CHAPTER 2

REVIEW OF LITERATURE
Seborrhoeic dermatitis (SD) of scalp (Dandruff) is a common condition affecting humans across the globe, yet there is very little work done on this subject, especially in India. The fungus belonging to Malassezia species has been widely accepted as the causative agent for the same. It is worth to look into the research and observations that have been reported with respect to this condition. This review of literature covers reported work on dandruff in the following sections:

- Skin Ecology
- Seborrhoeic Dermatitis & Dandruff
- Role of genus Malassezia in health and disease
- Host microbe interactions - The key players
- Seborrhoeic Dermatitis & Dandruff Therapy
- Challenges and opportunities ahead

2.1. Skin Ecology

2.1.1. The features of skin

The human skin is not a uniform structure and displays variations in form of structure, temperature, sebum concentration, moisture and pH. Moreover, the presence of glands like sebaceous, apocrine, eccrine also is variable from region to region. Usually, the skin is dry, acidic and cold, but distinctive habitats are determined by the density of hair follicles and glands apart from skin thickness and folds. Structurally, the epidermis is a formidable physical barrier, opposing infiltration by potential toxins and microorganisms while retaining moisture and nutrients inside the body. (Findley and Grice, 2014) The physical and chemical features of the skin opt for exclusive sets of microorganisms that are tailored to the niche they inhabit.

Trillions of bacteria, fungi, viruses, archaea and small arthropods colonize the skin surface, collectively forming the skin microbiome (Kong and Serge, 2012). In and on human body, microbes outnumber human cells by a factor of 10, while microbial genes outnumber human genes by a factor of 100. The term ‘microbiome’ is used to describe these microorganisms. Human microbiome is the entirety of the microorganisms and their genes on and in the human body (Findley and Grice, 2014). These microorganism have been classified as transient versus resident or beneficial versus pathogenic or collaborators versus adversaries.
by various researchers (Kong and Serge, 2012). Several methods are used for identifying the human skin microbial inhabitants (Gao et al., 2007).

2.1.2. Detection of microorganisms on skin

Up until the 1980s, microbiologists routinely relied on culture-dependent methods for identification, characterization, and microbial isolation. Colony morphology, stains (i.e. gram stain), motility tests, biochemical characteristics (i.e. coagulase test), antibiotic resistance profiles, and other characteristics guided identification of fungi and/or bacteria along with taxonomy. Recently, culture-independent methods of microbial identification are being used to identify the microbiota. These methods rely on a targeted amplicon strategy that does not depend on growing isolates in pure culture and utilizes highly conserved microbe-specific molecular markers. For bacterial identification, the 16S ribosomal RNA (rRNA) gene is used, while fungi and other micro eukaryotes are identified using either the Internal Transcribed Spacer (ITS) region or 18S rRNA gene. With the complementary approach of amplicon-based surveys, i.e. the whole genome shotgun metagenomics, one can identify the microbiota present and gain insight into the efficient potential of the microbiota in an untargeted manner (Findley and Grice, 2014)

a) The bacterial ecology of skin

While it is commonly assumed that microbes reside on the surface of the skin and within invaginations that open towards the surface (i.e. sebaceous and sweat glands), an innovative approach has demonstrated that microbial products are often found in the sub epidermal sections of the dermis and adipose tissue as well (Nakatsuji et al., 2013).

Studies on skin microflora suggest that skin feature is the topographical diversity of bacterial populations, with composition and variety of bacteria inhabiting the skin depending on the microenvironment. Variability between individuals is high, as is temporal variability within the same individual. However, the dominant types of bacteria that reside on the skin appear to be comparatively stable, with the occasional, less-abundant bacterial types, accounting for the variability. Indeed, site-specific colonization is a key feature of the human skin microbiome. Another important aspect is that longitudinal stability is dependent on the skin site, with sebaceous sites being the most stable and dry sites being the most variable over time. The findings of skin microbiome studies show that skin bacterial communities are
generally diverse between individuals (Gao et. al., 2007; Grice et. al., 2008) and may be influenced by ethnicity, lifestyle, and/or geography (Blaser et. al., 2013). Research on the skin microflora, suggests that the skin microbiota is diverse, but dominated by a small group of genera, in particular Staphylococcus, Propionibacterium, and Corynebacterium (Morgan and Huttenhower, 2012).

![Bacterial diversity of the skin. (Grice et. al., 2009)](image)

**Figure 2.1 - Bacterial diversity of the skin. (Grice et. al., 2009)**
Phylogenetic tree of the domain bacteria with each branch representing a phylum.

Black branches represent numerically abundant phyla on the skin, red branches represent rare phyla on the skin, and green branches represent phyla that are absent from the skin.
Table 2.1 - Summary of few recent studies on human skin bacterial microbiome in healthy subjects and in patients with skin disorders.

<table>
<thead>
<tr>
<th>Health status</th>
<th>Skin site studied</th>
<th>Methodology used</th>
<th>Organisms isolated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>Forehead</td>
<td>Culture Analysis</td>
<td>Propionibacterium acnes, Propionibacterium granulosum, Staphylococcus epidermidis</td>
<td>Dekio et. al. (2005)</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>Psoriatic lesions</td>
<td>16S rDNA PCR</td>
<td>Propionibacterium acnes, Corynebacterium tuberculostearicum, Staphylococcus hominis, Streptococcus mitis, Enhydrobacter aerosaccus, Staphylococcus capitis, Staphylococcus caprae, Staphylococcus epidermidis, Corynebacterium simulans, Dermacoccus</td>
<td></td>
</tr>
</tbody>
</table>
## Table 2.1 - Summary of few recent studies on human skin bacterial microbiome in healthy subjects and in patients with skin disorders

