Incidence and Antifungal Susceptibility Profile of Dermatophytes Associated with Superficial Lesions in Dogs and Cats in Abia State, Nigeria

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Abstract: Fungal infection occurs in dogs and cats which are companion to man. This study aimed at identifying the dermatophytes commonly associated with dogs and cats and their *in vitro*-antifungal susceptibility profile. Samples were collected from two pet animals (dogs and cats) in three local government areas of Abia State. A total of one hundred and fifty skin scrapings from suspected infected dogs (100) and cats (50) were screened. Sabouraud Dextrose Agar {SDA} was used for the culture and Needle Mount Technique was employed. Lactophenol Cotton Blue (LCB) was used for staining. Each of the fungal isolate was identified based on its colony characteristics, hyphal and conidial cells produced. The Clinical Laboratory Standard Institute Technique was used for sensitivity testing of the isolates. The most commonly isolated dermatophytes were *Microsporum* species (36%) and *Trichophyton* species (8.6%) which was significantly different among the cats and the dogs (P=0.05). *Microsporum* species were found to be 100% sensitive to terbinafine, fluconazole and aqusulfur, while *Trichophyton* species are more sensitive to terbinafine, fluconazole and tinidazole. Terbinafine, fluconazole and tinidazole are the most active drugs against *Microsporum* and *Trichosporum* spp and therefore recommended for use.
Keyword: Dogs, cats, Dermatophytes, Drugs, *in vitro*-antifungal sensitivity profile

INTRODUCTION

Dermatophytes are a distinct group of fungi that exhibit effect on keratinous tissues and penetrate the hair, skin and nails of animals or living host (Midgley e*t al*., 1994). The diseases caused by dermatophytes are called dermatophytosis (Garber, 2001). There are three important genera of dermatophytic fungi: Epidermophyton (infects skin and nail), *Microsporum* (attacks the hair and skin) and *Trichophyton* (invades hair, skin and nail) (DeBoer and Moriello**,** 2012). The clinical feature presented by dermatophytes in animals varies depending on the degree of infection. This includes; itching, inflammation, circular alopecia, dry scale and blister formation. Dermatophytes are the most frequently occurring infectious and highly contagious mycosis of man and animals (Pal, 2011). *Microsporum* specie is the most commonly isolated pathogen causing dermatophytosis in dog and cats, followed by *Trichophyton species* (Moriello *et al.*, 2017).The tineas (ringworm) are frequent in domestic and savage animals: the most affected are the small species, such as dogs, cats and rodents (Sparkles *et al.*, 2009). Infection occurs by direct transmission of infective spores to a susceptible host through cuts on the skin (Craig, 2018). Factors that predispose to infection of dermatophytes, include any pre-existing disease that will cause an increase in surface humidity, cause micro-trauma (cuts) to the skin and compromise host immune system (Ogawa *et al*., 1998). It has been estimated that dermatophytosis accounts for approximately 2% of all skin infection in which Microsporum species andTrichophyton species contributes a great deal (Scott *et al*., 2001). Prevalence of the disease tends to be more common in warm tropical/subtropical climates and or where there are large numbers of feral animals (Hasegawa *et al*., 1997). Although cat is regarded as the natural host, and even as a reservoir for *Microsporum* specie and *Trichophyton* species (Pinard *et al*., 1997), dogs are also incriminated though without obvious clinical symptoms. The presence of other diseases may also affect susceptibility to infection. Dermatophytosis is three times more prevalent in cats with feline immunodeficiency virus than in uninfected cats (Mancianti *et al*., 1992). The infections caused by this organism are hardly fatal but mostly debilitative and disfiguring that can give rise to permanent deformation if not treated (Yuanwu *et al*., 2009).

There have been increasing complaint by dog and cat owners of skin related infections in the study area and the poor management by animal health care givers, hence the need for this study. The aim of this study is to identify the dermatophytes responsible for causing infections in dogs and cats, and to determine the antifungal drug most sensitive to these organisms.

