2. REVIEW OF LITERATURE

2.1. Oral health: Health, as per World Health Organization (WHO) in 1946, is defined as "a condition encompassing total physical, psychological and communal, and not simply the lack of ailment or illness". Oral health is an important component in maintaining general health, being essential to assess it enduringly and setting up new methods of reinstating and sustaining it in normal limits. Oral health is an integral element of general health and wellbeing. Oral health is a state of being free from any abnormalities experienced by the mouth that affect the oral cavity. Mouth acts as a casement of our health by showing signs of nutritional deficit or general illness. WHO (2012) stated that systemic diseases are becoming apparent first in the form of mouth lesions or in the form of inconvenience of the mouth. All these scored for the importance of studying oral diseases as the need of the hour. Around the world, developed countries has seen marked development in their oral health but the developing countries like India needs to concentrate more on their oral health especially people belonging to disadvantaged society (Petersen, 2003).

2.2. Oral cavity: The human oral cavity consists of different elements such as teeth, tongue, gingiva, palate, lips, cheeks, and floor of the mouth (Figure 1). The oral cavities are flowed by saliva. A thin layer called pellicle, formed on the oral surfaces by the glycoproteins which include salivary mucins and secretory IgA (Gibbins et al., 2013). The mucosal and epithelial cells lined the shedding surfaces in the mouth, except the non-shedding surfaces (teeth and prostheses). Both the surfaces are more prone to microbial colonization since our body contains a tenfold higher number of microbial cells than human cells. (Bianconi et al., 2013; MacDougall, 2012).

The oral cavity harbors a diverse microbiome, and different microbial community harbors different niches in the mouth (Dewhirst et al., 2010; Huttenhower & Consortium, 2012). The presence of different microbial communities is also decided by various factors including salivary and crevicular fluids, a wide range of pH, nutrient availability, redox potential, shedding and non-shedding surfaces. In spite of having an alteration in composition...
and metabolic activity, they are responsible for the maintenance of homeostasis with the host (Marsh, 1994). Disruption of this delicate homeostasis caused by the changes in the oral environment (induced by aging, illness, diet, or medications) leads to the establishment of oral infectious disease (Abaci et al., 2010; Kleinegger et al., 1996; Schumann et al., 2005).

2.3. The oral cavity as a habitat of fungi: The oral cavity comprises diverse surfaces and microenvironments enabling colonization of a wide variety of microorganisms. Mucous membranes line the oral surfaces, which are pierced by teeth and ducts of the salivary glands. The flushing action of saliva removes non-adherent microorganisms from oral surfaces. Decreased saliva secretion (Meurman & Rantonen, 1994; Almstahl et al., 1999) may result in increased recovery of yeasts, due to decreased quantities of candidacidal molecules, including lysozyme (Tobgi et al., 1988), histatins (Jainkittivong et al., 1998), peroxidises (Lenander-Lumikari, 1992), and lactoferrin (Nikawa et al., 1993). On the other hand, saliva also contains microbial nutrients, such as glucose (Knight & Fletcher, 1971) which facilitates proliferation of yeasts (Samaranayake et al., 1986) and mucins which contribute to adhesion of yeasts to oral surfaces (Hoffman & Haidaris, 1993).

These human microbiome members include hundreds of different viral, archaeal, bacterial, fungal, and protozoan species (Dewhirst et al., 2010; Wade, 2013). The oral fungal microbiome is called mycobiome (Ghannoum et al., 2010). Several diseases associated viruses were found in the mouth. Among them Herpes viruses, most prevalent in saliva causes diseases of the oral mucosa (Slots & Slots, 2011; van der Beek et al., 2012). Human immunodeficiency virus (HIV), Human papilloma virus type 16, Hepatitis virus, Rabies virus, Mumps and Measles virus can also be detected in the oral cavity (Slots & Slots, 2011; Marur et al., 2010).

The predominant bacterial genera found in the oral cavity were Streptococcus, Lactobacillus, Actinomyces, Neisseria, Rothia, Veillonella, Prevotella, Gemella, Haemophilus, Porphyrromonas and Lepotrichia (Kraneveld et al., 2012). In an oral mycobiome study, 85 fungal genera including Candida, Cladosporium, Aureobasidium, Saccharomycetales, Aspergillus, Fusarium, and Cryptococcus species were found in the oral cavity of healthy individuals (Ghannoum et al., 2010). Candida albicans is the most prevalent fungal pathogen among them in oral mucosal and systemic infections (Pfaller, 2010). Non-albicans species such as C. parapsilosis, C. glabrata, C. kefyr, C. tropicalis, and C. krusei have also been detected in healthy and diseased individuals apart from C. albicans.
Two protozoan species include Entamoeba gingivalis, and Trichomonas tenax have been found among oral resident microorganisms (Wade, 2013; Wantland et al., 1958).

In healthy individuals, the oral recovery of yeasts is about 34% varying from 2% to 71% (Odds, 1988) probably due to the sampling method or site and study population (Arendorf & Walker, 1980, Brambilla et al., 1992). The numbers of yeasts isolated from the oral cavity of healthy carriers are usually low. In hospitalized patients, oral yeasts were recovered from about 55%, the oral recovery of yeasts varying from 13% to 76% (Odds, 1988).

"The normal oral flora" and "commensals" are terms used for microbes that are almost always present in high numbers in the oral cavity of healthy individuals (Liljemark & Bloomquist, 1988; Loesche, 1988), meaning that they live in the host, and derive benefit from the host without causing injury (Liljemark & Bloomquist, 1988; Asikainen & Chen, 1999). Persons infected with disease causing microbes are called carriers because they do not have symptomatic clinical disease, but can spread an infection to other persons. Yeasts can act as opportunistic pathogens, since can cause disease in the compromised host (Liljemark & Bloomquist, 1988; Cannon & Chaffin, 1999). The persistent presence and multiplication of commensal yeast in the oral cavity is called colonization (Salyers & Whitt, 1994). The term "infection" is regarded as successful colonization and multiplication by yeasts capable of causing damage to the host in the oral cavity (Liljemark & Bloomquist, 1988), and an infection that produces symptoms is known as disease. Infection can also be regarded as disruption of the host and production of a characteristic group of symptoms. For disease to occur, yeasts have to adhere and invade host tissues by passing through mucosal surfaces and spreading through the body, penetrating the host's defences.

2.4. Oral yeasts in health and disease: Yeasts are opportunistic pathogens but also regarded as members of the normal oral flora (Arendorf & Walker, 1979, Odds 1988). The dorsum of the tongue is the primary oral reservoir for yeasts (Arendorf & Walker, 1980), can also be recovered from other oral mucosae, tooth surfaces, and saliva (Arendorf & Walker, 1980, Borromeo et al., 1992, Töllonen et al., 1999). Smoking leads to localized epithelial alterations such as leukoplakic lesions which may enhance colonization of oral Candida. (Rindum et al., 1994). C. albicans is the most commonly isolated yeast species in the oral cavity both in health and disease (Odds, 1988). It accounts for about 47% to 75% of the oral yeast isolates,
while other medically important yeast pathogens, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida kefyr*, *Candida glabrata*, and *Candida guilliermondii*, each represent less than 10% of isolates (Odds 1988).

An equally complimentary condition is attained by the associated existence of all these microorganisms in the oral cavity. Their co-existence helps to resist the colonization of other pathogenic microorganisms in the oral cavity via a method called colonization resistance (He et al., 2013). These oral inhabitants’ micro flora has continual acquisition of nutrients for their growth, and produce inhibitory factors creating an unfavorable environment to restrict the adhesion and/or colonization by potential microbial invaders (Wilks, 2007; Wilson, 2005). The mutual relationship by these microorganisms also helps in maintaining the cardiovascular health by producing nitric oxide during their respiration process (Govoni et al., 2008; Petersson et al., 2009; Hezel & Weitzberg, 2013). It helps to keep the blood vessels flexible (Kapil et al., 2013; Hobbs et al., 2013). It is evidenced that the occurrence of a cross-talk between the resident oral micro flora and the host mucosal cells (Cosseau et al., 2008) by which the host exerts its defense mechanism to the pathogenic microorganism but not to the resident micro flora (Srinivasan, 2010).

