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Presence of filamentous fungi in powder and semiaqueous makeup

Presença de fungos filamentosos em maquiagens em pós e semissólidas

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ABSTRACT
Makeup is used by a large portion of humanity and often misused or stored incorrectly, leading to contamination by microbes. The water content in cosmetics influences susceptibility to microbial contamination, which can represent health risks, as well as deterioration. The aim of this work was to isolate and identify filamentous fungi in samples of face powder, foundation, blush, and lipstick of eight popular brands in Brazil. A study of their composition identified a total of 106 ingredients. Of the products, 75% of the samples showed contamination by filamentous fungi. *Penicillium* was the most prevalent, followed by *Rhizopus* and *Scopulariopsis*. The presence of these pathogens suggests misuse in the production and manipulation of these cosmetics as well as high risk to users' health.

keywords: Cosmetics, Secondary contamination, Microbiologically susceptible materials, *Penicillium.*

RESUMO
A maquiagem é usada por uma grande parcela da humanidade e geralmente é mal utilizada ou armazenada de forma incorreta, favorecendo a proliferação microbiana. O teor de água nos

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cosméticos também influencia a suscetibilidade à contaminação, podendo representar riscos à saúde e deterioração. O objetivo deste trabalho foi isolar e identificar fungos filamentosos em amostras de pó facial, base, *blush* e batom de oito marcas populares no Brasil. Um estudo de sua composição identificou um total de 106 ingredientes. Dos produtos, 75% das amostras apresentaram contaminação por fungos filamentosos. *Penicillium* foi o mais prevalente, seguido por *Rhizopus* e *Scopulariopsis*. A presença desses patógenos sugere uso indevido na produção e manipulação desses cosméticos, além de alto risco para a saúde dos usuários.

Palavras-chave: Cosméticos, Contaminação secundária, Materiais susceptíveis, *Penicillium.*

1 INTRODUCTION

Aesthetic beauty often presents itself as a primary factor for human beings, with an intense search for beauty stereotypes pre-established by society since ancient times (Hardy and Rollinson, 2011). The cosmetic, a word of Greek origin, whose meaning refers to the act of adorning, is defined by the Brazilian health regulatory agency as any preparation consisting of natural or synthetic substances, of external use on the different parts of the human body, skin, capillary system, nails , lips, external genitals, teeth and mucous membranes of the oral cavity, with the sole or main purpose of cleaning, perfuming, altering their appearance and/or correcting body odours and either protecting or maintaining them in good condition (ANVISA, 2015). Brazil ranks as the world's fourth largest consumer market for cosmetics, with an emphasis on makeup, responsible for a turnover of US$ 30 billion in 2018 (EUROMONITOR, 2018, Aslam et al., 2017).

Water-containing cosmetics, as well as soaps, deodorants and antiperspirants, are more microbiologically susceptible than alcoholic preparations. Susceptibility can be determined by the amount of water contained in a certain product. Semiaqueous materials are considered products of high susceptibility, while compact powders, pressed powders and makeup sticks are categorized as having medium susceptibility (Mitsui, 1997).

After being opened or partially used, cosmetics are subject to secondary contamination, a term used for events that promote microbial proliferation. The development of microbes in makeup, as well as in other cosmetics, occurs via two major factors. The first, is related to incorrect handling or failure to conduct good hygiene practices during the application of makeups. This permits the deposit of debris derived from the skin, which favours conditions for microbial establishment and proliferation, even if the products contain preservatives (Dadashi and Dehghabzedeh, 2016, Fiume et al., 2014).

The second and most important factor of microbial proliferation in makeup comes from the suspended particles in indoor air, including dust, microorganisms and spores (Irga and Torpy, 2016). Air quality plays a crucial role in microbial proliferation in makeup, especially in humid environments such as bathrooms, where most consumers store cosmetics (Picot-Guéraud et al., 2015,

Hamada and Abe, 2009). The investigation of indoor air as a vehicle for human pathogenic fungi is a subject that holds interest (Hayleeyesus and Manaye, 2014, Gutarowska et al., 2012), however the indoor contamination of materials is a problem difficult to prevent (Sattar and Bact , 2016).

The most studied microorganisms contaminating makeup are bacteria, particularly staphylococci and enterobacteria. Pathogenic filamentous fungi are an important object of study, especially since these organisms are associated with opportunistic infections, as well as mycotoxin poisoning (Peraica, et al., 1999; Hayleeyesus and Manaye, 2014). The present work aimed to isolate and identify filamentous fungi from powder and semiaqueous makeup.