MATERIALS and METHODS

Study Area

Three (3) Local Government Areas of Abia state were selected for the study. The study area wereUmuahia North, Umuahia South and Osisioma Local Government Area. Abia State in Nigeria is located in a tropical rainforest between latitude 543E, in the south eastern part of the country and longitude 752E. The average annual temperature and rainfall are 26.9 ^{o} C and 2193mm respectively (Kottek *et al*., 2006).

Sample Collection: The sample collection and study protocol used in this study were as described by Nwaze and Okafor (2005) with some modifications. This modification involves collection of samples in batch of ten and the use of gentamicin instead of cyclohexamide to inhibit bacteria growth in the preparation of Sabouraud dextrose agar. A purposive sampling method was employed. A total of 150 animals were sampled and this comprised of 100 dogs and 50cats.The sampling cut across the three selected local government areas of the State. Skin scraping were collected from obviously infected dogs presenting lesions. Relevant demographic data such as age, sex and breed were considered why collecting the samples. Only animals that had no history of antifungal or antibacterial therapy in the previous months were included in the study. Samples were collected from different clinics at different time. Sample collection lasted for 8months from April to November, 2019.

Samples were obtained during ambulatory services (House calls), while others were collected with the help of veterinarians working in different clinics. The animals with marked lesion was carefully examined for areas with loss of hair, erythema, scaling, or heavy crusts, which may or may not show suppuration beneath them. Animals of all ages showing areas of infection were sampled. The samples were collected aseptically by cleaning the affected area with 5% alcohol and using a sterile scalpel blade, areas of skin lesion were gently scrapped especially the advancing border of the lesion making contact with inner layers of the skin (blood) and placed into Bijou bottles. The bottles containing normal saline was transported to the laboratory in a cold-chain within 4 to 10hours and were processed. Culture and identification of the fungal genera was carried out in the Department of Veterinary Microbiology laboratory of Michael Okpara University.

Isolation, Microscopy and Biochemical Identification

Sabouraud Dextrose Agar (SDA) medium was used for the isolation of fungi from the skin scrapings. The medium was prepared aseptically following the manufacturer's description by dissolving 65grams in 1000mls of distilled water. In addition, gentamicin (2ml) was added to the media to inhibit bacteria growth and make the media selective for fungi organisms. The medium was autoclaved at 15pounds pressure, temperature of 121^{O}C for 15minutes and gradually dispensed into sterile Petri dishes in hood chamber and allowed to set cool.

Isolation: A sample was seeded into the already prepared media (SDA) and tightly sealed with a masking tape to avoid contamination and to create a humid environment necessary for growth. The sealed plates were incubated at room temperature for 7-14 days and observed at intervals for growth of the organism as described by Sykes and Rankin, (2014).

After incubation for 7days the sealed plates were opened, Microscopy: With the aid of a needle, a sample was picked and placed on a grease free glass slide. A drop of alcohol was then added with the aid of a pipette and allowed to stand for 5minutes. Afterwards, a drop of lactophenol cotton blue was added and covered with a cover slip. Isolates were then identified based on atypical colonial and microscopic morphology of the culture at 100-1000 magnification (Campbell *et al*., 1996). Microscopic examination of the isolates was done using direct microscopy and needle mount.

Biochemical Identification: Urease test was used to determine whether the organism utilizes urea as source of carbon in line with Clinical Microbiology Proceedings Handbook (2016). Broth made from urea was prepared following the manufacturers instruction. Then the organisms were introduced into broth and observed for change in colour. A positive test was indicated by colour change, while no change in colour implies negative reaction.

Antifungal Susceptibility Test: Antifungal susceptibility profiling was carried out using agar dilution method as described by Wiegand *et al.* (2008). The study was carried out to determine the antifungal sensitivity of isolated organisms. These antifungal agents were sourced from different pharmaceutical shop in Umuahia town and brought to the laboratory. The following drugs were used for antifungal susceptibility test.