2.5. Taxonomy: The genus *Candida* contains heterogeneous anamorphic yeasts and comprises about 196-200 species (Kwon & Bennet, 1992; Murray et al., 1995) that are physiologically related to ascomycetes or basidiomycetes. The name is derived from the custom in ancient Rome for a candidatus, a candidate for public office, to dress in white Albico means “to be white,” so the name *Candida albicans* is redundant. The more important pathogenic species, *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. lusitaniae* and *C. glabrata*, are phylogenetically related to the Ascomycetes. The genus *Candida* is classified as follows:

- **Phylum**: Deuteromycota
- **Class**: Blastomycetes
- **Order**: Cryptococcales
- **Family**: Cryptococcaceae
- **Genus**: Candida

Species within the genus *Candida* are characterized primarily by colonial morphology, carbon assimilation, and fermentation capabilities. *Candida* species grow well at 20°C to 38°C (Odds, 1988) and within the pH range from 2.5 to 7.5 (Odds, 1988), although a low pH (Arendorf & Walker, 1980) even below pH 2 (Odds & Abbott, 1980) especially
favours their proliferation. Candidal cells primarily multiply by budding. Yeast cells are ovoid, 3 x 5 mm in size.

*Candida albicans* was first called as *Oidium albicans* by Charles-Philippe Robin (1821-1885) in 1853 (Kwon & Benet, 1992). After further studies, Zopf in 1890 changed its name to *Monilia albicans*. The currently accepted name, *C. albicans*, was introduced by Berkhout in 1923 (Kwon and Benet, 1992). *C. albicans* is a simple diploid eukaryote organism lacking a sexual cycle. It grows in two forms, as a yeast (synonyms: blastospore; blastoconidium) and as a hypha (synonym: mycelium).

Aldo Castellani (1877-1971), while he worked in Ceylon (Sri Lanka), differentiated several species of Candida; including *Candida tropicalis* in 1910. He called it *Oidium tropicale*. Other names given to this species have been *Monilia tropicalis*, *Candida vulgaris*, *Mycotorula dimorpha*, *Candida paratropicalis*. 58 synonyms have been applied to *C. tropicalis*. Berkhout introduced the present name in 1923 (Kwon & Benet, 1992). It is germ tube negative. *C. tropicalis* is a diploid *Ascomycetous* yeast.

Castellani described *C. Krusei* in 1910 as *Saccharomyces krusei* and as *Endomyces Krusei* in 1912 (Castellani 1912). Chalmers (1913) named it *Monilia krusei*. 18 other synonyms were proposed before Berkhout renamed it in 1923 as *C. krusei*. Colonies of *C. Kruse* appear similar to *C. albicans* and other pathogenic *Candida* species on Sabouraud’s agar, but on cornmeal-Tween 80 agar *C. Krusei* form pseudo hyphae with elongated blastoconidia, giving the appearance of crossed match sticks or trees (Kwon & Benet, 1992; Guarro *et al.*, 1999). *C. Krusei* is inherently resistant to fluconazole.

### 2.6. Oral Candidiasis

It is defined as infection with the overgrowth of yeast *Candida* in mouth and esophagus. The *Candida* species is a dimorphic fungus which is normal microbial flora of the skin and mucous membranes of most of all the healthy individuals and if given opportunity, leads to infectious diseases (Williams and Lewis, 2011). The transformation of the yeast *Candida* species from a commensal to a pathogen rely on various factors such as, virulence of the infecting yeast strains, host resistance, and various environmental factors, including sugars and antibacterial drugs (Scully *et al.*, 1994), influence risk for the development of candidosis (Samaranayake, 1990). Intense research, no single particular virulence factor of any yeast species has been found to cause candidosis (Cutler, 1991;
Matthews, 1994; Odds, 1994). Rather, a combination of virulence mechanisms, such as
adhesins, rapid phenotypic switching, hyphal growth, and secretion of hydrolytic enzymes,
seems to be responsible for the development of candidosis, since at different stages of
candidosis different virulence factors are expressed (Cutler, 1991). An association exists
between candidosis and diseases leading to suppression in systemic and local host defences
(Samaranayake, 1990) additionally, altered nutritional status of the host, including iron
deficiency (Higgs, 1973; Fletcher et al., 1975) and folate deficiency (Samaranayake &
MacFarlane, 1981), are associated with oral candidiasis.

2.7. History of oral candidiasis: Candida infections are called candidosis or candidiasis,
terms used in the literature as synonyms. The International Society for Human and Animal
Mycology (1980) has suggested the term "candidosis", while the Council for International
Organizations of Medical Sciences (1982) recommends "candidiasis". The incidence of oral
candidiasis was evidenced from Hippocrates's Epidemics in which it is stated that two patients
with other underlying diseases had oral candidiasis in fourth century B.C (Jagadish Chander,
2002). An attempt to classify the disease into categories based on the severity and distribution
of the lesions. Bennett (1844) isolated the Candida albicans from the sputum of a
tuberculosis patient and isolated the organism from vaginal candidiasis (Parihar, 2011). The
first observation of concomitant thickening of the epithelium in lesions resembling thrush
was done by (Samaranayake & MacFarlane, 1990).

2.8. Prevalence of oral Candidiasis: Candida is the most frequent cause of oral candidiasis
with a total estimated prevalence between 15 and 75% (Ghannoum et al., 2010; Yang et al.,
2011), and up to 90% in elderly (De Resende et al., 2006; Kleinegger et al., 1996) especially
in denture wearers (Vanden et al., 2008). In the healthy people, 15% to 75% carriage rates
have been reported without any symptoms (Ghannoum et al., 2010; Yang et al., 2011). The
incidence of C. albicans isolated from the oral cavity of neonates has been reported to be
45% (Manning et al., 1985), 45%–65% in healthy children (Berdicevsky et al., 1980), 65%–
88% in those who are under acute and long term care amenities (Aldred et al., 1991;
Cumming et al., 1990), 90% in people undergoing chemotherapy especially acute leukemia
patients (Rodu et al., 1988), and in 95% of patients with HIV. These often occur early in the
course of HIV infection.

The extensive use of antifungals for prophylaxis in the patients became the leading
cause of colonization of Candida-non-albicans species and increasing resistance to antifungal
In India, *Candida tropicalis* is the most common cause of oral candidiasis. Epidemiological data from the Indian subcontinent showed that 17–20% of cases were due to species of which *C. tropicalis* was the most dominant (Kothari & Sagar, 2009; Verma *et al.*, 2003). In his comprehensive review on oral carriage of *Candida* sp., Odds, 1988 concluded that *C. Krusei* is the fifth most dominant species, with *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis* preceding it. In one large study, the most common yeast combinations isolated from oral samples comprised *C. albicans* with one or more of the following: *C. krusei*, *C. tropicalis*. These data indicate that *Candida* colonization mainly depends on the general health of an individual. Reported prevalence varies between epidemiological studies. This is most likely due to differences in the studied populations and the sampling and identification methods that are used.

