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Short Communication

Biofilm formation by zoonotic dermatophytes

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Abstract

Dermatophytes having clinical importance both in man and animals belong to the genus *Microsporum*, *Trichophyton* and *Epidermophyton*. Now a days, in most of the dermatophytosis cases, there is a common complaint of failure of treatment from different parts of the world. This may be due to the development of different drug resistance mechanisms of this organism, of which the formation of biofilm is important. With limited exception so far, the ability of dermatophytes to form biofilm along with proper characterization of the same has not been well studied. Therefore, the present study was conducted to determine the biofilm-forming ability of the isolated dermatophytes from pets in and around Kolkata. For this, skin scrapping samples collected from 40 clinically suspected cases of dermatophytosis presented at two different pet clinics in Kolkata were screened for cultural examination. Biofilm production of the isolates was determined by using tube method, where 10 mL of trypticase soy broth (TSB) was mixed with 1% glucose in each tube. Out of 40 skin scraping samples, 20 (50%) were found to produce dermatophytes on culture including 8 *Trichophyton rubrum*, 6 *Trichophyton mentagrophytes* and 6 *Microsporum canis*. Among the isolates, *Trichophyton mentagrophytes* and *Trichophyton rubrum* were found to produce strong biofilm, whereas *Microsporum canis* isolates were weak biofilm producers.

Key word: Biofilm, Dermatophytes, Pet animals

Highlights

- 45% (9/20) of all the isolates were found to produce one or the other form of biofilm *in vitro*.
- Among the three species of dermatophytes isolated in the present study, *T. mentagrophytes* produced the highest number (50%) and intensity (one strong and two moderate) of biofilm formation.

Fungal condition that affects the keratinized tissues of living organisms is termed as dermatophytosis (Pakshir *et al.*, 2009; Aboualigalehdari *et al.*, 2014). A homogenous group of keratinophilic fungi known as the dermatophytes is responsible for most infections of the skin, its appendages, the hair, and the nail. These infections can be treated with a variety of antifungal medications. Most of the time, fungal biofilm formation makes treatment more difficult, and some dermatophyte species reportedly develop biofilm, which has been linked to treatment failure (Burkhart *et al.*, 2002; Gupta *et al.*, 2016). Biofilms represent densely packed aggregates of sessile microorganisms encased in a self-produced matrix of extracellular polymeric substances, helping in their attachment to biotic and abiotic surfaces, and conferring them survival advantage in unfavourable conditions. (Vaishnavi *et al.*, 2019) and have higher antimicrobial and host defence resistance (Hassan *et al.*, 2011; Agarwal *et al.*, 2015). With limited exception so far, the ability of dermatophytes to form biofilm along with proper characterization of the same has not been well studied (Vlassova *et al.*, 2011). Therefore, it is crucial to find out the biofilm-forming ability of different dermatophyte isolates using some simple laboratory technique so that through their characterization further research can be targeted to develop some novel

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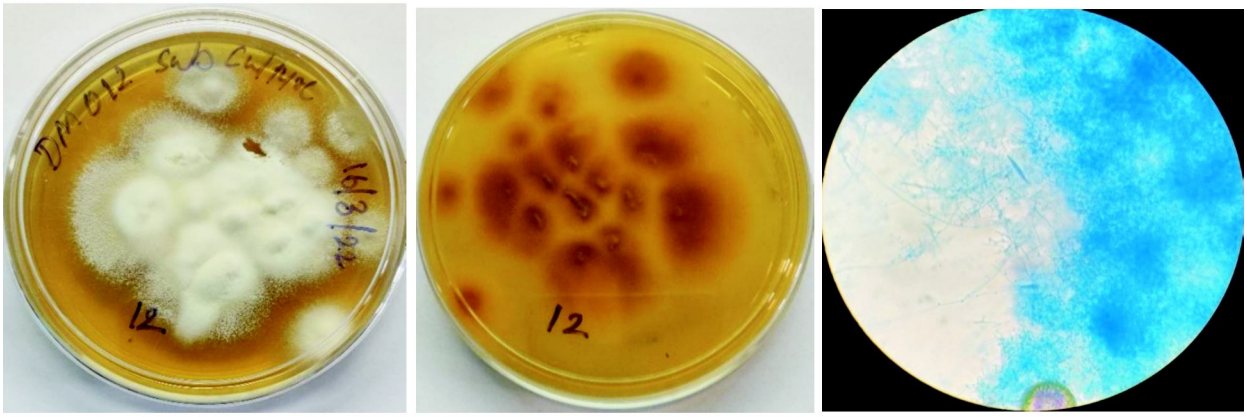
therapeutic strategies that can dissolve biofilm and effectively cure these mycoses (Costa-Orlandi *et al*., 2014). With this background, the present study was conducted for better understanding of the biofilm producing ability of dermatophytes.

