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Title: The best type of inoculum for testing the antifungal drug susceptibility of *Microsporium canis*: in vivo and in vitro results

Short title: The antifungal drug susceptibility of *Microsporium canis*: clinical outcomes and in vitro results

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Author contributions

C.C and A.C.I conceptualised the study and wrote the manuscript. P.C. performed the clinical evaluation of animals and collected the samples. W.R., A.C.I and C.C performed the experimental trial. C.C contributed to interpretation of the study data. C.C, C. Cz and D.O revised, edited and made intellectual inputs in the manuscript. All authors have read, revised and approved the final version of manuscript.

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Conflict of interests

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ABSTRACT

Background

Data correlating *in vitro* drug susceptibility of *Microsporum canis* with clinical outcomes of its infections are lacking as well as the most suitable inoculum and incubation time in broth microdilution assays.

Objectives and Methods

M. canis strains were collected from animal hosts that tested positive (Group I; n=13) and negative (Group II; n=14) to this pathogen following itraconazole (ITC) therapy. *In vitro* ITC susceptibility was assessed according to the Clinical Laboratory Standards Institute (CLSI M38-A2) methodology using conidia, hypha-conidia and arthroconidia at 3 and 7 days of incubation in order to assess the most suitable inoculum and incubation time. Successively, ketoconazole (KTC), voriconazole (VRC) terbinafine (TRB), posaconazole (PSZ), fluconazole (FLC) and griseofulvin (GRI) susceptibilities were assessed using the chosen inoculum.

Results

The MIC values of ITC after three day-incubation were equal than those recorded after seven day-incubation.

ITC MICs were $\leq 1\mu\text{g/ml}$ for strains from Group I and $>1\mu\text{g/ml}$ for those of Group II only when conidia were used. All strains showed high susceptibility to VRC, POS, TRB and low susceptibility to ITC, KTC, GRI and FLC regardless of the source and incubation time.

Conclusions and clinical importance

Results suggest that correlation between the *in vitro* results and clinical outcome was observed only by incubating conidia for 3 days at $30\pm 2^\circ\text{C}$. These conditions might be most suitable to assess *in vitro* susceptibility of *M. canis* and assist in determining the occurrence of drug resistance and cross-resistance phenomena.

Introduction

Microsporum canis is a zoophilic dermatophyte responsible for human and animal infections worldwide.^{1,2} In humans, *M. canis* is associated with *tinea capitis*, *tinea corporis*, *tinea pedis* and *onychomycosis*, whilst in veterinary species, infections cause multifocal alopecia, scaling and circular lesions.^{2,3} *M. canis* transmission occurs via direct contact with clinically or subclinically infected animals (mainly cats), or with spores that remain viable in the environment for up to 18 months.⁴

As *M. canis* infections are highly contagious, antifungal treatment should be systematically