### 2.9. Clinical manifestation:

Oral candidiasis can be divided into many different types, often by its clinical appearance in the mouth and sometimes by its predisposing factors (Cannon & Chaffin, 1999). In healthy individuals, *Candida* present in many sites of the oral cavity including the mid-line of the center and posterior thirds of the tongue, the cheek, or the palatal mucosa. The following are the most commonly described types of oral candidiasis;

**Table:** Clinical forms of oral candidiasis, description and location (adapted from Neville *et al.*, 2002).

<table>
<thead>
<tr>
<th>Clinical type</th>
<th>Pseudo-Membranous (thrush)</th>
<th>Erythematous</th>
<th>Median rhomboid glossitis</th>
<th>Angular Cheilitis</th>
<th>Denture Stomatitis</th>
<th>Hyperplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Creamy white removable plaques,</td>
<td>Red areas</td>
<td>Red mucosa</td>
<td>Red fissured lesions</td>
<td>Red on denture bearing area</td>
<td>White, nonremovable Plagues</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Burning sensation and bad taste</td>
<td>Burning Sensation</td>
<td>Asymptomatic</td>
<td>Sore</td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Common Sites</td>
<td>Buccal mucosa, tongue, palate</td>
<td>Hard palate, buccal mucosa, tongue</td>
<td>Midline of Tongue</td>
<td>Corners of lips</td>
<td>Opposing the denture surface</td>
<td>Buccal mucosa</td>
</tr>
</tbody>
</table>

#### 2.9.1. Primary oral candidiasis

**2.9.1.1. Pseudomembranous candidiasis:** It is also known as thrush, easily diagnosed and its clinical features include widespread white or yellow pseudomembranes resembling milk curds which contain desquamated epithelial cells, fibrin, and fungal hyphae (Figure 3a). The
pseudomembrane can be scrapable under which the mucosal cells will be inflamed commonly affected parts are oropharynx, hard and soft palate, periodontal tissues, labial and buccal mucosa and the tongue. This clinical form is most common with immunocompromised individuals such as infants and the elderly, those who use corticosteroid or people undergoing long term broad spectrum antibiotic therapy, those with poorly controlled diabetes mellitus, leukemia, and HIV infection (de Almeida & Scully, 2002; Akpan & Morgan, 2002).

2.9.1.2. **Erythematous candidiasis:** It is characterized by localized erythematous areas commonly on the dorsum of tongue and palate, and less commonly on the buccal mucosa usually associated with a burning sensation in the mouth or on the tongue. This is also known as “atrophic Candidiasis” (Figure 3b). The redness may be caused by reduced epithelial thickness (atrophy) and increased vascularity. Because it is mainly associated with the use of broad spectrum antibiotics which lower the oral bacterial population and facilitate subsequent overgrowth of *Candida*, it was initially known as the antibiotic sore mouth (Zegarelli, 1993; Muzyka, 2005).

2.9.1.2.3. **Hyperplastic candidiasis:** It is occasionally referred as Candidal leukoplakia, and is characterized by the appearance of well-demarcated, slightly elevated; adherent homogeneous or nodular white plaques that cannot be wiped away usually appears on the commissural region of the buccal mucosa, and less frequently on the dorsum of the tongue (Figure 3c). Smoking is the main predisposing factor associated with this disease. Since this condition has been associated with varying degrees of dysplasia and malignancy, it is important to recognize the lesion as early as possible (Budtz-Jorgensen, 1990; Sherman et al., 2002)

2.9.1.3. **Candida-associated Lesions**

2.9.1.3.1. **Denture stomatitis:** It is characterized by localized chronic erythema of tissues covered by dentures (Figure 3e). The occurrence of lesions on the palate and the upper jaw is usual but may also affect mandibular tissue. Though this condition is usually asymptomatic, patients may complain of slight soreness or burning sensation. Denture stomatitis is commonly associated with angular cheilitis and median rhomboid glossitis (Webb et al., 1998). Denture stomatitis is divided into three clinical types (Carmen et al., 2011), they are

- **Type I:** Localized pinpoint hyperemia
- **Type II:** Erythematous lesion involving denture covered mucosa
- **Type III:** Papillary type involving central part of hard palate & alveolar mucosa
2.9.1.3.2. Median rhomboid glossitis: Clinically, it appears as an erythematous, elliptical or rhomboid-like proportioned area on the tongue frontal to the circumvallated papillae (Figure 3d). Nearly in 85% cases, Candida yield was observed from the biopsy of this area. Smoking and the use of corticosteroid inhalers tends to be the major predisposing factors associated with this condition (Reichart et al., 2000).

2.9.1.3.3. Angular cheilitis: The appearance of this form is characterized by erythematous, fissured lesions affecting the corners of the mouth resulting in facial skin folds and wrinkling along the labial commissures and nasolabial folds (Figure 3f). In older individuals, it may cause saliva accumulation. Use of dentures, nutritional deficiency (iron or vitamin B₁₂) and a moist environment have been implicated in the development of angular cheilitis. Recently, it is accepted that they are mostly caused by Candida species and / or Staphylococci and Streptococci (Neville et al., 2002; Akpan & Morgan, 2002).

2.9.1.4. Keratinized Primary Lesions superinfected with Candida: As secondary invaders, Candida is present in the non-homogeneous leukoplaikia’s. Due to the structural changes of the epithelial surface or the cell-mediated immune response alterations against Candida, they cause secondary Candidal infection in conditions like oral lichen planus (Krogh et al., 1987; Budtz-Jorgensen, 1990).
2.9.2. Secondary oral candidiasis

2.9.2.1. Mucocutaneous Candidiasis: This form is commonly associated with a defect in cell-mediated immunity and its occurrence is mainly found with a variety of primary immunodeficiency, such as severe combined immunodeficiency syndrome (SCID), Nezelof syndrome (thymic lymphoplasia), DiGeorge syndrome, hyper-immunoglobulin E syndrome, myeloperoxidase deficiency, and endocrinopathy, especially Addison’s disease and hypoparathyroidism (Williams & Lewis, 2011).

2.10. Predisposing factors: There are factors other than the pathogenic attributes of the organism are involved in the transition of Candida species from endogenous commensal to the disease-causing pathogen (Marsh & Martin, 2009). It is evidenced by various reports that the development of the Candidal infection rely on predisposing host factors which increase the susceptibility of the host to oral candidiasis (Williams & Lewis, 2011). They are described as in Table 2.

2.10.1. Suppressed immune system: Oral candidiasis is a common manifestation in patients whose immune system is immune suppressed or immune compromised. It has been reported that the prevalence rate of oral candidiasis in HIV patients lies between 7% and 48% and more than 90% has been reported in those with advanced disease. In patients with severe immunosuppression, relapse rates are between 30% and 50% on completion of antifungal treatment. In HIV infection, T-helper lymphocytes are reduced due to immunodeficiency which makes the infected patients more susceptible to secondary infections, in particular opportunistic Candidal infections (Edgerton, 1999). Candidiasis is also found to be a common manifestation in a various forms of immunodeficiency disorders including severe combined immunodeficiency syndrome, DiGeorge syndrome and hereditary myeloperoxidase deficiency (Farah et al., 2000).

2.10.2. Endocrine disorders: Diabetes mellitus (DM) is one of the major predisposing factors in increasing the susceptibility to the development of Candidal infections. It leads to the immune system aberrations such as impaired opsonization and weakened activity of neutrophils and monocytes. The most prevalent clinical manifestations occurring in diabetes patients are Candida-associated lesions including denture stomatitis, median rhomboid glossitis, and angular cheilitis. If the host resistance is modified by some local factors like smoking and denture wearing, their susceptibility to the infection also seems to be more (Guggenheimer et al., 2000).

