EDITORIAL

GAINING MORE INSIGHT INTO THE DETERMINANTS OF *CANDIDA* SPECIES PATHOGENICITY IN THE ORAL CAVITY

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***Candida* infection (candidiasis) is potentially life threatening and can occur in almost all anatomical sites, including the mouth. *Candida* species are in fact the most common fungal pathogens isolated from the oral cavity and frequently cause superficial infections such as oral candidiasis and denture-associated erythematous stomatitis. Whilst systemic dissemination of *Candida* from intraoral foci is rare and largely due to severe deficits of the host immune defenses, the development of localized oral candidiasis is most commonly related to a variety of non-immune determinants such as *Candida* virulence factors and permissive oral microenvironment. In particular, phenotypic switching and dental biofilm have emerged as major determinants for the pathogenicity of *Candida* and are currently the subject of intense research. An understanding of the molecular aspects underlying the biological behavior of *Candida* will be the key to the development of effective preventive as well as therapeutic measures for invasive and oral candidiasis.**

Candida inhabits various parts of the human body including the epidermis, vagina, gastro-intestinal tract, nails and oral cavity (1). The diseases caused by *Candida* became common in the late 19th and 20th centuries and its prevalence is still increasing worldwide as a result of multiple factors which can facilitate the conversion of its commensal level to the parasitic level (2). According to Scardina et al. (3), the risk factors that enhance the severity of a candidal infection can be found widely in patients with impaired salivary gland, drug abusers, immunocompromised, high carbohydrate diet, smoking habits and Cushing's syndrome. Candidal infection can occur in almost all human organs. However, it is the systemic infection that can be much more severe and may lead to mortality. According to Leroy et al. (4), the mortality rate due to systemic infection of *Candida* is up to 60% and still increasing. The treatment

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of candidal infection can be difficult and most of the diagnoses can only be achieved by autopsy. With the current incidence in Europe on the rise, there have been reports of a 5-fold increase in candidemia in the last ten years (5).

Candida has been identified as the common member of the oral microflora and estimated to be present in approximately 40-60% of the general population. It can be present either as transient or permanent colonizer in the oral cavity (6). It is also recognised as an opportunistic microorganism that has the ability to cause oral diseases, such as oral candidiasis (7).

The most common oral condition caused by *Candida* is oral candidiasis (8). In a most recent study, candidal infection was also associated with oral cancer, burning mouth syndrome, endodontic diseases and taste disorder (1). *Candida albicans* is the main causative agent of oropharyngeal candidiasis. Researchers have, however, found that non-albicans species also contributed significantly to the development of oral candidiasis (9). Cases due to non-albicans species are increasing in number and this has raised great concern to society.

Candida is identified to colonise several types of host cells including epithelial, endothelial and phagocytic cells. In the oral cavity, *Candida* prefers to colonise several surfaces including the buccal and labial mucosa, dorsum or lateral borders of tongue, hard and soft palate regions, tooth surfaces and denture-bearing areas (10). This colonising ability is contributed by factors including the ability of oral *Candida* to produce specific enzymes such as agglutinin-like proteins and integrin-like proteins that lead to the formation of biofilm on oral surfaces. In addition, other factors that influence the colonisation of *Candida* are the reduction of salivary flow, low salivary pH, trauma, carbohydrate-rich diets and epithelial loss (11).

Here we distinguish between two categories of pathogenic determinants: extrinsic determinant, i.e. those provided by the host, which are permissive for growth and survival of *Candida*; and intrinsic determinants, i.e. those related to the characteristics of *Candida* species. Oral biofilm, which *stricto sensu* belongs to the first group, has been considered as an intrinsic determinant in that it relies on the ability of *Candida* to interact with the oral microflora.

GROWTH REQUIREMENT OF CANDIDA SPECIES

Availability of nutrients

Candida is a chemoheterotrophic organism that requires carbon and nitrogen for growth. According to Madigan and Martinko (12), the mutual interaction of carbon and nitrogen is important in the metabolism of microorganisms. Carbohydrates are the most readily utilised form of carbon in both oxidative and non-oxidative pathway. Thus, the presence of carbohydrates influences the colonisation of *Candida* in the oral cavity. Certain carbohydrates, such as sucrose and glucose, have been shown to increase the adhesion potential of *Candida albicans* towards hard and soft surfaces of the oral cavity. Glucose is an acid promoter that leads to the reduction of pH in the oral environment and as a consequence, activates acid proteinases and phospholipases, which enhance the adherence capability of *Candida*. In addition, the production of mannoprotein surface layer in an environment where glucose is present has been shown to assist the adherence capability of *Candida* including *C. krusei* in the oral cavity (7).