The study was conducted in the Department of Veterinary Public Health, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences. After taking written consent from pet owners, 40 skin scrapping samples from clinically suspected pet dogs with lesions like scaling, crust, annular plaques, hair loss or desquamation on anywhere of their body surfaces were collected from two different pet clinics in Kolkata. Before collection, the lesion was lightly disinfected with alcohol in gauze and then scrapped from centre to edge, crossing the lesion margin, using a sterile scalpel blade or equivalent. Isolation and identification of different dermatophyte species from the collected samples were made following the protocol described by Robert and Pihet (2008) and detection of the biofilm producing ability by the isolated dermatophytes was carried out. The study was conducted for a period of 6 months between January to June 2022. The samples were cultured on Sabouraud dextrose agar (SDA), and dermatophyte species isolated from the samples were identified by both macroscopic and microscopic morphology. Macroscopically, suspected colonies were studied for colony characteristics both on the obverse and on the reserve of each plate for identifying the colour, texture of the colonies as well as the presence or absence of pigment. For microscopical study of colonies, lactophenol cotton blue preparation of the matted mycelium mass was done on a glass slide and was examined under a microscope both using low and high-power objectives after putting a cover slip on it. The microscopic identification was made on the basis of the presence of hyphae, microconidia, macroconidia and other accessory structures of vegetative hyphae. Biofilm production of dermatophytes was detected by using the tube method described by Karim *et al*. (2017) with slight modification. Briefly, a loopful of test organism was inoculated in 10 mL of trypticase soy broth (TSB) with 1% glucose in test tubes. The tubes were incubated at room temperature for 72 hours. After incubation, the tubes were decanted, washed with phosphate buffer saline (pH 7.3), and dried. Tubes were then stained with crystal violet (0.1%) solution. Excess stain was washed with deionised 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. The biofilm producing capability of the isolate was classified based on the intensity of crystal violet staining. The presence of adhering film in the tube was used to visually evaluate the formation of biofilm by the respective isolates.

