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In vitro study of the sensitivity of terbinafine in clinical isolates of 38 oncological patients. About the use and practice in podiatry

Estudio in vitro *de sensibilidad de la terbinafina en aislamientos clínicos de 38 pacientes oncológicos. A propósito del uso y praxis en podología*

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Keywords:

Antifungals, in vitro susceptibility, oncologic patient, resistance, podiatric practice.

Abstract

Introduction: Yeast infections have become very important in recent decades. An increase in the frequency and morbidity and mortality of fungal infections has made it necessary to have new antifungals. In the last decade, the consumption of antifungals has increased by 12 % per year. The increase in resistance to a large number of antimicrobials has led to an increasing demand for new antifungal agents for topical or oral application for prophylaxis and treatment.

Material and methods: A descriptive and observational study was carried out to know the in vitro sensitivity of 38 species of Candida spp. The method used was Kirby-Bauer or Sabouraud® agar diffusion method. This method has allowed to characterise the resistance levels of terbinafine and other antifungals related to clinical podiatric practice. The antifungals studied were: terbinafine, ketoconazole, fluconazole, itraconazole, nystatin and cyclopirox-olamine.

Results: All strains were sensitive to ciclopirox-olamine and clotrimazole. 2.6 % of C. albicans species were resistant to ketoconazole and 2.6 % in C. glabrata. 5.3 % of the cases of C. albicans were resistant to fluconazole and 10.5 % in C. glabrata, C. guillermondii and C. parapsilosis. In the study group of terbinafine, 65.8 % of C. albicans were resistant to this antifungal, 10.5 % of all C. tropicalis and 13.2 % for the rest of species. In summary, there is a broad percentage of resistance of yeast forms to terbinafine.

Conclusions: It is interesting to analyse the amount of strains studied resistant to azoles, especially fluconazole and terbinafine. Interestingly, a large majority of the species are resistant to terbinafine, despite being the antifungal agent that is most prescribed in onychomycosis, as evidenced in most of the studies conducted.

Palabras clave:

Antifúngicos, susceptibilidad *in vitro*, paciente oncológico, resistencia, práctica podológica.

Resumen

Introducción: Las infecciones por levaduras han adquirido una gran relevancia en las últimas décadas. Este incremento de la frecuencia y morbimortalidad de las infecciones fúngicas ha hecho necesario disponer de nuevos antifúngicos. En la última, el consumo de antifúngicos se ha incrementado en un 12 % al año. El aumento de resistencias frente a una gran cantidad de antimicrobianos ha llevado a una creciente demanda de nuevos agentes antifúngicos de aplicación tópica u oral para profilaxis y tratamiento.

Material y métodos: Se ha realizado un estudio descriptivo y observacional para conocer la sensibilidad in vitro de 38 especies de *Candida* spp. El método utilizado fue Kirby-Bauer o método de difusión en agar Sabouraud®. Este método ha permitido caracterizar los niveles de resistencia de la terbinafina y otros antifúngicos afines a la práctica clínica podológica. Los antifúngicos estudiados fueron: terbinafina, ketoconazol, fluconazol, itraconazol, nistatina y ciclopirox-olamina.

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Resultados: Todas las cepas fueron sensibles a ciclopirox-olamina y a clotrimazol. El 2.6 % de las especies de C. albicans fueron resistentes a ketoconazol y un 2.6 % en C. glabrata. El 5.3 % de los casos de *C. albicans* fueron resistentes a fluconazol y el 10.5 % en *C. glabrata, C. guillermondii* y *C. parapsilosis*. En el grupo de estudio de la terbinafina, el 65,8 % de *C. albicans* fueron resistentes a este antifúngico, un 10.5 % de todas las C. tropicalis y un 13.2 % para el resto de especies. En resumen, existe un porcentaje amplio de resistencia de formas levaduriformes a la terbinafina.

Conclusiones: Es interesante analizar la cantidad de cepas estudiadas resistentes a los azoles, especialmente a fluconazol y terbinafina. Curiosamente, una gran mayoría de las especies son resistentes a terbinafina, a pesar de ser el antifúngico que más es prescrito en onicomicosis, como se evidencia en la mayor parte de los estudios realizados.

INTRODUCTION

Yeast infections have become very important in recent decades. This may be due not only to increased frequency in immunosuppressed patients or in patients undergoing aggressive drug therapies, but also to their high prevalence in terms of morbidity and mortality. The frequency and morbidity and mortality increase in fungal infections has made it necessary to have new antifungals.

In the last decade, the use of antifungals has increased by 12% per year and, according to data from the European Medicine Agency, the annual global cost in antifungals amounts to 3,600 million euros[1]. In parallel to this, an increasing percentage of strains with resistance to these antimicrobials is being detected[2].