Candida has a nitrogen content of around 10% of their dry weight (7). The source of nitrogen is usually provided by organic compounds which can be easily found in the oral environment. Nitrogen is also determined as the main stimulatory factor in yeast extract as it encourages bio-stimulation of microbial growth.

Influence of oral fluids

Saliva provides moisture and helps in lubricating the oral cavity. Furthermore, it also provides indigenous organic constituents including antimicrobial factors such as lysozyme, lactoferrin, calprotectin, lactoperoxidase, cystatins, histatins, VEGh and SLPI and chromogranin A which inhibit the growth of oral pathogens (13). The presence of cytokines, such as IL-17 and immunoglobulins, in saliva are also beneficial to the oral cavity as they inhibit the dissemination of oral microorganisms especially *Candida* species (14).

Saliva also introduces the formation of a thin film approximately 0.1 mm deep over all external surfaces in the oral cavity. The major role of the whole saliva is to maintain the integrity of teeth by

clearing off food debris and buffering the potential damaging acids produced by oral biofilm or dental plaque. The chemical composition of secretions from each gland is different. Bicarbonate, phosphates and peptides are examples of buffering agents in the saliva that give normal saliva a mean pH of 6.75 to 7.25 (7).

The flow rate of saliva is under the influence of circadian rhythms where the lowest flow rate has often been recorded during sleeping. Low flow rate of saliva reduces the protective function of saliva and increases the colonisation and development of microorganisms including *Candida*. Salivary composition is also affected by circadian rhythms, for example the total concentration of protein in whole saliva during resting time is estimated at 220 mg/100 mL, whereas the total protein in stimulated saliva is estimated at 280 mg/10 mL. The difference in the amount of protein may affect the distribution of the normal microflora in the oral cavity, as some proteins are known to serve as receptors in the colonisation of microorganisms to the saliva-coated surfaces of the teeth (7). Proteins and glycoproteins such as mucin in the saliva act as the primary source of nutrients for resident microflora including *Candida*. In addition to adherence, some proteins are also involved in the host's defence mechanism by aggregating exogenous microorganisms, hence, facilitating their clearance from the mouth during swallowing or spitting.

In addition to saliva, the gingival crevicular fluid (GCF) in the oral cavity can also influence the colonisation of oral *Candida* species. The flow of GCF is slow at healthy sites but increases drastically at areas with gingivitis by 147% and up to 30-fold at areas with advanced periodontal diseases. GCF also has a role in the development of subgingival plaque around and below the gingival margin. Moreover, it contains higher total protein compared to saliva which is capable of providing nutrient to several commensal microorganisms in the oral cavity (7). Among the host defence components in GCF are IgG and neutrophils which are directed specifically against important periodontal microorganisms and inhibit the colonisation by the action of opsonisation or activation of complement cascade (15).

Role of body temperature

The optimum growth temperature for *Candida* species including *C. albicans* has been shown to range from 30°C to 37°C (16). This range of temperature is also the optimum temperature of various pathogenic microorganisms in the oral cavity. Any alteration in the normal body temperature may influence the competitiveness among the normal microflora to survive which will then enhance the development of opportunistic microorganisms such as *Candida*. Nonetheless, many experimental assays were conducted at 37°C and this is generally accepted as the standard incubation temperature for *Candida* species (7).

Intrinsic pathogenic determinants of Candida species

The virulent factors of each different *Candida* species are not similar and can be a competitive factor between each different species. Among the important virulent factors of *Candida* species are phenotypic switching, adhesion (both to extracellular matrix and dental biofilm), cell surface hydrophobicity, and enzyme production.

