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Review Article

In vitro Susceptibility Patterns of Non-dermatophyte Fungal Agents of Onychomycosis

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Abstract

Onychomycoses are nail infections caused by three types of fungi: dermatophytes, yeasts, and non-dermatophyte molds. Members of the latter are *Aspergillus versicolor, Fusarium spp. Neoscytalidium dimidiatum* and *Scopulariopsis* spp., which are often confused as laboratory contaminants because their colonies reach maturity in less than a week and are susceptible to cycloheximide. However, *N. dimidiatum* and *Fusarium spp.* produce keratinases and thus are considered primary pathogens of nails and skin. These fungi are resistant to fluconazole and present a distinctive susceptibility pattern to the commonly used treatments available. Therefore, the correct identification of the etiological agent is necessary to ensure the proper treatment for the patients. The aim of this review is to compile and analyze the studies carried out on the determination of the minimal inhibitory concentrations to several antifungal agents of the different non-dermatophyte molds causing onychomycosis to provide the clinician with a reference document.

Keywords: *Aspergillus versicolor; Fusarium; Neoscytalidium dimidiatum; Scopulariopsis*

Abbreviations

KOH: Potassium Hydroxide; MIC: Minimal Inhibitory Concentration

Introduction

Onychomycoses are superficial fungal infections that affect the fingernails and/or toenails and account for about 50% of onychodystrophies [1]. These infections constitute a serious public health problem because they are contagious, the etiological agents are found in frequently visited environments (swimming pools or public bathrooms) and are difficult to treat [2]. In addition to the aesthetic affectation, psychological stress and low self-esteem, onychomycosis can cause discomfort, pain, paresthesia, nail deformities and difficulties in fitting shoes [3]. Also, if they are accompanied by lesions in the interdigital spaces or soles of the feet, these superficial mycoses could evolve to an invasive form because of immunosuppressive treatments, primary or secondary immunodeficiencies and hematopoietic and solid organ transplants [4-6].

The etiological agents of onychomycosis can be classified into three groups: dermatophytes, yeasts, and non-dermatophyte mycelial fungi such as *Acremonium* spp., *Aspergillus* spp., *Fusarium* spp., *Neoscytalidium dimidiatum* and *Scopulariopsis* spp. [7]. In Costa Rica, for example, 23.8 % of onychomycosis of the toenails are caused by this last group of fungi [2]. Regarding diagnosis, for the isolation of non-dermatophyte molds, 20 inoculations should be made on Sabouraud glucose agar without cycloheximide, and the following diagnostic criteria should be employed: (1) nail abnormalities consistent with the pathology, (2) direct smear with potassium hydroxide (KOH) positive for the presence of mycelium in the nail keratin, (3) non-isolation of dermatophytes and (4) growth in at least five inoculation points in two consecutive samplings [8]. It is important to remember that these fungi are usually fast-growing and sensitive to cycloheximide [9], so they are often considered as contaminants. On the other hand, although one of the criteria for their diagnosis is the non-isolation of dermatophytes, there is a possibility that the patient has a mixed infection [10].

As previously mentioned, nail infections are difficult to treat, and the therapeutic schemes are dependent on the etiological agent, since each fungus presents a characteristic *in vitro* susceptibility profile [11-15]. For example, *Trichophyton rubrum* (the main etiological agent of onychomycosis worldwide) presents high susceptibility to fluconazole [16-19], which is why it is one of the most widely used antifungals for the treatment of these infections. However, as presented below, non-dermatophyte filamentous fungi are usually resistant to this drug, making it essential to correctly characterize the etiological agent prior to the start of treatment. Therefore, the present literature review aims to compile and analyze the studies carried out on the determination of the minimal inhibitory concentrations (MIC) of the different non-dermatophyte molds causing onychomycosis to provide the clinician with a reference document.

Materials and Methods

A review of published literature regarding susceptibility patterns of non-dermatophyte fungal agents of onychomycosis was performed using databases such as PubMed (NCBI), Scopus, Google Scholar, Redalyc and Science Direct. For this purpose, we use the following key words: "*Aspergillus versicolor*", "*Fusarium*", "*Neoscytalidium dimidiatum*", "*Scopulariopsis*", "onychomycosis", "susceptibility patterns" and "antifungals". The articles found were classified according to the year of publication. Only original and research studies were included. Finally, articles published between 1984 and 2021 were taken into account, so that classic works and the most recent advances on the subject were analyzed.

