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Viabilidade de fungos causadores de onicomicose em esmaltes de unha

Viability of onychomycosis-causing fungi on nail polish

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RESUMO

Introdução: Infecções nas unhas causadas por fungos são chamadas de onicomicoses, e são cada vez mais frequentes. Esmalte é um verniz quimicamente complexo aplicado para fins decorativos ou para proteger a lâmina ungueal. No entanto, esmaltes usados podem ser colonizados por microrganismos e, consequentemente, constituir um risco para doenças nas unhas. **Objetivo:** Considerando a importância da onicomicose e o crescente uso de esmaltes, o objetivo deste estudo foi avaliar a sobrevivência de fungos em esmaltes de diferentes cores e marcas. **Métodos:** As cores testadas foram base incolor, branca, e vermelha, de quatro marcas, totalizando 12 amostras de esmaltes. Os frascos foram contaminados individualmente pelo imprint do aplicador em culturas de quatro espécies fúngicas (isolados clínicos): *Trichophyton rubrum*, *T. mentagrophytes*, *Candida albicans*, e *C. parapsilosis*. A partir do momento da contaminação, a viabilidade fúngica foi observada a cada 60 min, por oito horas. Análises sucessivas foram realizadas após 7, 14, 21, e 28 dias. **Resultados:** As quatro espécies mostraram-se capazes de sobreviver dentro dos frascos nas primeiras oito horas de inoculação. A viabilidade por mais tempo variou de acordo com a cor e marca. *Trichophyton rubrum* sobreviveu em todas as marcas, em pelo menos uma das cores, por 28 dias. **Conclusão:** Os principais agentes de onicomicose podem permanecer viáveis em frascos de esmalte por dias. Esmaltes devem ser considerados como possíveis veículos de contaminação, e medidas devem ser tomadas para reduzir os riscos de transmissão. Análises adicionais são necessárias para revelar os mecanismos de sobrevivência dos fungos neste ambiente.

Palavras-chave: Cosméticos; Doenças da Unha; Dermatomicoses; Dermatófitos; Leveduras.

ABSTRACT

Introduction: *Nail infections caused by fungi are called onychomycosis, which are increasingly frequent worldwide. Nail polish is a chemically complex lacquer applied for decorative purposes or to protect the nail plate. However, used nail polishes may be colonized by microorganisms and, consequently, constitute a risk of nail diseases.* ***Objective:*** *Considering the importance of onychomycosis and the increasing use of nail polish, the objective of this study was to evaluate the survival of fungi in nail polish from different colors and commercial brands.* ***Methods:*** *The colors tested were colorless basecoat, white, and red, from four brands, totaling 12 samples. The flasks were individually contaminated by imprinting the applicator on cultures of four fungal species (clinical isolates): Trichophyton rubrum, T. mentagrophytes, Candida albicans, and C. parapsilosis. Considering the moment of contamination, fungal viability was observed every 60 min, until the first eight hours. Further observations were after 7, 14, 21, and 28 days of the inoculation.* ***Results:*** *The four species showed the ability to survive in the flasks on the first eight hours after inoculation. The viability for longer periods varied by brand and color. Trichophyton rubrum survived in all brands, in at least one of the colors, for 28 days.* ***Conclusion:*** *The main agents of onychomycoses can remain viable in nail polish for several days. Nail polishes should be considered as potential contamination vehicles, and measures should be taken to reduce risks of transmission. Further analyses are necessary to reveal the mechanisms of fungal survival in this environment.*

Keywords: *Cosmetics; Nail Diseases; Dermatomycoses; Dermatophytes; Yeasts.*

INTRODUCTION

The cult of nail beauty has its origins in the antiquity, around 3,500 B.C. In ancient Egypt, women would already apply black henna dye to their nails. The first nail polish formulation was created in China and had a limited durability of just one day on the nails[1].

Considering the current status of the cosmetics industry, Brazil is the second country that sells more nail polish in the world. This segment is one of the main responsible for the global recovery of the beauty and personal hygiene markets[2].