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<thead>
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</table>
| Healthy       | 20 skin sites represented by 3 microenvironments:  
(i) **Sebaceous** (glabella, alar crease, external auditory canal, occiput, manubrium, and back)  
(ii) **Moist** (nare, axillary vault, antecubital fossa, interdigital web space, inguinal crease, gluteal crease, popliteal fossa, plantar heel, and umbilicus)  
(iii) **Dry** (volar forearm, hypothenar palm, and buttock) | 16S rRNA gene phylotyping | **Sebaceous regions**  
Propionibacterium, Staphylococcus spp.  
**Moist sites**  
Corynebacterium, Staphylococcus spp.  
**Dry sites**  
β-Proteobacteria, Flavobacteriales | Grice et. al. (2009) |
| Healthy       | 11 body locations;  
Forehead, left and right axillae, left and right inner elbows, left and right forearms, left and right forelegs, and behind the left and right ears. | Quantitative PCR | Corynebacterium, Propionibacterium, Streptococci, Staphylococci | Gao et. al. (2010) |
| Healthy       | Retroauricular creases, antecubital fossae, anterior nares. | 16S rRNA gene phylotyping | Actinobacteria (Propionibacterineae, Corynebacterineae, Micrococcineae)  
Firmicutes (Staphylococcus spp.) | Curtis et. al. (2012) |
| Atopic Dermatitis | Skin | 16S ribosomal RNA bacterial gene sequencing | Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Corynebacterium, Propionibacterium | Kong et. al. (2012) |
| Acne vulgaris | Skin microcomedone | 16S rDNA PCR | Propionibacterium acnes, Staphylococcus epidermidis, Propionibacterium humerusii, Propionibacterium granulosum | Fitz-Gibbon et. al. (2013) |
b) The fungal ecology of skin

Just until some time ago, the human microbiome was correspondent to the bacterial community. Fortunately, recent studies have revealed the human microbiome to be complex and comprise of viruses fungi, and bacteria. Describing “mycobiome”, in health, as the fungal community (relating to the oral cavity, skin, and gastrointestinal (GI) tract body sites), is paving the way to define the variations that occur in this community in the state of disease. Studies directed at defining the skin mycobiome are important since it is recognized that fungi, specifically dermatophytes and yeast (Candida and Malassezia) are known fungal skin pathogens.

Cultivation-based studies identified the major component of the skin fungal community as Malassezia (formerly known as Pityrosporum) genus, consisting primarily of seven of the 14 known species (Gaitanis et. al., 2012). These findings have been confirmed by molecular community analysis. In the review on skin microbiome, Grice & Serge (2011) have described the factors that affect the microbiome/mycobiome of skin as follows: topography, invaginations and appendages, various glands in the skin, host factors such as age, sex and environmental factors (occupation, clothing choice and antibiotic usage). They cite density of sebaceous glands as an example that influences the skin microbiota, depending on the area. Regions having an elevated density of sebaceous glands, including the face, chest and back, encourage the growth of lipophilic microorganisms like, Malassezia spp. The fungal microbiota in sebaceous areas tends to be less diverse than bacterial communities and is generally dominated by Malassezia spp., specifically M. restricta and M. globosa (Grice, 2014). According to Gao et. al., (2010) Malassezia account for up to 80% of fungi present, depending on the anatomical location sampled.

In a recent article, Ghannoum (2016) has taken stock of work on mycobiome and expressed disappointment at the lack of literature on ‘mycobiome’. He states: “As of November 2015, only 269 of more than 6,000 Web of Science search results for the word “microbiome” also acknowledge “fungus,” and the scientific search engine returns only 55 papers pertaining to “mycobiome.”

This comment underlines the need for study on fungal ecology in health and disease like dandruff/SD.
Figure 2.2 - Human skin fungal diversity
The fungus Malassezia (purple) dominates the majority of the body sites. The feet harbour the greatest diversity of fungi. (Source: http://i.kinja-img.com/gawker-media/image/upload/s--WK4QSKFw--/c_scale,fl_progressive,q_80,w_800/18of2y8yg4tocjpg.jpg)
<table>
<thead>
<tr>
<th>Health status</th>
<th>Skin site studied</th>
<th>Methodology used</th>
<th>Organisms isolated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot disease in soccer athletes</td>
<td>Foot skin</td>
<td>Culture and morphologic observation</td>
<td><em>Trichophyton, Candida</em></td>
<td>Purim <em>et. al.</em> (2005)</td>
</tr>
<tr>
<td>Dandruff</td>
<td>Scalp</td>
<td>Pyro sequencing</td>
<td><em>Filobasidium, Penicillium, Malassezia, Eupenicillium, Acremonium, Cryptococcus, Didymella, Rhodotorula,</em></td>
<td>Park <em>et. al.</em> (2012)</td>
</tr>
<tr>
<td>Healthy</td>
<td>Scalp</td>
<td>Pyro sequencing</td>
<td><em>Acremonium, Didymella, Cryptococcus, Malassezia</em></td>
<td>Park <em>et. al.</em> (2012)</td>
</tr>
<tr>
<td>Healthy</td>
<td>Skin</td>
<td>Culture and morphologic observation</td>
<td><em>Malassezia, Penicillium, Aspergillus, Alternaria, Candida, Rhodotorula, Cladosporium and Mucor</em></td>
<td>Findley <em>et. al.</em> (2013)</td>
</tr>
</tbody>
</table>
2.2. Dandruff and Seborrhoeic Dermatitis

Dandruff and SD are of an uninterrupted range of the identical disease that affects the seborrhoeic areas of the body (Table 2.3). They share many common features and respond to similar treatments. Dandruff is a chronic non-inflammatory scaling of the scalp, which is often grouped with SD. Seborrhoeic dermatitis unlike dandruff, is inflammatory in nature. In addition to scaling, SD is characterized by erythema and sometimes crusting (Ghannoum and Mukherjee, 2013).

Table 2.3 Comparison of seborrhoeic dermatitis and dandruff

<table>
<thead>
<tr>
<th></th>
<th>Seborrhoeic Dermatitis</th>
<th>Dandruff</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epidemiology</strong></td>
<td>Up to 40% of infants within 3 months of age, 1-3% of the general adult population.</td>
<td>50% of adult population</td>
<td>• Gupta et. al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Schwartz et. al., 2010</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Rosso, 2011</td>
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<td></td>
<td></td>
<td></td>
<td>• Manuel and Ranganathan, 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Sampaio et. al., 2011</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td>Scalp, retro-auricular area, face (nasolabial folds, upper lip, eyelids, eyebrows), upper chest.</td>
<td>Scalp</td>
<td>• Rosso, 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Clark et. al., 2015</td>
</tr>
<tr>
<td><strong>Presentation</strong></td>
<td>Erythematous patches, with large, oily or dry scales.</td>
<td>White to yellow flakes dispersed on the scalp and hair; without erythema.</td>
<td>• Rosso, 2011</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Sampaio et. al., 2011</td>
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<td></td>
<td></td>
<td></td>
<td>• Bukvic et. al., 2012</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td>Acanthosis, hyperkeratosis, spongiosis, parakeratosis, Malassezia yeasts.</td>
<td>Vasodilation and perivascular and perifollicular inflammatory infiltration; “shoulder parakeratosis”.</td>
<td>• Schwartz et. al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Sampaio et. al., 2011</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Antifungal shampoos and topical.</td>
<td>Topical corticosteroids, immune modulators, phototherapy, systemic treatment.</td>
<td>• Naldi and Rebora, 2009</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>• Berk and Scheinfeld, 2010</td>
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<td></td>
<td></td>
<td></td>
<td>• Rosso, 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Bukvic et. al., 2012</td>
</tr>
<tr>
<td><strong>Predisposing Factors and causes</strong></td>
<td>Sebaceous gland activity, fungal colonization, and individual susceptibility (epidermal barrier integrity, host immune response, genetic factors, neurogenic factors and stress, nutrition, etc.).</td>
<td></td>
<td>• Gupta et. al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Ro and Dawson, 2005</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>• Rosso, 2011</td>
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<td></td>
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<td></td>
<td>• Sampaio et. al., 2011</td>
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<td></td>
<td>• Bukvic et. al., 2012</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Turner et. al., 2012</td>
</tr>
</tbody>
</table>
2.2.1. Seborrhoeic dermatitis

