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Original Article

Study of Dermatophytes and their Biofilm Production

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ABSTRACT:

Background: Biofilms are sessile microbial communities surrounded by extracellular polymeric substances (EPS) with increased resistance to antimicrobial agents and host defences. Detection of biofilm by easy lab method is essential. Characterization of biofilms formed by dermatophytes may contribute to the search of new drugs which can break down biofilm for the effective treatment of these mycoses. **Aim of study:** To study biofilm production by dermatophytes. **Materials and methods:** The study was conducted in the Department of Microbiology, D.Y. Patil Hospital and Research centre, Kadamwadi, Kolhapur. Sample Size for the study was 50. The duration of Study was 1 year. Fifty clinically suspected samples with dermatophytosis were included. Biofilm production of dermatophytes was detected by tube method. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube. **Results:** 22 out of 50 samples were found KOH positive. Out of 50 samples 28 (56%) dermatophytes species were isolated. Most common isolate was T.rubrum (12%), followed by T.mantographyte (10%). **Conclusion:** T.rubrum, T.mantographyte and E.floccosum are common dermatophyte causing dermatophytosis and T.rubrum, T.mantographyteare also capable to produce biofilm. Major group of dermatophytes which are associated with skin infection are able to produce biofilm that may interfere with treatment.

Keywords: Biofilms, Dermatophytes, Fungal infection.

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INTRODUCTION:

Dermatophytes are fungi that have the ability to invade keratinized structures of humans and animals, producing a condition called dermatophytosis. There are three anamorphic genera: Trichophyton, Microsporum and Epidermophyton, which share certain microscopic features despite the taxonomic distance between them. Soil is a natural reservoir of dermatophytes; keratins present in soil are used as nutrients, so these fungi are adapted to various environments. Keratin is a protein of high molecular weight, relatively insoluble and present in the skin, hair, nails and debris deposited in soil. Dermatophytosis is a common fungal disease which involves the keratinized tissue.[1-4] Most infections of skin and its appendages, the hair and nail are caused by a homogenous group of keratinophilic fungi called the dermatophytes. Several antifungal agents can be used to manage these infections. Some species produce biofilm which is also reported a cause of treatment failure. Biofilms are sessile microbial communities surrounded by extracellular polymeric substances (EPS) with increased resistance to antimicrobial agents and host defences.[3,5,6] Detection of biofilm by easy lab method is essential. Characterization of biofilms formed by dermatophytes may contribute to the search of new drugs which can break down biofilm for the effective treatment of these mycoses.[3] Hence, the present was designed to study biofilm production by dermatophytes.

MATERIALS AND METHODS:

The study was conducted in the Department of Microbiology, D.Y. Patil Hospital and Research centre, Kadamwadi, Kolhapur. The objective of the study were: Isolation of common dermatophytes by routine culture methods from skin samples received at Microbiology laboratory; Identification of different dermatophyte species isolated from these samples; and Detection of the biofilm production by dermatophyte species.

Sample Size for the study was 50. The duration of Study was 1 year. For the study, fifty clinically suspected samples with dermatophytosis were included. Skin scrapings were

collected from the Department of Dermatology OPD at D.Y. Patil Hospital and Research centre, Kadamwadi, Kolhapur. The samples were cultured on Sabouraud dextroseagar and dermatophyte species isolated from the samples were identified by macroscopic and microscopic morphology.

Biofilm production of dermatophytes was detected by tube method. A loopful of test organisms was inoculated in 10 mL of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at room temperature for 72 h. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube.

Interpretation of Biofilm

Biofilm production was interpreted visually by presence of adherent film in the tube.

Statistical analysis

The statistical analysis of the data was done using SPSS program (version 20.0) for windows. Student's t-test and Chi-square test were used to check the statistical significance of the data. A p-value <0.05 was predetermined as statistically significant.

RESULTS:

In the present study, 50 skin scraping were received from outpatient clinic of dermatology department. 22 out of 50 samples were found KOH positive **[Figure 1]**. Out of 50 samples, 28 (56%) dermatophytes species were isolated. **Table 1** shows the number of samples found positive with Dermatophyte species and other species. Number of samples positive for T.rubrum was 6; for T.mentagrophyte was 5; for E.floccosum was 5; for T.violaecium was 4; for T.verrucosum was 2; for M.audoini was 2; for M.canis was 2; and for T.tonsurans were 2. Most common isolate was T.rubrum (12%), followed by T.mantographyte (10%). All strains of T.mantographyte and T.rubrum found positive for biofilm production **[Table 1; Figure 2, 3, 4]**. Only 33% strains of T.rubrum were found sensitive for Itraconazole. 40% strain of T. mantographyte was found sensitive for itraconazole and 20% for Ketoconazole.