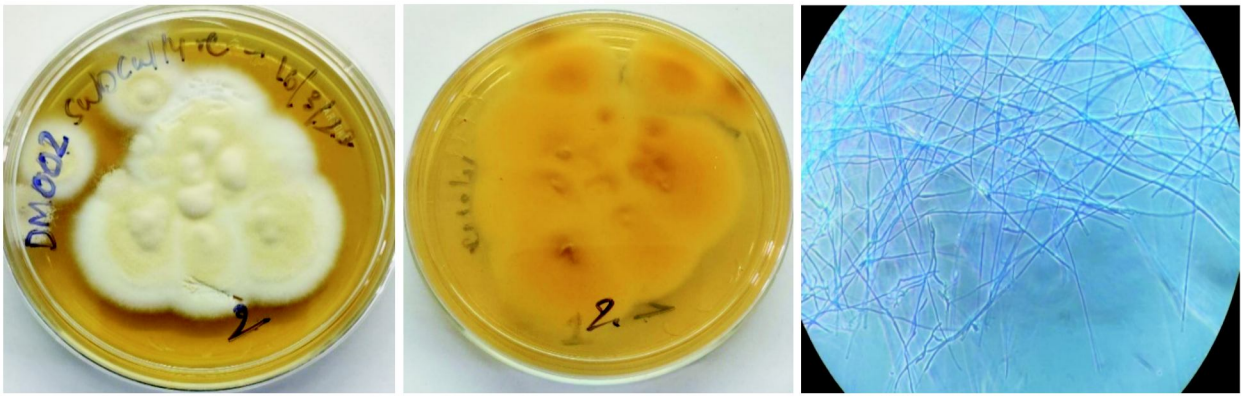
In this study, among 40 skin-scraping samples, 20 (50%) were found to produce dermatophytes on culture. Number of isolates identified were *T. rubrum* (8), *T. mentagrophytes* (6) and *M. canis* (6); thereby, the most common isolate was *T. rubrum*, followed by *T. mentagrophytes* and *M. canis*. Furthermore, 16.67% (1/6) and 12.5% (1/8) of the *T. mentagrophytes* and *T. rubrum* isolates, respectively, were found to be detected as strong biofilm producers, whereas 33.33% (2/6) of *T. mentagrophytes* isolates and 16.67% (1/6) of *M. canis* isolates were detected as moderate biofilm producers (Table 1). A total of 45% (9/20) of all the isolates were found to produce one or the other form of biofilm *in vitro* (Fig. 4, 5, 6, 7).

Infections with dermatophytosis are frequent in canine species. Although most of these illnesses are not life threatening, immune-compromised individuals may nonetheless experience morbidity (Sowmya *et al*., 2015). These outbreaks can occur in kennels, households, and institutional settings. Infections can spread mostly through close contact with an infected person or animal. They can also spread through contaminated clothing, bedding, and towels. Skin infections caused by dermatophytes can affect almost any part of the



(a) (b) (c)

Fig. 1. ***Microsporum canis*** **(a) Forward (b) Reverse (c) Microscopic**



(a) (b) (c)

Fig. 2. ***Trichophyton mentagrophytes*** **(a) Forward (b) Reverse (c) Microscopic**