Seborrhoeic dermatitis as the name suggests is an inflammatory disease of skin affecting mainly areas of skin rich in sebum secretion. It shows a bimodal occurrence affecting infants (cradle crap) and post puberty. The prevalence is reported to be between 1-3% in general population (Sampio et. al., 2011)

a. Clinical Presentation

Rosso (2011) has described clinical presentation of adult SD in a status report on its management. It presents as erythematous patches. The affected areas usually are face and/or scalp with fine scaling. Itching (pruritus) is often present when scalp is involved but may not be present otherwise. The sites commonly affected are scalp, anterior hairline, eyebrows, forehead (glabella region), nasal creases, ears, and chest.

Two forms of SD of chest have been described. Petaloid form is common and pityriasis form is rare. The follicular and perifollicular papules take a patchy form that resembles shape of petals of a flower in petaloid form of SD. In pityriasis form of SD fine scaling which is generalised in nature is seen (Janinger and Schwartz, 1995).

When SD appears on the face, it usually affects the nasolabial folds and the lateral sides of the nose in addition to the eyebrows and glabella (Berk and Scheinfeld, 2010)

b. Factors that play role in Seborrhoeic dermatitis

Despite quite a high prevalence, the exact cause of this condition is is not clearly understood. However, several factors (Malassezia yeasts, hormones, sebum levels, immune response, neurogenic factors, external factors) seem to be involved in SD aetiopathogenesis, but the exact pathogenic mechanism still remains controversial. Genetic pre-disposition has never been found to be associated with SD.
Though *Malassezia* are seen commonly on the surface of the skin, in SD they are seen within the stratum corneum. The yeasts are closely associated with the flakes and parakeratotic cells and the number of yeasts correlates with flaking severity. In SD, there is increased turnover rate of the epidermis and this hyper-proliferative nature of the epidermis is the cause of parakeratotic cells.

In a review on role of hyphae in fungal pathogenesis, Brand (2012) sees dimorphism of *Malassezia* as a mechanism to help the *Malassezia* to defend themselves against shedding and form colonies deeper into the skin using an example of *M. globosa*. Growth of *Malassezia* as commensals in yeast form is tolerated by immune system. *Malassezia* yeasts are considered to be taken up by keratinocytes and survive as facultative intracellular parasites by actively suppressing the inflammatory response. *Malassezia* respond to increased sebum level...
by morphogenesis and acquire hyphal forms. Hyphae produce lipases and proteases to lead to inflammatory molecules like oleic acid and arachidonic acid.

2.2.2. Dandruff

Dandruff is seen commonly in human population and nearly 50% of the population would have experienced dandruff in some time in their lives. It presents itself as whitish flakes that fall off from the affected area. It is usually seen once an individual attains puberty and thereafter. Dandruff affects only scalp and terminal hair (Turner et al., 2012).

a. Clinical presentation

Dandruff flakes are clusters of corneocytes having a large degree of cohesion with one another that get detached as such from the surface of the stratum corneum. Parakeratotic cells frequently make up an element of dandruff. Their number is related to the severity of the clinical manifestations that might also be predisposed by seborrhoea (Pierard et al., 2000; Pierard et al., 2006)

The condition presents itself in the form of light, white to yellow and dispersed flaking on the scalp and hair without erythema. Visual scoring is the most frequently used method for assessing the severity of dandruff (Pierard et al., 2006). Dandruff quantification using bio instrumental methods like photography and squamometry are also employed (Ranganathan and Mukhopadhyay, 2010).

b. Development of dandruff

Despite the high prevalence, the pathogenesis of dandruff is not well understood. However, research has acknowledged numerous predisposing factors, together with fungal colonization, sebaceous gland activity, as well as several factors that confer individual susceptibility (Ro and Dawson, 2005; DeAngelis et al., 2005).

A recent study by Thayikannu et al. (2015) suggested that alterations in sebum secretion as well as its breakdown by Malassezia play an important role in the aetiology of dandruff. Human sebum is a rich complex mixture of lipids (triglycerides, esters of various kinds, cholesterol and squalene) and as Malassezia are lipidophilic in nature they survive on sebum. They utilise sebum to produce diglycerides, monoglycerides and free fatty acids (FFA).
Rudramurthy et. al. (2014) report factors other than *Malassezia* that contribute to the pathogenesis of dandruff. These factors listed are stress, fatigue, use of shampoos, weather extremes, immunosuppressed status (AIDS), oily nature of skin, in addition to neurological disorders.

Some ancillary non-microbial causes of dandruff may operate through physical or chemical irritants (Pierard et. al. 2006). They consider excessive exposure to sunlight, daily minimal cumulative insults to the scalp including hard brushing, over shampooing and hair friction and certain cosmetic hair products.

Though *Malassezia* spp. have been considered to be the major cause of dandruff, recent work has pointed out probability of disequilibrium or imbalance between the bacterial and fungal populations colonising the scalp, which may be one of the contributing factors in its aetiology. A study by Wang et. al. (2015) in Chinese patients clearly shows ratios of *Malassezia* to *Propionibacterium* and *Propionibacterium* to *Staphylococcus* were significantly higher in dandruff patients as compared to normal.