The increase in resistance to a large number of antimicrobials has led to an increasing demand for new topical or oral antifungal agents for prophylaxis and treatment; therefore, they have generated the need to implement new antifungals in health systems, although it is true, many of them present serious disadvantages: high cost, high rate of adverse effects, ineffectiveness against new species of fungi and rapid development of resistances[3].

Nowadays, fungal infections that cause mycosis generate significant health costs[4]. It is estimated that approximately 5 to 10% of dermatologic visits are caused by mycosis[4]. Antifungal treatments have been continuously evolving and improving its efficacy and spectrum of fungal action over time.

Candida spp. (Figure 1) is one of the agents most frequently implicated in the serious mycoses of immunosuppressed and critical patients. In the last 30 years we have seen a notable increase in the incidence of candidiasis[5]. The rational and early use of antifungals must be combined with microbiological diagnostics and imaging procedures[6].

The purpose of our publication was to evaluate the efficacy of terbinafine and other antifungals used in podiatric practice.

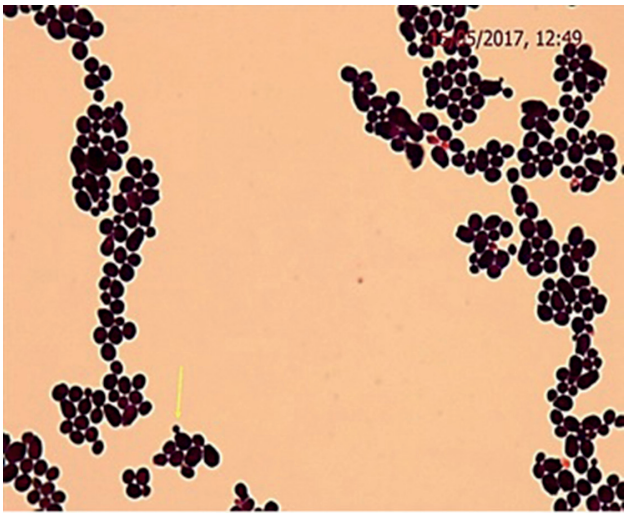

Figure 1. Gram stain of yeast forms (100 X). Image courtesy Dr. Tomás García-Lozano, assistant physician in microbiology, Oncology Institute of Valencia Foundation (FIVO), PDI Catholic University of Valencia "San Vicente Mártir".

PATIENTS AND METHODS

A descriptive and observational study was carried out to know the in vitro sensitivity of 38 species of Candida spp. (Figure 2), from 38 cancer patients and 38 wound exudates or soft tissues of the lower limb. The total number of samples processed for suspicion of fungal infection was 38, so that the selection of patients was clinically directed and confirmed in the microbiology department by gram staining, thus there is a total of 100% of positive wound exudates for levaduriform forms. The target population was patients admitted to the Hospital Oncology Institute of Valencia Foundation (FIVO) of Valencian Community. The studied patients presented clinical and microbiological infection due to candidiasis. The inclusion criteria were: patients with solid tumor-based pathologies, admitted patients and patients with any type of infection (cellulitis, fasciitis, radiodermatitis) with obvious clinical suspicion of fungal infection and microbiological confirmation through gram staining. Exclusion criteria were: non-oncological patients, not admitted and with lower limb infection without suspicion of mycosis in soft tissues. Samples were taken in the period of

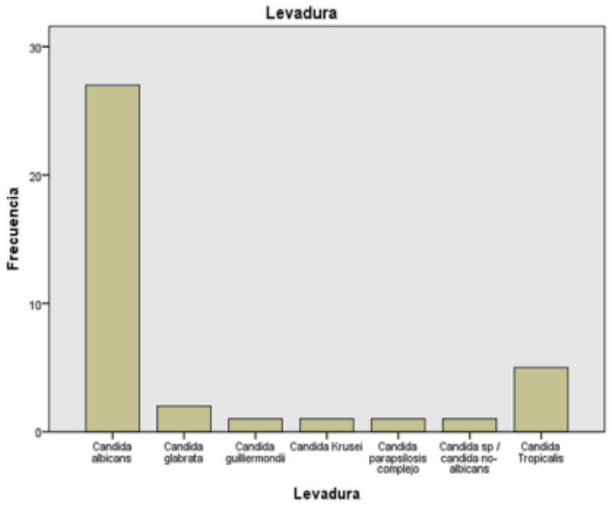

Figure 2. Fungal Species.

