INTRODUCTION

Fungi are free-living, eukaryotic organisms that exist as yeasts (round fungi), moulds (filamentous fungi), or a combination of these two (dimorphic fungi). Oral candidiasis is one of the common fungal infection, affecting the oral mucosa. These lesions are caused by the yeast Candida albicans. Candida albicans are one of the components of normal oral microflora and around 30% to 50% people carry this organism. Rate of carriage increases with age of the patient. Candida albicans are recovered from 60% of dentate patient’s mouth over the age of 60 years. There are many types of Candida species, which are seen in the oral cavity.[1,2] Species of oral Candida are: C. albicans, C. glabrata, C. guilliermondii, C. krusei, C. parapsilosis, C. pseudotropicalis, C. stellatoidea, C. tropicalis.[3]

Proposed revised classification of Oral Candidosis[4]

Primary oral candidosis (Group I)

- Acute
  - Pseudomembranous
  - Erythematous

Secondary oral candidoses (Group II)

Oral manifestations of Systemic mucocutaneous. Candidosis (due to diseases such as thymic aplasia and candidosis endocrinopathy syndrome).

RISK FACTORS

Pathogen

Candida is a fungus and was first isolated in 1844 from the sputum of a tuberculosis patient.[5] Like other fungi, they are non-photosynthetic, eukaryotic organisms with a cell wall that lies external to the plasma membrane. There is a nuclear pore complex within the nuclear membrane. The plasma membrane contains large quantities of sterols, usually ergosterol. Apart
from a few exceptions, the macroscopic and microscopic cultural characteristics of the different candida species are similar. They can metabolize glucose under both aerobic and anaerobic conditions. Temperature influences their growth with higher temperatures such as 37°C that are present in their potential host, promoting the growth of pseudohyphae. They have been isolated from animals and environmental sources. They require environmental sources of fixed carbon for their growth. Filamentous growth and apical extension of the filament and formation of lateral branches are seen with hyphae and mycelium and single cell division is associated with yeasts. Several studies have demonstrated that infection with candida is associated with certain pathogenic variables. Adhesion of candida to epithelial cell walls, an important step in initiation of infection, is promoted by certain fungal cell wall components such as mannose, C3d receptors, mannoprotein and saccharins. Other factors implicated are germ tube formation, presence of mycelia, persistence within epithelial cells, endotoxins, induction of tumor necrosis factor and proteinases. Phenotypic switching which is the ability of certain strains of C. albicans to change between different morphologic phenotypes has also been implicated.

**Host**

**Local factors**

Impaired salivary gland function can predispose to oral candidiasis. Antimicrobial proteins in the saliva such as lactoferrin, sialoperoxidase, lysozyme, histidine-rich polypeptides and specific anti-candida antibodies, interact with the oral mucosa and prevent overgrowth of candida.

Drugs such as inhaled steroids have been shown to increase the risk of oral candidiasis by possibly suppressing cellular immunity and phagocytosis. The local mucosal immunity reverts to normal on discontinuation of the inhaled steroids. Dentures predispose to infection with candida in as many as 65% of elderly people wearing full upper dentures. Wearing of dentures produces a microenvironment conducive to the growth of candida with low oxygen, low pH and an anaerobic environment. This may be due to enhanced adherence of Candida sp. to acrylic, reduced saliva flow under the surfaces of the denture fittings, improperly fitted dentures, or poor oral hygiene. Other factors are oral cancer/leukoplasia and a high carbohydrate diet. Growth of candida in saliva is enhanced by the presence of glucose and its adherence to oral epithelial cells is enhanced by a high carbohydrate diet.

**Systemic factors**

Extremes of life predispose to infection because of reduced immunity. Drugs such as broad spectrum antibiotics alter the local oral flora creating a suitable environment for candida to proliferate. Immunosuppressive drugs such as the antineoplastic agents have been shown in several studies to predispose to oral candidiasis by altering the oral flora, disrupting the mucosal surface and altering the character of the saliva. Other factors are smoking, diabetes, Cushing’s syndrome, immunosuppressive conditions such as HIV infection, malignancies such as leukemia and nutritional deficiencies – vitamin B deficiencies have been particularly implicated.