Due to various reasons, identification of the microbial species actually related to dandruff or even colonizing the normal scalp surface has been controversial in previous studies. Two major factors responsible for such a varied data are: i) different origin of the sampled populations (North America, Korea or France) having a large environmental and ethnicity variation and ii) the difficulty and variation in culturing methods. A comparison of studies reported by Gupta et. al. (2001), Gemmer et. al. (2002), Zaidi et. al. (2002), Lee et. al. (2011), Park et. al. (2012) and Clavaud et. al. (2013) have been represented in Table 2.4.
Table 2.4 – Comparison of reported studies on dandruff involving populations from different origins

<table>
<thead>
<tr>
<th>Reference</th>
<th>Population Studied</th>
<th>Sample size</th>
<th>Method used</th>
<th>Organisms isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemmer et. al. (2002)</td>
<td>American</td>
<td>70</td>
<td>tFLP analysis</td>
<td>• Malassezia restricta • Malassezia globosa</td>
</tr>
<tr>
<td>Zaidi et. al. (2002)</td>
<td>Pakistani</td>
<td>70 (20N – 50 D)</td>
<td>Microscopic analysis</td>
<td>• Malassezia</td>
</tr>
<tr>
<td>Lee et. al. (2011)</td>
<td>Korean</td>
<td>140 (100N – 40D)</td>
<td>26S rDNA PCR – RFLP analysis</td>
<td>• Malassezia restricta</td>
</tr>
<tr>
<td>Park et. al. (2012)</td>
<td>Korean</td>
<td>7 (3N – 4D)</td>
<td>Mass prosequencing</td>
<td>• Acremonium spps. • Penicillium spps. • Filobasidium spps. • Malassezia spps. • Cryptococcus spps. • Rhodotorula spps.</td>
</tr>
<tr>
<td>Clavaud et.al. (2013)</td>
<td>French</td>
<td>49 (20 N – 29 D)</td>
<td>Quantitative PCR</td>
<td>• Propionibacterium acnes • Staphylococcus epidermidis • Malassezia restricta</td>
</tr>
<tr>
<td>Rafiq et. al. (2014)</td>
<td>Indian</td>
<td>NA</td>
<td>Culture dependent methodology</td>
<td>• Malassezia furfur • Candida albicans • Other Candida spp • Aspergillus niger • Aspergillus flavus • Aspergillus fumigatus • Penicillium spp • Microsporum spp • Trichophyton spp</td>
</tr>
</tbody>
</table>

Key: N – Normal, D – Dandruff, SD – Seborrhoeic Dermatitis
2.2.3. The dandruff–SD connection

Dandruff can be distinguished from SD on the basis of inflammation and extension of scaling outside the scalp (Ro and Dawson, 2005). Considering the fact that there exists a very fine line of difference between the two, controversies with respect to their relationship still exists.

Dandruff has been described as a mild form of SD by some investigators. Many reports propose a clear association amongst the two clinical entities - the mildest form of the clinical presentation of SD as dandruff, where the inflammation is minimal and remains subclinical. Histologically, there is scattered presence of lymphoid cells and squirting capillaries in the papillary dermis with indications of spongiosis and focal parakeratosis (Pierard et. al., 2000; Pierard et. al., 2006)

Others believe that dandruff is an altogether separate condition involving flaking of the scalp (Schwartz et. al., 2012). Conceptually, dandruff is dander and represents nothing more than physiologic scaling (Ranganathan and Mukhopadhyay, 2010).

In summary, multiple predisposing factors have been identified in the pathogenesis of SD and dandruff. Various intrinsic and environmental factors, including sebaceous secretion, *Malassezia* yeast, immune response, host epidermal conditions, and the interactions between these factors, may all contribute to the pathogenesis.

2.3. Role of *Malassezia* in health and disease

2.3.1. *Malassezia*

*Malassezia* are yeasts that are lipophilic dimorphic and are probably the major fungal representative of the fungal microflora (mycobiome) of humans. Presently, *Malassezia* are classified into 14 species based on their physiological and biochemical properties and phenotypic characteristics as follows:

Kingdom - Fungi

Phylum - Basidiomycota

Sub-phylum - Ustilaginomycotina
Class - Exobasidiomycetes

Order - Malasseziales

Family - malasseziaceae

The species belonging to *Malassezia* are as follows:

* M. furfur, *M. sympodialis*, *M. nana*, *M. equine*, *M. globosa*, *M. restricta*, *M. cuniculi*, *M. obtuse*, *M. sloofiae*, *M. dermatis*, *M. japonica*, *M. caprae*, *M. pachydermatis*, *M. yamatoensis*

*Malassezia* commonly reside in oily areas such as the face, scalp, and back. They live in the infundibulum of the sebaceous glands where they nourish on lipids present in human sebum. According to Kim *et. al.* (2015), Eichstedt in 1846 was the first to report an association between *Malassezia* yeasts and skin diseases based on the presence of yeasts and filaments in sample from the infected scales of patients with Pityriasis versicolor (PV).

### 2.3.2. When do *Malassezia* colonize skin?

Del Rosso and Kim (2009) have stated that, skin colonization with *Malassezia* spp. is as high as 13 to 50 percent in the first week of life. The source of newborn skin colonization is the exposure to transient flora from the maternal genital tract. Breast feeding has also been assumed to correlate with skin colonization in newborns. The frequency of bathing, lubricants, use of skin care products, and use of any occlusive agents are all linked with colonization of infantile skin with *Malassezia* spp. At puberty, a friendly environment is provided to *Malassezia* spp. due to increased activity of sebaceous glands as they are lipophilic organisms. After this, the association of *Malassezia* in the human host becomes a stable one. One may label the ‘mycobiome’ in respect to *Malassezia* skin diseases as ‘Malasseziome’!

### 2.3.3. *Malassezia* on healthy skin

There are number of studies carried out across the world including India which demonstrate that *Malassezia* species are associated with healthy skins on various body sites and there could be a preponderance of a particular species on a given body site.
In one study, Aspiroz et al. (1999) examined three body sites (back, chest and scalp) and detected the presence of *M. restricta*, *M. globosa* and *M. sympodialis*, of which *M. sympodialis* was mostly associated with back.

Nakabayashi et al. (2000) studied scalp, face and trunk for the prevalence of *Malassezia* species in 35 healthy subjects. The frequency of isolation of *M. globosa* was 22%, *M. sympodialis* 10% and *M. furfur* 3%. *M. sloofiae, M. pachydermatis, M. restricta* and *M. obtusa* were occasionally isolated from normal skin.

Gupta et al. (2001), after studying 20 healthy individuals, for the presence of *Malassezia* spp. from five sites (scalp, forehead, arms, legs and trunk) observed that, *M. globosa* was uniformly distributed in scalp, forehead and trunk. *M. restricta* and *M. sloofiae* were recovered more frequently from upper body (scalp and forehead), while *M. sympodialis* was more likely to inhabit the forehead.