<table>
<thead>
<tr>
<th>Local factors</th>
<th>Systemic factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal barrier</td>
<td>Physiological states</td>
</tr>
<tr>
<td>Ill-fitting dental appliances</td>
<td>Infancy</td>
</tr>
<tr>
<td>Trauma</td>
<td>Old age</td>
</tr>
<tr>
<td>Changes in oral environmental conditions</td>
<td>Endocrine disorders</td>
</tr>
<tr>
<td>Inadequate home care of oral dental appliances</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Endogenous epithelial changes</td>
<td>Hypothyroidism</td>
</tr>
<tr>
<td>Atrophy</td>
<td>Nutritional deficiencies</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>Iron deficiency</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>Folate deficiency</td>
</tr>
<tr>
<td>Saliva</td>
<td>Vitamin B12 deficiency</td>
</tr>
<tr>
<td>Quantitative changes</td>
<td>Malignancies</td>
</tr>
<tr>
<td>Xerostomia</td>
<td>Acute leukaemia</td>
</tr>
<tr>
<td>Hypo salivation</td>
<td>Agranulocytosis</td>
</tr>
<tr>
<td>Qualitative changes Immune defects</td>
<td>Immune defects</td>
</tr>
<tr>
<td>pH</td>
<td>HIV infection</td>
</tr>
<tr>
<td>Glucose concentration</td>
<td>Thymic aplasia</td>
</tr>
<tr>
<td>Bacteria-yeast coaggregation</td>
<td>Pharmacotherapy</td>
</tr>
<tr>
<td>High-carbohydrate diet Broad-spectrum antibiotics</td>
<td>Broad-spectrum antibiotics</td>
</tr>
<tr>
<td>Tobacco smoking</td>
<td>Corticosteroids</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Cytotoxic drugs</td>
</tr>
</tbody>
</table>

In addition, factors viz., reduced salivary flow rates, reduced salivary pH, and increased salivary glucose levels are highly exhibited by the diabetic patients, which helps in the facilitation of oral Candidal growth and colonization (Manfredi et al., 2002). There are some contradictory findings also reported with reduced rates of Candidal carriage in diabetic patients compared with healthy controls (Belazi et al., 2005).

2.10.3. Malignancies: The host defense mechanism is generally impaired in people with solid organ or hematology related malignancies and also in people who are undergoing treatment by radiotherapy or chemotherapy. In such case, the homeostasis of the oral cavity will be favorable for the adhesion and penetration of the *Candida* pathogen. They are much prone to the development of oral candidiasis (Farah et al., 2010).

2.10.4. Dentures: Around 65% of denture wearing elderly people are susceptible to oral *Candida* infections. A microenvironment with acidic and anaerobic conditions is developed by dentures where it decreases the flow of oxygen and saliva to the underlying tissue.
(Jeganathan & Lin, 1992). Such an environment is more favourable for the \textit{Candida} to secrete extracellular hydrolytic enzymes which play a crucial role in the development of infection. Other conditions which facilitate the infection are overnight denture wearing and poorly fitting dentures. They contribute to promote the infection by increasing irritation from denture and mechanical trauma whereby it reduces the tissue resistance and increases the permeability of the epithelium to soluble \textit{Candida} antigens and toxins (Budtz-Jorgensen, 2000).

\subsection*{2.10.5. Nutritional factors:} The host defense mechanism is directly correlated with the availability of host nutrients. Deficiencies of vitamin B_{12}, folic acid and iron are proposed as important predisposing conditions for the oral \textit{Candida} infections. Among them; Iron deficiency anemia contributes to the main factor in the etiology of oral \textit{Candidiasis} by depressing cell-mediated immunity (Paillaud \textit{et al.}, 2004). A study showed that people who are having iron deficiency showed reduced lymphocyte response to the \textit{C.albicans} antigens which is correlated with increased colonization of \textit{C. albicans} in the oral cavity (Sweeney \textit{et al.}, 1994).

\subsection*{2.10.6. Xerostomia:} It is one of the important predisposing factors responsible for the establishment of oral candidiasis in elderly, those who undergo head and neck radiotherapy, medications and Sjogren’s syndrome. It is the clinical condition developed as a result of impaired salivary gland function. The saliva possesses several biological functions such as dilutional effect which removes organisms from the mucosa to maintain the normal oral micro flora. The presence of salivary antibodies and several non-specific antimicrobial factors viz., histatins, lysozyme and lactoferrin plays important role in decreasing the fungal adherence and colonization (Wu \textit{et al.}, 1993; Akpan & Morgan, 2002).

\subsection*{2.10.7. Medications:} People those who use broad-spectrum antibiotics, glucocorticoids (systemic or topical), several immune modulatory and cytotoxic drugs to prevent rejection following blood and solid organ transplants, are more prone to oral Candidal infections. It eliminates the normal symbiosis between bacterial and yeast flora, lower the resistance to fungal overgrowth by suppressing the cell mediated immunity and inducing neutropenia (Ellepola & Samaranayake, 2001). There are variety of prescribed drugs available including anti-depressants, anti-psychotics, anti-cholinergic, diuretics, anti-hypertensives and anti-adrenergic which elicit xerostomicside effects, thereby increasing susceptibility to oral Candidal infection (Scully, 2003).
2.10.8. High-carbohydrate diet: Recently, intake of diet containing high carbohydrate content has been assumed to predispose to oral candidiasis. The dietary sugars improved the adhesion of *Candida* to oral epithelial cells and to acrylic surfaces. It was reported from the study that glucose was exposed to be the most effective dietary sugar implicated on Candidal adhesion and biofilm formation, followed by galactose and sucrose (Jin *et al.*, 2004).

2.10.9. Smoking: Smoking has been proposed as an important predisposing factor in the progress of oral Candidiasis. Several possible mechanisms of action by which smoking leads to infection has been established. One among that could be localized epithelial alterations, results in Candidal colonization. In addition, it is assumed that *Candida* species covert the aromatic molecule present in the cigarette into carcinogen which is used as a nutrient source by the organism for its growth (Soysa & Ellepola, 2005).

2.11. Virulence properties: The survival and ability of *Candida* to establish infections within humans are mainly associated with certain pathogenic variables related to its ability to establish infection. A number of putative virulence factors (Table 3) have been proposed that in the incident of host debilitation which contribute to tissue damage and perseverance of the organism within the host. A schematic representation of selected *Candida* pathogenicity mechanisms are shown in figure 4.

**Table 3: Virulence factors and its pathogenicity** (Williams & Lewis, 2011)

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adherence</strong></td>
<td></td>
</tr>
<tr>
<td>Cell surface hydrophobicity</td>
<td>Promotes retention in the mouth</td>
</tr>
<tr>
<td>Expression of cell surfaces adhesions</td>
<td>Nonspecific adherence</td>
</tr>
<tr>
<td></td>
<td>Specific adherence</td>
</tr>
<tr>
<td><strong>Evasion of host defense</strong></td>
<td></td>
</tr>
<tr>
<td>Phenotypic switching</td>
<td>Promotes retention in the mouth</td>
</tr>
<tr>
<td>Hyphal development</td>
<td>Antigenic modification</td>
</tr>
<tr>
<td>Secreted aspartyl proteinase production</td>
<td>Reduces phagocytosis</td>
</tr>
<tr>
<td>Binding of complement</td>
<td>Secretory IgA destruction</td>
</tr>
<tr>
<td></td>
<td>Antigenic masking</td>
</tr>
<tr>
<td><strong>Invasion and destruction of host tissue</strong></td>
<td></td>
</tr>
<tr>
<td>Hyphal development</td>
<td>Enhances pathogenicity</td>
</tr>
<tr>
<td>Hydrolytic enzyme production</td>
<td>Promotes invasion of the oral epithelium</td>
</tr>
<tr>
<td></td>
<td>Host cell and extracellular matrix damage</td>
</tr>
</tbody>
</table>
**2.11.1. Polymorphism:** In relation to the virulence of *Candida*, an important attribute is its ability to switch between the diverse morphological forms (Sudbery et al., 2004). *Candida* is a polymorphic fungus that exhibits a number of unusual morphological forms viz., ovoid-shaped budding yeast, elongated ellipsoid cells with constrictions at the septa (pseudo hyphae), and parallel walled true hyphae. In addition, it exhibits some other morphological forms in various environmental conditions such as chlamydospores formation and white and opaque cells formation while phenotypic switching under different environmental circumstances also occurs (Berman & Sudbery, 2002).