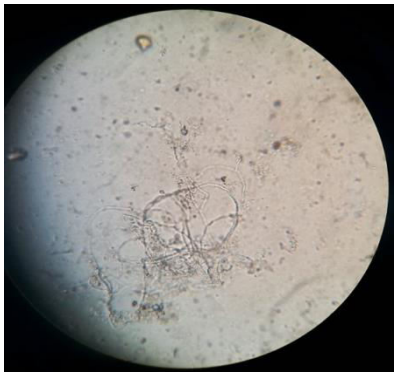


Figure 1: KOH mount showing fungal elements SDA culture of dermatophyte

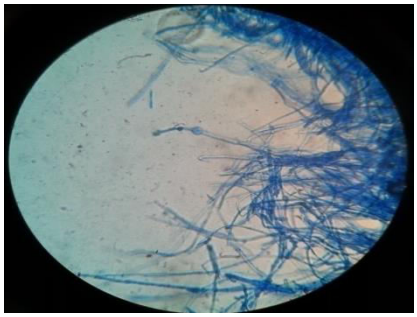


Figure 2: LPCB Mount showing E.floccosum

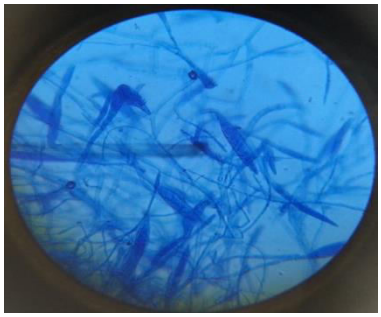
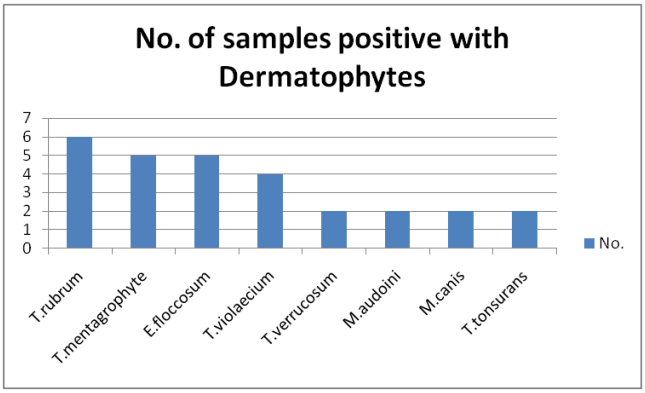


Figure 3: LPCB Mount showing M.canis

Table 1: Number of samples found positive with Dermatophyte species and other species

Dermatophytes	***No.***	***Biofilm producer***	***Others species***	
T.rubrum	**6**	*Yes*	*A.niger*	5
T.mentagrophyte	**5**	*Yes*	*A.flavus*	8
E.floccosum	**5**	No	No growth	9
T.violaecium	4	No		
T.verrucosum	2	No		
M.audoini	2	No		
M.canis	2	No		
T.tonsurans	2	No		

Figure 4: Number of samples found positive with Dermatophyte species



DISCUSSION:

Cutaneous dermatophyte infections are common in the general population with up to 20% of people being infected at any time. However, adults are generally less susceptible to skin infection than are children owing to the fungistatic properties of fatty acids in the sebum. Most of these infections are not life threatening, but they can cause morbidity in immunocompromised, diabetic patients, people who use communal baths, and people who are involved in contact sports such as wrestling.[2] Outbreaks of infections can occur in schools, households and institutional settings. Such infections can spread usually through direct contact with an infected person or animal, clothing, bedding and towels can also become contaminated and spread the infection. Dermatophyte infections can affect the skin on almost any area of the body, such as the scalp, legs, arms, feet, groin and nails.[7, 8] These infections are usually itchy, redness, scaling, or fissuring of the skin, or a ring with irregular borders and a cleared central area may occur.[9] In the current study, we studied biofilm production by dermatophytes by isolation of common dermatophytes by routine culture methods from 50 skin samples received at Microbiology laboratory; identification of different dermatophyte species isolated from these samples; and detection of the biofilm production by dermatophyte species. We observed that out of 50 samples, 28 (56%) dermatophytes species were isolated. Most common isolate was T.rubrum (12%), followed by T.mantographyte (10%). All strains of T.mantographyte and T.rubrum found positive for biofilm production. Only 33% strains of T.rubrum were found sensitive for Itraconazole. 40% strain of T. mantographyte was found sensitive for itraconazole and 20% for Ketoconazole. The results were consistent with other studies.