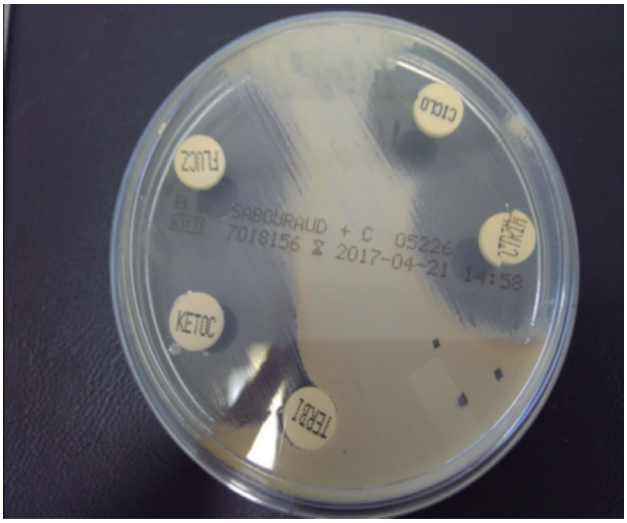

Figure 3. Kirby-Bauer Method. Sabouraud agar plate with colonization of Candida albicans, urine sample. Halo measurements: cycle: 21, ctrim: 30, ketoc: 28, terb: 6, fucz: 28. Own source. In vitro image of the study courtesy of Dr. Tomás García-Lozano. Medical Assistant Microbiology (FIVO).

approximately three months, from February 3rd to April 27, 2017. The study was included in a pilot project and collateral to the analysis of MRSA colonisations (prior authorization from ethics committe in FIVO, approved in 2012) and other species in cancer patients.

The sampling procedures were carried out through swab (Amies-Viscosa, Deltalab®, Barcelona, Spain) taken from wound and direct sowing with triple stria on Sabouraud® agar medium (Becton Dickinson®, BD, Québec , Canada). The sensitivity study was carried out using the Kirby-Bauer method (Figure 3) or Sabouraud® agar diffusion method (BD®). This method has allowed to characterize the resistance levels of terbinafine and other antifungals related to clinical podiatric practice. The antifungals studied were: terbinafine, ketoconazole, fluconazole, itraconazole, nystatin and cyclopirox-olamine. The antifungals used belongs to Rosco © (Taastrup, Denmark), in the form of standardized 9 mm diameter tablets (NeoSesitabs ©) with diffusible antifungal loads of 1 µg. To make the interpretation of sensitivity and / or resistance, the EUCAST and / or CLSI standards were used in their absence by measuring the halos obtained in mm from 2014. The study population consisted of 38 cancer patients with different types of tumor-based pathologies (Figure 4). The sensitivity study was carried out using the agar diffusion technique. Fungal inocula (by means of saline) were obtained from 24-hour cultures on Sabouraud® agar. The inocula included 5 x 105 colony forming units (CFU / ml) equivalent to 0.5 of the McFarland scale. The incubation was performed at 37° C for 24 hours and the reading of the inhibition zones allowed the interpretation of in vitro sensitivity. The obtained values were processed by descriptive statistics through the statistical package SPSS PC © version 19.0

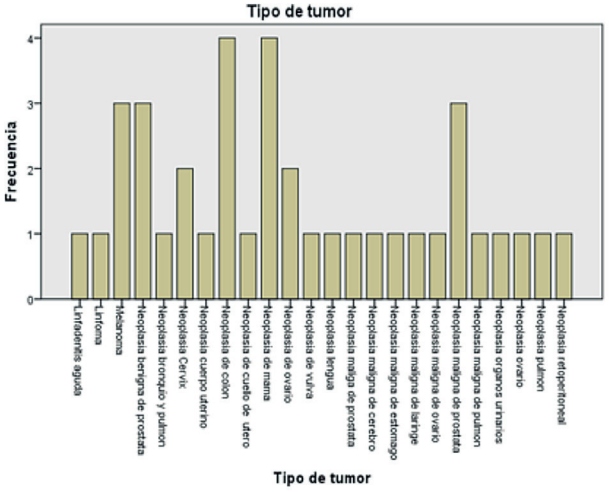

Figure 4. Tumoral disorders.

RESULTS

38 samples were obtained from 38 patients, 50% men and 50% women. The average age of the patients was 65.3 years. The distribution of the pathologies of tumor base is represented in Figure 2. The study of the fungal species is represented in Figure 1. Regarding to the analysis of antifungal sensitivity, the reason for our publication, several

aspects of great importance stand out: first, all strains were sensitive to ciclopirox-olamine (Figure 5) and clotrimazole (Figure 6); 2.6% of C. albicans species were resistant to ketoconazole and 2.6% in C. glabrata. 5.3% of the cases of C. albicans were resistant to fluconazole and 10.5% in C. glabrata, C. guillermondii and C. parapsilosis. In the study group of terbinafine, 65.8% of C. albicans were resistant to this antifungal, 10.5% of all C. tropicalis and 13.2% for the rest of the species. In summary, all strains were sensitive to clotrimazole and ciliopirox-alamin and a large percentage of yeast forms (Candida spp.) were resistant to terbinafine (Figure 7).