**2.11.2. Phenotypic switching:** The shift between yeast and hyphal form is termed as dimorphism and it has been proposed that both forms are essential for pathogenicity. The yeast form has been shown to be less invasive than the hyphal form. On the other hand, the smaller yeast form is believed to signify the form mainly implicated in dissemination (Saville...
et al., 2003). Mutants that are not capable of forming hyphae under in vitro conditions are commonly attenuated in virulence. Conversely, hypha development is associated with the expression of a subset of genes programming virulence factors that are not considered in hyphal formation. A number of proteins named the agglutinin-like sequence protein (Als3), hyphal wall protein (Hwp1), hypha-associated proteins (Ece1 and Hyr1) and the secreted aspartic proteases (Sap4, 5 and 6) are involved in this process (Jacobson et al., 2012).

2.11.3. Quorum-sensing: It is the mechanism of microbial communication via which microbes perceive their population density through diffusible signal molecules. Morphogenesis has also been shown to be synchronized by quorum sensing. This mechanism is used by microbes to regulate their gene expression in response to fluctuations in the cell population density (Albuquerque & Casadevall, 2012). In Candida, the main quorum sensing molecules include farnesol, tyrosol and dodecanol control the morphological switch between the yeast and filamentous forms in response to environmental cues (Sharma and Prasad, 2011). Yeast growth is promoted by cell densities (> 10^7 cells ml^-1) whereas low cell densities (< 10^7 cells ml^-1) favor hyphal formation due to quorum sensing.

2.11.4. Environmental factors: An array of environmental cues including pH, the presence of serum or N-acetyl glucosamine, starvation, physiological temperature and CO₂ endorse the configuration of hyphae and affect C. albicans morphology. For instance, at low pH (< 6) Candida cells largely grow up in the yeast form, while at a high pH (> 7) hyphal growth is induced (Odds, 1988).

2.11.5. Cell wall: Adhesion of Candida to epithelial cell wall, an essential step in initiation of infection, is promoted by certain fungal cell walls constituents (Figure 5) such as mannose, Csd receptors, mannoprotein, and saccharins (Tronchin et al., 2008) and the plasma membrane is selectively permeable to the molecules required by Candida for survival. It contains free fatty acids, glycolipids, phospholipids, sphingolipids, and sterols. The fungal cell wall is made up of 90% carbohydrate and 10% protein and acts as an exocytoskeleton. The association of the cell wall from the cell membrane outward starts with the chitin layer followed by a network of β-1, 3 glucan.
molecules coupled together by hydrogen bonds. The dry weight analysis of the cell wall revealed that it consists of approximately: 40% mannoproteins, 40% β-1, 3 glucans, 20% β-1, 6 glucans and 1-2% chitin. The composition of the cell wall of the yeast and hyphal forms varies in polysaccharide content (more chitin in the hyphal form) and is influenced by environmental factors such as the presence of antifungal agents (Klis et al., 2001). β-1, 6 glucan is present as a side chain connected to the β-1, 3 glucan layers. Interspersed within the β-1, 3 glucan layer are cell wall proteins (CWPs) that are either bound to chitin or to the β-1,3 glucan layer via a glycosyl-phosphatidyl-inositol (GPI) anchors. The mannans are attached to the terminal ends of the CWPs via a covalent linkage.

2.11.6. Cell Wall Proteins (CWP): These are highly glycosylated and their carbohydrate side chains possess negatively charged phosphate groups (Ruiz-Herrera et al., 2006). They play a major role in the restriction in permeability of the cell wall, protection against degrading enzymes (chitinases and glucanases) and antifungal substances; determination of hydrophobicity and the antigenicity of the cell wall. The CWPs are also thought to be responsible for remodeling of the cell wall as well as to cellular adhesion, which would also affect the organism’s virulence. There are three classes of CWPs (Chaffin, 2008). This first class includes GPI proteins (e.g. adhesins) and is linked to β-1, 6 glucans. The second sets of proteins are referred to as ‘proteins with internal repeats’ (PIR) which are crucial for maintaining the structural design of the cell wall. The third classes of proteins are those secreted into the extracellular space including hydrolytic enzymes like phospholipases and proteases.

2.11.7. Adhesins: Candida has a specialized set of proteins (adhesins) called agglutinin-like sequence (Als) proteins which are numbered as Als1 – Als7 and Als9. They facilitate the adherence of the Candida to other Candida cells and to the host cells (Murciano et al., 2012). The Als genes codes for GPI (glycosylphosphatidylinositol) linked cell surface glycoproteins. Among the eight Als proteins, the hypha-associated adhesion protein (Als3) is particularly important for adhesion. Also, in biofilms, hypha also expresses adhesion proteins Hwp1p and Ywp1p which help in biofilm retention and biofilm dissolution respectively. Hwp1 also acts as a substrate for mammalian transglutaminases by which Candida hyphae covalently linked to host cells. In addition, several other morphology independent proteins such as GPI-linked proteins, cell-surface associated proteases, non-covalent wall-associated proteins and integrin-like surface protein are also involved in the adhesion (Wachtler et al., 2011).
2.11.8. Invasion: *Candida* invades into the host cells by two possible mechanisms. They are induced endocytosis mediated by Als3 and Ssa1 but their active penetration is mediated by yet indeterminate molecular mechanisms (Phan *et al.*, 2007). Ssa1 is a cell-surface expressed member of the heat shock protein 70 and Als3 (agglutinin-like sequence 3) also functions as an adhesin.

2.11.9. Secreted hydrolases: *Candida* hyphae secrete hydrolases after adhering to host cell surfaces and hyphal growth to assist the active penetration into the host cells. These secreted hydrolases are concerned to augment the effectiveness of extracellular nutrient acquisition. They secrete three different classes of hydrolases include proteases, phospholipases and lipases.

2.11.10. Secreted aspartyl proteinase (SAPs): There are ten Saps (Sap1–10) present in this family of enzymes. The Sap1–8 is secreted out into the medium, while Sap9 and Sap10 still bound to the cell surface (Hube *et al.*, 1997). The Sap1, Sap2 and Sap3 was found to be essential to cause damage to the reconstituted human epithelium from some *in vitro* and *in vivo* studies. On the other hand, a recent study on mouse model of disseminated candidiasis specify that Saps are not requisite for invasion into reconstituted human epithelium and that Sap1–6 is not necessary for virulence (Lermann & Morschhaufer, 2008).

2.11.11. Phospholipases: The family of phospholipases consists of four different classes (A, B, C and D). Among them, class B (PLB1–5) was found to be extracellular which contribute to pathogenicity by disrupting the host membranes (Niewerth & Korting, 2001). These enzymes confine to the hyphal tips or growing end of the yeast form. Though based on the location phospholipase genes are expressed, phospholipase B1 (PLB1) is found more recurrently in candidiasis patients.

2.11.12. Biofilms formation: It is an important virulence factor of *Candida*. They are capable of developing biofilms on abiotic (Catheters and dentures) or biotic (mucosal cell surfaces) surfaces (Fanning & Mitchell, 2012). The sequence of biofilm formation starts from yeast cell adherence to the substrate, proliferation, hyphal cells formation, gathering all the extracellular matrix material and ends with dispersing the yeast cells from the biofilm complex. The dispersed cells from developed biofilm have been found to be more virulent in a mouse model of disseminated infection, so yeast cell dispersion is directly correlated with the virulence (Finkel & Mitchell, 2011). From a recent study, it was identified that the heat shock protein Hsp90 plays a vital role in dispersion of *C. albicans* biofilms.
Biofilms are greatly resistant to antimicrobial agents and host defense mechanism when they mature than the planktonic cells. The reason behind this resistance may be due to factors including the complex architecture of biofilms, augmented expression of drug efflux pumps, the biofilm matrix, and metabolic plasticity (Robbins et al., 2011). Along with, the biofilm formation is also controlled by several transcription factors include Bcr1, Tec1, Efg1, Zap1, Gca1, Gca2, Bgl2, Phr1 and Xog1. Evidence from reports suggests that the presence of β-glucans in the extracellular matrix protect *Candida* sp., from the host defense. Among the above-mentioned transcription factors, Zap1 is identified as negative regulator of β-1,3 glucan synthesis whereas Gca1, Gca2, Bgl2, Phr1 and Xog1 are positive regulators of β-1,3 glucan production (Nobile et al., 2009).