recommended to shorten the course of the infection, to reduce dissemination of infective material into the environment and to prevent spread to other animals and people.^{2,5} Several antifungal agents were employed into clinical practice for the treatment of *M. canis* infections in veterinary and human medicine. In particular, griseofulvin (GRI), itraconazole (ITC) and terbinafine (TRB) were used in veterinary medicine and fluconazole (FLC) in human medicine.^{2,5,6} The activity spectrum of these compounds is variable and treatment failure is recorded in 25-40% of treated human patients, as a consequence of poor patient compliance, lack of drug penetration into tissue, medication bioavailability, drug-drug interactions and/or the occurrence of antifungal resistance.⁷ Particularly, the latter is considered an emerging threat involving many fungal species (i.e. *Aspergillus* and *Candida* spp.), but antifungal resistance in dermatophytes has been only described for *Trichophyton rubrum*,^{6,8} *Trichophyton mentagrophytes*⁹ and, rarely in *M. canis*.¹⁰ The frequency of azole treatment failures of *M. canis* infections in animals is scant. However, recently a study reported TER treatment failure in *M. canis* infections of animals.¹⁰ Nevertheless, these observations have led to an increased interest in antifungal susceptibility testing for dermatophytes. However, till date, methodological inconsistencies mainly related to the standardization of the inoculum preparation and incubation times impair unequivocal interpretations of *in vitro* susceptibility assays.¹¹ Several studies have been conducted on antifungal susceptibility of *M. canis* using inocula consisting of both conidia and hyphae-conidia, and different incubation times (from three to seven days).¹¹ In some dermatophytes (*T. rubrum* and *T. mentagrophytes*), the wall of the macroconidia is considerably thicker than the hyphae, thus affecting the overall antifungal susceptibility profile of the fungus.^{12,13} However, dermatophytes also produce arthroconidia, a cellular structure presumably more resistant to antifungals, which may be responsible for therapeutic failure.^{7,14} No data is currently available on the antifungal susceptibilities of these three fungal structures (i.e., conidia, hyphae-conidia and arthroconidia) of *M. canis*. Since data correlating *in vitro* drug susceptibility with clinical outcomes are lacking the information on the most suitable inocula and incubation time to use in antifungal broth microdilution susceptibility assays for *M. canis* was never assessed. Therefore, the aims of this study were to i) determine the *in vitro* ITC susceptibility of *M. canis* conidia, hyphae-conidia and arthroconidia following incubation for three and seven days and ii) compare the ITC MICs of *M. canis* strains obtained from animals that tested positive and negative to the fungus following ITC therapy in order to assess the most suitable inoculum and incubation time to use in antifungal broth microdilution susceptibility assays for *M. canis*.

Using the best condition, in this study the antifungal susceptibility of *M. canis* to ketoconazole (KTC), FLC, posaconazole (POS), voriconazole (VRC), GRI and TRB and iv) the occurrence of a probable drug resistance or cross resistance phenomena were also assessed.

Materials and Methods

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

Microsporum canis strains

Hair and skin scraping samples were taken from the skin lesions of animals using a sterile lancet or pliers.¹⁵ A total of 27 selected clinical strains of *M. canis* were divided into two groups according to the clinical outcome to once daily dose of ITC oral therapy. Group I included 13 strains from 13 dogs treated with ITC (10mg/kg) that tested positive to this fungus following a consecutive 3-monthly oral therapy. Group II included 14 *M. canis* strains from dogs (12) and cats (2) treated with ITC (10mg/kg) following a consecutive 3-monthly oral therapy that subsequently tested negative to this fungus. The strains were identified based on colonial morphology and microscopic features of the hyphae, macroconidia and microconidia,¹⁶ and were molecularly identified by an improved molecular diagnostic assay as previously reported.¹⁷ Isolates were stored at -80°C at the Department of Veterinary Medicine, University of Bari (Italy). Prior to testing, each strain was sub-cultured at least twice onto Potato Dextrose agar (PDA, Liofilchem, Italy) plates at 30°C for 10 days to ensure strain purity and viability.

Medium for antifungal test

A broth microdilution assay was performed in RPMI 1640 medium (Sigma-Aldrich, St Louis, MO 63103, USA) with L-glutamine but without sodium bicarbonate and buffered with 0.165M morpholine propane sulfonic acid (MOPS) (Sigma-Aldrich, St Louis, MO 63103, USA) at pH 7.0.

Antifungal agents

The following drugs were obtained in their standard powder state: TRB and GRI (Sigma-Aldrich, Milan, Italy), KTC and VRC (Novartis, Basel, Switzerland), FLC (Pfizer, UK), ITC (Janssen Research Foundation, Beerse, Belgium), PSZ (Schering Plough Research, NJ, USA). Stock solutions of FLC (10mg/ml), KTC (10mg/ml), ITC (10mg/ml), VRC (10mg/ml), PSZ (10mg/ml), TRB (10mg/ml), GRI (50mg/ml), were prepared by dissolving the powders in their respective solvents. FLC was dissolved in distilled water, while the other

compounds were dissolved in 100% dimethyl sulfoxide (DMSO, Sigma-Aldrich). The stock solutions were stored at -20 °C until use.