Nail polish is a lacquer applied for decorative purposes or protection of the nail plate. Several formulations have been studied to improve the anatomy and physiology of the nail, as well as enhance the decorative effects. In this sense, many chemical compounds are present in the formulations. The basic polymer of the nail polish, which is responsible for the coat structure, is the nitrocellulose, which is dissolved in a volatile organic solvent, butyl acetate or ethyl acetate. Added to this basic formulation, there are plasticizers (for example, dibutylphthalate), dyes and pigments (chromium oxide, chromium hydroxide, ferric ferrocyanide, stanic oxide, titanium dioxide, iron oxide, carmine, ultramarine, manganese violet, mica, bismuth oxychloride, natural pearls, aluminum powder, among others), and a resin, historically composed of tosylamide-formaldehyde, which ensures the adherence of nitrocellulose to the nail plate. Additionally, a thickener, stearalconium hectorite, which keeps the particles in suspension, and color stabilizers to ultraviolet radiation, such as benzophenone-1, are included[2-4].

The nail plate consists of three layers: an inner layer (ventral nail); a hard, intermediate keratin layer; and the outermost layer (dorsal nail). All layers can be easily colonized or infected by microorganisms, such as bacteria, dermatophyte fungi and/or yeasts[5]. Dermatophytes, which include *Trichophyton*, *Microsporum*, and *Epidermophyton*, are responsible for 80-90% of onychomycoses, especially on toenails[6]. Yeast, in particular *Candida, Trichosporon,* and *Malassezia* species, represent 5-17% of onychomycoses[7-8]. Less prevalent, but clinically relevant, there are the non-dermatophyte filamentous fungi, such as: *Fusarium* sp., *Scytalidium* sp., among others[9-10]. Etiology and frequency of these infections vary worldwide, mainly due to geographical and climatic conditions. The individual's clinical condition, such as underlying diseases and immunodeficiencies, is another crucial characteristic. Rates of onychomycoses have been reported between 0.5% and 24%, depending on the population[11-12].

The onychomycoses most common manifestations include discoloration of the nail, onycholysis, and onychauxis. In severe cases, it may be socially embarrassing and have adverse effects on quality of life. It may also disseminate to other parts of the body and increase the risk for bacterial infections[13-14].

The origin of the nail infection is facilitated by trauma, and the agents can reach the nail directly or indirectly, by several fomites, such as bedding, clothing, shoes, and utensils contaminated with fungal propagules[11].

In Brazil, nail hygiene and beautification practices have been established by podiatrists and the National Health Surveillance Agency (ANVISA). However, in most beauty salons, the hygiene procedures adopted are varied. For example, it may occur sharing of scissors, pliers, and spatulas, with or without chemical cleaning; reuse of nail files; and sharing of nail polishes. These factors may contribute for a greater risk of nail colonization by fungi and, consequently, nail infections.

Considering the importance of this disease and the increasing use of nail polishes in beautification services, a preliminary pilot study demonstrated that nail polishes used in beauty salons are capable of harboring potentially pathogenic fungal species (unpublished data). Based on these results, this current study aimed to evaluate the survival of fungi within nail polishes of different colors and commercial brands, for a period up to 28 days.

METHODS

Four brands of nail polishes commonly sold in Brazil were tested, being identified by the letters A, B, C, and D. Colorless basecoat, white, and red nail polishes were tested for each brand, being inoculated with four species of fungi, totaling 48 flasks. The mycological tests were performed with fungi isolated from human nails (clinical isolates), of the following species: *Trichophyton rubrum, Trichophyton mentagrophytes, Candida albicans*, and *Candida parapsilosis*. They were obtained from the culture collection of the Microbiology Laboratory of the São José do Rio Preto School of Medicine (FAMERP), Brazil.

To prove the sterility of the polishes, recently opened flasks were inoculated directly into plates with Sabouraud Dextrose agar (SDA, DIFCO®), which were incubated at 30ºC, for 10 days.