**Figure 6**: Biofilm formation. *Candida* infection starts with biofilm formation and this requires attachment of *Candida* cells to ‘a surface’. After attachment, multiplication follows and the biofilm grows, matures, and is embedded in an extracellular polysaccharide (EPS) matrix. The final stage of biofilm formation is dispersion, biofilm cells detach and, subsequently, spread and colonize new surfaces. Courtesy: Karaneveld, 2011.

2.11.13. Metabolic adaptation: An interesting feature of *Candida* is metabolic adaptability by which they assimilate alternative nutrients from dynamic environments. Host-derived glucose, lipids, proteins and amino acids are the most essential nutrient sources for *Candida* during infection based on the site where they reside. Moreover, the ability of *Candida* to respond rapid and dynamic to the host and pathogen-induced changes during the acquisition of nutrients in diverse anatomical niches contributes to pathogenicity. There are long-lasting reports suggesting that expression of key virulence factors of *Candida* is modulated by the
influence of metabolic adaptation (Brock, 2009). For example, hyphal morphogenesis is triggered by glycolytic genes. The phenotypic switch between white to opaque cells are up regulated by metabolic genes; white cells up regulate glycolytic genes whereas opaque cells up regulate genes involved in respiratory metabolism. The availability of nitrogen and carbon sources controls the expression of secreted aspartic proteinase (SAP) genes (Brown et al., 2012).

2.11.14. Environmental stress response: A strong stress response seems to be responsible for the survival and virulence by adapting to varying conditions and shielding it against host-derived stresses, oxidative and nitrosative stresses produced by phagocytic cells of the immune system and pH stress occurs in the gastrointestinal and urogenital tract (Brown et al., 2012). These stress adaptability is regulated by stress-responsive regulatory pathways as well as downstream targets.

2.11.15. Heat shock proteins: These are special set of proteins synthesized by the cells in order to protect the cells from stressful conditions viz., high temperature, starvation and oxidative stress. As a result of such stress, the cells undergo protein unfolding and nonspecific protein aggregation; eventually it leads to cell death. But the presence of these Hsps acts as chaperones and prevent the cells from undergoing such detrimental changes by binding to their clients and stabilizing them (Lindquist, 1992). There are totally six Hsps have been identified in Candida sp., (Hsp104, 90, 78, 70 (Ssa1 and Ssa2) and Hsp 60). Of which Hsp90 is considered to be major Hsp in C. albicans which contributes to drug resistance, morphogenesis, biofilm formation and virulence of the pathogen. Small Hsps (sHsps) are additional low-molecular-mass chaperones present along with the major Hsps. There are six sHsps gens (Hsp31, Hsp30, Hsp21, two Hsp12 proteins and Hsp10) encode for these proteins (Singh et al., 2009). Under stressful conditions, they are expressed by the cells and transits from oligomeric form to a multimeric form and held together with aggregated proteins (Robbins et al., 2011). Followed by, the major Hsps involve in the disaggregation and refolding of the proteins.

2.11.16. Metal acquisition: Trace metals are indispensable for the growth and survival of all living organisms including humans, animals, plants, bacteria and fungi (Hood & Skaar, 2012). Among the most important metals iron, zinc, manganese and copper, all of which are crucial for the appropriate function of a large number of proteins and enzymes. These trace metals are acquired by Candida using different mechanisms including a reductive system, a siderophore uptake system, a heme-iron uptake system, and zincophore uptake system.
(Almeida et al., 2009). In addition, they also acquire Copper and manganese for their growth (Citiulo et al., 2012).

2.12. Currently available treatment: In general, several antifungal drugs exists for the treatment of oral candidiasis (Table 4) which are classified into three main categories; the polyenes which cause disruption of cell membrane; azoles which inhibits ergosterol synthesis, echinocandins which inhibits beta 1, 3-D glucan synthesis in addition to that allylamines, and the DNA analogue 5-flucytosine are also included. Nystatin is used as an ointment or oral suspension, Amphotericin B is used as lozenge, Miconazole is used as an oral gel or cream, Clotrimazole is used as a cream or pessary where as others available as tablets and capsules (Williams & Lewis, 2011; Garcia et al., 2014).

There are primarily two goals in the treatment of candidiasis, interfering with Candida proliferation in the body and the reduction of the factors providing favorable environment for growth of Candida. A priority is given to the alleviation of any identifiable predisposing factor in the treatment of oral Candidiasis. It is possible to prevent oral candidiasis in many cases with the periodic oral examination of complete oral and dental hygiene for which it becomes essential to make the patient aware of oral hygiene measures viz., cleaning of the teeth, buccal cavity, tongue, and dentures. The use of anti-Candida mouth rinses such as Chlorhexidine which can penetrate those areas where the brush does not. Denture wearers should be aware of removing the dentures at night and wash it deliberately, keeping it flooded in a disinfectant solution like Chlorhexidine (Akpan & Morgan, 2002).

2.13. Antimycotic drug resistance: Antimicrobial resistance is the ability of microbes to resist the effects of drugs. Antimycotic resistance developed by the fungi is an emerging public health problem where Candida is becoming increasingly resistant to antifungal drugs, named fluconazole and echinocandins (anidulafungin, caspofungin, and micafungin) (WHO, 2014). Approximately 7% of all Candida bloodstream isolates tested at CDC were found to be resistant to fluconazole. In addition, resistance to echinocandin also appears to be increasing, from the CDC surveillance data where nearly 1% of Candida isolates showed echinocandin resistance (CDC, 2013).

2.13.1. Mechanism of drug resistance: Resistance to a variety of antifungal agents was reviewed recently (Niimi et al., 2010). Antifungal resistance and its mechanism have been well documented and elucidated through in vitro studies.
Table 4: Antimycotic drug in management of Candidiasis

<table>
<thead>
<tr>
<th>Antifungal agents</th>
<th>Mode of action</th>
<th>Administration</th>
<th>Frequently recommended treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polyenes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nystatin</td>
<td>Binds to ergosterol and disrupts fungal cell membrane</td>
<td>Topical</td>
<td>CEC, CEC</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Azoles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Inhibits ergosterol biosynthesis</td>
<td>Systemic</td>
<td>PMC, AEC, CHC</td>
</tr>
<tr>
<td>Micazolozole</td>
<td></td>
<td>Topical/systemic</td>
<td>CEC</td>
</tr>
<tr>
<td>Ketocozolozole</td>
<td></td>
<td>Topical</td>
<td>CEC</td>
</tr>
<tr>
<td>Clofurozolozole</td>
<td></td>
<td>Systemic</td>
<td>PMC, AEC, CHC</td>
</tr>
<tr>
<td>Itraconzolozole</td>
<td></td>
<td>Systemic</td>
<td>CEC</td>
</tr>
<tr>
<td>Vorizolozole</td>
<td></td>
<td>Systemic</td>
<td>PMC, AEC, CHC</td>
</tr>
<tr>
<td>Posaconzolozole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5-fluycytosine</strong></td>
<td>Inhibition of DNA/protein synthesis</td>
<td>Systemic, often in combined therapy with Amphotericin</td>
<td></td>
</tr>
<tr>
<td><strong>Echinocandins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspofungin</td>
<td>Inhibits β 1, 3 D-glucan synthesis</td>
<td>Intravenous</td>
<td></td>
</tr>
<tr>
<td>Micafungin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anidulafungin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*CEC- Chronic erythematous candidiasis; PMC- pseudomembranous candidiasis; AEC- Acute erythematous candidiasis; CHC- Chronic hyperplastic candidiasis. (Courtesy: Williams & Lewis, 2011).

