CHAPTER 9

SUMMARY AND CONCLUSION
9.1. Summary

The scalp is unique among the varied skin areas in humans, with high follicular density and a high rate of sebum production. The comparatively warm and dark environment on the surface of the scalp provides a welcoming environment for superficial mycotic infections (Grimalt 2007). Many scalp conditions have similar symptoms and clinical features, complicating diagnosis; but an exact diagnosis is critical in determining proper treatment. This study describes the microbial aetiology and treatment strategies for seborrhoeic dermatitis (SD) of scalp (dandruff).

Dandruff is a scalp condition occurring in about 17–50% of humans depending on the population tested (Xu et al., 2007; Clavaud et al., 2013). It is characterized by an abnormal flaking of the scalp, related to mild inflammatory reaction, where the stratum corneum is altered exhibiting disrupted cohesion between the corneocytes and cell hyperproliferation (Pierard et al., 2000; Warner et al., 2001; Kerr et al., 2011). Although previous reports have suggested that dandruff is associated with the presence of Malassezia species, discrepancies exist between studies (Ashbee, 2002; Gemmer et al., 2002; Pierard et al., 2006). In addition to fungi, human scalp is also colonized by bacterial populations (Grice and Segre, 2011) but their association with dandruff has not been studied (Clavaud et al., 2013). This study is the first comprehensive data of microorganisms (bacteria as well as fungi) present on the human scalp in the Indian population.

In the present study, the bacterial and fungal communities associated with dandruff scalp, non–dandruff scalp, SD skin lesion and SD skin non-lesion areas were investigated in Indian subjects under study. The major bacterial and fungal species inhabiting the skin and scalp were isolated and identified. On the basis of the prevalence of species isolated from the 4 regions under study, the microorganisms were classified into normal flora and pathogenic flora. On the basis of the Shannon diversity indices, obtained for variation in species in the four different regions, a comparison of the diversity of microflora amongst these regions was performed in order to determine the dandruff-causing microorganisms. Further, the variation in the microflora amongst the male and female populations was analysed to ascertain any association between gender and dandruff. Since a large number of bacteria as well as fungi were isolated from all the four regions under study, the fungal and the non-fungal population were correlated to obtain the composite pathogenic flora associated with dandruff.
Considering the role of altered scalp microflora in the pathogenesis of dandruff, the search for an efficient and target specific treatment strategy appropriate for the Indian population was undertaken. The first step towards the treatment strategy was to develop a microbial system that would mimic the natural dandruff scalp microflora. Thus, a consortium of selected clinical isolates (both bacterial & fungal) was prepared. This consortium was tested for any intra-consortium antagonistic activity and further subjected to antibacterial, antifungal as well as an antibacterial-antifungal combination testing.
9.2. Major Findings of the Study

1. Microorganisms isolated and identified:

The data obtained in this study reveals that a total of 1,767 microorganisms were isolated from 50 volunteers. These isolates were found to be from 51 different genera and 116 different species. The microorganisms included gram-positive cocci, gram-positive rods, gram-negative rods and fungi.

The sesame oil incorporated medium, developed in this study, was successfully used for the primary isolation of lipophilic yeasts (Malassezia spp.) from clinical samples as well as for sub culturing and maintaining of stock cultures. This medium was used in solid as well as in liquid form. Thus, this medium can be used in the conventional microbial procedures such as isolation of pure culture by streaking and enumeration (viable count) using spread plate technique.

2. Characterisation of isolated microorganisms:

On the basis of the volunteer category and the site of sample collection, the percentage prevalence of each isolate was calculated. Upon analysis of this data, the isolated organisms were distributed into the 4 study populations. The results obtained reveal the presence of Pseudomonas spp., Klebsiella spp., Enterobacter spp., Bacillus spp., Staphylococcus spp., Candida spp., Aspergillus spp. as the most prevalent organisms across the study population.

Further, the data obtained in the form of percentage prevalence of the microorganisms isolated from human skin and scalp was analysed in order to classify the microflora of the human scalp, into normal and pathogenic microflora. The normal microflora of the human scalp under study was found to mainly comprise of gram-positive cocci, bacilli and Malassezia species, whereas the pathogenic microflora did not include fungi. In addition, some opportunists of the scalp involved in the pathogenesis of dandruff were also identified.