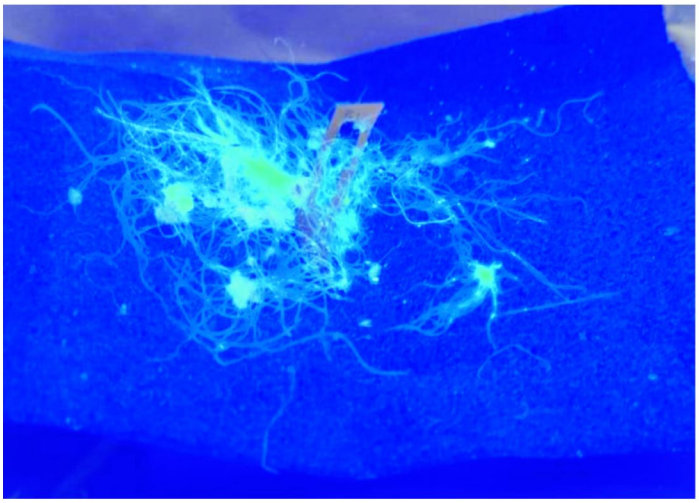


Fig. 3. Fluorescence by the presence of fungal elements under UV light

Biofilm production by dermatophytes

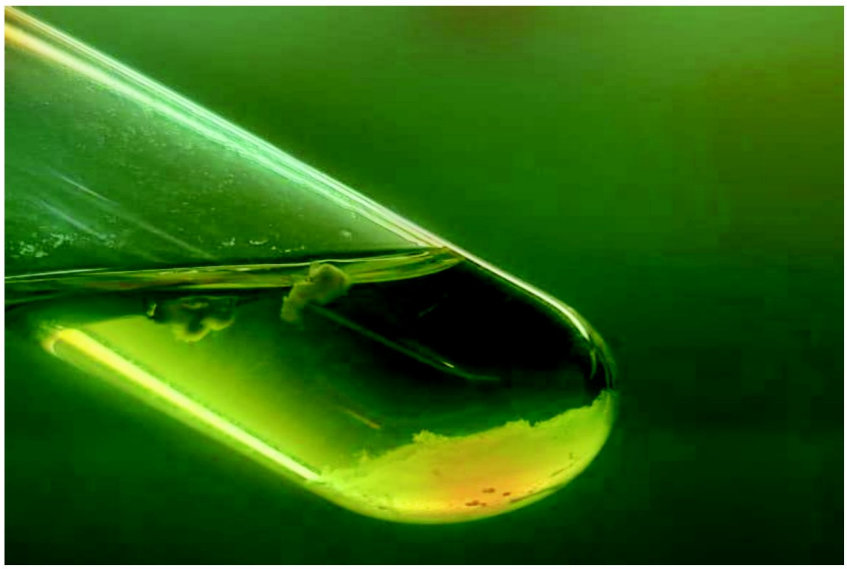


Fig. 4. Biofilm production in TSB

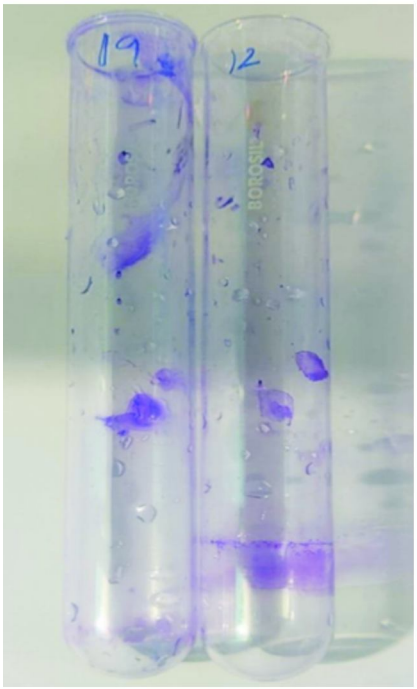


Fig. 5.

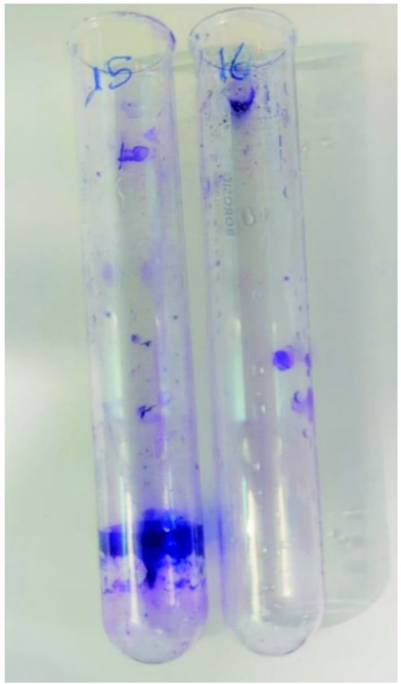


Fig. 6.

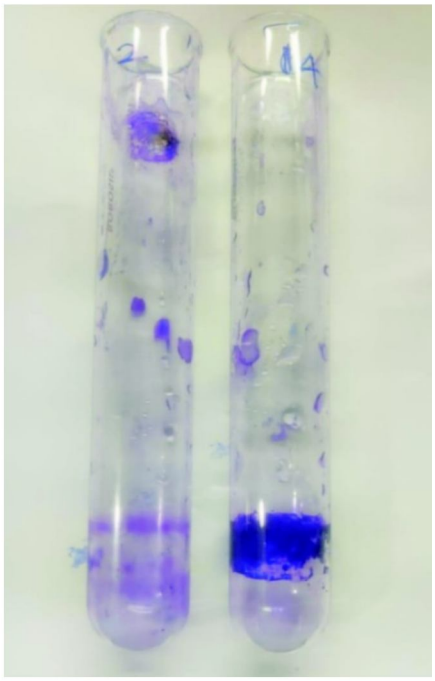


Fig.7.