Phenotypic switching

Two mechanisms are postulated to be involved in the ability of *Candida* to survive and adapt in a suppressed environment. The first is by undergoing mitotic recombination and the second is by carrying out phenotypic switching. A direct consequence of mitotic recombination is the loss of heterozygosity throughout the entire genome. This deletion of genome, however, affects the viability of *Candida* especially in the multiple changing conditions (17). Phenotypic switching, on the other hand is a phenomenon that occurs as a result of changes in the growth environment. A severely suppressed growth condition may lead to high frequency switching in candidal cells (Fig. 1). This adaptation is associated with the alteration of gene expression which eventually may lead to alteration of adhesiveness, susceptibility and the resistance of candidal cells to phagocytosis and polymorphonucleur (PMN) leukocyte. This mechanism of action does not involve deletion of any candidal genome, thus, the heterozygosity of the entire genomic are well maintained (7).

Phenotypic switching in *Candida albicans* was first defined in 1985 as the capacity to undergo

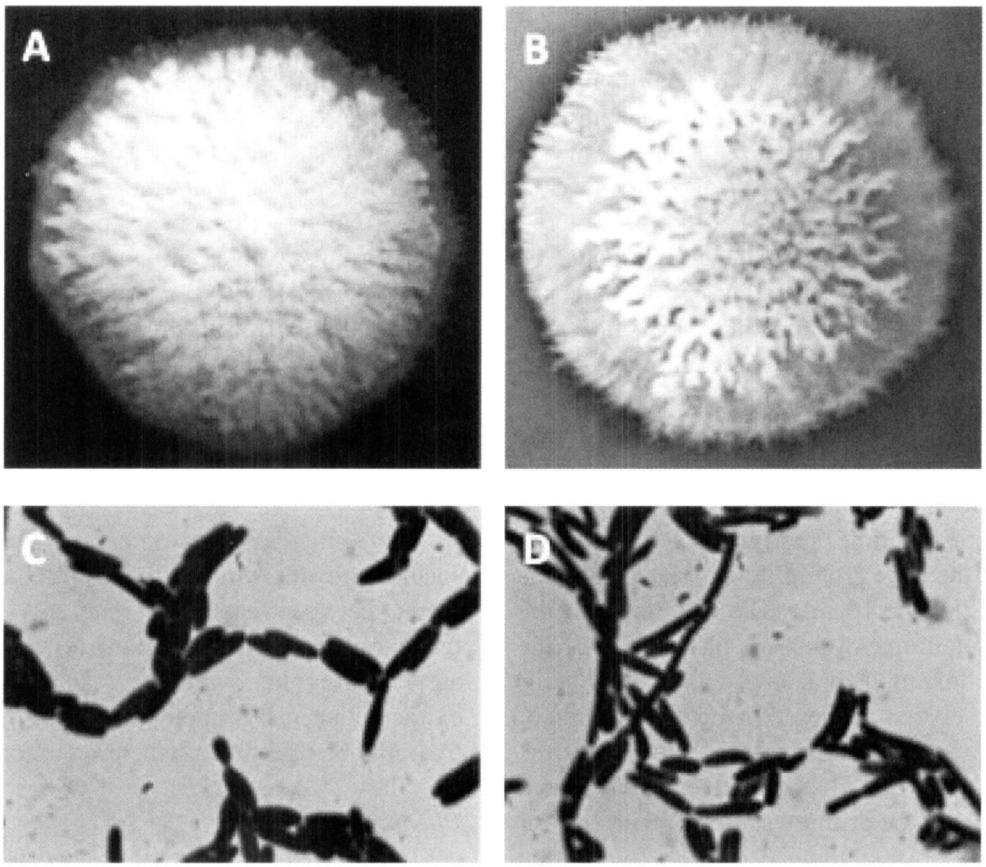

Fig. 1. ***A, B)*** *Colony morphology of the unswitched and switched Candida krusei at 10x magnification using stereoscope. A) Unswitched, (B) switched generation.* ***C, D)*** *Unswitched (C) and switched* ***(D)*** *Candida krusei examined at 100x magnification using a light microscope; note Blastoconidia and Pseudohyphae.*

spontaneous, reversible transitions between a set number of colony morphologies (18). This phenomenon is now recognized as an important technique for the survival of *Candida* within an environment such as the oral cavity. This mechanism enables *Candida* to adapt in a suppressed environment and to develop as dominant in the host. *Candida* can undergo reversibly high frequency of phenotypic switching which increases the survivability of the pathogen (17).

