to a new investigation with biopsy for direct research and culture for fungi, being identified *Prototheca Wicherhamii*, by Maldi-Tof®, with sensitivity to itraconazole and ampfotericin B. PCR amplification of the genetic material obtained in the clinical isolate was performed with purification of its product, and sequencing showed genetic similarity of 97,46% with *Prototheca Wickerhamii*. The sequence obtained was deposited in Genbank under number MZ409514. In the absence of therapeutic response to itraconazole (400 mg/day), and significant worsening of the lesion, with presentation of a secondary infection caused by *Staphylococcus haemoliticus*, treatment with Clindamycin (900 mg/day for 10 days) and Liposomal Amphotericin B (4 mg/kg/day for 45 days) were performed. After suspension of Liposomal Amphotericin B, the lesions recurred in 15 days, and voriconazole (200 mg 12/12 h) was prescribed for 6 months, with complete regression of the lesions. Currently, he is free of injuries, having been followed up every 6 months.

Conclusion: Rare disease caused by chlorophyllous algae may be surprising due to the severity and lack of response to antifungals that show sensitivity *in vitro*.

**P099**

**Molecular identification of dermatophyte species from Eastern Assam, Northeast India**

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: Dermatophyte infections occur worldwide both in developing as well as developed countries. However, species of dermatophytes may vary based on geographical region. Studies on dermatophytes from northeast India are rare. This study was done to know the various species of dermatophytes that are commonly associated with infection in this part of the country.

Methods: This study was done from 2020-2021. A total of 49 consecutive isolates of dermatophytes isolated from clinically suspected cases attending Assam Medical College and Hospital, a tertiary care hospital were subjected to molecular identification by using PCR and sequencing of the ITS region of the ribosomal RNA gene as well as using MALDI-TOF (VITEK MS). Samples from active margin of lesions from skin, nail, and hair were collected and primary identification was done by culture and microscopy as well as conventional phenotypic tests. Culture was done in Sabouraud Dextrose agar, Sabouraud Dextrose agar with chloramphenicol and cycloheximide, and dermatophyte test medium which was followed by genotypic confirmation by PCR of the ITS region and sequencing of PCR amplicons using already published protocols.

Results: The species isolated were *T. rubrum* (36.7%), *T. interdigitale* (32.6%), *T. mentagrophytes* complex (14.2%), *T. tonsurans* (8%), *M. gypseum* (6%), *T. violaceum* (2%). The cases were clinically found to be *T. corporis* (44.89%), *T. manuum* (12.24%), *T. pedis* (12.24%), *T. cruris* (10.20%), *T. faciei* (8.16%), *T. capitis* (8.16%), and *T. unguium* (4.08%).

Conclusion: *T. rubrum*, *T. interdigitale*, *T. mentagrophytes*, and *T. tonsurans* complex were the predominant species isolated.

**P100**

**Potent inhibition of dermatophyte fungi by Australian native jarrah honey**

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: Honey has been used as a remedy for multiple ailments, and the antibacterial activity of many different floral honeys has been extensively explored. The capacity of honey to inhibit fungi is much less well understood. Here we investigate the inhibition of dermatophyte species by native Australian jarrah honey.

Methods: Jarrah honey was sourced from beekeepers and commercial suppliers. Artificial honey, made from glucose (22.9%), fructose (20.7%), and sucrose (1.6%), was used to control for osmolarity. Hydrogen peroxide production by honey was assessed using horseradish peroxidase (HRP)/o-dianisidine colorimetric test. Dermatophytes included *Microsporum canis*, *M. nanum*, *Nannizzia gypsea*, *Trichophyton interdigitale*, *T. rubrum*, and *T. tonsurans*. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) for honey were assessed using CLSI methods. Fluorescent and scanning electron microscopy were used to visualize the effect of honey on fungal conidia and hyphae.