Inoculum preparation

Three types of inocula (i.e., conidia, hyphae-conidia and arthroconidia) were prepared (Fig.1). Hyphae-conidia and conidial suspensions were obtained from 14-day-old *M. canis* cultures on PDA incubated at 28°C. For arthroconidial suspensions, *M. canis* was cultured onto 2% yeast extract + 1% peptone agar at 12% CO₂ at 30±2°C for 21 days as previously described.⁷ Mature colonies were submerged with approximately 3ml of sterile saline solution (0.85% w/v) and the surface was scraped with the tip of a Pasteur pipette. The resulting mixture was transferred into 5ml sterile tubes.

For hyphae-conidial suspension, heavy particles of mixture were allowed to sediment for 15 minutes at room temperature. The supernatant was transferred into another sterile tube and adjusted to an optical density of 2.4 McFarland using a turbidimeter (DEN- 1 McFarland Densitometer, Biosan, Riga, Latvia) which is equivalent to $1- 5 \times 10^6$ colony forming units (CFU)/mL, as inferred by quantitative plate counts of CFU in PDA.

For conidial suspension, the mixture of conidia and hyphal fragments were allowed to sediment for 15 min at room temperature and the supernatant was collected and filtered using sterile filter paper (Whatman filter model 40, pore size, 8 µm), which retains hyphal fragments.¹⁸ The density of the filtered suspension was adjusted to an optical density of 2.4 McFarland as inferred above.

The resulting mixture of arthroconidia was agitated for 1h at 25° C. The supernatant was filtered using sterile filter paper (Whatman filter model 40, pore size, 8 µm). The density of the filtered suspension was adjusted to an optical density of 2.4 McFarland as reported above.

In vitro susceptibility testing

The antifungal susceptibility of the *M. canis* inocula to ITC was tested using the reference CLSI BMD assay¹⁹ with some modifications. Antifungal drug stocks and the inoculum suspensions were prepared as described above.

The concentration of each antifungal drug ranged from 0.008 to 16 µg/ml, with the exception of FLC and GRI, whose concentration ranged from 0.06 to 64 µg/ml. Visual reading of plates was performed after three and seven days of incubation, respectively, at 30±2°C. The MIC of each strain was defined as the lowest concentration of the agent producing a predominant decrease in turbidity (i.e., 100% of inhibition) when compared to the control growth, as previously described.^{20,21} Each plate was run in triplicate and each

drug dilution was tested in duplicates in each plate. Quality control strains (*Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258; American Type Culture Collection, Manassas, VA) were included to ensure accuracy of the drug dilutions and reproducibility of results.¹⁹

The results were reported as MIC range, MIC mean with standard deviation, MIC₅₀ (MICs at which 50% of the strains were inhibited) and MIC₉₀ (MICs at which 90% of the strains were inhibited).

Statistical analysis

The Student's t-test was used to evaluate the differences among MIC mean values of different antifungal drugs within and between Group I and II. A value of $P \leq 0.05$ was considered statistically significant.

Results

All quality control MIC values were within the ranges established by the CLSI.¹⁹ The ITC MIC values of *M. canis* strains from animals of Group I and II obtained using different inocula and incubation times are reported in Table 1. The MIC values of ITC recorded after three day-incubation, were equal or marginally lower ($p > 0.05$; Table 1) than those recorded after seven day-incubation. The ITC MIC values obtained with arthroconidia were significantly lower than those recorded by using conidia and hypha-conidia, regardless of incubation time and *M. canis* source (Table 1, $p < 0.05$). The ITC MIC values (MIC₅₀, MIC₉₀, mMIC) of hypha-conidia and conidia varied according to *M. canis* source being higher for strains from Group I. The lowest value of ITC MIC values in Group II samples was recorded using conidia and three day-incubation (Table 1).

