, impaired immunity or in the patient that smoke the tobacco and duration of hospitalization (4-6).

A small number of antifungal agent is now available for treating *Candida* deep infections, based on their chemistry and action , including polyene , triazole , cell wall inhibitor and nucleotide inhibitors. Among the polyenes group, which interact with orgosterol in fungal membrane and leading to alter their membrane permeability, amphotericin B is the most widely used agent for dee-p-seated fungal infection (6). Flucytosine inhibit DNA and RNA synthesis in pathogenic yeast such as *Candida* and *Cryptococcus* spp. And has been available for many years for use in combination with polyene or triazole.(7). But both of these agents have a side effect, amphotericin B is associated with many toxicities and requires intravenous administration, while flucytosine always cause the spontaneous mutation leading to resistance (8). For the triazole group, which specfically inhibit fungal ergosterol biosynthesis , fluconazole , itraconazole and ketoconazole,are used to treat various types of life-threatiening fungal disease (7).

Fluconazole, a water-soluble traizole with greater than 90% bioavaiability after oral administration,has been used extensively to treat a wide range of *Candida* infection worldwide (9).

The repeated use of azoles, especially fluconazole , in treatment of HIV-positive pateints with fungal infection in the period proceding the introduction of highly active antiretroviral therapy has favored the acquistion of azole resistance in several fungal pathogens . These were mostly *Candida* species, including *C. albicans, C. glabrata, C. dubliniensis* and *C. tropicalis*, in the order of decreasing importance and less frequently *Cryptococcus* species (10).

In the recent, the resercher suggest that up to 33% of AIDS patients have and oral commensal strain of *Candida* that is azole resistant (11). Mechanisms of azole resistance have been most extensively investigated in recent years. The resistant mechanisms including over expression of efflux pumps that reduce drug accumulation, alteration of the structure or concentration of antifungal target enzyme and alteration of fungal membrane sterol composition (12).

There are two groups of efflux pump; the major facilitator superfamily (MFS) such as Ben and the ATP binding cassette (ABC) transporter family such as *CDR1* and *CDR 2* (13). Many evidence has been presented that this efflux pumps is associated with azole resistance. From 17 clinical isolated from HIV – infected patients have increased mRNA level of *ERG11, CDR1* and *MDR1*. The increasing of mRNA level were correlated with increased in fluconazole resistance. Recently, there are a report that show the genetic knockouts of these pump in *Candida* result in hypersensitivity to antifungal drug. And the transformed *S. cerevisiae* that was inverted the efflux pump gene show the higher MIC of fluconazole compared with wild type (14). The over expression of efflux pump was up to 85% in *Candida* fluconazole resistant in the same series of same isolates (15).

Other mechanisms of resistance involve the target enzyme of azole, cytochrome P – 450 catalyzing the 14 α demethylation of lanosterol. Alteration in drug target caused by mutations point substitution, which alter the binding affinity to the drug but not the native substrate. (12). In general, azole resistant isolates displaying an alterations near the active site of the enzyme (16). In addition, associated with azole resistant *ERG 11* from matched pairs of azole susceptible and azole resistant *C. albicans* isolates have shown mutations resulting in amino acid changes. The expression of *S.cerevisiae* taransformed by PCR amplified *Erg11* alleles showed mutations coupled with the development of azole resistance (6). In addition, a gene dosing effect of *ERG 11* is a likely cause of azole resistant and has been observed in *C. albicans* and *C. glabrata*

clinical isolates in several reports. (15). But it probably has only a modest effect on the development of resistant. Because the apregration of *ERG 11* dose not exceed factor of three to five in azole resistant isolates (17).

Alteration in sterol composition is one of mechanism that contribute to the azoles resistant *Candida*, although there were very few cases until now. Loss of function mutation in *Erg 3* allels in a known Darlington strain, *C. albicans,* azole resistant isolates was characterized recently (18). And in this case the azole resistant is coupled with resistance to amphotericin B, because ergoterol is absent from these cells (6).

In this study, we cultured a single cell of fluconazole susceptible *Candida albicans* , which colonized the oral cavities of HIV-infected patient, in the medium plus fluconazole. The purpose of this study was to investigate the fluconazole resistant mechanisms in the study strain, *Candida albicans*. The MIC level were detected. The resistant mechanisms were determined by *ERG11* gene sequencing for detecting the point mutation and measure the expression of mRNA of resistant related gene, *CDR1, CDR2, MDR1* and *ERG11.*