Results and Discussion

Aspergillus versicolor

Aspergillus spp. are opportunistic fungi with universal distribution but predominate in climates with alternating dry and wet seasons. Aspergillosis can vary among different presentations such as pulmonary forms (aspergilloma), mycetoma, disseminated aspergillosis, cutaneous forms (onychomycosis), otomycosis and keratomycosis [20-23]. Onychomycosis caused by *Aspergillus* spp. most often occurs in the toenails. The most frequent form is the distal subungual onychomycosis, similar to the ones produced by dermatophytes. Additionally, cases of lateral or superficial white subungual onychomycosis have been described [23]. The species involved are A. *terreus, A. flavus, A. niger* [8], *A. fumigatus, A. sidowii*, and *A. versicolor* (the most prevalent) [20].

A. versicolor is a fast-growing fungus ([3 – 5] days), susceptible to cycloheximide. On Sabouraud agar it produces yellow, brown, or green colonies with a yellowish, orange, or reddish pigment (Figure 1A). At the microscopic level, it produces hyaline and septate mycelium, and a conidiophore with a biseriated vesicle (Figure 1B) [24]. As shown in Table 1, *A. versicolor* is susceptible to terbinafine and itraconazole but resistant to fluconazole and amorolfine.

Fusarium spp

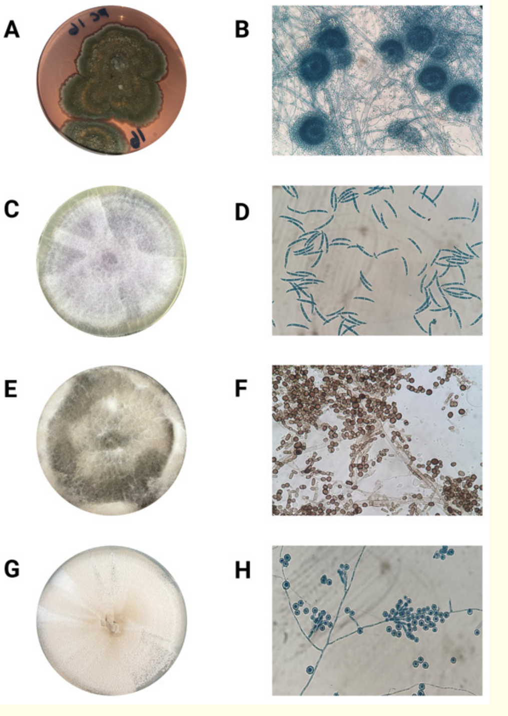

Figure 1: Macroscopic and microscopic morphology of the non-dermatophyte molds that cause onychomycosis. A and B. *Aspergillus versicolor*; C and D. *Fusarium* spp., E and F. *Neoscytalidium dimidiatum* and G and *H. Scopulariopsis* spp. The cultures were grown in Sabouraud glucose agar and the wet mounts were made with clear lactophenol for *N. dimidiatum* and blue cotton dye with lactophenol for the other three fungi. The microscopic photographs were taken with a light microscope at 400 X. Created with BioRender.

Fusarium spp. are fast-growing fungi that produce velvety or slightly cottony surface colonies that vary in color: white, pink, purple, orange, gray and brown with a light reverse or with pink or violet pigment (Figure 1C). Microscopically its mycelium is hyaline and septate. They can present two or three types of sporulation depending on the species: hyaline, multiseptated, fusiform shape macroconidia measuring between (2 - 6) x (14 - 80) µm (Figure 1D) and/or non-septate or uniseptate microconidia, which measure between (2 - 4) x (4 - 8) µm and can vary from an ovoid to cylindrical and even pyriform shape; also, they can produce chlamydospores [27]. Among the fungal species described as agents of onychomycosis are *F. solani* and *F. oxysporum*. The affected nails are often yellow-white coloration with painful periungal inflammation [8]. As shown in Table 2 the MICs of *Fusarium* spp. to the different antifungals are high in all cases.

Neoscytalidium dimidiatum

Table 1: *In vitro* susceptibility patterns of *Aspergillus versicolor* to antifungal compounds used to treat onychomycosis, by country.

Country	n	Minimal inhibitory concentration (µg/mL)		Reference
		Mean	Range	
Amorolfine				
Costa Rica	13	> 64.00	NA*	[14]
Ciclopiroxolamine				
Costa Rica	13	4.9	1.6 - 8.0	[14]
Fluconazole				
Spain	12	64.0	64.0 - > 64.0	[25]
Itraconazole				
Costa Rica	13	0.9	0.1 - 2.0	[14]
Spain	12	2.0	0.5 - 4.0	[25]
Ketoconazole				
Spain	12	2.8	1.0 - 4.0	[25]
Terbinafine				
Spain	9	NC**	0.03 - 1.0	[26]
Spain	12	0.4	< 01 - 1.0	[25]
Costa Rica	13	0.55	0.1 - 1.6	[14]

*NA: not applicable. **NC: not calculated.

Table 2: *In vitro* susceptibility patterns of *Fusarium* spp. to antifungal compounds used to treat onychomycosis, by country.