In another study, Gupta et al. (2004) included 245 healthy subjects from six different age groups. When they looked for *Malassezia* species on four body sites (scalp, forehead, chest and back), they recovered *Malassezia* from 70% of individuals from one or more body sites. The most common species were *M. sympodialis* (56.9%) and *M. globosa* (31.8%) followed by *M. furfur* (6.1%), *M. sloofiae* (2.7%), *M. obtusa* (1.8%), and *M. restricta* (0.7%).

A similar study by Tarazoioae et al. (2004) reports that, the most commonly identified species in healthy individuals were *M. globosa, M. sympodialis, M. furfur, M. sloofiae* and *M. restricta* which made up 41.7%, 25.0%, 23.3%, 6.7% and 3.3% respectively of the isolated *Malassezia* flora. *M. globosa, M. furfur* and *M. sympodialis* were equally found on the scalp, chest and back.

Six body sites (right and left forearm, upper & lower back, forehead & scalp) were studied for presence of *Malassezia* species. *M. restricta* was the most abundant species in the majority of samples, accounting for 57–100% of all *Malassezia* spp. identified in samples from the healthy subjects as compared to *M. sympodialis* which was present solely on the upper back (Paulino et al., 2008).

Scalp, forehead, cheeks and thighs were the sites examined by Oh et al. (2009) for the presence of *Malassezia* spp using nested PCR and RFLP. They detected seven
Malassezia spp. of which *M. globosa* and *M. restricta* were predominant followed by *M. sympodialis*, *M. furfur*, *M. dermatis* and *M. sloofiae*.

In a study of healthy subjects for the presence of *Malassezia* spp. on their upper backs, carried out in India by Kaur et al. (2013), *M. sympodialis* was the major isolate followed by *M. obtusa* and *M. globosa*.

In a recent study by Prohic (2014), *M. sympodialis* was found to be the predominant species on trunks in older subjects, *M. restricta* on scalps in age groups 21-35 years, while *M. globosa* was identified as a common species in adults (36-50 years), both from scalp and trunk. From the trunks, *M. furfur* was the most frequent in children.

Thus various studies across the world, though not large in numbers, show variable distribution of *Malassezia* spp. on various body sites in healthy individuals, confirming that *Malassezia* are a ubiquitous component of the human skin microbiome.

### 2.3.4. *Malassezia* in Disease

*Malassezia* has been implicated as the causative organism in dandruff, SD, PV, atopic eczema and *Malassezia* folliculitis. It has also been involved in systemic infections (Gaitanis, 2012).

Velegraki et al. (2015) have stated multi-faceted interactions between *Malassezia* and human host in health and disease. They have put forward the following five types of relations:

- Commensalism
- Subtle alterations in skin melanocytes resulting into hypo- or hyper-pigmented areas with absence of clinical inflammation (PV)
- Inflammation without generation of antibody mediated immunity (dandruff and SD)
- Induction of specific dermatitis (atopic dermatitis)
- Invasion and inflammation of hair follicles (*Malassezia* folliculitis)
2.3.5 *Malassezia* in Seborrhoeic Dermatitis and Dandruff

The microbial origin of dandruff centers on the causal role of yeasts of the genus *Malassezia*. The vast majority of recent data supports a direct causal link between *Malassezia* fungi and dandruff. Growing evidence indicates that *Malassezia* spp. are a major etiologic factor in SD development. The number of *Malassezia* spp. decreases after antifungal therapy with disappearance of skin lesions. This is probably the most robust proof that *Malassezia* spp.s have a significant role in the development of SD.

Studies have detected *Malassezia* on the scalp of dandruff patients (DeAngelis *et. al.*, 2005; Rudramurthy *et. al.*, 2014), and higher numbers of *Malassezia* (*M. restricta* and *M. globosa*) show a relationship with SD appearance/severity (Heng *et. al.*, 1990; McGinley *et. al.*, 1975). *Malassezia* is known to have lipase activity, which hydrolyses human sebum triglycerides and releases unsaturated fatty acids like arachidonic and oleic acid (DeAngelis *et. al.*, 2007). These metabolites cause aberrant keratinocytes differentiation, leading to stratum corneum abnormalities such as parakeratosis, intracellular lipid droplets, and irregular corneocyte envelope (Warner *et. al.*, 2001). Such changes lead to disrupted epidermal barrier function and activate inflammatory response, either with or without visible local inflammation. In addition, these metabolites persuade keratinocytes to manufacture pro-inflammatory cytokines such as IL-1α, IL-6, IL-8 and TNF-α, thus prolonging the inflammatory response (Faergemann *et. al.*, 2001; Schwartz *et. al.*, 2013). Furthermore, arachidonic acid can be a source of prostaglandins that are pro-inflammatory mediators that can cause inflammation via neutrophil recruitment and vasodilation. Interestingly, *Malassezia* infection has also been reported in goats, dogs and monkeys with seborrhea (dry or greasy) and dermatitis (Uzal *et. al.*, 2007; Borda and Wikramanayake, 2015). Studies reported on the correlation of *Malassezia* with SD and dandruff have been represented in Table 2.5.
Table 2.5 - Studies correlating *Malassezia* with SD and dandruff

<table>
<thead>
<tr>
<th>Disease condition</th>
<th>Site of sample collection</th>
<th>Study outcome</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Seborrhoeic dermatitis   | Face                      | *M. furfur* (35%)  
*M. globosa* (22%)  
|M. restricta  
*M. sympodialis* | Nakabayashi et. al. (2000) |
| Dandruff                 | Scalp                     | *M. restricta*  
*M. globosa* | Gemmer et. al. (2002) |
| Seborrhoeic dermatitis   | Trunk                     | *M. sympodialis*  
*M. globosa* | Sandstrom et. al. (2005) |
| Seborrhoeic dermatitis   | Face and trunk            | *M. globosa* (63%, 20%)  
*M. furfur* (29.6%, 80%)  
*M. restricta* (7.4%, 0) | Hedayeti et. al. (2010) |
| Dandruff                 | Scalp                     | *M. globosa* (55.5 %)  
*M. furfur* (28.8%)  
*M. restricta* (11.1%)  
*M. sympodalis* (2.2%)  
M. japonica (2.2%) | Hedayeti et. al. (2010) |
| Dandruff                 | Scalp                     | *M. restricta* (47.5%)  
*M. globosa* (27.5%)  
*M. furfur* (7.5%)  
*M. sympodalis* (2.5%) | Lee et. al. (2011) |
| Dandruff                 | Scalp                     | *M. globosa* (40.7)  
*M. pachydermatis* (22.2%)  
*M. furfur* (11.1%)  
*M. restricta* (7.4%) | Mahmoudabadi et. al. (2013) |
| Dandruff                 | Scalp                     | *M. restricta* (97%)  
M. sloofiae (4%)  
*M. globosa* (<1%)  
*M. sympodialis* (<1%) | Clavaud et. al. (2013) |
| Seborrhoeic dermatitis   | Forehead                  | *M. globosa* (42.3%)  
*M. furfur* (26.9%)  
*M. obtusa* (15.4%). | Arsenijevic et. al. (2014) |
| Dandruff                 | Scalp                     | *M. globosa*  
*M. furfur*  
*M. restricta*  
*M. sympodialis*  
M. sloofiae | Rudramurthy et. al. (2014) |
| Dandruff                 | Scalp                     | *M. globosa* (46.5%)  
*M. furfur* (27.0%)  
*M. restricta* (12.7%)  
*M. sympodalis* (6.5%)  
*M. sloofiae* (0.8%) | Zareei et. al. (2016) |