3. Comparative analysis of the microorganisms isolated from skin and scalp

On comparing the distribution of microflora across the study population, it was observed that the diversity as well as the prevalence of cocci is highest on the dandruff scalp and least on SD skin lesion. The cocci showed similar distribution on skin non–lesion area and non-dandruff scalp. These observations suggest an important role played by these cocci in dandruff aetiologies. In case of gram-negative rods, a significantly high diversity of
species was found to be present on the dandruff scalp, whereas the non–dandruff scalp, skin lesion area and skin non-lesion area exhibited similar diversity, suggesting that the gram-negative rods form an important part of the dandruff scalp pathogenic microflora. Further, the scalp showed a higher diversity of bacilli as compared to the skin. In comparison with the bacterial microflora, the fungal microflora shows highest diversity on SD skin lesion area. The distribution of fungi on the dandruff scalp, non-dandruff scalp and SD skin non–lesion area is similar in terms of diversity of species. This reveals the fact that fungal microflora is an important aetiological agent in SD of skin, but not dandruff.

Further, analysis of the overall gender variation in the microflora on skin and scalp, revealed a similar diversity of species found in both the genders, with a higher prevalence rate on the female skin and scalp.

Also, the association between bacteria and fungi was found to be reduced on the dandruff scalp as compared to the healthy scalp, indicating an alteration in the healthy scalp microflora resulting in dysbiosis.


The test consortium prepared, comprised of 6 different organisms including Enterobacter cloacae, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Malassezia globosa and Candida parapsilosis. The gram-negative organisms showed high levels of antagonistic activities against other organisms except bacillus.

5. Antimicrobial spectrum of the test consortium

The consortium prepared was further subjected to different antimicrobial agents to test for its susceptibility pattern. The antimicrobial data obtained shows that the antifungal agents do not possess potential ability to inhibit the test consortium, while 15 antibiotics were found to exhibit impending activity against the test consortium, of which 4 antibiotics Amikacin, Gentamycin, Tetracycline and Chloramphenicol showed cidal activity against the test consortium at 60 – 120 minutes. Further, these 4 most antibiotics were used in combination with the most preferred antidandruff agent i.e. Ketoconazole to check for combination activity of antibacterial and antifungal agents. Tetracycline – Ketoconazole combination was found to exhibit maximum inhibition of the test consortium. Results obtained for antibacterial treatment alone, antifungal treatment alone and the combination treatment were compared and it was found that antibacterial treatment and combination treatment showed better inhibition of the consortium as compared to antifungal treatment.
9.3. Conclusion

The data obtained in the above study has confirmed that the human scalp is home to a rich community of microorganisms. In a healthy state, scalp skin peacefully co-exists with commensal bacteria and at the same time fending off potentially dangerous invaders. Disruption of this equilibrium can result due to alterations in the composition of our skin bacteria, an altered immune response to them, or both. Identification of the cutaneous bacterial species has time and again been related to skin diseases, but the role of a mixed bacterial population on clinical skin syndromes has not been adequately investigated (Ursell et al., 2012).

This study of human skin and scalp microbiota will serve to direct future research addressing the role of skin microbiota in health and disease. The data obtained will help in understanding the role of scalp microflora in the pathogenesis of dandruff and ultimately facilitate in the development of novel translational therapies. The two major findings obtained in the above study are as follows:

- The present study on the complete microflora of healthy and dandruff-affected scalps both confirms previous findings and provides new information. Other than Malassezia, we detected several fungal and bacterial species on the samples from the dandruff-affected scalps, indicating that Malassezia is not the sole causative agent of dandruff. It is an association of the complete microbial community inhabiting the human scalp, which is responsible for the diseased condition. It can be said that dandruff may be associated with the differences in the balance between the fungal and bacterial populations on the scalp, and not just Malassezia (Clavaud et al., 2013). Hence, treatment strategies targeting bacteria as well as fungi need attention.