Brilhante SN et al evaluated the in vitro and ex vivo biofilm-forming ability of dermatophytes on a nail fragment. Methodology: Initially, four isolates of Trichophyton rubrum, six of Trichophyton tonsurans, three of Trichophyton mentagrophytes, ten of Microsporum canis and three of Microsporum gypseum were tested for production biomass by crystal violet assay. Then, one strain per species presenting the best biofilm production was chosen for further studies by optical microscopy (Congo red staining), confocal laser scanning (LIVE/DEAD staining) and scanning electron (secondary electron) microscopy. Results: Biomass quantification by crystal violet assay, optical microscope images of Congo red staining, confocal microscope and scanning electron microscope images revealed that all species studied are able to form biofilms both in vitro and ex vivo, with variable density and architecture. M. gypseum, T. rubrum and T. tonsurans produced robust biofilms, with abundant matrix and biomass, while M. canis produced the weakest biofilms compared to other species. Conclusion: This study sheds light on biofilms of different dermatophyte species, which will contribute to a better understanding of the pathophysiology of dermatophytosis. Further studies of this type are necessary to investigate the processes involved in the formation and composition of dermatophyte biofilms. B Costa-Orlandi et al analyzed biofilm formation by light microscopy, scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) as well as by staining with crystal violet and safranin. Metabolic activity was determined using the XTT reduction assay. Both species were able to form mature biofilms in 72 h. T. rubrum biofilm produced more biomass and EPS and was denser than T. mentagrophytes biofilm. The SEM results demonstrated a coordinated network of hyphae in all directions, embedded within EPS in some areas. Research and characterization of biofilms formed by dermatophytes may contribute to the search of new drugs for the treatment of these mycoses and might inform future revisions with respect to the dose and duration of treatment of currently available antifungals. [10, 11]

ARAÚJO CR et al tested the antifungal activities of fluconazole, itraconazole, ketoconazole, terbinafine and griseofulvin by broth microdilution technique, against 60 dermatophytes isolated from nail or skin specimens from Goiania city patients, Brazil. In this study, the microtiter plates were incubated at 28 oC allowing a reading of the minimal inhibitory concentration (MIC) after four days of incubation for Trichophyton mentagrophytes and five days for T. rubrum and Microsporum canis. Most of the dermatophytes had uniform patterns of susceptibility to the antifungal agents tested. Low MIC values as 0.03 µg/mL were found for 33.3%, 31.6% and 15% of isolates for itraconazole, ketoconazole and terbinafine, respectively. Yadav A et al examined patients with Tinea infections clinically by dermatologist. Isolation, confirmatory test were done as per the standard procedure, and Antifungal Susceptibility test was done by Disc diffusion method. A total of sixty six patients of dermatophytosis were studied. Tinea unguium was more common in the age group of 31-40 years with 6 cases (37.5%) and in males with 10 cases (62.5%) than females with 6 cases (37.5%).Tinea cruris was more common in the age group 51-60 years with 2 cases

(40%) and was more common in males with 5 cases (100%).In tinea pedis, one case was seen in the age group of 11-20 years and the other in the age group of 41-50 and 51-60 years, and was more common in males with 3 cases (100%).Tinea barbae was more common in the age group 21-30 years with 2 cases (66.66%) .Tinea capitis was more common in the age group of 31-40 years with 2 cases (66.66%) and was more common in females with 3 cases (100%). Tinea manuum was more common in the age group of 31-40 years and in males with 1 case (100%). In males, commonest infection was T. corporis while in female commonest infection was T.corporis.rate of direct microscopy and culture (78.79%). About 89.47% of the dermatophytes grew faster in DTM with compare to SDA, so the growth rate of dermatophyte is better in DTM. A total of thirty five species of dermatophytes were isolated and identified. T.rubrum 15(42.85%) is commonest among other isolates.Ketoconazole showed best susceptibility i.e 26 (74.28%). The study suggested that every patient of tinea infection should be properly studied for mycological examination and should be treated accordingly. This study revealed that Ketaconazole highest susceptibility.[12,13]

CONCLUSION:

T.rubrum, T.mantographyte and E.floccosum are common dermatophyte causing dermatophytosis and T.rubrum, T.mantographyteare also capable to produce biofilm. Major group of dermatophytes which are associated with skin infection are able to produce biofilm that may interfere with treatment.