After this step, the flasks were contaminated with fungi obtained from a 24-hour culture for yeasts or 5-days culture for dermatophytes, with an imprint of the applicator on a colony forming unit. Each flask was contaminated with a single fungal species. The flask was then homogenized and 30 µl of the polish was applied directly to the surface of a SDA plate, to check the fungal viability at zero hour. Every 60 minutes, a new aliquot of each nail polish was inoculated on the plate, totaling 8 consecutive hours of analysis (new nail polishes). In addition, the experiments were repeated after 7, 14, 21, and 28 days (old nail polishes). The cultures of the four species were individualized for each color and brand. Once inoculated, the plates with dermatophytes and yeasts were incubated at 25ºC and 30ºC, respectively. They were checked daily for the presence or absence of fungal growth.

RESULTS

The four species tested (*C. albicans, C. parapsilosis, T. rubrum* and *T. mentagrophytes*) showed the ability to survive in new nail polish flasks for the first 8 hours after inoculation. However, the ability to remain viable for more than one day varied according to the brand.

Figure 1 shows the results of fungal survival in each nail polish brand and color, on days 7, 14, 21 and 28. Brand D was the only one that all species tested remained viable, in at least one of the colors. In relation to the colors, red exhibited greater growth. In 50.0% of the tests, viability was maintained for at least 7 days. This result is mainly due to *T. rubrum*, which maintained its viability in all brands of red polish. In contrast, *C. parapsilosis* was unable to maintain its viability in any red polish. The white color showed the greatest level of inhibition. In 62.5% of the tests, the species did not maintain viability for 7 days or more. Considering the fungi, yeast species showed greater ability to survive in light nail polishes (basecoats and white). For filamentous species, the greatest survival rates occurred in red polishes (Figure 1).

Trichophyton rubrum was the only species that could survive on all brands, in at least one color, in old nail polishes (Figure 1c). *Candida albicans*, on the other hand, despite having survived on three brands for at least 7 days, in none of them it maintained its viability longer than 21 days (Figure 1a). *Candida parapsilosis* was the only species which did not maintain its viability for at least 7 days in red polishes of any brand, showing that it is more capable of surviving on colorless basecoats (Figure 1b). Finally, *T. mentagrophytes* showed the lesser ability to maintain viable, exhibiting growth in only 4 of the 12 old nail polishes tested (Figure 1d).