Fig. 5. Non & weak biofilm producer *Microsporum canis;* Fig. 6. Strong & non biofilm producer *Trichophyton rubrum*; Fig. 7. Moderate & strong biofilm producer *Trichophyton mentagrophytes*

Table 1. Distribution of biofilm producing ability of isolated dermatophytes

Dermatophytes species	Strong biofilm producer	Moderate biofilm producer	Weak biofilm producer	Non-biofilm producer
M. canis (n= 6)	0	1	2	3
T. mentagrophytes (n= 6)	1	2	0	3
T. rubrum (n= 8)	1	0	2	5

Table 2. Distribution of sample

No. of sample tested	Dermatophytes positive				Percentage
	M. canis	*T. mentagrophytes*	*T. rubrum*	Total	
40	06	06	08	20	50%

body, including the legs, arms, feet, groin area, and nails (Singh and Bina, 2003; Peerapur *et al*., 2004). These infections typically cause itching, redness, scaling, or fissures in the skin. They can also cause a ring with irregular borders and a cleared central area (Burkhart *et al*., 2002). The present study has documented a prevalence of dermatophytoses in 50 percent (Table 2) of the suspected animals, where the most common isolate was *T. rubrum* followed by *T. mentagrophytes* (Fig. 2) and *M. canis* (Fig.1). The higher prevalence of anthropophilic *T. rubrum* in the samples collected from pet animals is quite unusual, and it may be due to the fact that most of the pets were from the indoor housing with very close and frequent contact with their owners. Among the three species of dermatophytes isolated in the present study, *T. mentagrophytes* produced the highest number (50 %) and intensity (one strong and two moderate) of biofilm formation.

Brilhante *et al*. (2017) evaluated *in vitro* and *ex vivo* biofilm-forming ability of dermatophytes on nail fragments. They evaluated four isolates of *Trichophyton rubrum*, six of *Trichophyton tonsurans*, three of *Trichophyton mentagrophytes*, ten of *Microsporum canis* and three of *Microsporum gypseum* by a crystal violet assay for producing biomass. Then, one strain per species that produced the best biofilm was chosen for additional optical microscopy by Congo red staining, confocal laser scanning and scanning electron microscopy. Their study indicated that every species under study could create biofilms with variable density and architecture, both *in vitro* and *ex vivo*. *M. gypseum*, *T. rubrum and T. tonsurans* produced strong biofilms, abundant in biomass and abundant in matrix, whereas *M. canis* produced the weakest biofilms compared to other species. The present study was consistent with the study conducted by Brilhante *et al*. (2017), where it illuminates the biofilm-forming ability of different dermatophyte species, which will again contribute to a better understanding of the pathophysiology of dermatophytosis in animals and man.

Costa-Orlandi *et al*. (2014) also analysed biofilm forming ability of dermatophytes by using different methods like light microscopy, scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) as well as by staining with crystal violet and safranin. They observed that compared to *T. mentagrophytes* biofilm, *T. rubrum* biofilm produced more biomass and extracellular polymeric substances. Another study conducted by Mustafa *et al*. (2017) revealed the prevalence of biofilm-forming dermatophytes in 51.85% (28/54) of all the isolates obtained from the nail specimens of onycomycotic patients, which is also in line with the finding of the present study. Further research in this

line and analysis of the biofilm produced by dermatophytes from different angles may help in the hunt for new therapeutic options to treat these mycoses as well as direct future changes to the dosage and course of treatment for already existing antifungal (Costa-Orlandi *et al*., 2014; Brilhante *et al*., 2017).

To conclude the present study, it can be said that among the majority of the dermatophytes, *T. mentagrophytes* is capable of producing strong biofilm. As a significant group of skin pathogen with zoonotic potentiality, the biofilm-forming ability of dermatophytes should be well studied to prevent further therapeutic failure and to find out novel avenues to tackle these common ailments of man and animals.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: YM, CD: Planned, designed, conducted the collection of samples, analysis, and prepared the manuscript; CD: Provided inputs in the design and further developed the manuscript; AP, AR, IS, RB, AB, RB: Helped in the planning of the study and revised the manuscript. All authors read and approved the final manuscript.

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