REFERENCES:

1. KeyvanPakshir, Leila Bahaedinie, Zahra Rezaei, ManuchehrSodaifi, Kamiar Zomorodian.2009. In vitro activity of six antifungal drugs against clinically important dermatophytes.Jundishapur Journal of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz,Iran 2(4): 158-163.
2. Sowmya N, Appalaraju. , Srinivas , Surendran. 2015. Antifungal susceptibility testing for dermatophytes isolated from clinical samples by broth dilution method in a tertiary care hospital. The Journal of Medical Research 2015; 1(2): 64-67
3. C.B. Costa-Orlandi, J.C.O. Sardi, C.T. Santos, A.M. Fusco-Almeida & M.J.S. Mendes-Giannini. 2014In vitro characterization of Trichophyton rubrumand T. mentagrophytes biofilms. The Journal of Bioadhesion and Biofilm Research 30(6): 719-727.
4. ElhamAboualigalehdari, Ali Rezaei-Matehkolaei, MaralGharaghani, Ali ZareiMahmoudabadi . 2014.The Suseptibility Patterns of dermatophyte species from AHVAZ to several antifungals.International Journal of analytical, pharmacutical, and biomedical sciences 3: 30-33.
5. R.K.Agarwal, S.Gupta, G.Mittal, F.Khan, S.Roy and A.Agarwal.2015 Antifungal Susceptibility Testing of Dermatophytes by Agar Based Disk Diffusion Method.international journal of current microbiologyand applied science 4(3): 430-436
6. Afreenish Hassan; Javaid Usman; Fatima Kaleem; Maria Omair; Ali Khalid; Muhammad Iqbal.2011. Evaluation of different detection methods of biofilm formation in the clinical isolates. Brazilian journal of infectious diseases 15(4):305-311.
7. Singh S, BeenaPM . Comparative study of different microscopic techniques and culture media for isolation of dermatophytoses. Indian Journal of Medical Microbiology. 2003; 21:21-24
8. Peerapur BV, Inamdar AC, Pushpa PV, Srikant K. Clinicomycological study of dermatophytosis in Bijapur. Indian Journal of Medical Microbiology. 2004; 22(4):273-274.
9. Craig N. Burkharta Craig G. Burkhart, Aditya K. Gupta, 2002. Dermatophytoma: Recalcitrance to treatment because of existence of fungal biofilm, Journal of American academy of dermatology 47(4):629-631.
10. Brilhante SN, Correia EM, de Melo Guedes M, Pereira S, Oliveira V, Bandeira P, Alencar S, Lucas & Raquel Colares de Andrade, Ana & Castelo-Branco, Débora & de Aguiar Cordeiro, Rossana & Pinheiro, Adriana & Jackson Queiroz Chaves, Lúcio & de Aquino Pereira Neto, Waldemiro & Júlio Costa Sidrim, José & Fábio Gadelha Rocha, Marcos. (2017). Quantitative and structural analyses of the in vitro and ex vivo biofilm-forming ability of dermatophytes. Journal of medical microbiology. 66. . 10.1099/jmm.0.000528.
11. B Costa-Orlandi, C & Sardi, Janaina & Santos, Claudia & Almeida, Ana & Mendes Giannini, Maria Jose. (2014). In vitro characterization of Trichophyton rubrum and T. mentagrophytes biofilms. Biofouling. 30. 1-9. 10.1080/08927014.2014.919282.
12. Araújo, C.R.; Miranda, K.C.; Fernandes, O.F.L.; Soares, A.J. & Silva, M.R.R. In vitro susceptibility testing of dermatophytes isolated in Goiania, Brazil, against five antifungal agents by broth microdilution method. Rev. Inst. Med. trop. S. Paulo, 51(1): 9-12, 2009.
13. Yadav A, Urhekar AD, Mane V, Danu MS, Goel N, Ajit KG. Optimization and Isolation of Dermatophytes from Clinical Samples and In Vitro Antifungal Susceptibility Testing By Disc Diffusion Method. Research and Reviews: Journal of Microbiology and Biotechnology, Volume 2, Issue 3 ,July – September, 2013.

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