**LABORATORY DIAGNOSIS OF ORAL CANDIDIASIS**

**Specimen collection**

The specimen should be collected from an active lesion; old ‘burned out’ lesions often do not contain viable organisms.

Collect the specimen under aseptic conditions.

Collect sufficient specimen.

Use sterile collection devices and containers

Label the specimen appropriately; all clinical specimens should be considered as potential biohazards and should be handled with care using universal precautions.

The specimen should be kept moist or in a transport medium and stored in a refrigerator at 4°C. Due to variety of clinical forms of oral candidiasis, a number of different types of specimens may be submitted to the laboratory.

**Smear**

Smears are taken from the infected oral mucosa, rhagades and the fitting side of the denture, preferably with wooden spatulas. Smears were fixed immediately in ether/alcohol 1:1 or with spray fix. Dry preparations may be examined by Gram stain method and periodic acid Schiff (PAS) method.

**Swabs**

Swabs are seeded on Sabouraud’s agar (25°C or room temperature), on blood agar (35°C), on Pagano-Levin medium (35°C) or on Littmann’s substrate (25°C). Incubation at 25°C is done to ensure recovery of species growing badly at 35°C. Sabouraud’s dextrose agar is frequently used as a primary culture medium. Since mixed yeast infections are seen in the oral cavity more frequently than previously thought, particularly in immunocompromised or debilitated patients, Pagano-Levin agar or Littmann’s substrate, are useful supplements, because they enable distinction of yeasts on the basis of difference in colony color.

**Biopsy**

Biopsy specimen should in addition be sent for histopathological examination when chronic hyperplastic candidosis is suspected.

**Imprint culture technique**

Sterile, square (2.2 × 2.5 cm), plastic foam pads are dipped in peptone water and placed on the restricted area under study for 30-60 seconds. Thereafter the pad is placed directly on
Pagano-Levin or Sabouraud’s agar, left in situ for the first 8 hours of 48 hours incubation at 37°C. Then, the candidal density at each site is determined by a Gallenkamp colony counter and expressed as colony forming units per mm² (CFU mm⁻²). Thus, it yields yeasts per unit mucosal surface. It is useful for quantitative assessment of yeast growth in different areas of the oral mucosa and is thus useful in localizing the site of infection and estimating the candidal load on a specific area (Budtz-Jorgensen, 1978, Olsen and Stenderup A, 1990).\[16,18\]

**Impression culture technique**

Taking maxillary and mandibular alginate impressions, transporting them to the laboratory and casting in 6% fortified agar with incorporated Sabouraud’s dextrose broth. The agar models are then incubated in a wide necked, sterile, screw-topped jar for 48-72 hours at 37°C and the CFU of yeasts estimated.\[19\]

**Saliva**

This simple technique involves requesting the patient to expectorate 2 ml of mixed unstimulated saliva into a sterile, universal container, which is then vibrated for 30 seconds on a bench vibrator for optimal disaggregation. The number of Candida expressed as CFU/ml of saliva is estimated by counting the resultant growth on Sabouraud’s agar using either the spiral plating or Miles and Misra surface viable counting technique. Patients who display clinical signs of oral candidiasis usually have more than 400 CFU/mL.\[19\]

**Oral rinse technique**

It was first described by Mekendrik, Wilson and Main (1967) and later modified by Samaranyake et al. (1968).\[20\]

**Paper Points**

An absorbable sterile point is inserted to the depth of the pocket and kept there for 10 sec and then the points are transferred to a 2 ml vial containing Moller’s VMGA III transport medium, (which also facilitates survival of facultative and anaerobic bacteria).\[16\]

**Commercial identification kits**

The Microstix-candida (MC) system consists of a plastic strip to which is affixed a dry culture area (10 mm \(\times\) 10 mm) of modified Nickerson medium (Nickerson, 1953) and a plastic pouch for incubation. The O Yeast-I dent system is based on the use of chromogenic substances to measure enzyme activities. Ricult-N dip slide technique is similar to, but of higher sensitivity than MC system.\[21\]

