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Comparison of disk diffusion method and broth microdilution method for antifungal susceptibility testing of dermatophytes

A. ESTEBAN, M. L. ABARCA & F. J. CABAÑES

Departament de Sanitat i d'Anatomia Animals (Microbiologia), Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona, Spain

The use of the agar diffusion Neo-Sensitabs® method to determine antifungal susceptibility of 59 isolates of dermatophytes, namely *Epidermophyton floccosum*, *Microsporum canis*, *M. gypseum*, *Trichophyton mentagrophytes*, *T. rubrum* and *T. tonsurans* to Clotrimazole (CLZ), Itraconazole (ITZ) and Terbinafine (TBF) is described. Results obtained are compared to the minimum inhibitory concentrations (MIC) determined by an adaptation of the NCCLS-M38-A procedure. Using the diffusion method, all strains showed a broad zone of inhibition at the first available reading time (3 or 7 days). Using the broth microdilution method, the geometric mean MIC (μg/ml) with regard to all isolates was ≤ 0.03 for TBF, ≤ 0.069 for CLZ and ≤ 0.919 for ITZ. In both methods, TBF was the most active antifungal agent against all isolates tested. The two methods evaluated were able to detect the resistance of the quality control strains of *Aspergillus fumigatus* to ITZ. Even though a reference method for testing dermatophytes still has not been developed, our data suggest that the Neo-Sensitabs® diffusion method could provide a simple procedure for the antifungal susceptibility testing of dermatophytes in the routine clinical laboratory.

Keywords agar diffusion, broth microdilution, dermatophytes, *in vitro* susceptibility testing, Neo-Sensitabs®

Introduction

Dermatophytes are responsible for the great majority of fungal infections of the skin, hair and nails, and comprise a group of closely related genera with numerous species. They are cited as being among the most frequent causes of dermatological problems in domestic animals [1]. In recent years, the number of human infections caused by this group of fungi has increased considerably and is of particular concern in immunocompromized patients [2]. Many dermatophytoses respond well to topical antifungal treatment but more extensive or severe disease may require systemic therapy. Imidazole derivatives are available in many formulations for topical use. Among oral antifungal agents, itraconazole and terbinafine are presently widely used for treatment of dermatophytoses [3,4]. Nevertheless, a *Trichophyton rubrum* strain exhibiting primary resistance to terbinafine has been recently reported [5].

With an increasing variety of drugs available for the treatment of dermatophytoses, the need for a reference method for testing antifungal susceptibilities of this group of fungal pathogens has become apparent. Following the principles established for testing yeasts, a reference method for antifungal susceptibility of molds was published by the NCCLS as M38-P [6], which was recently approved as M38-A [7]. However, dermatophytes were not included in either document.

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Correspondence: F. J. Cabañes, Departament de Sanitat i d'Anatomia Animals (Microbiologia), Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain. Tel: +3493 5811749; Fax: +3493 5812006; E-mail: javier.cabanes@uab.es

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Multiple efforts are under way to devise a standardized method for the evaluation of the antifungal susceptibility of these fungi. In that sense, different adaptations of the NCCLS method for use with dermatophytes have been described [8–13], and a recent multicenter study was conducted to define optimal antifungal susceptibility testing conditions for these fungi [14].

However, NCCLS methods may not be the most practical procedures for use in the routine clinical laboratory. Of the several commercial systems now available for antifungal susceptibility testing, the disk diffusion Neo-Sensitabs® method, developed by Rosco® (Taastrup, Denmark) [15] is of potential value for dermatophytes. This method has proven to be useful in testing yeasts [16–19], and it has been modified here to evaluate dermatophyte fungi.

The present report describes the use of the agar diffusion Neo-Sensitabs® procedure to determine antifungal susceptibility of dermatophytes to three antifungal agents: Clotrimazole (CLZ), Itraconazole (ITZ) and Terbinafine (TBF). Results obtained are compared to minimum inhibitory concentrations (MIC) determined by an adaptation of document M38-A [14].