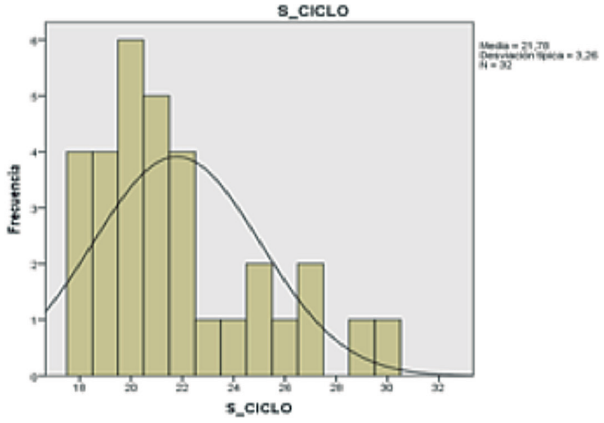

Figure 5. Inhibition halos are around 20 mm, considering the large number of species with cyclopirox-olamine sensitive

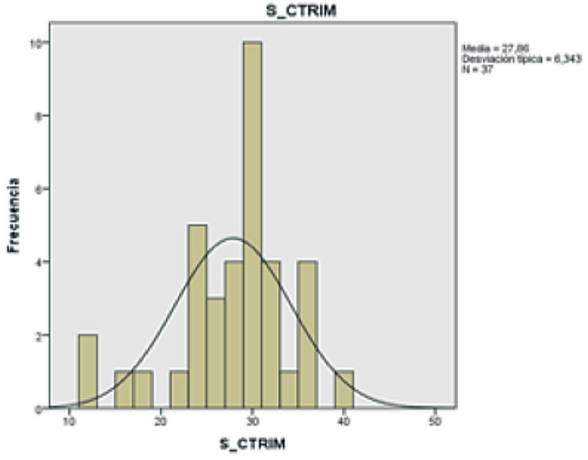

Figure 6. The inhibition halos are around 30 mm, considering that the large number of species with clotrimazole sensitive.

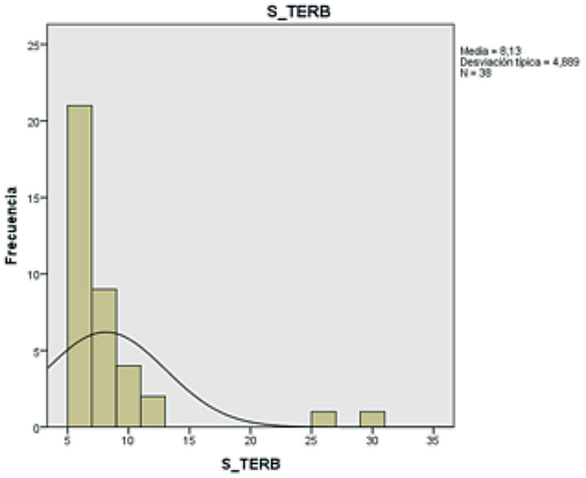

Figure 7. Halos of inhibition are around 5 mm, considering that the large number of species are resistant to terbinafine.

DISCUSSION

The fungal infection most frequently described in the oncological or immunosuppressed patient is due to the Candida spp genus[7], and especially C. albicans. Increases in other levaduriform and emerging species are also being observed.

Currently, one of the problems that are causing more resistance is the excessive use of oral antifungals and topical application, especially in azoles and terbinafine, whose resistance, the objective of our analysis, is due to one or several mutations in the ERG17 genes.

Despite the multitude of factors involved in the in vitro activity of antifungals, sensitivity study techniques are being studied with some interest, as well as standardization and standardization measures[8]. There are many demographic factors on published resistance rates, such as resistance to amphotericin B with previous use of orally azoles[9] or the influence of the fungal inoculum or the pH of the culture medium in vitro sensitivity to azoles reading and interpretation[8]. At this point, it is interesting to analyze the number of strains studied resistant to azoles and alylamines, especially to fluconazole and terbinafine respectively (Pearson chi-square, p = 0.001 and Cramer's V test, p = 0.001 respectively) and interestingly, almost all species are resistant to terbinafine, despite being the most prescribed antifungal for onychomycosis, as evidenced in most studies.