Thus whether it is dandruff or SD, there are a number of factors that interplay for causation of the same. The frequency of *Malassezia* associated with these two entities varies depending upon the geographical region, location of the lesions on skin, methods of detection and individual susceptibility.
2.4. Host microbe interactions

2.4.1. The microbiome

The term microbiome was coined by Joshua Lederberg (2001) to “signify the ecological community of symbiotic, commensal, and pathogenic microorganisms that literally share our body space and have been all but neglected as elements of health and disease”.

The “microbiome”, contributes to genetic diversity, regulates disease, persuades metabolic processes, in addition to being essential for immunity. The human microbiome is also dynamic, and the changes associated with health and diseases have been described and mechanistically investigated (Grice, 2014). According to Grice & Segre (2012), the human microbiome is a source of genetic diversity, a modifier of disease, a crucial constituent of immunity, and a functional unit that influences metabolism and modulates drug interactions. As was clear from the Human Microbiome Project study (Peterson et al., 2009), each body site is a highly specialized niche distinguished by its individual microbial consortia, community dynamics, and interaction with host tissue.

2.4.2. How microbes influence immunity

In humans and animals, microbes provide protection against foreign invaders, stimulate and educate the immune response, aid in digestion, produce antimicrobials vitamins, among a host of other functions (Findley and Grice, 2014). Each body site is a home to a unique microbial community and shifts in microbial communities can result due to numerous factors, including genetic and environment variations, lifestyle and hygienic factors, in addition to the immune system, and have been associated with many diseases (Findley and Grice, 2014).

Immune function also can be influenced by the skin microbiome. *S. epidermidis* can upregulate production of the AMP human β-defensin 2 via activation of TLR2 and magnify the immune response of keratinocytes to pathogenic bacteria (Yuki et. al., 2011). Conversely, *S. epidermidis* can hinder TLR3-dependent inflammation subsequent to skin wounding via activation of TLR2 by surface lipoteichoic acid (Scharschmidt and Fischbach, 2013). Colonization with a human commensal skin microbe, namely, *S. epidermidis*, adjusts T-cell homing and play a role in an IL-1–dependent manner in mice (Naik et. al., 2012).
Sanford and Gallo (2013) referred to the breakdown of sebum by *Propionibacteria* that generates FFA, which work to control microbial colonization along with, β-defensins, antimicrobial histones, and sebocyte-derived cathelicidin. Wang et. al. (2012) demonstrated that TLR2 activation, interfered by LTA from *S. epidermidis*, leads to larger numbers of mast cells being recruited to sites of viral challenge in the skin. Furthermore, the release of the AMP cathelicidin by these recruited mast cells was amplified by this TLR2 stimulus, ending up in amplified antiviral immunity.

Thus, it appears that cutaneous microbial communities are closely associated with skin adaptive and innate immune functions.

### 2.4.3. Microbe-microbe interactions

The results of Chiller *et. al.* (2001) reveal that skin enhances the growth of commensal bacteria, which protect the host from pathogenic bacteria directly as well as indirectly. The direct effects consist of bacteriocin production, production of toxic metabolites, stimulation of a low-reduction oxidation potential, inhibition of translocation, prevention of adherence of competing bacteria, depletion of essential nutrients, and deterioration of toxins. Commensal bacteria compete for nutrients, niches, and receptors. Referring to Bibel *et. al.* (1983), they state that *S. epidermidis* bind keratinocyte receptors and may inhibit adherence of virulent *S. aureus*. Commensals can release bacteriocins. The authors cite example of *P. acne* and how they inhibit *Streptococcus pyogenes* by breakdown of lipids to produce fatty acids that acidify the site and inhibit *S. pyogenes*.

As discussed in Chapter 1, resident skin bacteria provide the first line of defence against potentially hazardous pathogens besides producing minute molecules that influence growth and behaviour of their microbial neighbours (Scharschmidt and Fischbach, 2013). Certain strains of *S. epidermidis* secrete Esp, a protease that inhibits biofilm formation and colonization by *S. aureus* in the anterior nares (Iwase T. *et. al.*, 2010). *Staphylococci* also produce AMPs of the antibiotic and phenol-solubin modulin classes and have activity against skin pathogens, though their clinical relevance remains undetermined. The abundance of *Corynebacterium* spp. in the nares is inversely correlated with that of *S. aureus* and *Corynebacteria*, as they inhibit colonisation of anterior nares by *S. aureus*, indicating that they may also play a protective function against skin pathogens.
In view of Schommer and Gallo (2013), the fungus-bacterium interaction can be very important in causation and treatment of a disease. They cite that mixed communities have resistance and virulence characteristics considerably different from those of single species communities. To support this, a study by Peleg et. al. (2010) showed that biofilms containing *S. epidermidis* and *C. albicans* in medical device-associated infections are significant more resistant to antimicrobials as single-organism biofilms.

Schommer and Gallo (2013) explore how commensal microorganisms can become pathogens stating that the most prominent and best studied examples are *S. epidermidis* and *C. albicans*. Others are less understood organisms like *M. restricta* and *M. globosa*.

But how does a commensal becomes a pathogen? And even more importantly, what might be the unique factors that allow a commensal to be tolerated by the host? These questions still await an answer. A better understanding of interactions between microbes, disease-causing organisms, and host may bring about better strategies against skin diseases.