Phenotypic switching is identified as one of the important virulent factors in *C. albicans* (17) *C. glabrata* (19) and *C. krusei* (20). The significance of the switching strategy is in a way similar to the human immunity function whereby it is aimed to counter threats in the host's environment. Therefore, scientists have suggested that phenotypic switching mechanism does enhance the survivability of *Candida* by rapidly changing its phenotype as an adaptive response to the suppressed environment (21).

Phenotypic switching may influence the normal physiological growth of *Candida* species such as *C. albicans* (17). Under the smooth white and wrinkle phenotypes, *C. albicans* has been shown to exhibit faster growing colonies compared to when it is in the form of heavy myceliated with ring phenotype. In addition, phenotypic switching is also discovered to be able to alter the adhesive properties of *Candida*. Findings by our group (20) demonstrated

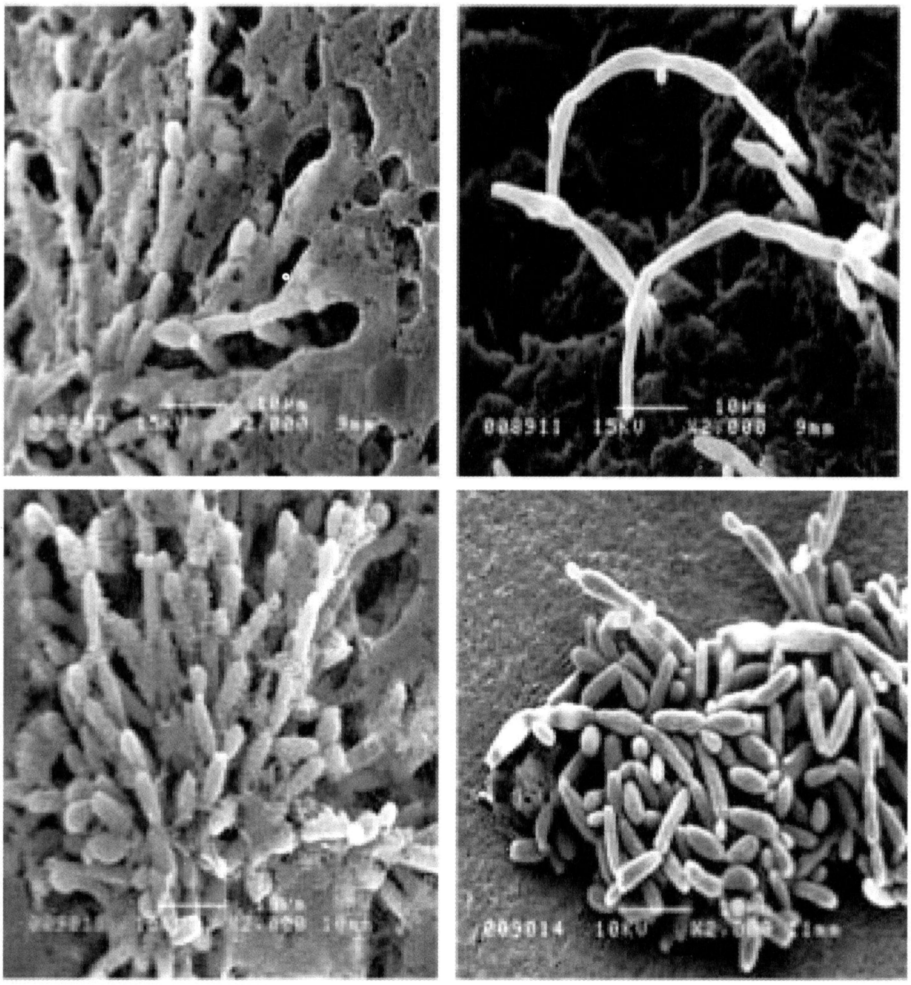

Fig. 2. *SEM micrographs of Candida krusei observed for the various growth generations at 2000x magnification. Note pseudohyphae (upper right panel) and attachment on extracellular matrix (lower right panel).*

that the adherence ability of second generation switched *C. krusei* was increase significantly in flow cell supplemented with unstimulated saliva. Furthermore, this virulence attribute may also induce the formation of tube and pseudohyphae in *Candida,* which enhances the adherence capacity of the candidal strains (19).

