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

Sumita Boro[2,3], Anindita Bagchi, Reema Nath
[1]Assam Medical College and Hospital, Dibrugarh, India
[2]Assam Medical College and Hospital, Dibrugarh, India
[3]Assam Medical College and Hospital, Dibrugarh, 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

Kenya Fernandes[1,2], Annabel Guttentag[1,2], Daniel Susantio[1,2], Dee Carter[1,2]
[1]University of Sydney, Sydney, Australia
[2]Sydney Institute for Infectious Diseases, Sydney, Australia

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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

Anupam Das[1] Bikash Baishya[2], Suresh Chandra Ahirwar[1], Vikramjeet Singh[1], Manodeep Sen[1], Vineeta Mittal[1], Jyotsna Agarwal[1]
[1]Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India
[2]Centre of Bio-Medical Research, Lucknow, India

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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

Seema Gangar, SHUKLA DAS, Vishal Gourav, S.N. Bhattacharya, Deepika Pandhi, N.P. Singh
UCMS and GTB Hospital, Delhi University, Delhi, India

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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**

Jyoti Gupta, Sunil Dogra, Sendhil Kumaran, Archana Angrup, Amit Arora, Harsimran Kaur, Anup Ghosh, Arunaloke Chakrabarti, Shivaprakash M Rudramurthy
PGIMER, Chandigarh, India

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.

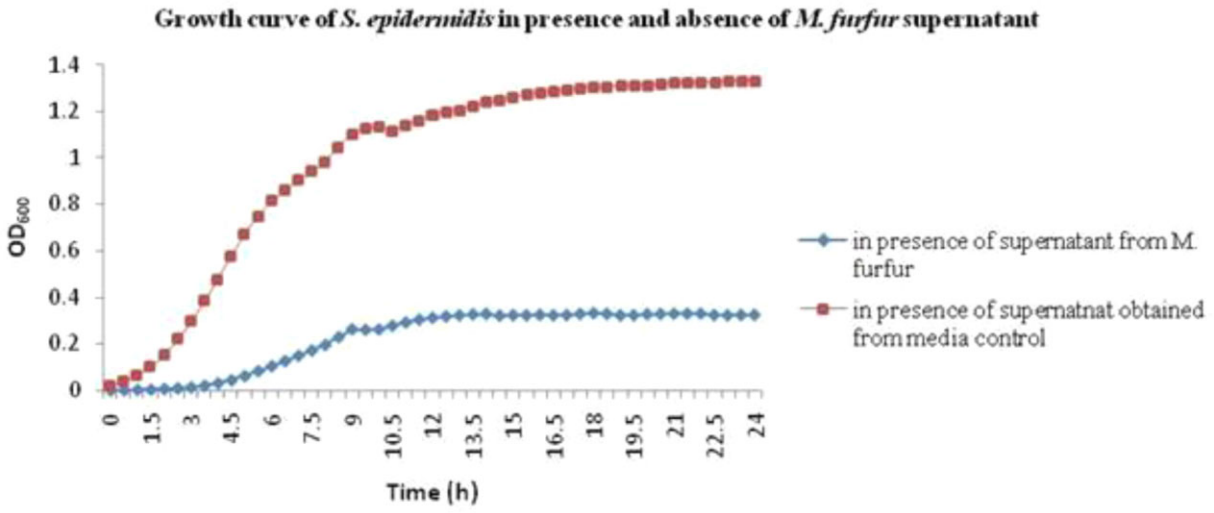

Figure 1. Growth curve of *S. epidermidis* in presence and absence of *M. furfur* supernatant

P106

A dermoscopic finding of *Tinea capitis* caused by *Microsporum canis*

Eunhye Jeong, Jeongeun Yim, Hyeongmok Kwon, Donghoon Shin, Jongsoo Choi
Yeongnam University Hospital, Daegu, South Korea

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

Objectives: Tinea capitis is a relatively common disease, and the mycological examination is the gold standard for diagnosis. However, the probability of false negative on the KOH test is up to 40% and culture examination takes a long time for diagnosis. The characteristic pattern of dermoscopy not only aids in diagnosis, but also enables early treatment.