### 2.4.4. Microbiome disequilibrium

It is likely that skin disorders presenting at specific anatomical sites are correlated to the skin physiologic characteristics, for example whether it is moist or oily, or also regarding the bacteria that preferentially reside at those specific skin sites (Kong, 2011). Microbe–host dysbiosis refers to a state of imbalance with the microbiota that negatively impacts the host, which can take place primarily due to exogenous factors that alter the composition of the flora toward a more pro-inflammatory population. Alternatively, host susceptibility factors such as polymorphisms in innate or adaptive immune elements can result in increased inflammation prior to any significant microbial shift. Either case can lead to a state where both the host immune response and microbiota are changed and contribute to a vicious cycle of detrimental inflammation in skin diseases (Figure 2.4).
vanRensberget. *et al.*, (2015), based on their experimental *H. ducreyi* infections in volunteers, suggest that the pre-existing microbiome of the skin could influence the outcome of infection. As per their study, in volunteers who had a stable microbiome consisting of higher levels of *Actinobacteria* and *Propionibacteria* the *H. ducreyi* infection resolved.

Wang *et. al.* (2015), in their study on microbial populations in scalps of dandruff and normal subjects, found that there is a microbial disequilibrium in scalps of dandruff afflicted population.

Clavaud *et. al.* (2013), in their study in French subjects, suggested that dandruff is linked to the balance between bacteria and fungi of host scalp.
Bertrandt et. al. (2015) underline the role of disequilibrium in skin microbiome in development of skin diseases such as psoriasis, atopic dermatitis and acne vulgaris without castigating it as the causal event.

These studies suggest that the interplay between skin bacteria, fungi as well as the host factors can be considered important in the development of skin disorders/diseases. To maintain healthy skin conditions, there needs to be a subtle balance that allows symbiosis with our commensal microbes while fending off potentially dangerous invaders. Disruption of this equilibrium or ‘dysbiosis’ can result from a change in the composition of skin microflora, or an alteration of the host immune response, or both; in either case the end result is excessive inflammation that is associated with skin diseases.

2.5. Seborrhoeic dermatitis and dandruff therapy

Treatment of SD and dandruff focuses on clearing signs of the disease; ameliorating associated symptoms, especially pruritus; and maintaining remission with long-term therapy. Because the main underlying pathogenic mechanisms involve the proliferation of Malassezia in addition to local skin irritation and inflammation, the most common treatment is topical antifungal and anti-inflammatory agents (Table 2.5). Other widely used therapies are coal tar, lithium gluconate/ succinate and phototherapy (Table 2.5). New therapies have also emerged including immune modulators like topical calcineurin inhibitors, and metronidazole; however their efficacy still remains controversial (Dessinioti and Katsambas, 2013). Alternative therapies such as tea tree oil have also been reported (Satchell et. al., 2002; Pazyar et. al., 2013). Few factors that need to be kept in mind before choosing a treatment regime include efficacy, side effects, ease of use/compliance, and age of the patient (Dessinioti and Katsambas, 2013). Systemic therapy is needed only in widespread lesions and in cases that do not respond to topical treatment (Sampaio et. al., 2011; Bukvic et. al., 2012).
Table 2.6 - Treatment of seborrhoeic dermatitis and dandruff

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dose/Formulation</th>
<th>Regimen</th>
<th>Mechanisms</th>
<th>Side Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOPICAL Antifungals</strong></td>
<td></td>
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</tr>
<tr>
<td>Ketoconazole</td>
<td>2% Shampoo, cream, gel or foam.</td>
<td>Scalp or skin: Twice/week x 4 weeks, then once/week for maintenance.</td>
<td>Inhibition of fungal cell wall synthesis.</td>
<td>ICD in &lt;1% of patients. Itching, burning sensation and dryness in 3% of patients.</td>
<td>• Pierard et. al. (2002) • Koc et. al. (2009) • Naldi and Rebora (2009) • Rosso (2011) • Bukvic et. al. (2012) • Hald et. al. (2015) • Okokon et. al. (2015)</td>
</tr>
<tr>
<td>Bifonazole</td>
<td>1% shampoo, cream or ointment</td>
<td>Scalp: every other day or once daily. Skin: once daily.</td>
<td></td>
<td>ICD in 10% of patients.</td>
<td>• Segal et. al. (1992) • Naldi and Rebora (2009) • Bukvic et. al. (2012) • Okokon et. al. (2015)</td>
</tr>
<tr>
<td>Ciclopirox Olamine</td>
<td>1.5% shampoo, cream, gel or lotion</td>
<td>Scalp: 2-3 times/week x 4 weeks, then once/week for maintenance. Skin: twice daily.</td>
<td>Inhibition of metal-dependent enzymes.</td>
<td>ICD in &lt;1% of patients. Itching, burning sensation in 2% of patients.</td>
<td>• Naldi and Rebora (2009) • Berk and Scheinfeld (2010) • Hald et. al. (2015) • Okokon et. al. (2015)</td>
</tr>
<tr>
<td>Selenium sulfide</td>
<td>2.5% shampoo</td>
<td>Scalp: Twice/week x 2 weeks, then once/week x 2 weeks. Repeat after 4-6 weeks.</td>
<td>Cytostatic and keratolytic</td>
<td>ICD in ~3% of patients. Orange-brown scalp discoloration.</td>
<td>• Naldi and Rebora (2009) • Gilbertson et. al. (2012) • Hald et. al. (2015)</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td></td>
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<tr>
<td>Zinc Pyrithione</td>
<td>1% shampoo</td>
<td>Scalp: 2-3 times/week.</td>
<td>Increased cellular copper interferes with iron-sulfur proteins.</td>
<td>ICD in ~3% of patients.</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>1% cream</td>
<td>Skin: 1-2 times daily.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betamethasone dipropionate</td>
<td>0.05% lotion</td>
<td>Scalp and skin: 1-2 times daily.</td>
<td>Anti-inflammatory, anti-irritant.</td>
<td>Risk of skin atrophy, telangiectasias, folliculitis, hypertrichosis, and hypopigmentation with prolonged use.</td>
<td></td>
</tr>
<tr>
<td>Desonide</td>
<td>0.05% lotion, gel</td>
<td>Scalp and skin: 2 times daily.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluocinolone</td>
<td>0.01% shampoo, lotion or cream</td>
<td>Scalp or skin: Once or twice daily.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunomodulators</td>
<td>1% cream</td>
<td>Skin: 1-2 times daily.</td>
<td>Inhibition of cytokine production by T-lymphocyte.</td>
<td>Risk of skin malignancy and lymphoma with prolonged use.</td>
<td></td>
</tr>
<tr>
<td>Pimecrolimus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.1% ointment</td>
<td>Skin: 1-2 times daily x 4 weeks, then twice/week for maintenance.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.6 - Treatment of seborrhoeic dermatitis and dandruff (…..Continued)
### Table 2.6 - Treatment of seborrhoeic dermatitis and dandruff (…..Continued)