### Histological identification

Demonstration of fungi in biopsy specimens may require several serial sections to be cut.\[16\] Fungi can be easily demonstrated and studied in tissue sections with special stains. The routinely used Hematoxylin and Eosin stain poorly stains Candida species. The specific fungal stains such as PAS stain, Grocott-Gomori’s methenamine silver (GMS) and Gridley stains are widely used for demonstrating fungi in the tissues, which are colored intensely with these stains.\[17\]

### Physiological tests

The main physiological tests used in definitive identification of Candida species involve determination of their ability to assimilate and ferment individual carbon and nitrogen sources.\[17,22\]

The assimilation reactions and fermentation reactions of Candida species are tabulated in Tables 1 and 2.

#### Phenotypic methods\[22,23\]

#### Serotyping

Serotyping is limited to the two serotypes (A and B), a fact that makes it inadequate as an epidemiologic tool. It has recently been shown that there can be wide discrepancies in the results obtained with different methods of serotyping.

#### Table 2: Fermentation reactions

| Candida species | Glucose | Maltose | Sucrose | Lactose |
|-----------------|---------|---------|---------|---------|
| C. albicans     | AG      | AG      | –       | –       |
| C. tropicalis   | AG      | AG      | –       | –       |
| C. kefyr        | AG      | AG      | –       | –       |
| C. guilliermondii | AG    | –      | AG      | –       |
| C. parapsilosis | AG      | –       | –       | –       |
| C. krusei       | AG      | –       | –       | –       |
| C. glabrata     | AG      | –       | –       | –       |

\+: Positive reaction, –: Negative reaction, A: Acid production, G: Gas production

### Table 1: Assimilation reactions

| Candida species | Glu | Mal | Suc | Lac | Cel | Gal | Tre | Raff | Mel | Xyl | Ino | Dul |
|-----------------|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|
| C. albicans     | +   | +   | +   | +   | +   | +   | +   | –    | –   | +   | –   | –   |
| C. tropicalis   | +   | +   | +   | –   | +   | +   | +   | –    | –   | –   | +   | –   |
| C. kefyr        | +   | –   | +   | +   | +   | +   | +   | –    | +   | –   | +   | –   |
| C. parapsilosis | +   | +   | +   | –   | –   | +   | +   | +    | +   | –   | +   | –   |
| C. guilliermondii | +  | +   | +   | +   | –   | –   | +   | –    | +   | +   | –   | –   |
| C. krusei       | +   | –   | +   | +   | –   | –   | –   | –    | +   | +   | –   | –   |

Glu: Glucose, Mal: Maltose, Suc: Sucrose, Lac: Lactose, Cel: Cellobiose, Gal: Galactose, Tre: Trehalose, Raf: Raffinose, Mel: Melibiose, Xyl: Xylose, Ino: Inositol, Dul: Dulcitol, +: Positive reaction, –: Negative reaction
Resistogram typing
Resistograms do not correlate with pathogenic potential and even though improvements have been made in the method growth end-points often present problems because of inoculum size, interpretation and reproducibility.

Yeast ‘Killer Toxin’ typing
These authors initially used nine killer strains, developing a triplet code to distinguish between 100 strains of *C. albicans* and found 25 killer-sensitive types. This method was expanded by using 30 killer strains and three antifungal agents, which appeared to discriminate between sufficient numbers of strains of *C. albicans*.

Morphotyping
This method has been used in a study of the morphotypes of 446 strains of *C. albicans* isolated from various clinical specimens.

Biotyping
Williamson (1987) has proposed a simpler method. This system comprised three tests, the APIZYM system, the API 20C system and a plate test for resistance to boric acid. This system was found to distinguish a possible 234 biotypes, of which 33 were found among the 1430 isolates of *C. albicans* taken from oral, genital and skin sites.

Protein typing
Non-lethal mutations of proteins during the yeast cell cycle yield proteins of differing physical properties between strains, which may be distinguishable by one or two dimensional gel electrophoresis. These methods have been used to separate *C. albicans* at the subspecies level.