Methods: We evaluated six patients who were diagnosed with tinea capitis through clinical and dermoscopic findings. The images of the lesions were taken with a digital camera (Nikon, HB-42) and photographed with dermoscopy (Dermlite Foto 2 Pro) from the patients. The pictures were obtained by taking multiple focal points with dermoscopy. The comma, corkscrew, Morsecode-like, zig-zag, and bent hairs were observed as the main findings.

Results: The dermoscopic finding was seen with overlapping of various findings in each of the patients. Upon dermoscopy, the most common findings were the corkscrew hair (66%) and the bent hair (66%). The comma hair (33%) and the proximal white shaft hair (33%) were less frequently observed and zigzag hair and Morse-code like hair were not seen in six patients. In the photograph taken with a camera, findings considered to be dermoscopic features such as corkscrew hair or comma hair were not observed.

Conclusion: It is important for dermatologists to consider that abnormal findings in dermoscopy can play an important role in diagnosing *Tinea capitis*. And it will help in early treatment and prevent the progression of complications. Here in, we report specific dermoscopic findings which can narrow down the differential diagnosis.

P107

Spectrum of Dermatophyte infections and drug susceptibility pattern of Dermatophytes in patients visiting to tertiary care hospital in Chhattisgarh state of India

Archana Keche[1], Padma Das[1], Pratibha Sharma[1], Satyaki Ganguly[1], Namrata Chhabra[1], MR Shivaprakash[2]
[1]AIIMS RAIPUR, Raipur, India
[2]PGIMER, Chandigarh, India

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Objectives: 1. To isolate and identify various species of Dermatophytes from clinical specimens 2. To perform and analyze the antifungal susceptibility testing of isolated Dermatophytes for commonly used antifungal agents; terbinafine and itraconazole.

Methods: A prospective study was conducted from December 2019 to October 2021. Clinical specimens (skin, hair, and nail) from suspected cases of dermatophytosis were received and processed in the department of microbiology. All the samples were subjected to microscopic examination and culture by standard techniques. Their clinico-demographic profile was obtained. Specimen were processed for KOH and fungal culture. Dermatophytes were identified by studying macroscopic and microscopic characteristics of the isolates. The conidium-forming dermatophyte isolates were processed for antifungal susceptibility testing for terbinafine and itraconazole by Microbroth dilution testing following the CLSI M-38A2 guidelines.

Results: Total 248 patients with male predominance (68%) were noted in the above-mentioned study period. Predominance of study population belonged to rural area. Maximum numbers of cases were from the age group 21-30 years. Majority of patients belong to poor socioeconomic status. Out of 248 samples, 178 (72%) had a positive KOH mount amongst which 72% had positive culture results. Amongst 2 4881% were skin scraping, 17% were nail, and 1.6% hair samples were processed. Out of culture-positive samples 52% were Dermatophytes. The most clinical form of dermatophytosis was combination of both *Tinea cruris* and *T. carporis* (31%) followed by *T. cruris* (22%), and *T. corporis* (17%) for which skin scraping was processed. The most common isolate was *Trichophyton tonsurans* (73%) followed by *T. mentagrophytes* (10%), and *T. verrucosum*. Onychomycosis was diagnosed in 17% patients of which 59% were positive by KOH 49% were culture positive.11.5% isolates from nails were dermatophytes.

Antifungal susceptibility testing was done by Microbroth dilution method and analyzed the range. The MIC range of major isolates, i.e., *T. tonsurans* showed MIC ranges against terbinafine <0.03-4 μg/ml and itraconazole 0.03-2 μg/ml. *Trichophyton mentagrophyte* for terbinafine <0.12-4 μg/ml and for itraconazole 0.12-2 μg/ml. Four isolates of *T. tonsurans* had higher MIC values for terbinafine and two isolates had higher MIC for itraconazole. One isolate of *T. mentagrophytes* had higher MIC values of itraconazole, and one another isolate had higher MIC for terbinafine.