Materials and methods

Fungal isolates

In total, 59 clinical isolates of the following dermatophytes were tested; *Epidermophyton floccosum* ($n = 10$), *Microsporum canis* ($n = 10$), *M. gypseum* ($n = 10$), *Trichophyton mentagrophytes* ($n = 9$), *T. rubrum* ($n = 10$) and *T. tonsurans* ($n = 10$). The test population were all well-characterized, clinical fresh isolates supplied by different Spanish Hospitals and stored in sterile distilled water at room temperature in the culture collection of the Barcelona Faculty of Veterinary, Spain. Two isolates of *Aspergillus fumigatus* (NCFP 7099 and NCFP 7100; National Collection of Pathogenic Fungi, Bristol, UK) resistant to itraconazole [20] and *Paecilomyces variotii* ATCC 36257 (American Type Culture Collection, Manassas, VA, USA) were used as quality control.

Neo-Sensitabs® susceptibility testing

Neo-Sensitabs® susceptibility testing is an agar diffusion method with the antifungal agent in 9-mm-diameter tablets. This method, initially described for yeasts, has been adapted here to assay dermatophyte fungi. All the isolates were subcultured on potato dextrose agar (PDA) at 28°C for 14 days. Dense inoculum suspensions of conidia and hyphal elements were prepared in tubes containing 10 ml sterile saline (0.85%). After heavy particles were allowed to settle for 15–20 min, the upper homogenous suspensions were transferred to sterile tubes and adjusted with a spectrophotometer at 530 nm to 65% transmittance (T). Inoculum quantitation was performed by plating 0.01 ml of a 1:100 dilution of adjusted inocula onto PDA plates to determine the viable number of colony-forming units (c.f.u.) per ml. The plates were incubated at 28°C and observed daily for the presence of fungal colonies. Inocula adjusted to 65% T were streaked on the surface of Sabouraud glucose agar (SGA) plates in four different directions to cover the entire surface. Using a flamed sterilized forceps, antifungal tablets of CLZ, ITZ and TBF (IZASA, Barcelona, Spain) were applied onto the surface of the inoculated agar plates, then incubated at 28°C and inhibition zone sizes were measured after 3 and 7 days of incubation.

Controls of growth on SGA plates were carried out without the placement of antifungal tablets. All isolates were run at least in duplicate and a new inoculum was prepared for each replicate.

Broth microdilution method

The broth microdilution method used is an adaptation of the method recommended by the NCCLS [7] according to the most suitable testing conditions reported for dermatophytes [14]. The inoculum was prepared and standardized spectrophotometrically as described above for the agar diffusion method and further diluted 1:50 in RPMI medium. Clotrimazole (Química Farmacéutica, Bayer, Barcelona, Spain), Itraconazole (Jansen Research Foundation, Beerse, Belgium) and Terbinafine (Novartis, Basel, Switzerland) were provided by the manufacturers as standard powders. Drug dilutions were prepared following the additive twofold drug scheme described in the NCCLS M38-A reference method [7]. Briefly, stock solutions were first diluted to $\times 100$ the final concentrations in 100% dimethyl sulfoxide and further diluted 1:50 in RPMI medium to obtain the $\times 2$ drug concentration. The drug concentrations finally assayed were 8–0.0078 μg/ml for CLZ and ITZ and 2–0.019 μg/ml for TBF.

Each microdilution well containing 100 μl of the $\times 2$ drug concentration was inoculated with 100 μl of the diluted ($\times 2$) inoculum suspension. The growth control wells contained 100 μl of the diluted inoculum suspension and 100 μl DMSO (2%). Microdilution trays were incubated at 28°C without agitation and examined after 3 and 7 days. Microplates were read visually with the aid of a reading mirror. The MIC for TBF and ITZ was defined as the lowest concentration showing 100%

growth inhibition [7,23]. For CLZ, the MIC was the lowest concentration showing prominent growth inhibition (approximately 50% of the growth control) [6]. Every strain was tested at least in duplicate and a new inoculum was prepared for each experiment. The significance of differences in mean values was determined by using Student's *t*-test with the statistical SPSS package (version 10.0). *P*-values of <0.05 were considered statistically significant.

Results and discussion

The final concentrations of the stock inoculum suspensions ranged from 1.0×10^4 to 6.6×10^6 c.f.u./ml, as shown by quantitative colony counts on PDA. Similar results have been obtained by other authors [9,10,13].