Biofilms cause modification to the metabolism of the microbes thereby developing antimicrobial resistance. On the other hand, the extracellular materials prevent the penetration of the antimicrobial agent into the cell. There is no particular gene involved in the resistance rather it can be a combination of 18 altered drug targets, drug modification, and restricting drug penetration and the combinations are ABC drug efflux pumps (CDR1-4, MDR4), modification of ergosterol synthesis (Erg1, Erg3, Erg11, and Erg25), synthesis of cell wall β-glucans (Kre1, Skn1, Fks1), and alteration of biofilm matrix proteins (Zap1, Gca1, Gca2, Adh5, Csh1).

The more specific examples of the basic drug resistance mechanisms are listed here:
1) Target enzymes are up-regulated to overcome the ability of antifungal agent to cause the desired effect. 2) Alteration of the drug binding site to make the drug non-functional. 3) Removal of the antifungal agent by drug efflux pumps of the cell. 4) The cell develops an altered physiological pathway and bypasses the target enzymes. 5) Through enzyme and diffusion, the cell wall blocks the antifungal agent. 6) Through sequestration in the extracellular matrix or enzymatic breakdown, the antifungal agent is metabolically (intracellular or extracellular) inactivated (Ghannoum & Rice, 1999).
2.13.2. Polyenes: Polyenes interact with fungal membrane sterols resulting in the production of aqueous pores in the membrane. All organisms that contain ergosterol in their cell membranes are susceptible to polyenes (*e.g.* yeast, algae, and protozoa). The development of these aqueous pores in the cell membranes leads to alterations in membrane permeability, which causes several problems including leakage of ions, a property that leads to cell death. Of two well-known polyenes, amphotericin B is more effective at killing fungal organisms than nystatin. However, the former has a narrow therapeutic window, which limits its usefulness in the clinic (Ghannoum & Rice, 1999).

2.13.3. Azoles: The target ofazole based antymycotic drugs (*e.g.* ketoconazole and fluconazole) is the fungal enzyme P450 cytochrome lanosterol 14α-demethylase. The function of this enzyme is the demethylation of the ergosterol precursor at the C4 position. Inhibition of this enzyme leads to depletion of ergosterol in the cell membrane and accumulation of sterol precursors, resulting in the formation of a plasma membrane with altered structure and function (Ghannoum & Rice, 1999).

2.13.4. Pyrimidine analogues: Flucytosin or 5-Fluorocytosine (5-FC) is a synthetic antymycotic compound and is administered intravenously for systemic candidal infections. 5-FC has no intrinsic antifungal capacity, but after uptake and transformation by susceptible fungal cells, its metabolites become incorporated into DNA and RNA molecules (Vermes *et al*., 2000). 5-FC enters fungal cells and is converted to 5-fluorouracil (5-FU), and then into 5-fluourouridylic acid (FUMP). This pyrimidine analog is then incorporated into RNA, resulting in disruption of protein synthesis (Ghannoum & Rice, 1999). 5-FU is also converted to 5-fluorodeoxyuridine monophosphate and interferes with DNA synthesis. The main adverse effects of 5-FU, hepatotoxicity and myelotoxicity, have been ascribed to the occurrence of significant plasma levels of 5-FU during treatment with 5-FC (Bellmann., 2007). An increased hematotoxicity is expected when 5-FC is administered together with cytostatic and immunosuppressive drugs (Vermes *et al*., 2000).

2.13.5. Echinocandins: Although few, there have been cases of echinocandin resistance. The mechanism behind this resistance entail mutations in the echinocandins target genes Fks1 and Fks2 which are alternativeβ (1, 3)-glucan synthase subunits. In addition, mutations in the Fks gene are heritable (Balashov *et al*., 2006). Moreover, *C. albicans* cell wall chitin biosynthesis augments by the mechanism of compensatory cell wall remodeling because yeast cells with elevated chitin levels are less susceptible to echinocandins (Denning, 2003).
2.14. **Vaccination:** A rational approach to the development of a vaccine against *Candida* is problematic because the specific immune system mechanisms responsible for protective immunity against infections have not yet been explored evidently (Lucia *et al*., 1999). Moreover, candidiasis is a versatile disease which may apparent itself at various levels, such as from mucocutaneous tissue to internal organs (Vecchiarelli *et al*., 2012).

2.15. **Recent approaches involved against oral candidiasis**

2.15.1. **Passive Immunization with Chicken Egg yolk antibodies (IgY):** Antibody based therapies is on the rise using the advancement in the genetic engineering of recombinant antibodies which allows for the development of novel drugs by understanding the immuno pathology of the diseases. Oral immunotherapy (passive immunization) with specific antibodies is an approach that has been actively practiced by the researchers for the last two decades. Passive immunization with the chicken egg yolk antibodies to neutralize specific pathogens is a potential alternative to overcome the issues of resistance to antimycotics. Specific antigens are used to immunize the chickens which trigger their immune responses against the antigens to generate antibodies against the specific antigen which are then maternally transferred to the egg yolk. Booster doses of immunization are given at specific time intervals to ensure continued transfer of antibodies to the egg yolk which are then purified and characterized. In the last few decades several commercial sources of egg yolk antibodies against different human and veterinary pathogens are available.

2.15.1.1. **Antibodies Biosynthesis:** There are three immunoglobulin classes (IgA, IgM and IgY) present in birds which are differentiated by their structure, concentration, and immunochemical function. The structure, molecular weight, and electrophoretic mobility of avian IgA and IgM are alike to mammalian IgA and IgM. Chicken IgY is as efficient as IgG (mammalian serum antibody) in its function which comprises of about 75% of the total antibody production (Carlander *et al*., 2000). The antibodies are transferred to the growing embryo for providing acquired immunity in chicken whereas in mammals, the maternal antibodies are transferred after birth (Sim *et al*., 2000). IgA and IgM antibodies are secreted into the developing egg follicle and are integrated into the egg white along with the
secretion of egg albumin in the oviduct (Figure 7). A receptor which is specific for IgY translocation is present on the yolk membrane surface on which the serum IgY is selectively transferred to the yolk (Loeken & Roth, 1983; Morrison et al., 2002; Tressler & Roth, 1987). IgY is present at a concentration of 5 to 25 mg/ml in egg yolk, however IgA and IgM are present in a concentration of 0.15 and 0.7 mg/ml respectively in egg white (Li et al., 1997). Chickens do not possess IgE and IgD antibodies as identified in mammals (Sharma, 1997).

2.15.1.2. Structure of Immunoglobin Y (IgY): The molecular weight of IgY (~180kDa) is heavier than the molecular weight of mammalian IgG which is ~150kDa. Two uniform heavy (H) chains and two uniform light (L) chains connected by a disulfide bridge makes the general structure of the IgY. Like IgG of mammals, one variable (V1) domain and one constant domain (C1) are present in the light chain of IgY but the intra chain disulfide connection between the V\textsubscript{L} and C\textsubscript{L} region is missing. This linkage in particular is important for the stabilization of L-chain of mammalian IgG, so the intra molecular forces of IgY are not as strong as mammalian IgG (Shimizu et al., 1993). There is one variable domain V\textsubscript{H}, four constant domains C\textsubscript{H}1, C\textsubscript{H}2, C\textsubscript{H}3 and C\textsubscript{H}4 present in the IgY heavy chain which are different from the heavy chain of mammalian IgG which consists of only three constant domains (C\textsubscript{H}1; C\textsubscript{H}2 and C\textsubscript{H}3).

In the heavy chain of IgY, potential switch regions (made up of proline and glycine residues) are present near the margins of C\textsubscript{H}1-C\textsubscript{H}2 and C\textsubscript{H}2-C\textsubscript{H}3 domains which gives limited flexibility to the heavy chain of IgY. On the other hand, the C\textsubscript{H}1 and the C\textsubscript{H}2 domains are divided by a region called hinge region, which provides substantial flexibility to the Fab fragment of the heavy chains of IgG. While comparing the C-domain sequences of IgG and IgY, the C\textsubscript{H}2 domain and C\textsubscript{H}3 domain of IgG was found to be equal to the C\textsubscript{H}3 domain and C\textsubscript{H}4 domain of IgY whereas the IgY C\textsubscript{H}2 domain equivalent is not there in IgG heavy chain. The β-sheet structure of C domains of IgY is lower in its content when compared to the mammalian IgG; this results in the chaotic conformation of IgY domains. The Fc part of IgY is considered to be the site of the majority

Figure 8: Structure of IgG and IgY antibodies (Losonczy & Batke, 1997)
of biological functions which consist of two carbohydrate side-chains whereas the IgG contains only one (Figure 8).