Country	n	Minimal inhibitory concentration (µg/mL)		Reference
		Mean	Range	
Amorolfine				
Costa Rica	29	19.3	0.1 - 64.0	[12]
China	6	24.7	4.0 - 64.0	[28]
Bifonazole				
Italy	5	64.0	NA*	[29]
Ciclopiroxolamine				
Costa Rica	29	15.4	1.0 - 32.0	[12]
Italy	5	4.0	2.0 - 8.0	[29]
Fluconazole				
Brazil	15	22.6	16.0 - >64.0	[30]
Colombia	8	64.0	NA*	[31]
Iran	27	64.0	64.0 - >64.0	[32]
Iran	14	64.0	64.0 - >64.0	[33]
United States of America	4	>64.0	NA	[34]
Turkey	2	>64.0	NA	[18]
Brazil	10	121.6	64.0 - >128.0	[35]
Colombia	14	128.0	NA	[16]
Spain	1	>256.0	NA	[17]
Itraconazole				
Brazil	15	5.65	4.0 - >16.0	[30]
United States of America	4	6.0	4.0 - 8.0	[34]

Italy	5	7.0	0.3 - 16.0	[29]
Iran	14	8.0	8.0 - >16.0	[33]
Brazil	8	8.7	4.0 - 16.0	[36]
Iran	27	13.4	0.25 - >16.0	[32]
Colombia	8	16.0	NA	[31]
Costa Rica	29	>16.0	NA	[12]
Iran	13	>16.0	16.0 - >16.0	[37]
Iran	2	>16.0	NA	[33]
Brazil	10	51.4	0.5 - >64.0	[35]
Colombia	14	95.0	8.0 - 128.0	[16]
Ketoconazole				
Brazil	15	8.9	4.0 - >16.0	[30]
Italy	5	24.2	2.0 - >64.0	[29]
Miconazole				
Iran	27	13.0	4.0 - >16.0	[32]
Italy	5	42.2	1.0 - >64.0	[29]
Terbinafine				
Iran	27	3.3	0.03 - >64.0	[32]
Turkey	2	0.6	0.3 - 1.0	[19]
Colombia	6	1.7	0.3 - 16.0	[31]
Brazil	15	4.0	4.0 - >16.0	[30]
Iran	14	4.0	0.03 - 4.0	[33]
Colombia	14	4.2	0.1 - 128.0	[16]
Iran	2	>4.0	NA	[33]
Spain	12	13.4	2.0 - >16.0	[26]
Colombia	2	16.0	NA	[31]
United States of America	4	>16.0	0.5 - >16.0	[34]
Italy	5	42.2	32.0 - >64.0	[29]
Brazil	10	52.8	16.0 - >64.0	[35]
Costa Rica	29	61.9	4.0 – 64.0	[12]
Brazil	3	64.0	NA	[35]
Tioconazole				
Italy	5	9.2	0.5 – 64.0	[29]
Iran	27	16.0	16.0 - >16.0	[32]

*NA: not applicable.

N. dimidiatum is found in soil, plants, and their detritus. The infection derives from direct contact with these elements since it has keratinases [20,38]. At the macroscopic level its colonies are grayish and cottony-like on the adverse, with a black reverse (Figure 1E). Microscopically it produces fuliginous and septate mycelium and chains of arthrospores (Figure 1F) [20]. Clinically, their infections are indistinguishable from those caused by dermatophytes. Hyperkeratosis is seen on soles and toes, and less often - on hands. Lesions on the palms are usually unilateral, while on the soles of the feet they are usually bilateral. The condition in the nails is of distal subungual type and lateral in the feet; of laterodistal type in the hands, both possibly associated with dark pigmentation. Paronychia and partial or total dystrophy may also be present. *In vivo*, resistance to almost all therapeutic approaches has been demonstrated. Occasionally, response has been reported with amorolfine in combination with terbinafine [38]. *In vitro*, this pattern is also observed since the lowest MICs correspond to amorolfine and terbinafine (Table 3).

Scopulariopsis spp.

Table 3: *In vitro* susceptibility patterns of *Neoscytalidium dimidiatum* to antifungal compounds used to treat onychomycosis, by country**.**