Results: Jarrah honey inhibited all of the dermatophyte species with MICs ranging from 1.5-3.5% w/v, and MFCs from 2-5% w/v. No antifungal activity was seen with the artificial honey indicating this was not due to osmolarity. Microscopy revealed honey treatment prevented the germination of conidia and caused hyphae to bulge and collapse. While the inhibitory action of jarrah honey was greatly reduced by the addition of catalase suggesting hydrogen peroxide production was responsible for inhibition and killing, microscopy revealed hyphae were still damaged suggesting there are agents within honey that augment antifungal activity. REDOX fluorophores failed to detect internal oxidative stress within hyphae, indicating that damage likely occurs on the hyphal surface.

Conclusion: Jarrah honey is a non-toxic agent that may have utility in the treatment of superficial fungal infections caused by dermatophyte fungal species.

**P102**

**Nuclear magnetic resonance -based identification of metabolites in dermatophytes**

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: Nuclear magnetic resonance (NMR) spectroscopy provides a holistic snapshot of the metabolome of an organism. There is a dearth of studies till date that had exploited NMR metabolomic platform to study dermatophytes, despite its potential for rapid identification and subsequent application of the knowledge in performing faster antifungal susceptibility of dermatophytes. Here we attempted to study the frequency of various species of dermatophytes in clinically suspected cases of dermatophytosis and perform NMR-based identification of metabolites in the culture suspensions/cell extracts of *T. mentagrophyte* and *T. rubrum*.

Methods: This was a hospital-based prospective study conducted in the isolates obtained from clinically suspected cases of Dermatophytosis in the patients. Skin, nails, and hair samples of patients suspected with superficial fungal infections were processed for dermatophytes using conventional microbiological methods. NMR-based identification of metabolites was carried out in cell extracts prepared from the culture suspensions of *T. mentagrophytes* and *T. rubrum* obtained during the study from a subset of the clinical isolates from the samples.

Results: Dermatophytes were isolated in 85.88% (219/255) cases, with *T. mentagrophyte* being isolated in 65% (143/219) of isolates, followed by *T. rubrum* in 31.5% (69/219) isolates. In NMR study was done in the standard ATCC strains (*T. mentagrophyte* ATCC9533 and *T. rubrum* ATCC28188) and representative clinical isolates of both the species. Overall, 24 metabolites were identified in *T. rubrum* and 23 metabolites in *T. mentagrophyte* amongst which 22 metabolites were common to both fungus, however, '4-hydroxyproline' and 'acetate' was found specific to *T. rubrum*, and 'allantoin' was found specific to *T. mentagrophyte*.

Conclusion: *T. mentagrophyte* was the predmominant dermatophyte species in the study. Amongst the number of metabolites detected in *T. rubrum* and *T. mentagrophyte*, '4-hydroxyproline' and 'acetate' was found specific to *T. rubrum*, and 'allantoin' was found specific to *T. mentagrophyte*. These specific metabolites could be useful for as early identification of these dermatophytes as well early determination of antifungal susceptibility by using metabolic endpoints, further large-scale study will be helpful in this regard.

**P103**

**Role of biofilm production in recalcitrant tinea**

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objective: To determine the role of biofilm production in dermatophytic isolates from tinea infections of recalcitrant skin lesions of study patients.

Methods: An observational study conducted in UCMS and GTB Hospital Delhi, in forty clinically diagnosed and mycologically confirmed cases of recalcitrant tinea infection of glabrous skin to analyze the role of biofilm production in dermatophytes.

After taking written informed consent from the study population sample collection (skin scraping) was done.

The scraping was then mounted in 10% potassium hydroxide (KOH) for direct microscopic examination followed by culture on Sabouraud Dextrose Agar (SDA) media with antibiotics (Chloramphenicol, Gentamicin, Cycloheximide).

The fungal growth was then subjected to LPCB mount (Lactophenol cotton blue).

The isolates were allowed to form *in-vitro* biofilms on polystyrene microtiter plates.

Quantification of biofilm biomass was done using crystal violet staining and measuring the optical density (OD) at 570 nm and classified as non-adherent/non-producer, weak moderate, and strong biofilm producers.

Results: Tinea corporis and cruris were the most common clinical types of dermatophytosis.