The ITC MIC values of conidia were $\leq 1\mu\text{g/ml}$ for strains from Group II and $> 1\mu\text{g/ml}$ for those of Group I. All the *M. canis* strains showed low MIC values to VRC, PSZ and TRB, and high MIC values to ITC, KTC, GRI and FLC, regardless of the source of collection and incubation time (Table 2). The MIC mean value for all compounds tested in Group I were higher than those from Group II. High FLC ($> 64\mu\text{g/ml}$) MICs was recorded using the strains that showed high ITC MIC values ($> 1\mu\text{g/ml}$).

Discussion

Results of this study suggest that the type of inocula used in *in vitro* antifungal susceptibility assays of *M. canis* affect the ITC MIC values, as previously reported for *T. mentagrophytes* and *T. rubrum*.²² In addition, the ITC antifungal susceptibility observed for samples obtained from animals infected by *M. canis* and that had tested positive (Group I) or negative (Group II) to this pathogen following ITC therapy was compared, thus allowing

us to evaluate the most suitable type of inocula or incubation time for *in vitro* susceptibility testing. No differences were observed in the MIC values obtained after three and seven days, thus supporting the hypothesis that the time of incubation does not affect the ITC MIC values as previously observed for *T. rubrum*.^{8,22,23} Therefore, we suggest that a 3-day incubation is optimal in *in vitro* antifungal susceptibility tests of *M. canis*, which considerably shortens the time needed to obtain reliable diagnostic results. ITC MIC values observed in *in vitro* antifungal susceptibility tests varied according to the inocula used, with arthroconidia displaying the highest susceptibility than the other structures tested, and hyphae-conidia the lowest. In contrast, arthroconidia of *T. rubrum*, *T. tonsurans* and *T. equinum* are less susceptible to antifungals,⁷ which is possibly linked to their thick conidia walls.²⁴ The MIC values observed using hyphae-conidia were higher than those recorded using conidia alone, particularly in comparative analyses of MIC values of *M. canis* obtained from Group I and II, respectively. Interestingly a correlation between the antifungal *in vitro* results and clinical outcome was observed only by incubating conidia for three days at 30±2°C.

Particularly, ITC MIC values lower and higher than 1 µg/mL was observed using *M. canis* conidia from Group II and Group I, respectively, thus suggesting that antifungal resistance may occur in *M. canis* strains from animals that tested negative to this pathogen following *in vivo* ITC therapy (Group I). Therefore, the *in vitro* results of MIC >1 µg/mL correlates well with the *in vivo* results of a negative clinical outcome following a consecutive 3-monthly ITC oral therapy, thus suggesting a probable *in vitro* resistance phenomena to ITC for *M. canis*.

Interestingly, ITC MIC>1 µg/mL represents the epidemiological cut off (ECV) for filamentous fungi (i.e., *Aspergillus* spp.) and the clinical breakpoint (CBP) for yeasts (i.e., *Candida* spp.).^{25,26} Accordingly, high ITC or FLC MIC values for *M. canis* strains were previously recorded suggesting the occurrence of resistance phenomena although the correlations between the *in vitro* antifungal results and clinical outcome were never recorded.²⁷

Our study supports the validity of the CLSI guidelines.²⁸⁻³⁰ However, these guidelines recommend separation of the fungal structures (hyphae and conidia) through sedimentation for 15-20 min, and the use of the upper part of the suspension for susceptibility testing.³⁰ The results of our study show that the sedimentation of the inoculum is inefficient for separating dermatophyte hyphae, and filtration might be required, similarly to *T. rubrum* and *T. mentagrophytes*.¹⁸ Using these test conditions, the

most effective drugs against *M. canis* strains obtained from Group I and II were VRC, PSZ and TRB, thus confirming previous reports.^{20,31} The highest FLC MICs (i.e., MIC > 64 µg/ml) were recorded using the strains with high ITC (MIC >1µg/ml), thus suggesting the occurrence of cross-resistance.