Results obtained with the diffusion Neo-Sensitabs® method are summarized in Table 1. After 3 days of incubation it was possible to read the diameters of inhibition zone of all *M. gypseum* and *T. mentagrophytes* isolates and good growth was obtained on the SGA control plates. Some isolates of *E. floccosum*, *M. canis*, *T. tonsurans* and *T. rubrum* required longer incubation. At the first available reading time (3 or 7 days), all the strains showed a broad zone of inhibition, with the largest zones obtained with TBF and the smallest with ITZ. One isolate of *T. rubrum* and one of *T. mentagrophytes* showed no inhibition zone (diameter 9 mm) around ITZ tablet after 7 days of incubation, although these isolates had shown, respectively, a 23- and a 29-mm inhibition zone after 3 days of incubation. As different isolates grow at different rates, the diameter of inhibition zone should be read on the first day that the SGA controls show good growth.

The *in vitro* activities with microdilution method of CLZ, ITZ and TBF at 3 and 7 days of incubation are shown in Table 2. Geometric mean MIC, MIC range, the MIC at which 50% of the isolates tested are inhibited (MIC_{50}), and MIC_{90} are provided for all the isolates tested. A significant ($P<0.05$) increase in MIC values was observed as incubation time increased. However, at 3 days of incubation, growth was scarce and reading was, in general, difficult. When all the results were considered together, the geometric mean MIC (μg/ml) was ≤ 0.03 for TBF, ≤ 0.069 for CLZ and ≤ 0.919 for ITZ. TBF was shown to be the most effective antifungal drug. Of the two azoles tested, the highest MIC values were obtained with ITZ.

Although the methodological conditions differed between the laboratories, in general, MIC values obtained are in the range reported by other authors [9–13,21–24]. In relation to ITZ, following the new criteria defined by document M-38A [7] (100% growth inhibition) we have obtained MIC ranges greater than those published in other studies that used the

Table 1 Diameter ranges of the inhibition zone (in mm) using the diffusion method Neo-Sensitabs®

Species (no. isolates)	Antifungal agent	Incubation time	
		3 days*	7 days
Epidermophyton floccosum[a] (10)	CLZ	72[c]	51–89
	ITZ	62[c]	49–89
	TBF	89[c]	89
Microsporum canis[b] (10)	CLZ	62–89[d]	61–89
	ITZ	33–52[d]	22–48
	TBF	84–89[d]	83–89
M. gypseum (10)	CLZ	51–66	48–61
	ITZ	30–39	19–31
	TBF	84–89	81–89
Trichophyton mentagrophytes (9)	CLZ	50–67	50–63
	ITZ	23–44	9–36
	TBF	75–89	74–89
T. rubrum[c] (10)	CLZ	47–72[b]	47–82
	ITZ	19–46[b]	9–46
	TBF	89[b]	70–89
T. tonsurans[d] (10)	CLZ	53–66[a]	55–85
	ITZ	33–45[a]	28–53
	TBF	78–81[a]	78–89

CLZ, Clotrimazole; ITZ, Itraconazole; TBF, Terbinafine. *When not all the isolates grew after 3 days of incubation, letters indicate the number of isolates developed: a, one isolate; b, nine isolates; c, six isolates; d, four isolates.

Table 2 Minimum inhibitory concentrations obtained by broth microdilution method of the three antifungal agents assayed: Clotrimazole, Itraconazole and Terbinafine against six different dermatophyte species

