Sensitivity of some strains of the genus *Epidermophyton* to different antifungal agents

F. J. Cabañes, L. Abarca, Ma . Bragulat & T. Bruguera

Dpt. Patología y Producciones Animales (Microbiología); Fac. de Veterinaria; Universidad Autónoma de Barcelona 08193; Bellaterra, Barcelona, España

Accepted 2 November 1988

Abstract

The inhibitory activity of some antifungal agents against 17 strains of genus *Epidermophyton* have been studied. The behaviour of *Epidermophyton stockdaleae* against antifungal agents tested is clearly different from that observed in the strains belonging to *Epidermophyton floccosum*, since all of the latter were sensitive to the antifungal agents used and the geophilic species showed resistance to griseofulvin, isoconazole, natamycin and nystatin, and intermediate sensitivity to ketoconazole and miconazole. The low sensitivity of *E. stockdaleae* (and other geophilic dermatophytes) to antifungal agents could be a problem in the current therapy if further studies or case reports demonstrate their pathogenic role.

Introduction

The sensitivity of *Epidermophyton floccosum* to antifungal agents such as imidazoles or griseofulvine has been mentioned by various authors [2, 3, 4, 7, 8]. On the other hand, in the bibliographical review carried out we were unable to find studies concerning the action of antifungal agents on the species *Epidermophyton stockdaleae*, due possibly to its geophilic nature and to the fact that it so far has not mentioned as being a causal agent of dermatomycosis. The inhibitory activity of some antifungal agents against 17 strains of genus *Epidermophyton* have been studied using the diffusion method described by Bou Casals [1].

Methods

Strains used

Seventeen strains of genus *Epidermophyton* were included in the investigation: 15 strains of *E. floccosum* provided by spanish hospitals, *E. flocco-*

sum ATCC E18397, E. floccosum var. nigricans ATCC 26072 and E. stockdaleae ATCC 28687.

In vitro susceptibility test and antifungal agents used

To carry out the assays the diffusion method as described by Bou Casals [1] was chosen. The *Epidermophyton* strains were grown on Sabouraud dextrose agar at 28 °C for 14 days and then, a suspension of each strain was made in sterile physiological saline. The suspension was pipetted by 0.2 ml on the surface of Shadomy's agar medium (Yeast nitrogen base additioned of phosphate buffer pH 7.0, 1% dextrose and 0.15% L-asparagine) and evenly spread.

Once the inoculation was completed, the antifungal tablets (NEO-SENSITABS.A/S ROSCO) were placed for assay using sterile forceps. The antifungal agents utilized were: Clotrimazole, Econazole, Isoconazole, Ketoconazole, Miconazole, Tioconazole, Griseofulvin, Natamycin and Nystatin.

At the same time, controls of growth of all strains in Shadomy's and Sabouraud dextrose agar media were carried out under the same conditions as explained above, without the placement of antifungal tablets.

Incubation temperature was 28 °C and assay readings were made at 7 and 14 days. Such readings consisted of measuring the diameter of the growth inhibition (in mm) observed around the antifungal tablet. The study was performed in duplicate.

The interpretation of results has been made following the criteria established by Bou Casals [1].

Results and discussion

Figure 1 shows the susceptibility spectra of *E. floccosum* strains (including *E. floccosum* var. nigricans) and *E. stockdaleae* to the antifungal agents studied. Following the sensitivity and the resistance criteria cited by Bou Casals [1], *E. stockdaleae* has shown resistance to the following antifungal agents: Griseofulvin, Isoconazole, Natamycin and Nystatin, with intermediate sensi-

tivity to ketoconazole and miconazole. It was sensitive only to the following imidazoles tested: Clotrimazole, Econazole and Tioconazole. Following critical review of the available references we have only found one paper concerning the action of antifungal agents on this species: Prochacki and E.-Zasada [6], when describing E. stockdaleae, mention the use of an organomercury fungicide in the methodology employed in order to isolate the fungus. The behaviour of the geophilic species in the presence of the tested antifungal agents is clearly different from that observed in the antropophilic strains belonging to E. floccosum, since all of the latter were sensitive to the antifungal agents used.