- Considering the role of bacteria along with fungi in the scaling of scalp epidermis resulting in dandruff, it is essential to modify dandruff treatment for the Indian population. As evident from the antimicrobial data obtained in this study, antifungal agents alone are not capable of eliminating all the pathogens. Ketoconazole in combination with antibacterials has shown increased antimicrobial activity. In our search for an efficient line of treatment for dandruff, Tetracycline in combination with Ketoconazole was found to be the most potent antimicrobial agent against the test consortium. Thus, antibacterials and combination of antibacterial and antifungal agent
needs to be explored further for the development of a target specific treatment against dandruff.

In conclusion, the present study has illuminated a pathway beyond *Malassezia*, towards exploring other microbial communities inhabiting the human scalp that may be associated with dandruff. This study provides a comprehensive data of microorganisms (bacteria as well as fungi) present on the scalp, describing the human scalp microflora especially for the Indian population.

Further this study brings forward a novel concept for dandruff research by developing an *invitro* microbial test consortium that can be tested for its susceptibility against antimicrobial agents. The antimicrobial data obtained suggests that the antibacterial treatment alone as well as in combination with antifungal agent proves to be more efficient than the currently used antifungal treatment. This will help in the development of an efficient and target specific treatment against dandruff especially for the Indian population.
9.4. **Limitations of the study**

- The first limitation of our study is the comparatively small sample size. The sample for the present study comprised of 50 volunteers. Although statistically significant, this sample is only a very small proportion of the entire population. Therefore, research studies with a greater sample size would be required to ensure appropriate generalization of the findings of the study.

- The second limitation is the use of only culture-dependent methodology. In this study culture-dependent methods have been used and optimized to detect and quantify microbiological diversity. Apparently, using the culture-dependent method, only the most abundant organisms or the organisms better adapted to the culture conditions were being screened. For this reason and due to the expected higher sensitivity, one would predict that the culture-independent methods may allow the detection of uncultivable taxa and also target the less abundant organisms.

- The third limitation of the study is that anaerobic microorganisms have not been studied. Even though anaerobic bacteria like *Propionibacterium acnes* are known to be associated in the pathology of dandruff, it was not possible logistically to include anaerobic cultures. The presence of these anaerobic organisms could not be estimated in the study.
9.5. **Future Prospects**

- This study is an important stepping stone in improving our understanding of dandruff related to the Indian population. It has illuminated the pathway towards our thinking about inflammatory skin disease, to include potential host–microbe interactions as factors that can potentiate or even initiate dandruff without the prerequisite of overt fungal infections. To date, acne rosacea, acne vulgaris, atopic dermatitis and psoriasis have received the most attention in this regard (Scharschmidt and Fischbach, 2013), but this thought needs to be applied to dandruff as well. Genetics and other host factors are of course central to pathogenesis in the above mentioned inflammatory conditions but our cutaneous immune system does not function in a vacuum. Shifts in the skin microbiota may act co-operatively with host susceptibility factors to propagate a pathogenic pro-inflammatory state. It will be now be essential to study the specific interactions within such a microbial community and the capability of the microbiota to interact with two other etiologic facets of dandruff- host susceptibility and sebum metabolism.

- As opposed to the conventional approach of antibiotic treatment, future therapies to tackle microbial involvement to disease will hopefully engross more subtle manipulation of the host–microbiome ecosystem. Whether topical probiotics to treat dandruff will ever become a reality remains unexplored (Krutmann, J., 2009), however engineering topical therapeutics to favourably influence the composition of scalp microflora and optimize their interaction with our skin is a real possibility. An alteration in the scalp microbial ecology, either because of chemicals or changes in the host skin general physiology, may improve the dandruff condition of the scalp.

- Cultured *in-vitro* human sebocytes have recently turned out to be a helpful tool in studying sebaceous gland activity and regulation, understanding the pathophysiological mechanisms and treatment of sebaceous gland related diseases. These cell lines need to be explored in dandruff studies as *in-vitro* model systems. They can further be used in search for biologically active ingredients, new pharmaceutical and cosmetic drugs for dandruff.