Species (no. isolates)	MIC parameter	MIC (μg/ml) at 3 days			MIC (μg/ml) at 7 days		
		CLZ	ITZ	TBF	CLZ	ITZ	TBF
Epidermophyton floccosum (10)	Range	0.0078–0.125	0.0078–0.5	0.0019–0.06	0.03–0.25	0.0625–1	0.0078–0.06
	GM	0.018	0.061	0.01	0.058	0.294	0.023
	MIC_{50}	0.01	0.125	0.01	0.0625	0.25	0.03
	MIC_{90}	0.0625	0.5	0.03	0.125	0.5	0.06
Microsporum canis (10)	Range	0.0078–0.0625	0.01–2	0.01–0.125	0.0078–0.125	1–8	0.03–0.25
	GM	0.012	0.447	0.033	0.022	1.954	0.065
	MIC_{50}	0.01	1	0.03	0.03	2	0.06
	MIC_{90}	0.03	1	0.06	0.0625	4	0.125
M. gypseum (10)	Range	0.03–0.5	0.5–2	0.0078–0.06	0.0625–0.5	0.5–8	0.03–0.125
	GM	0.154	0.776	0.018	0.233	1	0.055
	MIC_{50}	0.125	1	0.01	0.25	1	0.06
	MIC_{90}	0.5	1	0.03	0.5	2	0.06
Trichophyton mentagrophytes (9)	Range	0.01–0.25	0.125–1	0.0078–0.06	0.03–0.5	0.125–8	0.01–0.125
	GM	0.079	0.680	0.015	0.142	1.508	0.034
	MIC_{50}	0.0625	1	0.01	0.125	2	0.03
	MIC_{90}	0.25	1	0.03	0.5	8	0.06
T. rubrum (10)	Range	0.0078–0.25	0.03–1	0.0039–0.06	0.01–0.25	0.25–2	0.0078–0.06
	GM	0.021	0.444	0.008	0.057	0.912	0.019
	MIC_{50}	0.01	0.5	0.0078	0.0625	1	0.03
	MIC_{90}	0.0625	1	0.01	0.125	1	0.03
T. tonsurans (10)	Range	0.0078–0.25	0.0078–1	0.0019–0.03	0.01–0.25	0.01–2	0.0078–0.06
	GM	0.027	0.289	0.006	0.049	0.8	0.013
	MIC_{50}	0.03	0.5	0.0039	0.0625	1	0.01
	MIC_{90}	0.125	1	0.01	0.0625	1	0.03
Total (59)	Range	0.0078–0.5	0.0078–2	0.0019–0.125	0.0078–0.5	0.01–8	0.0078–0.25
	GM	0.034	0.352	0.013	0.069	0.919	0.03
	MIC_{50}	0.03	0.5	0.01	0.0625	1	0.03
	MIC_{90}	0.25	1	0.03	0.25	2	0.06

CLZ, Clotrimazole; GM, geometric mean; ITZ, Itraconazole; MIC, minimum inhibitory concentrations; TBF, Terbinafine. MIC_{50} and MIC_{90}, MIC at which 50 and 90% of the isolates tested are inhibited respectively.

Table 3 *In vitro* antifungal susceptibility of the quality control strains tested using the diffusion Neo-Sensitabs® method (in diameter of inhibition zones) and broth microdilution (minimum inhibitory concentrations values)

Quality control strain	Antifungal agent	Diameter range (mm)	MIC (μg/ml)	
			Range	Geometric mean
Aspergillus fumigatus NCPF 7099	CLZ	48–56	0.25–1	0.5
	ITZ	9	>8	>8
	TBF	44–47	1–>2	–
A. fumigatus NCPF 7100	CLZ	23–26	0.25	0.25
	ITZ	9	>8	>8
	TBF	28–35	>2	>2
Paecilomyces variotii ATCC 36257	CLZ	23–29	2	2
	ITZ	16–23	0.25–0.5	0.35
	TBF	46–54	0.25–0.5	0.35

CLZ, Clotrimazole; ITZ, Itraconazole; TBF, Terbinafine.

less stringent MIC determination criteria defined previously [8] (50% growth inhibition) [9,10,25,26].

In vitro antifungal susceptibility of the quality control strains as determined by diffusion method and broth microdilution method are shown in Table 3. Both methods were able to distinguish the *A. fumigatus* isolates resistant to ITZ. Thus the control strains *A. fumigatus* NCPF 7099 and 7100 showed no inhibition zones (diameter 9 mm) around the ITZ tablet and their MIC values were >8 μg/ml. MIC for *P. variotii* fell within the expected range [24,27].

The main problem in comparing the results obtained with the two methods evaluated is that a reference method for the antifungal susceptibility testing of dermatophytes is not available. Interpretative MIC breakpoints have not been established at present for molds but, according to the available data, MIC for the recently reported *T. rubrum* isolates resistant to TBF were >4 μg/ml [5] and the *A. fumigatus* isolates resistant to ITZ (NPCF 7099 and 7100) had MIC > 16 μg/ml [20]. These values are clearly higher than the MIC obtained for the dermatophyte isolates tested in this study.