Adhesion: key role of the extracellular polymeric matrix

The adherence ability of *Candida* is an important factor in the initiation of oral candidiasis. Adherence can occur either on the hard tissue surfaces, such as teeth and palatal surface, or on soft surfaces, such as the buccal and lingual surfaces (22). Characteristics of *Candida* that contribute to the adherence on these surfaces include the formation of pseudohyphae and extracellular matrix.

A single filament hypha (plural, hyphae) is a long branching filamentous structure of fungus which can be found easily in the developmental phase of *Candida* (12). It is classified as the main mode of vegetative fungal growth and consists of one or more cells that are surrounded by tubular cell walls made of chitin. Hyphae usually grow together to form compact tufts which are known as mycelium. Hyphae formation is usually referred to the germination phase of fungi. However, it is also involved in the colonisation of the target host. Pseudohyphae are distinguished from true hyphae by their method of

growth, which lacks cytoplasmic connection between the cells. The pseudohyphae of *Candida* are usually found to possess incomplete budding blastoconidia whereby cells remain attached to the mother cells after division. *C. albicans* and *C. krusei* have been recognised to develop pseudohyphae which adhere to the monolayer of human epithelial cells and hard surfaces (20).

In many cases, extracellular polymeric substance (EPS) matrix is also produced by oral microorganisms once they are adhered to the oral surfaces. EPS matrix is a network of non-living mass which provides support to cells including *Candida* (Fig. 2). The presence of EPS matrix, which has a slimy texture, provides a significant role to support attachment and proliferation of the cells (23). Furthermore, the synthesis of EPS has also been found to increase significantly when exposed to liquid flow (24). This anchorage property assists the colonisation of *Candida* to hard tissue surfaces and thus, contributes to the formation of biofilm. When in a biofilm, the resistance of candidal species towards various antifungal agents, including amphotericin B, voriconazole and ketoconazole, has been found to increase up to 1000-fold compared to planktonic stage (24, 25). Mitchell et al. (26) recently found that the presence of matrix ß-1,3 glucan in EPS matrix sequesters antifungal drug which then increases the resistance to fluconazole.

Dental biofilms

Biofilm production is considered a potential virulence factor of some *Candida* species (27). Dental biofilm is defined as a thin layer comprising of various communities of microorganisms including bacteria, fungi and yeast that are attached to oral surfaces and on the surface of prosthesis, including dental acrylic surfaces and human epithelial cells. Microorganisms in the biofilm are enclosed in a matrix of extracellular polymeric substance (EPS). This biofilm provides protection to the microorganisms and facilitates the interaction among each other with the contribution of enzymes such as catalase and superoxidase dismutase (11, 28). The development of biofilm is dependent on the dietary, salivary and oral environmental factors that interact with the microorganisms within the community of the biofilm.

The formation of biofilm has been shown to reduce the susceptibility of microorganism to antimicrobial agents, which may then lead to the increase in pathogenicity (11). This phenomenon is suggested to occur due to the restriction of the antimicrobial agents to penetrate the matrix of the biofilm which then reduces the susceptibility of the target microorganism (29). Furthermore, the presence of transcription factor Efg1 in *C. albicans* biofilm has also been reported to induce the tolerance toward miconazole, caspofungin and amphotericin B (30).

The development of dental biofilm involves several stages which are the acquired pellicle formation on the oral surface; adhesion, reversible and irreversible interactions between the pellicle and the colonising microbes; co-aggregation between microorganisms; and detachment of microbes from the oral surfaces. These sequences of events may eventually form a structural and functional organised microbial community that, if allowed to accumulate, may enhance the potential of periodontal disease and dental caries (28). Specifically, co-aggregation or co-adhesion has been suggested to involve *Candida* in the late stage of oral biofilm formation. This is a process of microbial adhesion involving the late colonisers on to the early colonisers of dental biofilm. It is a phenomenon of cell-to-cell recognition of genetically distinct partner cell types (31). The co-aggregation can be facilitated either through intragenerics such as the interaction between *S. sanguis* and *Actinomyces* sp. or intergenerics such as the interaction between *Streptococcus* sp. or *Actinomyces* sp. and *Prevotella* sp. *C. krusei* has been found to be involved in co-aggreagation with *S. mutans, S. sanguis* and *S. salivarius* in the presence of sucrose. *C. albicans* has also been reported to have high coaggregation with *S. sanguinis*, *S. oralis* and *S. gordonii* (32). Protein such as lectin is usually involved in co-aggregation. This carbohydrate-binding protein attaches to the carbohydrate-binding protein receptors of other cells which then contribute to the increased thickness of the dental biofilm.

