SHORT COMMUNICATION

Evaluation of Disc Diffusion Assay for Antifungal Susceptibility of *Microsporum canis* Strains of Animal Origin from India

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Abstract

Dermatophytes are keratinophilic fungi that cause skin infections in both humans and animals. In the current study, 72 *Microsporum canis* isolates were tested for antifungal susceptibility to seven commercially available antifungal discs (HiMedia), which included clotrimazole (10 µg), amphotericin B (20 µg), nystatin (50 µg), miconazole (30 µg), itaconazole (30 µg), ketoconazole (30 µg), and fluconazole (10 µg). The disc diffusion method was used to assess the antifungal susceptibility of animal dermatophyte isolates. Among all tested isolates fluconazole showed the lowest zone of inhibition, and miconazole as well as clotrimazole were having the highest mean zone of inhibition in *M. canis* isolates. Although the disc diffusion assays are not frequently used, they will give initial insights into the effective antifungal drugs prior to starting treatment. Antifungal sensitivity testing should be the basis of treatment since dermatophytes are increasingly showing signs of drug resistance to antifungal drugs.

Keywords: Animal, Antifungal agents, Disc diffusion, *Microsporum canis,*

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Introduction

Dermatophytes are keratinophilic moulds that cause skin infection in humans as well as animals (Gnat *et al.,* 2020). Dermatophytes are comprised of seven fungal genera: *Trichophyton, Microsporum, Epidermophyton, Nannizzia, Paraphyton, Arthroderma, and Lophophyton* (de Hoog *et al.,* 2017). Dermatophytosis or ringworm is a zoonotic skin mycoses that may be transmitted from animals to humans (Pasquetti *et al.,* 2017). Clinical manifestations may vary based on the species of dermatophyte and the site of infection (Gupta and Cooper, 2008). The most common pathogen among cats and dogs is *M. canis* with cat being the majorly infected host animal. The prevalence of dermatophytes varies depending on the environment and geographic regions. The disease is widespread throughout the world, but is more prevalent in tropical countries like India (Ramaraj *et al.,* 2016; Hubka *et al.,* 2018).

Dermatophytosis is treated with a variety of antifungal medications but not all dermatophyte species exhibit the same pattern of susceptibility, and resistance can be either absolute or relative (Fernandez-Torres *et al.,* 2002). Although there are a number of therapeutic options for cutaneous fungal infections, antifungal sensitivity testing should be the basis of treatment due to the growing tendency of antifungal medication resistance among dermatophytes. The greatest challenge in treating dermatophytosis in humans and animals is antifungal drugs resistance, which can result in refractory and recurrent infections (Yamada *et al.,* 2008; Salehi *et al.,* 2018). Thus, antifungal resistance is also a growing concern among dermatophytes, similar to antibiotic resistance (Yamada *et al.,* 2008; Salehi *et al.,* 2018; Hsiao *et al.,* 2018). The broth microdilution method is frequently used to determine the minimum inhibitory concentration values (MIC50 and MIC90) of antifungal drugs for the dermatophytes.. However, the procedure takes a long time, and individual variations may affect the observations. The Clinical Laboratory and Standards Institute (CLSI) do not provide guidelines for the disc diffusion assay, and it is not typically used for dermatophytes. Though antifungal disc diffusion assay is not a standard method for determining the antifungal sensitivity of dermatophytes, it has been widely used by many workers to have a gross idea about the sensitive and resistant drugs (Esteban *et al.,* 2005). In this study, we used

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the disc diffusion assay to assess the antifungal susceptibility of dermatophytes that were isolated from animals.

Materials and Methods

Collection of Samples and Isolates

A total of 72 *M. canis* strains isolated from 478 clinical samples of felines, canines and rabbits were used in the study. These isolates were obtained from the clinical samples collected at Teaching Veterinary Clinical Complex of the Veterinary Universities of five states of India including Uttar Pradesh, Punjab, Haryana, West Bengal and Tamil Nadu.

Preparation of Test Inoculums

In the disc diffusion assay for dermatophytes, the test inoculum was prepared from a suspension of conidia and hyphae. The isolates were sub-cultured on potato dextrose agar and incubated for 7-14 days at 30°C or until excellent conidial growth was observed. Sterile normal saline (0.85%) was used to flood the agar plates, and the colonies were softly scraped with the help of a sterile 10 µL tip to make a suspension. The conidia and hyphal suspension were collected in a 15 mL centrifuge tube The solution was allowed to settle for 15 min. A new 15 mL centrifuge tube was used to collect the upper homogeneous suspension. A heamocytometer was used to measure the number of conidia, and the concentration of conidia was set at 1 x 10^6 conidia/mL.