2 MATERIAL AND METHODS

2.1 MAKEUP AND LABEL ANALYSIS

Eleven powder and semiaqueous sorts of makeup from eight popular brands in Brazil were evaluated: two products from national brands and six foreign brands, kindly provided by users. The products were separated by class, receiving coding consisting of a letter, followed by Arabic numerals: face powder (A), foundation and/or blush (B) or lipstick (C). The packaging and respective products were analysed for condition, expiration date, and composition information on the label. The ingredients were listed and classified according to the information provided by The Cosmetic Ingredient Review (CIR) website.

Isolation and identification of filamentous fungi

In a laminar flow chamber previously sterilized, the makeups were swabbed at different points on the product surface and/or at the edges of the packages in contact with the products. The collected material was transferred to test tubes containing Sabouraud broth, to which was amoxicillin 50 mg/L (Sigma-Aldrich, USA) and then the material was incubated at 37°C for 72-96h until growth.

After this, the grown cells were spread on the Sabouraud-Dextrose Agar (SDA) surface and incubated again. Isolates were identified by the microculture method in SDA at room temperature for 15 days. Fragments of the grown fungi were inoculated in the center of a Petri dish, containing SDA. The microstructures were evaluated by the cover slip culture technique, with observations under an optical microscope every 24 h, using cotton blue lactophenol dye (Genhardt et al., 1994).

Determination of water contente

The test was based on the ANVISA gravimetric method (2008). Briefly, incubation was carried out at 87°C for 48h. The water content was calculated using the formula: U (%) = [(wet weight - dry weight) / wet weight) x 100].

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3 RESULTS

3.1 CHARACTERIZATION OF MAKEUP

All samples had already been opened and used, the labels, preserved and the packaging showed no damage. According to the information provided on the labels, for all the samples taken together, a total of 106 ingredients were involved, of different natures and functions. Many ingredients were common to most of the products, such as synthetic and natural polymers, mineral compounds, alcohol, carbohydrates, modified fatty acids, amino acids, aromatic compounds, essential oils, waxes and plant extracts. Six different preservatives were identified. The most prevalent was phenoxyethanol, followed by parabens or an association of two parabens (methylparaben and propylparaben).

Three of the samples were beyond expiration date: A1 (face powder), B1 (blush) and C1 (lipstick). These samples were analysed because they were still in use, in order to inform the users about possible contamination. The specific results of the expired samples are not presented in this study, however all samples were contaminated by *Penicillium* sp.

The number of filamentous fungi isolated and the water content in the samples are summarized in Table 1. Six of the eight samples analysed (75%) were contaminated by 19 phenotypically different isolates of filamentous fungi from 3 genera: 11 types of *Penicillium* sp., 5 types of *Rhizopus* sp. and 3 types of *Scopulariopsis* sp. The foundation blush samples were the ones that presented the highest contamination. The water content in the samples ranged from 1.1% to 73.0%. The naturally driest products, such as powder, had very low water content, ranging from 1% to 2%. Only two samples did not show contamination by filamentous fungi: samples A4 (blush) and C2 (lipstick).

Table 1. Fungal contamination and water content from samples of powder and semiaqueous makeup. The numbers in parentheses indicate the variety of morphological aspects that suggest different species of fungi

Sample	Water content (±0.1%)	Genera
A2 (face powder)	1.1	*Penicillium* sp. (2)
A3 (face powder)	2.1	*Penicillium* sp. (1)
A4 (face powder)	1.8	None
B2 (blush)	62.0	*Scopulariopsis* sp. (3) *Penicillium* sp; (3) *Rhizopus* sp.(3)
B3 (foundation)	42.0	*Penicillium* sp.(1)
B4 (foundation)	73.0	*Rhizopus* sp. (2) *Penicillium* sp. (2)
B5 (blush)	61.0	*Penicillium* sp. (2)
C2 (lipstick)	1.9	None