Considering the great importance of the use and antifungal spectrum of terbinafine as antifungal (choice for the treatment of dermatophytes, onychomycosis in feet and distal areas of the distal-lateral onychomycosis nails)[10,11], the main studies are basically based on comparing clinical efficacy, but few evidence mycological eradication. The vast majority conclude, and even

if there are substantial discrepancies between them, that the use of terbinafine is the most appropriate alone or in combination with other antifungals such as amorolfine. Even more so than with itraconazole, especially in infections by dermatophytes and non-dermatophytic forms or molds; although it is true, this controversy remains in a multitude of publications referenced with onychomycosis by Candida ssp., reason for our communication. Even so, and despite the discrepancies in the use of a multitude of drugs and physical treatments, new therapies or diagnostic methods are added to the market, rather "speculative" than real, ensuring clinical efficacy with different levels of evidence and absence of descriptions. Objective in vitro microbiological studies[12,13]. Regarding this point, despite the resistances found in the sample studied (n = 38), we must indicate that they are yeast forms and not dermatophytes, confirming that the use of terbinafine remains, until now, clinically effective in fungal infections due to dermatophytes, as it is supported in the vast majority of studies carried out.

In view of our results, we can affirm that there is a clear predominance of strains resistant to terbinafine and a phenomenon of cross-resistance between azole antifungals.

And to conclude, it is interesting to bear in mind that antimicrobial susceptibility studies, monitoring studies and genetic or taxonomy studies are necessary to be able to carry out an adequate practice care with a good complementary scientific base, especially in the management of antifungals orally and / or topically.

CONFLICT OF INTERESTS
The authors declare that they have no conflicts of interest.

FINANCING
None.

BIBLIOGRAPHY

1. Ausina Ruiz V, Moreno Guillén S. Treated SEIMC of Infectious Diseases and Clinical Microbiology. Madrid: Editorial Medica Panamericana; 2006
2. Aznar-Marín P, Galán-Sánchez M, Marín-Casanova P, García-Martos P, Rodríguez-Iglesias M. Candida nivariensis as a New Emergent Agent of Vulvovaginal Candidiasis: Description of Cases and Review of Published Studies. Mycophatologia. 2016; 181 (5-6): 445-9. DOI: 10.1007 / s11046-015-9978-y.
3. Rivas P. Antifungal sensitivity tests in clinical isolates of Candida spp. of patients with cancer. Rev Colomb Cancer. 2004; 8 (1): 22-8.
4. Master Vera J. R, Alou Cervera L. Advantages and disadvantages of topical antifungals. Rev Esp Quim. 2001; 14 (3): 340-4.
5. Fridkin SK. The changing face of fungal infections in health care settings. Clin Infect Dis. 2005; 41 (10): 1455-60.
6. Fortún J. Update in antifungal therapy: new drugs and indications. Enferm Infecc Microbiol Clin. 2011; 29 (Suppl 5): 38-44.
7. Díaz-López M.M, Oliveros-Garay O.A and Orozco-Díaz O. Analysis SSCP (ITS2, ERG11) of clinical isolates of Candida spp. of oral cavity in patients with cancer. Rev Iberoam Micol. 2004; 21: 182-6.
8. Rodríguez-Tudela JL, Cuenca-Estrella M. Has it been useful for a decade standardizing antifungal sensitivity tests? Enferm Infecc Microbiol Clin. 2002; 20 (5): 191-3. DOI: 10.1016 / S0213-005X (02) 72787-0.
9. Ruiz-Camps I, Cuenca-Estrella M. Antifungals for systemic use. Enferm Infecc Microbiol Clin. 2009; 27 (6): 353-62. DOI: 10.1016 / j.eimc.2009.04.001.
10. Fortún J. Update in antifungal therapy: new drugs and indications. Enferm Infecc Microbiol Clin. 2011; 29 (Suppl 5): 38-44. DOI: 10.1016 / S0213-005X (11) 70042-8.
11. Seebacher C. Action mechanisms of modern antifungal agents and resulting problems in the management of onychomycosis. Mycoses 2003; 46 (11-12): 506-10
12. El-Tatawy RA, Abd El-Naby NM, El-Hawary EE, Talaat RAZ. A comparative clinical and mycological study of Nd-YAG laser versus topical terbinafine in the treatment of onychomycosis. J Dermatol Treat. 2015; 26 (5): 461-4. DOI: 10.3109 / 09546634.2014.998607.
13. Crawford F, Hollis S. Topical treatments for fungal infections of the skin and nails of the foot. Cochrane Database Syst Rev. 2007; 18 (3): CD001434.