Country	n	Minimal inhibitory concentration (µg/mL)		Reference
		Mean	Range	
Amorolfine				
Costa Rica	20	0.4	0.1 - 1.0	[15]
Ciclopirox olamine				
England and Canada	3	NC	0.1 - 0.25	[39]
Costa Rica	20	1.2	0.75 - 3.0	[15]
Clotrimazole				
Malaysia	16	0.3	0.1 – 0.5	[40]
Fluconazole				
Malaysia	16	12.7	2.0 - 32.0	[40]
United States of America	2	16.0	NA	[34]
Spain	1	16.0	NA	[17]
Colombia	4	26.9	4.0 – 64.0	[31]
Turkey	2	>64.0	NA	[19]
Griseofulvin				
United States of America	2	2.5	1.0 - 4.0	[34]
Itraconzole				
Turkey	2	0.25	NA	[19]
United States of America	2	2.5	1.0 – 4.0	[34]
Colombia	4	3.32	0.03 – 16.0	[31]
Costa Rica	20	6.8	0.3 - >16.0	[15]
Malaysia	16	>16	NA	[40]
Ketoconazole				
Malaysia	16	>16.0	NA	[40]
Miconazole				
Malaysia	16	0.6	0.03 – 1.0	[40]
Terbinafine				
United States of America	2	0.04	<0.03 – 0.1	[34]
Colombia	4	0.1	0.03 – 0.5	[31]
Costa Rica	20	0.2	0.1 – 0.3	[15]
England and Canada	3	NC**	0.1 – 2.0	[39]
Turkey	2	1	NA	[19]

*NA: not applicable **NC: Not calculated.

The genus *Scopulariopsis* includes different species like *S. brevicaulis*, *S. brumptii* and *S. acremonium*. They are saprophytic fungi that can be isolated from soil. They are considered opportunistic pathogens associated with clinical manifestations such as onychomycosis, osteomyelitis, mycetoma, infection of the central nervous system, endophthalmitis, peritoneal infections, endocarditis [23] and keratitis [41]. *S. brevicaulis* is the species most isolated from onychomycosis. It usually affects the first toenail of patients with underlying disease or previous trauma of the nail [20]. Microscopically, its mycelium is septate and hyaline. They reproduce by branched conidiophores with anelidic and equinulated conidia ([4 – 9] µm in diameter) (Figure 1H). It is a fast-growing fungus that produce powdery colonies thar are initially whitish and, when mature, take on a brown or cinnamon color (Figure 1G) [24]. The susceptibility patterns for this genus are shown in Table 4.

Conclusion

Table 4: *In vitro* susceptibility patterns of *Scopulariopsis* spp. to antifungal compounds used to treat onychomycosis, by country.

Country	n	Minimal inhibitory concentration (µg/mL)		Reference
		Mean	Range	
Amorolfine				
United States of America	4	0.1	0.1-0.13	[42]
China	3	1.8	0.5 – 4.0	[28]
Costa Rica	11	8.1	0.1 – 64.0	[13]
Bifonazole				
Italy	10	6.5	0.1 - >64.0	[29]
Ciclopirox olamine				
Canada	1	0.1	NA*	[39]
Spain	14	0.4	0.1 – 0.5	[43]
United States of America	4	0.6	0.5 – 1.0	[42]
Italy	10	1.4	0.3 – 2.0	[29]
Costa Rica	11	5.1	2.0 – 12.0	[13]
Fluconazole				
Colombia	1	32.0	NA	[16]
Spain	14	>32.0	NA	[43]
Spain	11	64.0	NA	[17]
United States of America	2	>64.0	NA	[34]
Turkey	2	>64.0	NA	[19]
Costa Rica	11	128.0	NA	[13]
Itraconazole				
Colombia	1	0.02	NA	[16]
Spain	14	0.4	0.3 – 0.5	[43]
United States of America	2	4.0	NA	[34]
United States of America	4	>4.0	NA	[42]
Italia	10	5.3	0.1 - >16.0	[29]
Costa Rica	11	16.0	NA	[13]
EE. UU	97	26.7	1.0 – 32.0	[44]
Ketoconazol				
Italia	10	1.6	0.5 - 4	[29]
Miconazol				
Italia	10	5.0	0.5 - >64.0	[29]
Terbinafina				
España	14	0.4	0.1 – 0.5	[43]
EE. UU.	2	0.8	0.5 – 1.0	[34]
EE. UU.	4	1.0	0.5 – 2.0	[42]
Canadá	1	1.0	NA	[39]
España y Argentina	5	1.38	0.01-16.0	[17]
EE. UU	97	1.9	0.5 – 4.0	[44]
Colombia	1	4.0	NA	[16]
Costa Rica	11	7.7	1.0 – 64.0	[13]
España	21	12.3	1.0 – 16.0	[26]
Italia	10	22.6	16.0 – 32.0	[29]
Tioconazol				
Italia	10	5.7	2.0 – 16.0	[29]

*NA: not applicable.

Non-dermatophyte molds are important agents of onychomycosis worldwide, therefore, the usage of culture media without cycloheximide is mandatory when working with dermatological samples. Also, the correct identification of the etiological agent is important since the susceptibility patterns of each genus are different, and the correct selection of the antifungal drugs will impact in the outcome of the disease.

Conflict of Interest

The authors declare no competing interests.

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