2.15.1.3. Merits of IgY Technology: The chicken scores more in the production of polyclonal antibody than the traditional antibody production in mammals (Table 5). The simple precipitation methods are enough for the purification of IgY from the egg yolk since it contains only IgY class of antibody (Gassmann et al., 1990). The phylogenetic distance among mammals and chickens makes the possibility of antibody production in chickens and also very less antigen is enough for the stimulation of effective immune response (Larsson et al., 1988). Chicken antibodies are able to recognize diverse epitopes so it can provide access to various antibody ranges than mammalian antibodies (Carlander et al., 1999). Antibodies are obtained from the eggs of immunized chickens whereas in mammals antibodies are collected by bleeding of the animals which is a stressful procedure when compared with the less invasive method in chickens (Schade et al., 1991). Also, continued high titers of antibody production in chickens lessen the frequency of immunization (Gassmann et al., 1990). The animal maintenance costs for chicken are also economical as compared with the mammals such as rabbits (Carlander et al., 2000). Therefore, chickens are considered good experimental animal for polyclonal antibody production which offers more sterile, economical, suitable, and abundant source of antibodies when compared with the mammalian serum (Gassmann et al., 1990; Carlander et al., 2000). It was found that the antibody production in chickens is 18 times more than the antibody obtained from rabbits (Nakai et al., 1994). It was estimated around 2gm of IgY can be acquired from one egg per month per chicken (Akita & Nakai, 1992; Carlander et al., 1999). IgY is stable in the egg yolk for months and after purification it may be stable for years which can be refrigerated (Larsson et al., 1993). With all these advantages chicken eggs exhibit as ultimate alternative antibody source.

2.15.1.4. Physiochemical Properties of IgY: Polson et al., (1980) reported that the isoelectric point of IgY is lower than that of IgG which is in the range of 5.7 to 7.6 whereas that of IgG lies between 6.1 and 8.5. Since the Fc fragment, the most hydrophobic part of the IgY is bigger than that of the IgG, the IgY molecule is more hydrophobic than IgG molecule (Davalos et al., 2000).

2.15.1.5. Antimicrobial effects of IgY: It is evidenced from various studies that IgY antibodies exhibit antimicrobial activities against various bacteria including E.coli (Imberechts et al., 1997), Porphyromonas gingivalis (Hamajima et al., 2007; Tezuka et al.,
2006; Yokoyama et al., 2007), Salmonella (Lee et al., 2002), Staphylococcus aureus (Zhen et al., 2008), Helicobacter pylori (Shin et al., 2002), Streptococcus mutans (Hatta et al., 1997), Pseudomonas aeruginosa (Kollberg et al., 2003), fungi like Candida albicans (Ibrahim et al., 2008), and virus-like Rotavirus (Vega et al., 2012). There are several mechanisms of action proposed for IgY therapy, including prevention of adhesion of microbes to cell surfaces, prevention of cell-to-cell spread to suppress the viral colonization, agglutination of bacteria results in microbial immobilization, inhibition of enzyme activity and neutralization of toxin activity.

### Table 5: Comparison of mammalian IgG and Chicken IgY

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mammalian IgG</th>
<th>Chicken IgY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Sampling</td>
<td>Invasive</td>
<td>Non-invasive</td>
</tr>
<tr>
<td>Source of Antibody</td>
<td>Blood Serum</td>
<td>Egg Yolk</td>
</tr>
<tr>
<td>Antibody Amount</td>
<td>200mg/bleed</td>
<td>50-100mg IgY/egg (300 eggs/year)</td>
</tr>
<tr>
<td>Frequency of Collection</td>
<td>Every two weeks</td>
<td>Every Day</td>
</tr>
<tr>
<td>Quantity of antibody/year</td>
<td>5200mg</td>
<td>22,500mg</td>
</tr>
<tr>
<td>Amount of Specific Antibody</td>
<td>~5%</td>
<td>~2-10%</td>
</tr>
<tr>
<td>Protein A/G Binding</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Interference with mammalian IgG</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Interference with rheumatoid factor</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Activation of mammalian complement</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Adapted from Schade et al., 2005; Courtesy: Xu et al., 2011

2.15.1.6. **Application of anti-Candida sp., IgY antibodies:** The effect of anti-Candida albicans antibody (anti-CA IgY) was investigated by Ibrahim et al. (2008) in vitro and in vivo. They found that anti-CA IgY significantly reduced the adherence of Candida albicans to FaDu cells (human pharynx carcinoma cells) in vitro and also had a protective efficacy in immunosuppressed mice model with experimentally induced oral candidiasis. The efficacy of anti-Candida albicans IgY antibodies in the prevention of disseminated infection in BALB/c mice was evaluated (Abdelnoor et al., 2006) and the results indicated that no Candida albicans colonies could be detected and no abscesses were seen in kidneys obtained from anti-C. albicans IgY treated mice.

The IgY antibody production against Hsp90 of Candida albicans was evaluated in a study (Ziglari et al., 2009) in comparison with whole cell antigen. The results suggested that the purity, yield and the specific IgY levels were better with whole cell immunized antibodies.
than Hsp90. A study was conducted by Wang et al. (2008) to measure inhibition ability of anti-
C. albicans IgY against fluconazole (FLC) sensitive and resistant strains of C. albicans
and concluded that anti-Candida albicans IgY inhibited the growth of Candida albicans in
invitro and it has the potential to be used in the prevention and treatment of oral candidiasis.
A small feasibility study performed by Wilhelmson et al., (2005) indicated that IgY might
prevent Candida albicans infections in the mouth of children with lymphatic leukemia. None
of the children who received IgY treatment (0/4) had signs of C. albicans in their mouth,
compared to 7/13 in the untreated group. An invention has also been made by Larsson &
Kollberg, (2010) on local administration of chicken egg yolk immune globulins (IgY) to treat
and prevent fungal infections caused by Candida species and Aspergillus species.

The efficacy of anti-C albicans IgY in the prevention of C. albicans adherence to
human oral epithelial cells and biofilm formation was evaluated in vitro (Fujibayashi et al.,
2009) and it revealed that the adhesion and biofilm formation were significantly reduced. It
also found effective in preventing the adherence of Candida to denture base material thereby
preventing denture stomatitis and candidiasis (Kamikawa et al., 2014). In a study conducted
by Takeuchi et al. (2014), the efficacy of oral moisturizing gel containing anti-C albicans
IgY was evaluated in older patients for one month and concluded that C. albicans CFU was
significantly reduced.

2.16. Rationale of the study: The dramatic increase in fungal infections has intensified the
search for new, safer and more efficacious agents to combat serious fungal infections the
increasing incidence of the development of resistance against some of the traditionally used
antifungal, there is a constant need for research into new and effective agents to treat oral
candidiasis. The present research dealt with the ability of chicken egg yolk antibodies to
neutralize the virulence and development of Candida sp., was assessed in vitro and in vivo.
They were able to significantly reduce the adhesion ability of Candida sp., thereby reducing
the severity of the tongue lesions and the systemic dissemination. These results suggest that
anti-Candida sp., IgY could be used as a prophylactic measure against oral candidiasis. The
consortium of anti- C albicans ,C.tropicalis, C.krusei IgY antibody will be used together for
the development of a novel oral care product so they can exhibit combined anti-fungal
activity against the infection. These results have formed a strong platform for the formulation
of oral care products such as lozenges, chewing gum and mouth rinse comprising of yolk
derived antibody (IgY) developed against antymycotic resistant fungi on further investigations
in animal models and clinical trials involving humans.