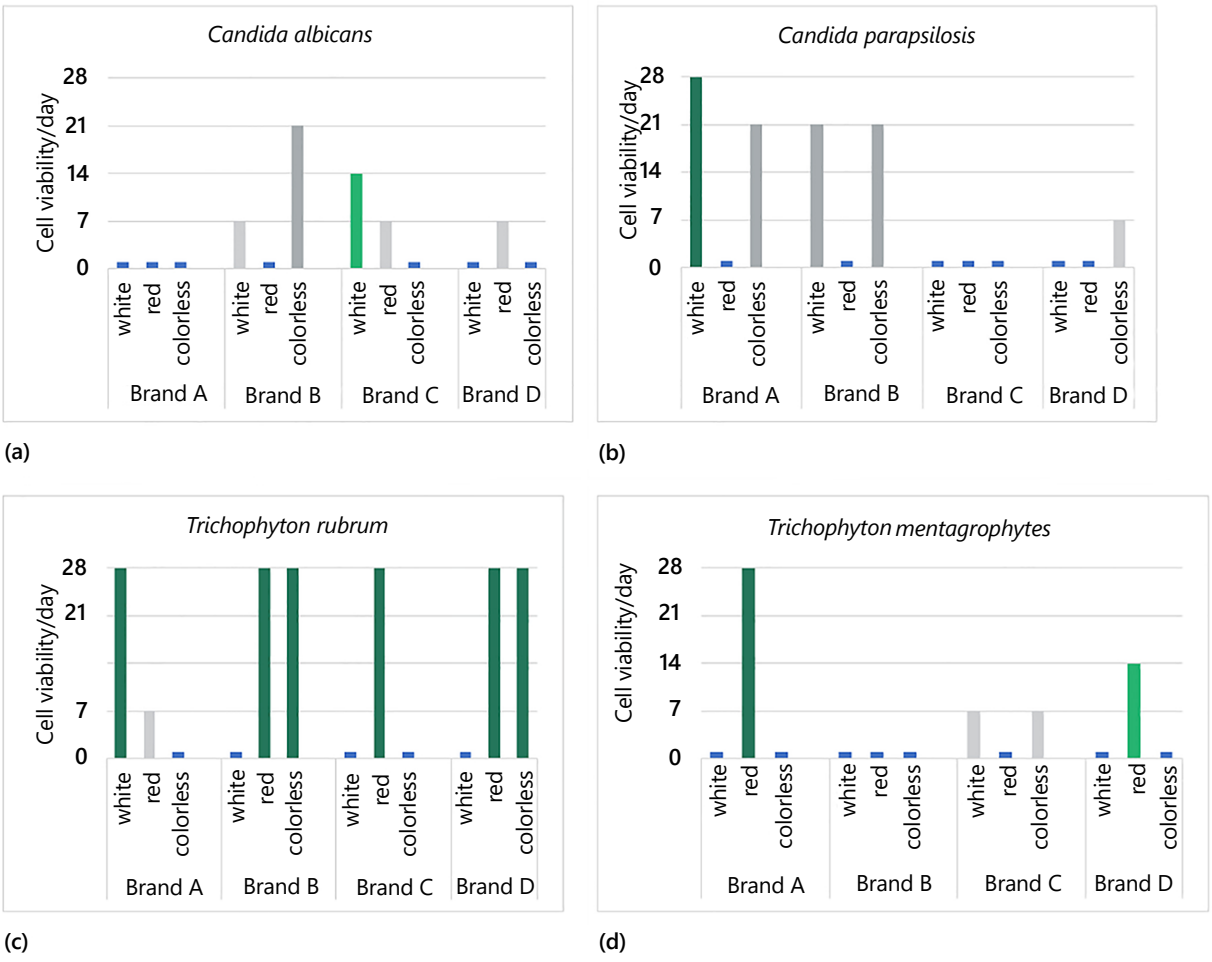

Figure 1. Qualitative fungal cell viability in each test, after 7, 14, 21, and 28 days in the nail polishes flasks, of: (a) *Candida albicans*; (b) *Candida parapsilosis*; (c) *Trichophyton rubrum*; and (d) *Trichophyton mentagrophytes*.

DISCUSSION

Currently, Brazil is the 4th largest market for personal care products, perfumes, and cosmetics. In 2018, the country held 6.2% of market share, totaling US$ 30 billion in consumer sales. Considering only nail polishes, the Brazilian market is considered the second largest in the world, behind the United States of America[14-15]. The use of nail polish has been increasing both for aesthetics and treatment of nail disorders[16-17].

This study showed that nail polishes used for aesthetics can be potential sources of fungi, and these organisms can remain viable for several days in this environment. Fungi, in contact with nails and under favorable conditions (trauma of the nail, for example), can cause onychomycosis, one of the most common types of diseases found on nails[18-19]. For a long time, onychomycosis was considered only an aesthetic issue. However, studies have shown a major impact on patients' quality of life and self-esteem[20]. Moreover, most cases affect individuals with predisposing factors, such as poor blood circulation, peripheral neuropathy, or immunosuppression, which can also be causes of serious conditions[21]. Therefore, onychomycosis can warn of risks that are possibly still silent. Therapeutic failures are common due to difficulties in patient adherence to treatment and low availability of topical medication in hyper-keratinized nails[22]. High recurrence rates (when there is no complete cure of the disease) and reinfection (when there is complete cure and new occurrence) are also typical in this type of infection[21].

The transmission of onychomycosis is linked to several factors, whether inherent to the hosts, environmental or behavioral. Nail beautification practices often concern about viral agents contaminating files, pliers, and scissors. However, a preliminary study showed the possibility of the occurrence of fungi in nail polishes used in beauty salons (unpublished data). This study has proved that the main species that cause onychomycosis can remain viable in the nail polish flasks for weeks. In fact, *T. rubrum* is the most common agent of onychomycosis in Brazil[23] and it was able to survive in all tested brands for 28 days in at least one color. These data reveal unexpected results, since it is believed that the chemical composition of the nail polish would not allow microorganisms to remain viable inside[24]. Unfortunately, discussion of these results is limited due to the lack of studies in the literature that evaluate the same criteria investigated here.