In conclusion, data herein reported suggest the importance of the inoculum type and incubation time in determining the *in vitro* antifungal susceptibility of *M. canis* using micro dilution assays. Based on our data, conidia and three day-incubation at 30±2°C are most suitable for testing *in vitro* antifungal susceptibility of *M. canis*. The above conditions may also assist in determining the occurrence of probable drug resistance phenomena. Whilst the suitability of these test conditions must be tested in further studies conducted in other laboratories to ensure reproducibility, data from our work represent a solid foundation for the development of standardized antifungal susceptibility tests for *M. canis*. Furthermore, complementary studies on *M. canis* resistance are advocated in order to investigate the molecular mechanisms of this phenomenon.

References

1. Ginter-Hanselmayer G, Weger W, Ilkit M *et al.* Epidemiology of tinea capitis in Europe: Current state and changing patterns. *Mycoses* 2007; 50:6–13.
2. Moriello KA, Coyner K, Paterson S *et al.* Diagnosis and treatment of dermatophytosis in dogs and cats. *Veterinary Dermatol* 2017; 28:266–268.
3. Degreef H. Clinical forms of dermatophytosis (ringworm infection). *Mycopathologia* 2008; 166:257–65.
4. Sparkes AH, Werrett G, Stokes, CR *et al.* *Microsporum canis*: Inapparent carriage by cats and the viability of arthrospores. *J Small Anim Pract* 1994; 3:397–401.
5. ESCCAP, European Scientific Counsel Companion Animal Parasites, Superficial Mycoses in Dogs and Cats. ESCCAP Guideline 02, 4th edition, 2019.
6. Ghannoum M. Azole Resistance in Dermatophytes: Prevalence and Mechanism of Action. *J Am Paediatr Med Assoc* 2016; 106:79–86.
7. Bueno JG, Martinez C, Zapata B *et al.* *In vitro* activity of fluconazole, itraconazole, voriconazole and terbinafine against fungi causing onychomycosis. *Clin Exp Dermatol* 2010; 35: 658–663.

- 267 8. Coelho LM, Ferreria RA, Maffei CM *et al.* *In vitro* antifungal drug susceptibilities
268 of dermatophytes microconidia and arthroconidia. *J Antimicrob Agents*
269 *Chemother* 2008; 62:758–761.
- 270 9. Yamada T, Maeda M, Alshahni MM *et al.* Terbinafine Resistance of
271 *Trichophyton* Clinical Isolates Caused by Specific Point Mutations in the
272 Squalene Epoxidase Gene. *Antimicrob Agents Chemother* 2017; 61:00115–17.
- 273 10. Hsiao YH, Chen C, Han HS *et al.* The first report of terbinafine resistance
274 *Microsporum canis* from a cat. *J Vet Med Sci* 2018; 80:898–900.
- 275 11. Fernandez-Torres B, Carrillo-Munoz A, Inza I *et al.* Effect of culture medium on
276 the disk diffusion method for determining antifungal susceptibilities of
277 dermatophytes. *Antimicrob Agents Chemother* 2006; 50:2222–4
- 278 12. Aneke CI, Otranto D, Cafarchia C. Therapy and Antifungal Susceptibility Profile
279 of *Microsporum canis*. *J Fungi* 2018; 4:107.
- 280 13. Mahajan S, Tilak R, Kaushal S *et al.* Clinico-mycological study of dermatophytic
281 infections and their sensitivity to antifungal drugs in a tertiary care center. *Indian*
282 *J Dermatol, Vener and Leprol* 2017; 83: 436–440.
- 283 14. Yazdanparast SA, Barton RC. Arthroconidia production in *Trichophyton*
284 *rubrum* and a new ex vivo model of onychomycosis. *J Med Microbiol* 2006; 55:
285 1577–1581.
- 286 15. Cafarchia C, Romito D, Sasanelli M, Lia R, Capelli G, Otranto D. The
287 epidemiology of canine and feline dermatophytoses in southern Italy. *Mycoses*.
288 2004; 47(11-12): 508-13.
- 289 16. de Hoog GS, Guarro J, Gené J, Figueras MJ. Atlas of Clinical Fungi. Amer
290 Society for Microbiology, 2000. Centraalbureau voor Schimmelcultures /
291 Universitat Rovira i Virgili, Utrecht / Reus
- 292 17. Cafarchia C, Gasser RB, Figueredo LA *et al.* An improved molecular diagnostic
293 assay for canine and feline dermatophytosis. *Med Mycol* 2013; 51:136–143.
- 294 18. Santos DA, Barros MES, Hamdan JS. Establishing a method of inoculum
295 preparation for susceptibility testing of *Trichophyton rubrum* and *Trichophyton*
296 *mentagrophytes*. *J Clin Microbiol* 2006; 144:98–101.
- 297 19. CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of
298 Filamentous Fungi. Approved Standard. CLSI Document M38-A2. 2nd ed.
299 Wayne, PA: 2008.