Conclusion: This study highlights the change in pattern of causative agents of dermatophytosis. The present study showed the predominance of *T. tonsurans*. More extensive studies are needed to evaluate the cut-off range of antifungal susceptibility testing of dermatophytes with clinical follow-up to see the response of respective antifungals and to guide the therapy.

P110

AIRE gene mutation predisposing chronic mucocutaneous Candidiasis in two kids from a Chinese family

Yubo Ma, Xiaowen Wang, Ruoyu Li
Peking university First Hospital, Beijing, China

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Objectives: Chronic mucocutaneous candidiasis (CMC) is a group of clinical syndromes characterized by chronic recurrent skin, nails, and mucosal superficial *Candida* infections. Various gene mutations have been reported to predispose individuals to CMC and its related syndrome. This study aims to study the clinical features and the genetic background underlying two kids of CMC from a Chinese family.

Methods: Clinical and laboratory findings of the two patients were studied, including physical examination, direct microscopic examination, and fungal culture. Genomic DNA of all family members was extracted from peripheral blood leukocytes, and whole-exome sequencing (WES) was performed.

Results: A 2-year-old boy and his sister were admitted to the hospital due to recurrent thrush and thickening of their nails. Direct microscopic examination of their nails and the brother's tongue showed branched pseudohyphae and yeast cells, and *Candida albicans* was identified through fungal culture. The brother also experienced a progressively impaired vision, which was diagnosed as retinitis pigmentosa, causing no light perception in one eye and light perception up to 0.1 in the other. Their parents belonged to the Hui population (a minority population in China) and had a history of consanguineous marriage. Chronic mucocutaneous candidiasis (CMC) was diagnosed, and oral fluconazole was prescribed. After continuous fluconazole treatment for 6 months, the nails and the tongue became normal. These patients are still under follow-up.

Due to the recurrent *Candida* infections and history of consanguineous marriage, genetic susceptibility was suspected. When we compared the WES data with all genes reported to be related to CMC, a homozygous mutation in the AIRE gene was noted (*C.* 769 C >T, p. Arg257Ter) in both patients. The parents were heterozygous carriers of the variant.

Conclusion: In this study, we identified two CMC patients of Chinese harboring AIRE mutations. These patients remind us the importance of genetic analysis in management of CMC, which then help to adjust the time of treatment, as well as to predict and early detect related complications.

P111

A case of nail discoloration due to topical treatment of onychomycosis with luliconazole 5% nail solution

Tomotaka Sato[1], Norihito Fukada[1] Akihiko Kinjo[1], Mariko Hatta[1], Ryota Akimoto[1], Hisashi Kobayashi[2], Satoshi Fukuzumi[2]
[1]Department of Dermatology, Teikyo University Chiba Medical Center, Ichihara, Chiba, Japan
[2]Department of Plastic surgery, Teikyo University Chiba Medical Center, Ichihara, Chiba, Japan

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We use efinaconazole 10% topical solution and luliconazole 5% nail solution for topical treatment of onychomycosis in Japan. We show a case of onychomycosis treated with nail debridement and topical luliconazole 5% nail solution to the nail and topical luliconazole 1% cream to the foot.

A woman in her seventies with chronic urticaria had a nail spike color change on her left big toe (Fig. 1).

We opened the spike lesions with a plastic nipper and KOH direct microscopic examination showed dermatophytoma. We treated with topical luliconazole cream on the toes and soles of the foot and 5% solution on the nail. Because of the summer season, she walked outside in sandals without socks during treatment and noticed the nail yellow color change (Fig. 2). We advised the patient to protect from sun light and not to walk outside without socks. Due to the report from the production company, the reason for nail color change to yellowish is photodegradation of luliconazole. After 1 year since first visit, the fungal infection of the big toe disappeared by our topical treatment. The nail yellow color change also disappeared. We recommended avoiding sunlight exposure on the treated nail during topical treatment of luliconazole 5% nail solution.