Figure 1. Aspects of the reproductive mycelia of some fungal isolates

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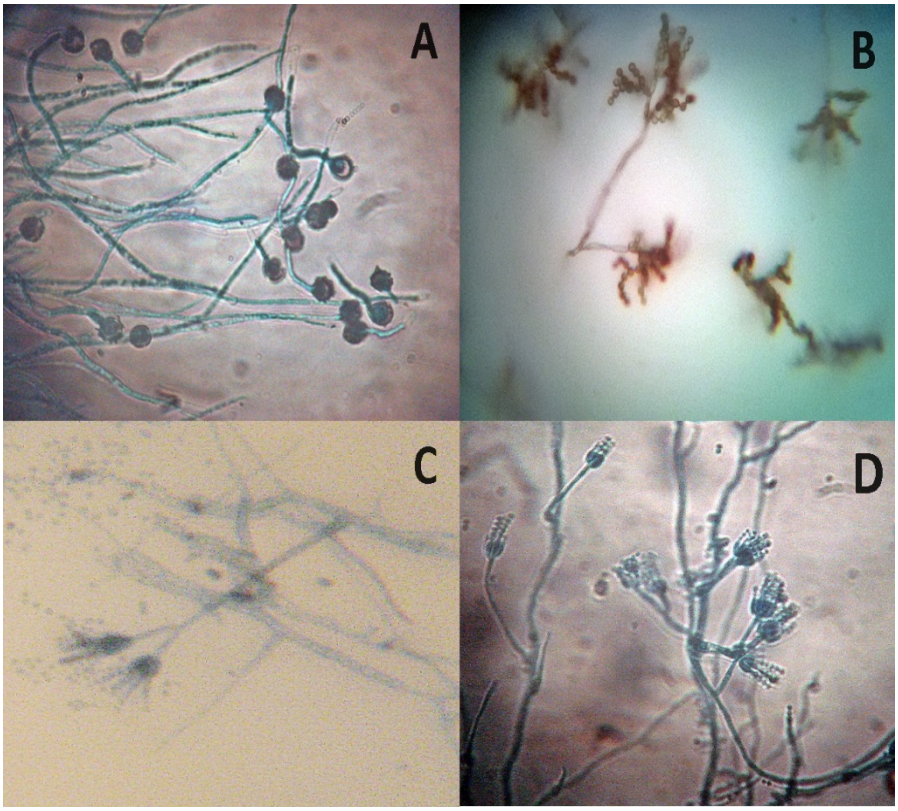

A- *Rhizopus* sp. (sample B4 – foundation), **B** - *Scopulariopsis* sp. (sample B2 – blush) **C** – *Penicillium* sp. (sample – foundation) e **D** - *Penicillium* sp. (sample A2 - face powder)

4 DISCUSSION

In the course of using makeup, microorganisms, especially filamentous fungi, can be introduced as direct contamination or cross-contamination and, consequently, fungal development may lead to biodeterioration, as well as causing changes in the physical-chemical properties, such as irregularities in texture and viscosity, phase separation, changes in pH, colour and odour (Zabielska et al., 2017).

Indoor environments may present a concentration of spores and fungal cells in the air ranging between 10^2-10^3 CFU/m^3 (Hayleeyesus et al., 2014). This may happen when the air circulation is irregular and hazarded (Irga and Torpy, 2016). Fungal contamination on the surface of makeup, related to exposure when manipulated, will depend on several factors, such as the supply of nutrients, temperature and water content, which are crucial to the development of fungi in makeup. The water content found in cosmetic formulations is intrinsically related to the growth of fungi. The higher the water content, the more susceptible the product is. As an example, in semiaqueous formulations, the presence of water in oil emulsions with high concentrations of solutes and low water activity creates favourable conditions for fungal growth (Elmorsy and Hafez, 2016).

Nineteen phenotypically distinct isolates from three genera of filamentous fungi were detected in the samples. *Penicillium* sp. was the most prevalent organism in the study, occurring in 75% of

samples. This finding may be due to the fact that *Penicillium* spp. are dominant in house dust (Ren et al., 1999) and thus well distributed throughout an environment (Bullerman, 2003). The degradation of makeup by *Penicillium* spp. results from the expression of different enzymes, such as: exo-β-1, 4-glucanase, endo-β-1,4-glucanase and β-glucosidase (Babalola and Eze, 2015). Certain species of *Penicillium* pose health risks, since they are associated with the production of mycotoxins, especially ocratoxin A and citrinine. In addition, micromolar concentrations of both mycotoxins are highly nephrotoxic (Geisen et al 2018). The presence of mycotoxins in makeup and their respective association with the user's health has not yet been well-elucidated. In addition, colonization by resident microbiota fungi, such as *Malassezia* spp, may not cause morbidity, but in certain circumstances they can cause irritability and dermatitis (Edwards et al., 2015). However, *Malassezia* was not identified in this study.