20. Fernández-Torres B, Cabañes FJ, Carrillo-Munõz AJ *et al.* Collaborative evaluation of optimal antifungal susceptibility testing condition for dermatophytes. *J Clin Microbiol* 2002; 40:3999–4003.
21. Ghannoum MA, Chaturvedi V, Espinel-Ingroff A *et al.* Intra- and inter-laboratory study of a method for testing the antifungal susceptibilities of dermatophytes. *J Clin Microbiol* 2004; 42:2977–2979.
22. Jessup CJ, Warner J, Isham N *et al.* Antifungal susceptibility testing of dermatophytes: Establishing a medium for inducing conidial growth and evaluation of susceptibility of clinical isolates. *J Clin Microbiol* 2000; 38:341–344.
23. Favre B, Hofbauer B, Hildering KS *et al.* Comparison of *in vitro* activities of 17 antifungal drugs against a panel of 20 dermatophytes by using a micro dilution assay. *J Clin Microbiol* 2003; 41:4817–4819.
24. Fernández-Torres B, Inza I, Guarro J. Comparison of *in vitro* antifungal susceptibilities of conidia and hyphae of dermatophytes with thick-wall macroconidia. *Antimicrob Agents Chemother* 2003; 47:3371–3372.
25. Espinel-Ingroff A, Diekema DJ, Fothergill A *et al.* Wild-type MIC distributions and epidemiological cutoff values for the triazoles and six *Aspergillus* spp. for the CLSI broth microdilution method (M38-A2 document). *J Clin Microbiol* 2010; 48:3251–7.
26. Pfaller MA, Diekema DJ. Progress in antifungal susceptibility testing of *Candida* spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. *J Clin Microbiol* 2012; 50:2846–56.
27. Galuppi R, Gambarara A, Bonoli C *et al.* Antimycotic effectiveness against dermatophytes: comparison of two *in vitro* tests. *Vet Res Commun* 2010; 34: 57–61.
28. CLSI. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-Forming Filamentous Fungi; Approved Standard M38-A, M51; Clinical and Laboratory Standards Institute: Wayne, PA, USA: 2002.
29. Nweze EI, Mukherjee PK, Ghannoum MA. Agar-based disk diffusion assay for susceptibility testing of dermatophytes. *J Clin Microbiol* 2010; 48:3750–3752.
30. da Silva Barros ME, de Assis Santos D, Hamdan JS. Evaluation of susceptibility of *Trichophyton mentagrophytes* and *Trichophyton rubrum* clinical isolates to

antifungal drugs using a modified CLSI microdilution method (M38-A). *J Med Microbiol* 2007; 56:514–518.

31. Korting HC, Schäfer-Korting M, Zienicke H *et al.* Treatment of tinea unguium with medium and high doses of ultra microsize griseofulvin compared with that with itraconazole. *Antimicrob Agents Chemother* 1993; 37:2064–2068.