Table 1: Drugs Used For Antifungal Susceptibility Testing

Drugs	Trade name	Concentration(mg/ml)	Minimum inhibitory concentration
Grioseofulvin	Rysovin	500mg/ml	0.50µg
Tarbinafine	Tarbifine	400mg/ml	0.45 µg
Ketoconazole	Sivoketoconazole	200mg/ml	0.55 µg
Tinidazole	Tinzol	500mg/ml	0.50 µg
Visita plus	Vista plus	10mg/ml	0.50 µg
Whitefield's oint.	Whitefield's oint.	10mg/ml	0.05 µg
Ppt. sulfur (10%)	Aquasulf	100mg/ml	0.50 µg
Fluconazole	Fluconazole	200mg/ml	0.50 µg

NB: oint. Stands for ointment; Ppt= Precipitated

A serial dilution was done to obtain 0.5µg/ml of the drugs in 100mls of sabouraud dextrose agar. Fluconazole (200mg) and ketoconazole (200mg) were dissolved in 100mls of distill water and a serial dilution of 1/40 was made, then 1ml was aspirated from it into 100mls of the media.The concentration for both drugs was 5mg/ml and the technique adopted was as described (CLSI, 2008)

Tinizol – 1tablet of tinizol of 500mg was also dissolved in distilled water and 1/100 serial dilution made and 1ml was aspirated into 100mls of the media. Griseofluvin – 1tablet of flusin500mg was dissolved in distilled water a serial dilution of 1/100 in 1ml was aspirated into 100mls of the media. Both drugs had a concentration of 10mg/ml. After cooling the media the drugs was added and gradually poured into Petri dishes and Whitefield ointment and visita plus a serial dilution of 1/20 were made 1ml added to 100mls of the media and the concentration used for both drugs was 1mg/ml

Precipitated aquasulfur– a serial dilution of 1/2000 was made. About 1ml was added to 100mls of the media and terbinafine 1/80 and 1ml dispensed to 100mls of the media. The paste was accurately weighted and dissolved in appropriate solvents. The tablet and paste was macerated with a homogenizer and dissolved in distilled water and appropriate solvent. 100mls of distilled water was dispensed in each conical flask (8 in all) and 6.5g of sabouraud dextrose agar was added to each flask and autoclaved.

allowed to set. The concentration for terbinafine was 10mg/ml while that of precipitated aquaslfur was 2.5mg/ml.

After setting, a growth culture of Microsporum and Trichyphoton organisms were seeded into the media separately with the help of a sterile wire loop and allowed to grow for 48hours. The reading was done visually in line with Clinical and Laboratory Standard Institute (CLSI, 2010).

RESULTS

The result of the study revealed that out of the 150dogs and cats screened for the presence of fungal infections, 67 (44.6%) were positive out of which (36%) were Microsporum spp and (8.6%) were Trichophyton spp (Table 2). Table 2 further indicated that, the prevalence of Microsporum spp was higher in dogs with a rate of (41%) than in cats (26%). Similarly the prevalence of Trichophyton spp was higher in dogs (9%) than in cats (8%). The incidence of Microsporum spp in female dogs was 32% while in the male, Trichophyton spp recorded 18%. The female dogs less than 1year old and above 1year old had incidence rate of 42% and 32%, while the male dogs of less than 1year old and those above 1year had 16% and 10% respectively for Trichophyton spp (Table 3). Table 3 also show that Mongrel breed of dogs had high occurrence rate at 66% for Microsporum spp while, the incidence rate in Alsatian breed for Trichosporum spp was 34% . The incidence of Microsporum spp in female cats was 24% compared to 10% of Trichosporum spp that occurred in male. Female cats of less than 1year and above 1year old had incidence rate of 14% and 10% for Microsporum spp, while male cats less than 1year old and above 1year had incidence rate of 6% and 4% respectively (Table 4). From the results obtain as shown in Plate 1, the growth of the fungal was even and rapid after 14 days and this is suggestive of Microsporum because the colonies were downy, wooly and powdery. The growth was slow to moderate in Sabouruad dextrose agar at 25^{O}C. The diameter of the colonies varies from between 1 to 7cm after 7days of incubation. Plate 2 revealed the growth of Microsporum spp as multicellular spores with thick rough walls. The macroconidia appear as hyaline with multi-septate and characteristically spindle to obviate shape, possessing between 5 to 7cells.