The three antifungal agents studied are successfully used for treatment of dermatophytoses. So in order to compare the results obtained with the evaluated methods, the three antifungal agents can be considered highly active *in vitro* against the isolates tested. This is supported by the low MIC values obtained using the broth microdilution method and by the broad inhibition zones (greater than 19–20 mm) obtained in all cases with the diffusion method at the first available reading time. These values are very similar to the interpretative standards provided by the manufacturer for yeasts [15], where susceptible isolates to CLZ and TBF are those with zone diameters of ≥20 mm, and for ITZ the zone diameter has to be ≥15 mm.

In both methods, the allylamine TBF was the most active agent against all dermatophyte isolates tested, showing the lowest MIC and the highest inhibition diameter. Finally, both methods were able to identify the *A. fumigatus* isolates resistant to ITZ.

Even though a reference method has still not been developed, our data suggest that Neo-Sensitabs® diffusion method could provide a simpler alternative for the antifungal susceptibility testing of dermatophytes in the routine clinical laboratory. Although this diffusion method has been commercialized for yeasts, it has been sporadically adapted to evaluate antifungal susceptibility of some mold genera such as *Fusarium* [28], *Alternaria* [29], *Scedosporium* [30], other dematiaceous fungi [31] and *Epidermophyton* spp. [32].

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References

1 Cabañes FJ. Dermatophytes in domestic animals. In: Kushwaha RKS, Guarro J (eds). *Biology of Dermatophytes and Other Keratinophilic Fungi*. Bilbao: Revista Iberoamericana de Micologia, 2000: 104–108.
2 Walsh TJ, Groll AH. Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. *Transpl Infect Dis* 1999; **1**: 247–261.
3 del Palacio A, Garau M, González-Escalada A, Calvo MT. Trends in the treatment of dermatophytosis. In: Kushwaha RKS, Guarro J (eds). *Biology of Dermatophytes and Other Keratinophilic Fungi*. Bilbao: Revista Iberoamericana de Micologia, 2000: 148–158.
4 Sheehan DJ, Hitchcock CA, Sibley CM. Current and emerging azole antifungal agents. *Clin Microbiol Rev* 1999; **12**: 40–79.
5 Mukherjee PK, Leidich SD, Isham N, *et al*. Clinical *Trichophyton rubrum* strain exhibiting primary resistance to terbinafine. *Antimicrob Agents Chemother* 2003; **47**: 82–86.
6 National Committee for Clinical Laboratory Standards. *Reference Method for Broth Dilution Antifungal Susceptibility Testing*