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Table 1: Itraconazole MIC values ($\mu\text{g/ml}$) of *Microsporium canis* from Group I (animals with a negative clinical outcome) and Group II (animals with a positive clinical outcome) using hyphae-conidia, conidia and arthroconidia at 3 and 7 days of incubation

Groups	N ^a Strains	Reading time	Hyphae and conidia				Conidia				Arthroconidia			
			range	MIC	MIC ₅₀	MIC ₉₀	range	MIC	MIC ₅₀	MIC ₉₀	range	MIC	MIC ₅₀	MIC ₉₀
				mean (SD)					mean (SD)					mean (SD)
Group I	13	3 days	1-16	4.4 ^f (5.2)	2	4	0.5-16	4.5 ^{a, e} (5.2)	2	8	0.25-4	1.5 ^{a, i} (1.4)	0.5	2
		7 days	1-16	5.5 ^{b, g} (5.9)	2	8	0.5-16	5.4 ^{c, h} (5.9)	2	8	0.008-8	1.8 ^{b, c} (2.2)	0.5	2
Group II	14	3 days	0.008-1	0.8 ^{d, f} (0.5)	1	1	0.008-1	0.4 ^{d, e} (0.3)	0.5	0.5	0.008-1	0.5 ⁱ (0.4)	0.5	1
		7 days	0.008-2	1.1 ^g (0.7)	1	2	0.008-1	0.7 ^h (0.5)	1	1	0.008-2	0.8 (0.6)	1	1

^{a-i} Students t-test—statistically significant differences ($p \leq 0.05$) are marked with the same letters.

Groups	Number	Drugs	Conidia at 3 days of incubation				Conidia at 7 days of incubation			
			MIC	MIC mean	MIC ₅₀	MIC ₉₀	MIC	MIC mean	MIC ₅₀	MIC ₉₀
			Range	(SD)			Range	(SD)		
Group I	13	ITC	0.5-16	4.5 (5.2) ^a	2	8	0.5-16	5.4 (6) ^d	2	8
		KTC	0.125-4	1.4 (1.2)	1	2	0.125-4	1.4 (1.26)	1	2
		VRC	0.008-1	0.14 (0.27)	0.03	0.125	0.016-1	0.14 (0.28)	0.03	0.125
		PZ	0.008-2	0.5 (0.6) ^b	0.25	1	0.008-2	0.5 (0.60)	0.25	1
		TRB	0.008-0.25	0.07 (0.09)	0.016	0.25	0.008-0.25	0.07 (0.10)	0.016	0.25
		FLC	4-128	36.9 (44.0) ^c	8	64	4-128	36.9	8	64

Table 2.
Itraconazole (ITC),

ketoconazole (KTC), voriconazole (VRC), posaconazole (POS), terbinafine (TRB), fluconazole (FLC) and griseofulvin (GRI) MIC (µg/ml) data of *Microsporium canis* from Group 1 and Group II at 3 and 7 days of incubation using conidia

397							(44.0) ^e			
398		GRI	0.125-2	1.0 (0.72)	0.5	2	0.125-2	1.0 (0.72)	0.5	2
399	Group 14	ITC	0.008-1	0.4 (0.3) ^a	0.5	0.5	0.008-1	0.7 (0.5) ^d	1	1
400	II	KTC	0.008-4	0.7 (1.0)	0.25	1	0.008-4	1.2 (1.4)	0.5	2
401		VRC	0.008-	0.03 (0.03)	0.016	0.06	0.008-	0.03 (0.03)	0.016	0.06
402			0.125				0.125			
403		PZ	0.008-1	0.1 (0.30) ^b	0.03	0.06	0.008-1	0.1 (0.3)	0.03	0.25
404		TRB	0.008-0.5	0.08 (0.14)	0.016	0.125	0.008-0.5	0.13 (0.14)	0.125	0.25
405		FLC	0.06-32	7.9 (10.5) ^c	4	8	1-32	8.2 (10.4) ^e	4	8
406		GRI	0.03-2	0.6 (0.51)	0.5	1	0.03-2	0.6 (0.52)	0.5	1

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416 ^{a-j} Students t-test—statistically significant differences ($p \leq 0.05$) are marked with the same letters.

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418 Figure 1: Hyphae-conidia (A), macroconidia (B) and arthroconidia (C) inocula used for in vitro antifungal susceptibility of *Microsporum*
419 *canis* at X40 magnification.

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The best type of inoculum for testing the antifungal drug susceptibility of *Microsporum canis*: In vivo and in vitro results

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