Genetic methods
The earliest molecular methods used for fingerprinting *C. albicans* strains were karyotyping, restriction endonuclease analysis (REA) and restriction fragment length polymorphism (RFLP). In arbitrarily primed polymerase chain reaction (AP-PCR) analysis (synonym: randomly amplified polymorphic DNA (RAPD) analysis), the genomic DNA is used as a template and amplified at a low annealing temperature with use of a single short primer (9 to 10 bases) of an arbitrary sequence.[22]

Serological tests[23]

Serological tests for invasive candidiasis
- Detection of antibodies
- Slide agglutination
- Immunodiffusion
- Phytohemagglutination
- Coelctosynsris
- Immunoprecipitation
- A and B immunofluorescence
- Nonspecific Candida Antigens
- Latex agglutination
- Immunoblotting
- Cell Wall Components
- Cell Wall Mannoprotein (CWMP)
- b-(1,3)-D-glucan
- Candida Enolase Antigen testing.

Immunodiagnosis[17]
The use of specific antibodies labeled with fluorescent stain permits causative organisms to be diagnosed accurately within minutes. However, the preparation of specific antisera and purified polyclonal or monoclonal antibodies entails a much more extensive technical outlay, so the application of these reagents need only be considered when a very precise diagnosis is of therapeutic consequence (Olsen and Stenderup, 1990). The usefulness of antibody testing in the diagnosis of oral candidosis when other simpler, sensitive and reliable techniques are available is questionable (Silverman *et al.*, 1990).

MANAGEMENT[4]
Assessment of predisposing factor plays a crucial role in the management of candidal infection. Mostly the infection is simply and effectively treated with topical application of antifungal ointments. However in chronic mucocutaneous candidiasis with immunosuppression, topical agents may not be effective. In such instances systemic administration of medications is required [Tables 3 and 4].[4]

CONCLUSION
Yeast-free diets, or people, are both impossible to come by. They can only be totally avoided in the diet by eating solely fresh dairy, meat, fish and peeled fresh fruits and vegetables. From a practical standpoint, this is neither feasible nor necessary. Total elimination of yeast from the body is also neither feasible nor desirable, considering that yeasts are beneficial to the body.

Table 3: Topical antifungal medications[4]

| Dosage form/strength | Indication         |
|----------------------|-------------------|
| Miconazole cream 2% (OTC) | Angular cheilitis |
| Clotrimazole cream 1% (OTC) | Angular cheilitis |
| Ketoconazole cream 2% (Prescription) | Angular cheilitis |
| Nystatin ointment 100,000 units/gram (prescription) | Angular cheilitis |
| Nystatin topical powder 100,000 units/gram (prescription) | Denture stomatitis |
| Nystatin oral suspension 100,000 units/gram (prescription) | Intraoral candidiasis |
| Betamethasone dipropionate clotrimazole cream (prescription) | Intraoral candidiasis |
| Clotrimazole troches 10 mg (prescription) | Intraoral candidiasis |
| Amphotericin B 100 mg/ml (prescription) | Intraoral candidiasis |
when a proper balance exists. Treatment of candida overgrowth does not seek the eradication of candida from the diet or the person, but rather a restoration of the proper and balanced ecological relationship between man and yeast.

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Table 4: Systemic antifungal medications of oropharyngeal candidiasis

| Generic name    | Formulation                          |
|-----------------|--------------------------------------|
| Amphotericin B  | 100 mg/ml oral suspension            |
| Clotrimazole    | 10 mg troche                          |
| Fluconazole     | 100 mg tablet                         |
|                 | 10 mg/ml oral suspension             |
|                 | 40 mg/ml oral suspension             |
| Itraconazole    | 100 mg capsule                        |
|                 | 10 mg/ml oral suspension             |
| Ketoconazole    | 200 mg tablet                         |
| Nystatin        | 100,000 units/ml oral suspension     |
|                 | 200,000 units/ml pastille            |
|                 | 500,000 units/ml tablet              |
|                 | 100,000 units/ml vaginal tablet      |

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