Generally, in the test effected with *E. floccosum* strains, larger inhibition zones were obtained with antifungal-tablets containing imidazoles or grise-ofulvine; inhibition zones were smaller when the tablets contained natamycin or nystatin. Approximately in 40% of tests done no growth was detected due to the high sensitivity of the *E. floccosum* strains to the antifungal tablets (Fig. 2). However all these strains did grow in the culture medium not having antifungal tablets and used as control. All the strains (*E. floccosum* and

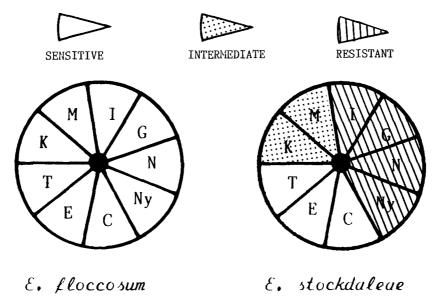


Fig. 1. Susceptibility spectra of E. floccosum (including E. floccosum var. nigricans) and E. stockdaleae to the antitungal agents studied. C, Clotrimazole; E, Econazole; I, Isoconazole; K, Ketoconazole; M, Miconazole; T, Tioconazole; G, Griseofulvin; N, Natamycin; Ny, Nystatin.



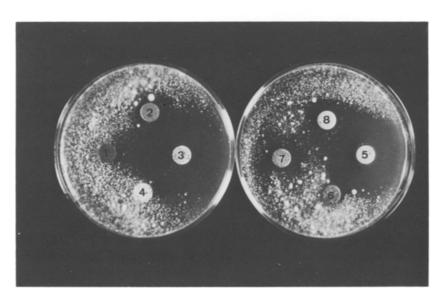


Fig. 2. Behaviour of a strain of E. floccosum and E. stockdaleae ATCC 28687 against (1) Isoconazole, (2) Natamycin, (3) Clotrimazole, (4) Griscofulvine, (5) Econazole, (6) Nystatin, (7) Ketoconazole, (8) Miconazole.

E. stockdaleae) showed development on control plates containing Shadomy's medium, although growth was more pronounced in Sabouraud dextrose agar. Although E. stockdaleae is categorized as geophilic, it is known that some geophilic strains of dermatophytes can produce clinical lesions, for instance Microsporum gypseum. This species has higher minimal inhibitory concentration values with imidazole derivates as fenticonazole, miconazole, econazole and clotrima-

zole than other zoophilic or antropophilic dermatophytes [2] and resisted concentrations of 100 mcg/ml of griseofulvin [5].

We think it would be interesting to point out that the low sensitivity of *E. stockdaleae* and other geophilic dermatophytes to antifungal agents could be a problem in the current therapy if further studies or case reports demonstrate their pathogenic role.

References

- Bou Casals J. Tablet sensitivity testing of pathogenic fungi. Journal of Clinical Pathology 1979; 32: 719–722.
- Costa AL. In vitro antimycotic activity of fenticonazole. Mykosen 1982; 25: 47–52.
- Gisslen H, Hersle K, Mobacken H, Nordin P. Comparative study of ketoconazole and griseofulvin in dermatophytosis in the groin. Current Therapeutic Research 1982; 32: 40-44.
- 4. Mahgoub ES. Clinical trial with clotrimazole cream (Bay b 5097) in dermatophytosis and onychomycosis. Mycopathologia 1975; 56: 149–152.
- Minami PS, Mendes MJS, Cuce LC, Salebian A. Crescimento de dermatófitos em meio contendo altas concentraçoes de griseofulvina. Anais Brasileiros de Dermatologia 1980; 55: 121-124.

- Prochacki H, Zasada E. Epidermophyton stockdaleae sp. nov. Mycopathologia 1974; 54: 341–345.
- Van Cutsem J. The antifungal activity of ketoconazole. The American Journal of Medicine 1983; 24: 9-15.
- Van Cutsem J, Janssen PAJ. Le spectre antifungique in vitro et in vivo du ketoconazole. Bull Soc Myc Med 1984; 13: 9-30.

Address for offprints:

F. J. Cabañes
Universidad Autonoma de
Barcelona, Facultad de
Veterinaria
08193 Bellaterra, Barcelona, Spain.