of Conidium-forming Filamentous Fungi. Proposed standard M38-P. Wayne, PA: National Committee for Clinical Laboratory Standards, 1998.
7 National Committee for Clinical Laboratory Standards. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. Approved Standard M38-A.* Wayne, PA: National Committee for Clinical Laboratory Standards, 2002.
8 Barchiesi F, Arzeni D, Camiletti V, *et al*. *In vitro* activity of Posaconazole against clinical isolates of dermatophytes. *J Clin Microbiol* 2001; **39**: 4208–4209.
9 Fernández-Torres B, Carrillo AJ, Martín E, *et al*. *In vitro* activities of 10 antifungal drugs against 508 dermatophyte strains. *Antimicrob Agents Chemother* 2001; **45**: 2524–2528.
10 Fernández-Torres B, Vázquez-Veiga H, Llovo X, *et al*. *In vitro* susceptibility to Itraconazole, Clotrimazole, Ketoconazole and Terbinafine of 100 isolates of *Trichophyton rubrum*. *Chemotherapy (Basel)* 2000; **46**: 390–394.
11 Jessup CJ, Ryder NS, Ghannoum MA. An evaluation of the *in vitro* activity of terbinafine. *Med Mycol* 2000; **38**: 155–159.
12 Favre B, Hofbauer B, Hildering K, Ryder NS. Comparison of *in vitro* activities of 17 antifungal drugs against a panel of 20 dermatophytes by using a microdilution assay. *J Clin Microbiol* 2003; **41**: 4817–4819.
13 Fernández-Torres B, Inza I, Guarro J. *In vitro* activities of the new antifungal drug eberconazole and three other topical agents against 200 strains of dermatophytes. *J Clin Microbiol* 2003; **41**: 5209–5211.
14 Fernández-Torres B, Cabañes FJ, Carrillo-Muñoz AJ, *et al*. Collaborative evaluation of optimal antifungal susceptibility testing conditions for dermatophytes. *J Clin Microbiol* 2002; **40**: 3999–4003.
15 Anon. *Neo-Sensitabs® Susceptibility Testing. User's guide*. Denmark: Rosco Diagnostica, 2002 (available at http://www.rosco.dk).
16 Cantón E, Pemán J, Carrillo-Muñoz A, *et al*. Fluconazole susceptibilities of bloodstream *Candida* sp. isolates as determined by National Committee for Clinical Laboratory Standards Method M27-A and two other methods. *J Clin Microbiol* 1999; **37**: 2197–2200.
17 Carrillo-Muñoz AJ, Abarca L, Quindós G, *et al*. Multicenter evaluation of Neo-Sensitabs, a standardized diffusion method for yeast susceptibility testing. *Rev Iberoam Micol* 1999; **16**: 92–96.
18 Faiad Soni LM, Nascimento Burattini M, Campos Pignatari AC, *et al*. Comparative study of agar diffusion test and the NCCLS macrobroth method for *in vitro* susceptibility testing of *Candida* spp. *Mycopathologia* 1999; **145**: 131–135.
19 Quindós G, Abarca L, Carrillo- Muñoz AJ, *et al*. Multicenter survey of *in vitro* antifungal resistance in yeasts of medical importance isolated from Spanish patients. *Rev Iberoam Micol* 1999; **16**: 97–100.
20 Denning DW, Venkateswarlu K, Oakley KL, *et al*. Itraconazole resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 1997; **41**: 1364–1368.
21 Jessup CJ, Warner J, Isham N, *et al*. Antifungal susceptibility testing of dermatophytes: Establishing a medium for inducing conidial growth and evaluation of clinical isolates. *J Clin Microbiol* 2000; **38**: 341–344.
22 Oyeka CA, Gugnani HC. In vitro activity of seven azole compounds against some clinical isolates of non-dermatophytic filamentous fungi and some dermatophytes. *Mycopathologia* 1990; **110**: 157–161.
23 Hofbauer B, Leitner I, Ryder NS. *In vitro* susceptibility of Microsporum canis and other dermatophyte isolates from veterinary infections during therapy with terbinafine or griseofulvin. *Med Mycol* 2002; **40**: 179–183.
24 Fernández-Torres B, Carrillo-Muñoz A, Ortoneda M, *et al*. Interlaboratory evaluation of the Etest® for antifungal susceptibility testing of dermatophytes. *Med Mycol* 2003; **41**: 125–130.
25 Perea S, Forthergill AW, Sutton DA, Rinaldi MG. Comparison of in vitro activities of voriconazole and five established antifungal agents against different species of dermatophytes using a broth microdilution method. *J Clin Microbiol* 2001; **39**: 385–388.
26 Pujol I, Capilla J, Fernández-Torres B, *et al*. Use of the Sensititre Colorimetric Microdilution panel for antifungal susceptibility testing of dermatophytes. *J Clin Microbiol* 2002; **40**: 2618–2621.
27 Espinel-Ingroff A, Dawson K, Pfaller M, *et al*. Comparative and collaborative evaluation of standardization of antifungal susceptibility testing for filamentous fungi. *Antimicrob Agents Chemother* 1995; **39**: 314–319.
28 Cabañes FJ, Alonso JM, Castellá G, *et al*. Cutaneous Hyalohyphomycosis caused by *Fusarium solani* in a loggerhead sea turtle (*Caretta caretta* L.). *J Clin Microbiol* 1997; **35**: 3343–3345.
29 Cabañes FJ, Abarca L, Bragulat MR, Bruguera T. Phaeohyphomycosis caused by *Alternaria alternata* in a mare. *J Med Vet Mycol* 1988; **26**: 359–365.
30 Cabañes FJ, Roura X, García F, *et al*. Nasal granuloma caused by *Scedosporium apiospermum* in a dog. *J Clin Microbiol* 1998; **36**: 2755–2758.
31 Cermeño-Vivas JR, Torres-Rodríguez JM. Sensibilidad de hongos miceliares dematiáceos a diez antifúngicos empleando un método de difusión en agar. *Rev Iberoam Micol* 2001; **18**: 113–117.
32 Cabañes FJ, Abarca L, Bragulat MR, Bruguera T. Sensitivity of some strains of the genus *Epidermophyton* to different antifungal agents. *Mycopathologia* 1989; **105**: 153–156.