Plate 3 is a pictoral view of Trichophyton colonial growth. The colonies are initially slow but later rapid in growth. The texture is waxy, glabrous, and cottony. The colour is white to bright yellow in front, while the reverse is pale, yellow, brown or reddish brown. In Plate 4, the presence of septate hyphae, microconidia, macroconidia, arthroconidia and conidiophores is visible. However, the conidiophores are poorly differenciated from the hyphae.

The urease test carried out showed that Microsporum species were urease negative as there was no change in colour when the organism was introduced into the test medium. Trichophyton specie was urease positive as indicated by visible change in color from yellow to pink. Griseofulvin, Fluconazole and Precipitated aqulsuf were found to be effective against Microsporum spp as the organism exhibited 100% susceptibility to all the three drugs. The susceptibility of Microsporum spp to terbinafine, ketoconazole, visita plus and whitefield ointment was also recorded at 90% for all the three drugs, while tinidazole was 80% (Figure 1). The antifungal susceptibility test against Trichophyton spp indicates that tarbinafine, fluconazole, and tiniadzole exhibited a higher activity against the organism with 100% activity by all the three drugs. This is followed by griesofulvin and ketoconazole whose activity was recorded at 90%, while visita plus, whitefield ointment and precipitate aqusulf recorded 80%(Figure 2).

Table 2: Occurrence of *Microsporum* spp and *Trichophyton* spp in dogs and cats

Type of Animals	**No Examined**	**No Positive**	
		Microsporum spp	*Trichophyton* spp
Dogs	100	41(41%)	09(9%)
Cats	50	13(26%)	04 (8%)
Total	150	54(36%)	13(8.6%)

Table 3: Occurrence of *Microsporum* spp and *Trichophyton* spp in dogs

Demograph	*Trichophyton* spp	*Microsporum* spp
Sex	Male 18(18%)	Female 32(32%)
Age<1year	8(16%)	21(42%)
Age>1year	5(10%)	16(32%)
Breed	Alsatian 17(34%)	Mongrel 33(66%)

Table 4: Occurrence of *Microsporum* spp and *Trichophyton* spp in cats

Demograph	*Trichophyton* spp	*Microsporum* spp
Sex	Male 5(10%)	Female 12(24%)
Age<1year	3(6%)	7(14%)
Age>1year	2(4%)	5(10%)