Application of Antifungal Discs

The disc diffusion assay was carried out using antifungal assay agar (HiMedia). The commercially available antifungal discs like fluconazole (FLC 10 µg), itaconazole (IT 30 µg), ketoconazole (KT 30 µg), miconazole (MIC 30 µg), nystatin (NS 50 µg), amphotericin B (AMB 20 µg), and clotrimazole (CC 10 µg) purchased from HiMedia were used. The antifungal assay agar plates were uniformly swabbed using a concentration solution of 1 x 10^6 conidia/ mL. The process was carried out three times, with extra attention paid to streaking the agar plates' edges.. The plates were allowed to dry for 10-15 min. Sterile forceps were used to apply the discs to the agar surface. . Four discs were placed on one plate, and three discs on another. The plates were then incubated for four to seven days at 28 °C (Nweze *et al.*, 2010). The measurement and expression of the inhibitory zone were done in millimetres.

Results and Discussion

The disc diffusion assay was performed for 72 *M. canis* isolates. Among the tested isolates, fluconazole (FLC 10; 0.59±0.17 mm) exhibited the least zone of inhibition. Miconazole (MIC 30; 17.22±0.25mm) and clotrimazole (CC 10; 14.25±0.41 mm) had the highest mean zone of inhibition among all tested isolates (Fig. 1A&B). Table 1 lists the mean zone of inhibition.

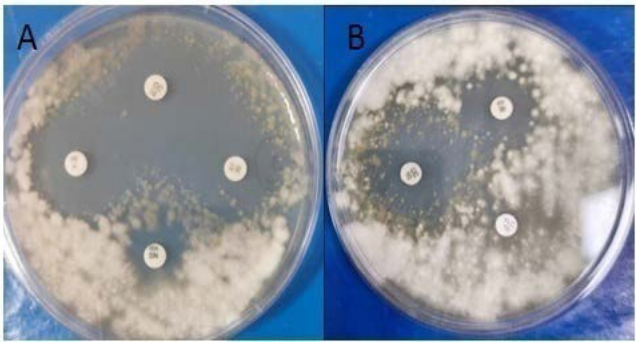

Fig. 1 (A&B): Disc diffusion assay of *M. canis* isolates

Table 1: Zone of inhibition of antifungal discs against *M. canis* isolates (Mean ±SE)

Antifungal agents	MIC 30	NS 50	KT 30	CC 10	FLC 10	IT 30	AP 20
Zone of inhibition (mm)	17.22±0.25	4.62±0.18	9.51±0.27	14.25±0.41	0.59±0.17	10.41±0.40	1.15±0.23

Begum and Kumar (2021) also reported similar findings regarding the antifungal susceptibility of dermatophytes using the disc diffusion method. In a similar study by Esteban *et al.* (2005), clotrimazole was reported to have a high zone of inhibition following terbinafine. A similar study conducted by Khadka *et al.* (2017) demonstrates that miconazole is the most effective antifungal drug against dermatophytes. Our current findings are consistent with these three studies. Fluconazole (FLC) showed the lowest zone of inhibition among all the isolates that were examined. Similar findings were also identified by other researchers (Singh *et al.,* 2007; Galuppi *et al.,* 2010; Agarwal *et al.,* 2015). *In vitro* antifungal sensitivity testing is a crucial method or procedure that aids in clinical efficacy prediction. Therefore, miconazole as well as clotrimazole and azole medication other than fluconazole can be suggested for the treatment of small animal dermatophytosis.

In conclusion, miconazole and clotrimazole were found to be efficient against Microsporum canis based on disc diffusion assay. The major difficulties in performing the disc diffusion assay for dermatophytes are the varying growth rate of conidia production. Since there are no standard procedures for interpreting the disc diffusion assay results for dermatophytes, the isolates cannot be categorized as sensitive, intermediate sensitive, or resistant. However, the disc diffusion method is a useful and easy way to assess dermatophytes' susceptibility to antifungals and may provide early findings regarding the most effective antifungal medicine for ringworm or tinea treatment.

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