Once a climax community is achieved in the biofilm, detachment of some microbes may occur in the final stage of the oral biofilm development The microorganism is released from the matrix of the biofilm to the fluid surrounding the biofilm, a

process which has been reported to be facilitated by several enzymes such as proteases, fluid shear stress, multivalent cross-linking cations and microbial growth status (33). This process of detachment will, however, help the microorganism colonise other surfaces in the oral cavity. An example of a microorganism involved in the detachment process from the oral biofilm is *Prevotella loescheii* which produces proteases that hydrolyse adhesion-associated fimbriae which is important in its co-aggregation with *S. oralis* (31). Furthermore, the detachment stage can also be initiated due to the presence of certain quorum sensing molecules such as farnesol, which has been found to be related to biofilm-self-limitation. The level of farnesol increases proportionally to the number of *Candida* cells until threshold where the molecule starts to suppress the yeast-to-mycelium conversion of newly budding cells. As a result, the adherence is reduced within the architecture of biofilm, and releasing yeast forms *Candida* during the dissemination stage (34).

Cell surface hydrophobicity

The virulence factor of *C. krusei* can also be observed from the cell surface hydrophobicity characteristic. This factor is classified as one of the most important adherence mechanisms in the colonisation of the host surface, as well as in denture-related candidiasis (22). In fact, one of the key properties contributing to the initial adherence to the solid surfaces of acrylic dentures are hydrophobic interactions, and this feature has salient clinical implications for prevention and therapy of denture-related candidiasis. Various experimental approaches have been used to examine the mechanisms of hydrophobic interactions between *Candida* species and solid surfaces. The hydrophobic nature of the denture surface has been cited as a factor in the development of new bactericidal materials (35).

C. krusei is more hydrophobic compared to other medically important *Candida* species (22). *C. krusei* was reported to possess the same hydrophobicity level as *C. glabrata* and *C. Tropicalis,* but is more hydrophobic compared to *C. albicans* and *C. parapsilosis.* Super-hydrophilic surfaces have been reported to accept few bacterial or fungal cells (35) and could be a potent method for the reduction of the adherence of relatively hydrophobic fungal cells, particularly the hyphal form of *C. albicans* which causes denture stomatitis and related infections.

Enzymatic activity

Hydrolytic enzymes of *Candida* have been reported to contribute to its pathogenicity in causing oral diseases such as oral candidiasis. The enzymes include aspartyl proteinase, phospholipases, lipases, phosphomonoesterase and hexosaminidase (1). Among these enzymes, aspartyl proteinase has attracted most interest and is widely considered to be central in the development of candidal infection. Aspartyl proteinase is a hydrolytic enzyme which is secreted by the transcription and translation of sphingolipid activator protein (SAP) gene. This enzyme has the ability to invade host and also contributes as a defence system of yeast. Examples of candidal species possessing this enzyme are *C. albicans* and *C. krusei* (22).

Another important hydrolytic enzyme is phospholipase which is identified as an enzyme that invades the host tissue. This enzyme activity has been observed in many fungal pathogens including *Candida.* There are 4 types of phospholipases, namely A, B, C and D. Phospholipase A and C can be found in *C. albicans*; however, there is no evidence that shows the presence of phospholipase B and D in candidal species (22). Phospholipase A can attack cell membranes and can be easily found on the cell surface especially at the sites of bud formation. Hence, the enzyme activity can be enhanced when the hyphae are in direct contact with the host tissue (1).

CONCLUSIONS

Candidiasis is an ubiquitous infectious disease and its incidence has been increasing over the last few years, not only in immunocompromised patients, thus becoming a public health problem. Knowledge of factors that affect the virulence of the *Candida* strains is essential, and the oral cavity provides an ideal environment to study not only the intrinsic characteristics of *Candida*, but also their interactions in a complex environment such as the oral biofilm. An understanding of the molecular aspects underlying the biological behavior of Candida will be the key to the development of effective preventive as well as

therapeutic measures for invasive and oral candidiasis.

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