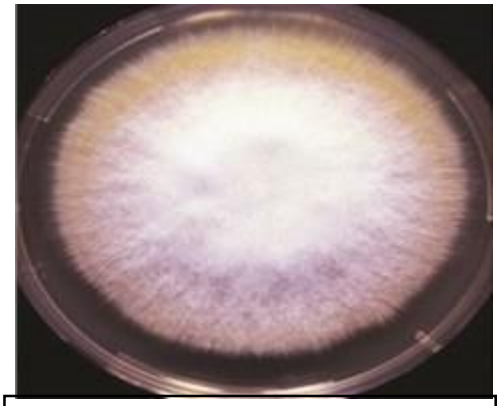

Plate 1; *Microsporum* colonies after 14days incubation

Plate 2; *Microsporum* spp at microscopy ×10

Plate 3; *Trichophyton* colonies 14days incubation

Plate 4: *Trichophyton* spp at microscopy ×10

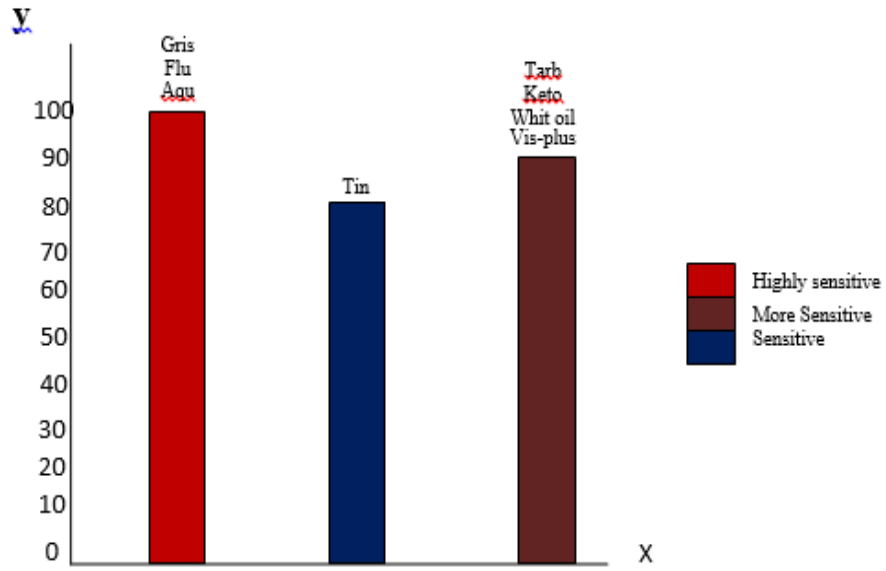

Figure 1: Susceptibility of Trichophyton specie to antifungal agents.

Key:Where Ket = Keteconazole, Flu = Fluconazole, Pre.Aqu =Precipitated aqusulf, Tin = Tinidazole, Tarb = Tarbinafine, Gri = Griseofulvin, Whit = Whitefield's ointment, Vis = Visita plus.

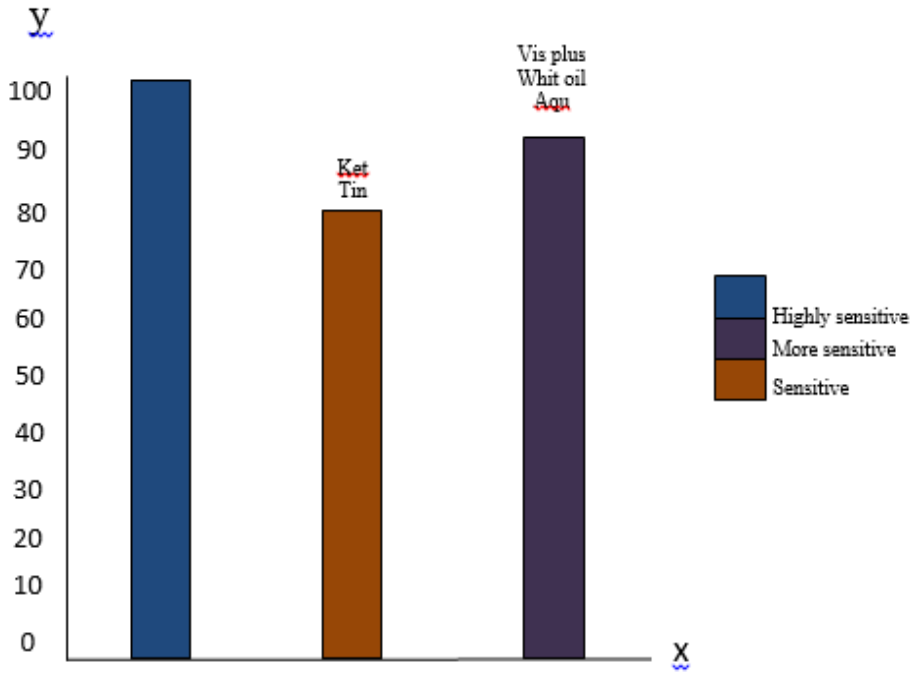

Figure 2: Susceptibility of Microsporum specie to antifungal agents
Key:WhereKet = Keteconazole, Flu = Fluconazole, P.aqu = Precipitated aquasulf, Tin = Tinazole, Terb = Terbinafine, Gri = Griseofulvin, Whit = Whitefield's ointment, Vis = Visita plus.