*T. mentegrophytes*-complex was the most common dermatophyte isolated from the clinical specimens.

Majority (86.84%) of isolates formed strong (OD >4 ODc) biofilms.

Conclusion: There has been an increase in the incidents of chronic and recalcitrant dermatophytosis of skin.

The predominance of *T. mentegrophytes*-complex as observed in our study highlights the importance of the pathogen in causation of current and chronic and recalcitrant dermatophytosis in India.

High rate of *in-vitro* strong biofilm formation by the isolates indicates that these organisms might be forming biofilms *in-vivo* leading to chronicity and poor response to therapy.

**P105**

***In vitro* interaction of *Malassezia* and commensal *Staphylococcus* species**

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objective: *Malassezia* is the most abundant fungal skin commensal organism, representing 50%–80% of total fungi present on the skin. It has been associated with many skin disorders such as pityriasis versicolor (PV) and seborrheic dermatitis/dandruff (SD/D). The role of *Malassezia* in disease manifestation is not discerned. It is important to understand its interaction with bacterial flora such as *Staphylococcus* epidermidis and S. capitis *in vitro*. We have studied the interaction of *Malassezia* and *Staphylococcus* species isolated from skin flora.

Methods: *Malassezia restricta*, *M. globosa* ($n = 5$) isolated from patients with SD and *M. furfur* ($n = 5$) isolated from PV were sub-cultured on Modified Dixon's agar (MDA). Staphylococcus epidermidis and S. capitis were isolated from patients with SD and sub-cultured on brain heart infusion (BHI) agar. *Malassezia* species requires media supplemented with lipids (MDA) for its growth. Bacteria and *Malassezia* were quantified on MDA and BHI agar by Miles and Mishra method to perform interaction between them. For direct interaction, suspensions (100 $\mu$l) of *M. restricta*, *M. globose*, and *M. furfur* were prepared in normal saline and added to wells on the plates of lawn cultures containing *S. epidermidis* and *S. capitis* (107 CFU/ml). Plates were incubated for 12 h at 35°C and observed for zone of inhibition. To investigate the release of antibacterial compounds into the extracellular environment, *M. furfur* was inoculated in modified Dixon's broth (MDB) and incubated at 35°C for 5 days. Supernatant was collected at 12 h, 24 h, 48 h, 72 h, 96 h, and 120 h of incubation and evaluated for antibacterial activity by agar-well diffusion assay. Effect of cell-free supernatant of *Malassezia* on growth of bacteria was also monitored by growth kinetics of S. epidermidis for 24 h in the absence and presence of *M. furfur* supernatant using Epoch-2 microplate spectrophotometer.

Results: MDA supported the growth of bacteria at different cell densities (107-103 CFU/ml count) and incubation time of *S. epidermidis* and *S. capitis* was similar on MDA and BHI. Zone of inhibition (ZOI) was witnessed with *M. restricta* (20.6 ± 3 mm, 21 ± 3 mm), *M. globosa* (21 ±1 mm, 22.6 ±2 mm) and *M. furfur* isolates (16.5 ± 1 mm, 18 ± 2 mm) against *S. capitis* and *S. epidermidis* respectively by direct interaction. Inhibition of bacteria by *M. furfur* was noted from 48-120 h as ZOI (21.7 ±5.1 mm) was observed on bacterial lawn cultured plate. When growth kinetics of *S. epidermidis* was monitored in presence of M. furfur supernatant, maximum value reached upto 0.26 ± 0.019 only from 0.01 ± 0.001 at OD600 in 9 h including lag phase of 4 h (Fig. 1). However, OD600 value reached upto 0.97 ± 0.005 in 8 h including lag phase of 1.5 h in absence of supernatant. Doubling time calculated from logistic growth equation was 76.6 ± 4.4 and 65.2 ± 2.9 minutes in the presence and absence of supernatant respectively.

Conclusion: Inhibition of bacteria by *Malassezia* species noted in our study has not been reported earlier. The possible production of antibacterial compounds by *Malassezia* might be responsible for dysbiosis leading to disease.