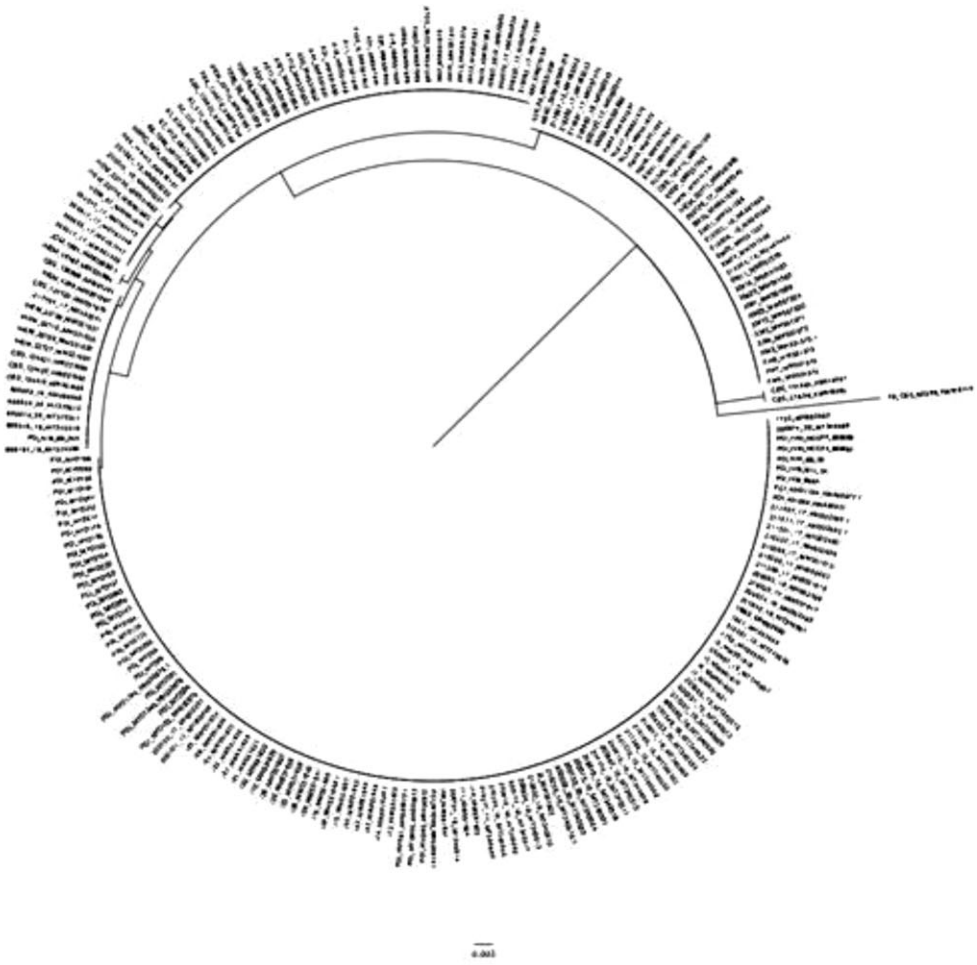

Figure 2. Phylogenetic tree based on *TEF 1-α* sequences Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). The evolutionary distances were computed using the Kimura 2-parameter method. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA X.

P097
Epidemiology of dermatophytes related infections in Kuwait: a retrospective study

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Introduction and Objective: Dermatophytes are a common cause of cutaneous infections that affect a large number of healthy individuals throughout their lives. Although such infections are classically benign, they have a negative impact on patient's physical and psychological health. We aim to explore the epidemiology of dermatophytes infections at a national level.

Methods: The study was conducted retrospectively. Demographic and microbiological data were obtained from laboratory information system in the Mycology Reference Laboratory in the year 2021. Dermatophytes were either isolated from clinical samples in mycology reference laboratory or sent from other laboratories for species identification. The clinical samples were divided into two parts. The first half was examined microscopically, and the second half was inoculated on Sabouraud agar media with and without cycloheximide and then incubated at 30°C for at least 2 weeks. Dermatophytes were identified by colonial morphology and microscopic characteristics.

Results: During the year 2021, 60 dermatophytes were found. The male to female ratio was 2:1. A total of 60% of patients were children. Half of the cases were isolated from hair specimens and the second half were from the skin. Only one dermatophyte was isolated from nail cultures. Regarding dermatophytes distribution, *Microsporum* species were the commonest and involved mostly *M. canis* (26). Other less common species included two *M. audouinii* and two *M. praecox*. A total of seven other *Microsporum* species were not identified to species level. On the other hand, 23 *Trichophyton* species were found including 5 *T. tonsurans*, 4 *T. interdigitale*, 3 *T. rubrum*, 1 *T. simii*, and 1 *T. erinacei*. A total of 9 other *Trichophyton* species were not identified to species level.

Conclusions: Higher rates of infection were seen in males compared to females. Phenotypic identification has failed in identifying a significant number of isolates. As in other types of molds, the phenotypic examination may also result in inaccurate identification, especially among uncommon and evolving species. Hence, molecular testing is essential for accurate identification and better understanding of the epidemiology of dermatophytes-related infections. The following species were reported for the first time in Kuwait, namely: *T. erinacei*d, *T. simii*, and *M. praecox*.

P098
Human prototothecosis: Acase report in Northeastern Brazil

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

Introduction: Prototothecosis is an emergent disease caused by members of the genus Prototheca. Most such infections probably occurred by traumática inoculation into subcutaneous tissues.

Objectives: It is to report a case of human cutaneous prototothecosis identified in the state of Maranhão, northeast Brazil. Case report: 75-year-old patient, Merchant, from the municipality of São Luís Island, Northeastern Brazil. He sought care referring to an erythematous and painful lesion on the left arm that started 6 months before the treatment. On examination, he presented an infiltrative, hyperemic lesion with burning pain throughout the upper limb (Fig. 1). The patient reported that a week before the onset of the condition, he suffered trauma on the arm, with a laceration in the skin, while cleaning a sewage system with clay pipes. During the healing process, he noticed a hyperemic, slightly pruritic lesion measuring 2 cm which did not improve. He sought medical assistance at the dermatological service, who suspected dermatophytosis, initiating treatment with terbinafine (250 mg, once a day), evolving with worsening of the lesion. A lesion biopsy was indicated, to histopathological examination, which showed circular, moniliform structures, diagnosed as prototothecosis (Fig. 2). Treatment with itraconazole (200 mg/day) was started, with no therapeutic response and the lesion spread throughout the patient's left upper limb. Submitted

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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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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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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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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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 μl) 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.