In general, fungi are well adapted to live in a variable range of environments and situations and can colonize an infinite number of niches. For example, *T. rubrum* and the other dermatophyte species can produce enzymes which allow them to invade keratinized tissues[25]. The beauty salon environments are frequently exposed to these microorganisms, by conidia carried through the wind, contaminated tools, or infected nails, which can contaminate the nail polishes.

The nail polishes were formulated to coat the nail with a quick drying film, resistant to peeling and with shiny and pearly appearance[1]. The standard composition of nail polishes includes basic elements, such as: a film former, a plasticizer, a thermoplastic resin, solvents, and

pigments. Solvents are important to dissolve soluble components and regulate characteristics such as viscosity, drying time, and rigidity. Generally, it is the largest portion of the final product. Most often used as solvents are ethyl, propyl, and butyl acetates, alone or in a mixture[26]. In a study by Lens et al. (2016)[24], they showed that the most common solvents used in cosmetics have antimicrobial activity against three bacterial species (*E. coli*, *S. aureus*, and *P. aeruginosa*) e two fungi (*C. albicans* and *T. rubrum*), suggesting that they are an inhospitable medium for microorganisms. Nevertheless, it is worth mentioning that these tests were carried out on reference strains and on the isolated solvents. Furthermore, no data were found in the literature to prove that the antimicrobial activity of the isolated solvents is the same as the final product.

Beside solvents, pigments are other constituents that may have antimicrobial activity. They can be inorganic or organic. Inorganic pigments are metallic oxides, for example iron or titanium. Organic pigments are produced by precipitating an organic dye with an inorganic substrate. These inorganic substrates are insoluble metallic salts such as barium sulphate, calcium sulphate, aluminum hydroxide, or aluminum oxide[26]. Regarding the metallic oxides, there are reports of antimicrobial activity in the literature. However, they are usually in the form of nanoparticles[27]. Due to the higher surface/volume ratio, nanoparticles have different properties compared to larger particles[28]. Colorless polishes are formulated differently, because they do not contain the pigments nor the suspending agents necessary to prevent the pigments from settling[26]. The results observed in this study suggest that color is a determining factor for maintaining viability.

Some of the compounds that could grant antimicrobial activity to nail polishes are no longer commonly used due to their toxicity. For example, dibutyl phthalate, used as a plasticizer, and the toluenesulfonamide/formaldehyde compound used as a resin[26]. For patent reasons, it was not possible to determine the exact composition of the polishes used in this study and, consequently, to assess the reason for the different results among the brands and the colors analyzed.

Another point worth considering is that the properties of the components in nail polish may change over time or in contact with air. Knowledge of the safety and efficacy of nail products remains limited due to the scarcity of data in the literature. In addition, because of the lack of adequate regulations, little is known about the safety and efficacy of marketed products[29]. Recently, a study has shown that the application of nail polishes and the methods used to remove these products, can damage the nails, leaving them fragile and brittle[30], which constitutes a risk factor for fungal infections.

CONCLUSION

In conclusion, it has been proven that the most common onychomycosis agents can remain viable in nail polish for weeks. These results are highly relevant, since, due to the chemical composition of this cosmetic, it has been suggested that fungi and other microorganisms could not survive in this environment. Brand, polish color and fungal species are important variables that determine the time of viability. In all tested brands, fungal viability was maintained. Although the data obtained in this work is qualitative, it opens for the possibility of future studies. Further investigation is needed to demonstrate the mechanisms involved in the survival of fungi in this environment, and to develop formulations that do not allow their proliferation, without changing their structural properties. Meanwhile, nail polishes should be considered as vehicles for the contamination and dispersion of fungal pathogens. Due to the high frequencies of onychomycosis, measures must be taken to reduce the risk of transmission of fungi to other nails and other individuals. For example, individual use of nail polish flasks and avoid the use of nail polish on diseased nails.

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