Rhizopus was the second most prevalent genus found in the makeup, specifically in the foundation and blush samples. *Rhizopus* species are described as lipophilic, producing different lipases, which are associated with the deterioration of makeup with a certain lipid content, such as foundations and blush (Ghosh et al., 1996). As primary pathogens they are responsible for the most common human zygomycosis, affecting healthy individuals, whose infections can evolve to the lungs and brain (Ribes et al., 2000).

Scopulariopsis was the third prevalent genus. It was identified in only one sample. This genus is important in cases of distal and proximal onychomycosis (Tosti et al., 2003), and may also be responsible for systemic mycoses (Gariano and Kalina, 1997; Patel et al., 1993). There is no confirmation about the relationship of *Scopulariopsis* sp. to the deterioration of cosmetics, although the genus has already been recovered from some of these products (Korsten and Anelich, 2006).

Face powders have a higher content of mineral compounds and low water content. Half of the samples, however, were contaminated by filamentous fungi. It is important to note that these products have between 10-20% of organic agents, usually in the form of polymers (Mohuddin, 2019). As well, frequency of use may allow the deposit of residues of keratin, sebum and other skin debris onto the product, providing essential factors for fungal growth (Hamada and Abe, 2009). Non-preferred substrates, such as talc and kaolin, may allow fungal growth. In addition, filamentous fungi can use the humidity of the environment to their advantage, ensuring growth on the surfaces of cosmetics containing low-water (Omorodian et al., 2014; Dashen et al, 2011; Kulkarni et al., 2011).

The average humidity in the foundation and blush samples was 59.2%, categorizing them as semiaqueous preparations. This implies that humidity is a favouring path for filamentous fungi growth. It is noteworthy that in 90% of the foundation and blush samples, contamination was observed. Even though high water content was found in those preparations, these cosmetics contained

substances of animal origin as well as vitamins and proteins, essential factors for promoting deterioration as consequence of microbial proliferation (Rabasco and Rodrígues, 2000). Additionally, microbial growth may occur via two mechanisms. The first is related to the use of these molecules as a nutritional source; and the second mechanism, when the ingredients interact with the conservation agents, leads to their inactivation (Smart and Spooner, 1972).

Of the preservative agents identified in the samples, the most prevalent was phenoxyethanol. This compound is not considered so effective compared to other preservative families, such as parabens (Lundov et al., 2009). Based on this premise, the sample B4 was expected to be absent or have less contamination, since the preservative in the formulation was methylparaben. However, in both of the samples, two isolates with distinct phenotypes of *Rhizopus* sp. and *Penicillium* sp. were identified.

Fungal contamination was also detected in samples A2 and B5, where the preservatives were a combination of two parabens (meylparaben and propylparaben), inferring that the presence of parabens in makeup may not guarantee efficiency in preventing microbial proliferation. This finding was also reported in a previous study (Glavač and Lunder, 2018).

Preservatives in cosmetics are used in low concentrations to keep the product microbiologically pure during manufacture, packaging, storage and especially during use by consumers (Herman et al., 2019). Some organisms may develop resistance and cross-resistance to antibiotics, however, bacteria have been more investigated, to the detriment of filamentous fungi (Martins et al., 2018, Shaqra et al., 2014, Shaqra and Al-Grom, 2012). This may reflect that bacteria and some yeasts are recognized as parameters for the microbiological control of cosmetics in most health legislation in different countries, including Brazil (ANVISA, 1999).

It is the industry's responsibility to ensure product quality. From the time of purchase on, the user has the responsibility to store the product in suitable conditions in order to prevent contamination (Cornélio and Almeida, 2020). This knowledge is important for the dermatologist who treats patients suffering from dermatological infections of unknown origin (Michalek and John, 2019).

The present work highlights the fact that different makeup products may be vehicles for mycoses. The filamentous fungi detected in the samples may also represent health risks, especially since filamentous fungi are not indicator pathogens in cosmetic control. In addition, we suggest that the cosmetics industry provides information about good hygiene practices for the handling of makeup, including information that sharing makeup should be avoided, and that storage must occur correctly, aiming to guarantee the conservation and safety of these products after opening.

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5 CONCLUSION

Three important genera of filamentous fungi, *Penicillium*, *Rhizopus* and *Scapulariopsis*, were identified in powder and semiaqueous cosmetics, including all those with a water content less than 10%, suggesting misuse by consumers, as well as representing a potential health risk. We propose that further studies to adjust the concentration of preservatives or to design novel conservative agents be tested with these three genera, since they may also be associated with product deterioration

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