DISCUSSION

The findings in this study revealed the presence of some pathogenic dermatophytes among dogs and cats in the area under study and this is very important because of the public health implication, since humans live with these animals as pet. The possibility of these animals transmitting these fungal diseases from animals to human is high. The occurrence of dermatophytes in dogs and cats were high, similar to what was obtained in studies carried out in Italy and Indonesia. The incidence of dermatophytes was high at 36.0% and 8.6% for *Microsporum* spp and *Trichosporum* spp respectively and this is in agreement with Galluppi *et al.*, (2013) who reported an incidence rate of 20.5% in dogs and 33.7% in cats in a research carried out in Italy. The findings in the study agrees with Cabanes (2000). Cabanes in his study reported an incident rate of 37.4% for *Microsporum* spp in dogs and 6.0% in other domestic animals. Also, the findings in the study agrees with Debnath *et al.* (2016) who reported 21% of *Mycrosporum* spp in dogs and companion animals in eastern district of India. The findings in this study is also, consistence with the report (Beigh *et al.,* 2014 ; Ranganathan *et al* 1997), who reported in their separate studies that the predominant dermatophytes isolated was *Microsporum* spp followed by *Trichophyton* spp. From the study, female dogs and cats had high incidence rate for *Microsporum* spp and *Trichosporum* spp across the age range (<1year and >1year), and this may be due to stress occasioned by the physiological cycles which the female undergo during pregnancy as well as poor nutrition. These factors may compromise their immunity, making them more susceptible to infection. Younger female dogs and cats had more incidences of dermatophytes at 42% and 14% than the adults because they are more at risk due to low immune system and poor resistance level and this is in agreement with Almuzaini *et al.*, (2016). The high incidence of *Microsporum* spp in Mongrel breed may be due to lack of adequate care and exposure to environmental hazard as they often stroll away or roam on the streets in search of food.

Also, the macroscopic appearance of the colonies as white, light yellow, cottony to powdery suggests *Microsporum* and *Trichophyton* spp. The microscopy shows presence of large, spindle shape, thick walled spores with six or more internal cells and often with a terminal knob. Colonies of *Trichophyton* spp appear waxy in texture, glabrous and cottony. The colour is whitish to bright yellow in the front while the reverse is pale, yellowish- brown or reddish brown. Similar findings were observed by Vetlab **(**2010 ; Ciesielsk and Staczek, 2020; Cano *et al*. 2005; Larone, 1995; Kim *et al*. 2018)

Microscpically, the presence of septate hyphae, conidiophores, microconidia, macroconidia and arthroconidia were visible in *Trichophyton* spp. The change in colour when urease test was conducted to pink colouration suggests urease positive and confirms *Trichophyton* spp. Similar confirmatory features was observed by Frias-De Leon *et al.*(2020).

All the isolated *Microsporum* spp (100%) were found to be sensitive to terbinafine, fluconzole and tinadazole, and this action could be attributed to the fact that the drug exhibits its efficacy by inhibiting fungal sterol biosynthesis to a greater extent than mammalian sterol biosynthesis and this is consistent with the findings of Darkes *et al.* (2003). They are of the view that the high sensitivity of *Microsporum* spp to terbinafine was because it irreversibly inhibits the membrane bound enzyme squalene peroxidase in a concentration dependent manner which prevents the conversion of lanosterol to cholesterol. Also, the mean inhibitory concentration of terbinafine for *Microsporum* spp and *Trichophyton* spp is very low and this is in agreement with Favre *et al*.(2003). Fluconazole exhibited high activity on *Microsporum* spp and *Trichophyton* spp. because this drug is easily absorbed since they are water soluble and this support the report of Dubey *et al*.(2005). They found that fluonazole dose of 5mg/kg taken orally once daily bring about clinical

cure in 4weeks. *Trichophyton* spp was most sensitive to griseofulvin similar to the report of Guptal *et al*.(2009). The high sensitivity of Griseofulvin may be due to the interference this drug has with the function of spindle microtubules and this causes changes in morphology in fungal cells and this agrees with Panda *et al*. (2005). It was observed from the study, that griseofulvin was more effective than fluconazole when their Mean Inhibitory Concentration (MIC) is considered and this is in agreement with Hofbauer *et al*. (2002).

CONCLUSION

The study reveals that *Microsporum* and *Trichophyton* spp are the dermatophyte agents associated with superficial infections among dogs and cats in the study area. *Microsporum* species are the most commonly encountered isolates. The dermatophyte were sensitive to all the drugs used in the study, with most of them exhibiting 100% sensitivity especially terbinafine, griseofulvin and fluconazole.

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