<table>
<thead>
<tr>
<th>Category</th>
<th>Treatment</th>
<th>Route</th>
<th>Frequency</th>
<th>Duration</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coal tar</strong></td>
<td>4% shampoo</td>
<td></td>
<td>Scalp: 1-2 times/week.</td>
<td>Antifungal, anti-inflammatory, keratolytic, reduces sebum production.</td>
<td>Local folliculitis, ICD on fingers, psoriasis aggravation, skin atrophy, telangiectasias, hyper-pigmentation. Risk of squamous cell carcinoma with prolonged use.</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lithium gluconate/succinate</td>
<td>8% ointment or gel</td>
<td>Skin: twice daily x 8 weeks.</td>
<td>Anti-inflammatory via increased IL-10 and decreased TLR2 and TLR4 in keratinocytes.</td>
<td>ICD in &lt;10% of patients</td>
</tr>
<tr>
<td></td>
<td>Metronidazole</td>
<td>0.75% gel</td>
<td>Skin: twice daily x 4 weeks.</td>
<td>Anti-inflammatory via inhibition of free radical species.</td>
<td>Rare contact sensitization with prolonged use.</td>
</tr>
<tr>
<td></td>
<td>Phototherapy</td>
<td>UVB: Cumulative dose of 9.8 J/cm²</td>
<td>Three time/week x 8 weeks or until clearing.</td>
<td>Immuno-modulation and inhibition of cell proliferation.</td>
<td>Burning, itching sensation during/after therapy. Risk of genital tumour with prolonged use.</td>
</tr>
<tr>
<td><strong>SYSTEMIC</strong></td>
<td>Itraconazole</td>
<td>Oral: 200 mg</td>
<td>Once daily x 7 days, then once daily x 2 days/month for maintenance.</td>
<td>Inhibition of fungal cell wall synthesis. Anti-inflammatory via inhibition of 5-lipoxygenase metabolites.</td>
<td>Rare liver toxicity.</td>
</tr>
<tr>
<td></td>
<td>Terbinafine</td>
<td>Oral: 250 mg</td>
<td>Once daily x 4-6 weeks or 12 days monthly x 3 months.</td>
<td>Inhibition of cell membrane and cell wall synthesis.</td>
<td>Rare tachycardia and insomnia.</td>
</tr>
</tbody>
</table>

- Schwartz *et. al.* (2006)
- Naldi and Rebora (2009)
- Berk and Scheinfeld (2010)
- Hald *et. al.* (2015)
- Ballanger *et. al.* (2008)
- Stefanaki and Katsambas (2010)
- Bukvic *et. al.* (2012)
- Koca *et. al.* (2003)
- Dessinioti and Katsambas (2013)
- Hald *et. al.* (2015)
- Lee *et. al.* (2005)
- Hald *et. al.* (2015)
- Das *et. al.* (2011)
- Hald *et. al.* (2015)
- Gupta *et. al.* (2014)
2.6. Challenges and opportunities ahead (Fungi alone or others too?)

It is an accepted fact for more than a century, that *Malassezia* play a major role in the causation of dandruff and SD and treatment modalities are designed accordingly. However, as *Malassezia* reside on healthy skin as well, there are factors other than *Malassezia* that could play a role in development of these entities.

Earlier studies have tried to determine the role of bacteria in dandruff formation. However, these studies could not establish clearly any link between scalp bacteria flora and dandruff. Recently studies in French and subsequently Chinese populations have opened the possibility of role of bacteria in dandruff from a different perspective. A comparison of the scalp microbiota of dandruff affected and healthy individuals reveals that there exists disequilibrium in the microflora of dandruff scalp as compared to that of healthy scalp. This suggests a disturbance in the microbiota of scalp which could be related to development of dandruff.

Ghannoum (2010), in his report, has suggested that the alteration of one component of the microbiome influences other component. This is evident from the use of antibiotics which replace the bacterial flora with fungal one citing vaginal candidiasis arising out of tetracycline use. The report further emphasises on studies that cover all components of the microbiome of the body site, as such studies will help in understanding the role these communities play in health and disease.

Gaitanis (2012), in a review, has speculated that on the skin surface there are complex interactions between *Malassezia* species and other commensal or pathogenic microbiota. These interactions may affect survival or virulence of either and may play an important role in pathogenesis of all *Malassezia* related skin diseases. Thus study of both, mycobiome and bacteriome, of the *Malassezia* affected site is necessary. This thought of studying and understanding the relation between fungi and other micro-organisms on scalp for prevention and treatment of dandruff was further emphasised by Park *et. al.* (2012).

Further support for the interaction and role of different biomes in disease progression is stated by Cui *et. al.* (2013). Referring to interactions between *Mycobacterium tuberculosis* superinfection in Aspergillosis and suppression of *Candida* by *Pseudomonas aeruginosa* in cystic fibrosis, they state that the interaction between a bacteriome and mycobiome has the
potential to affect response to medical therapy and hence these interactions are critical in disease progression.

Clavaud *et. al.* (2013) are probably the first to report that dandruff is not only associated with a particular species of *Malassezia* and their numbers. Rather, dandruff is also associated with the disturbance in the balance (disequilibrium) between the mycobiome and bacteriome of the scalp. They further suggest that this could have implications on treatment of dandruff as a new concept. They postulate modification of scalp microbial ecology by use of chemicals or by improving host skin general physiology to be beneficial in dandruff.

Bacterial-fungal interactions are seen as areas of future exploration and it is hypothesized by Grice (2015) that disruption of these stable interactions could lead to disequilibrium or dysbiotic state. This could in turn act as predisposing cause of skin diseases.

Thus, dandruff/SD should not only be looked at form a ‘*Malassezia*’ angle. There should be an attempt to comprehend both ‘mycobiome’ and ‘bacteriome’ to understand its presence, pathogenesis, progression and treatment.

Considering the lack of reported data on the complete scalp microflora for the Indian population, the present study has been undertaken and designed to determine the scalp microflora associated with the human scalp in healthy and diseased state with respect to the Indian population. The rationale behind this research work, along with the aims and objectives, developed towards this microflora exploration has been discussed in the forthcoming chapters.