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Microbiological Contamination of In-Store Lipstick Testers Available to the Consumer

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Abstract

The microbiological contamination of pharmaceutical and cosmetic products represents a public health problem because it can cause infections. Before purchasing a particular cosmetic, many consumers test it on their own skin, increasing the susceptibility to microbiological contaminations. We hypothesize that these lipstick testers available to pharmacy consumers represent a potential source of microbiological contamination. This study analyzed the microbiological quality of 30 lipstick samples, randomly collected, from different manufacturers, available to the consumers for trials in pharmacies in southern Brazil (fifteen samples were collected in Casca, five in Ciríaco and ten in Passo Fundo). The microbiological quality of the lipsticks was evaluated by: total count of viable bacteria and mold and yeast, and presence of pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It was verified that amounts ranged from 1.0 x 10^1 to 1.9 x 105 CFU/g of viable bacteria and from 1.0 x 10^1 to 7.3 x 10^3 CFU/g of molds and yeasts on the lipsticks. 54.33% and 40% of the samples were disapproved by the total count of viable bacteria and molds and yeasts, respectively. *S. aureus*, *Aspergillus* sp. and *Cladosporium* sp. were also found. Although the waxy composition of the lipsticks hinders microbial contamination this research reveals a large number of microorganisms. Our hypothesis that the lipsticks have a high microbiological load was confirmed. Opportunistic pathogenic microorganisms can become infectious agents in patients with compromised immune systems. The use of disposable applicators is proposed as an alternative way to avoid microbiological contamination of the cosmetic products.

Keywords: Fungi. Microbial contamination. Preservatives. Quality control. Staphylococci.

INTRODUCTION

Cosmetics, personal care products and perfumes are preparations consisting of natural or synthetic substances, for external use in the 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 odors and/or keeping them in good condition1.

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reports that Brazil is the fourth largest global consumer market for personal hygiene, perfumery and cosmetics, with 6.2% market share and it is responsible for a total of USD 30 billion in consumer sales in 2018, according to a study by Euromonitor. Brazil is only behind the USA (18.3% with USD 89.5 billion), China (12.7% with USD 62 billion) and Japan (7.7% with USD 37.5 billion). In Brazil, the consumption of makeup is fifth in a worldwide ranking[2].

The physicochemical and microbiological tests performed by manufacturer or importer of the cosmetics guarantee the safety, the promotion and protection of the users' health avoiding the transmission of diseases[3,4]. Cosmetovigilance monitors problems related to use, quality defects and undesirable effects after the marketing of the products[5,6]. Excessive microorganisms go beyond to implicate health risks. The endotoxins and metabolites produced by microorganisms may cause abrasion, irritation and allergies on the skin and may lead to product deterioration altering the smell, color, viscosity, and performance[7]. Contaminated cosmetics (moisturizing body milk and mouthwash) led to outbreaks of nosocomial infections[8–11].

Besides that, cosmetic products are rarely suspected as the cause of skin infections, for example in individuals with prior skin diseases such as allergic dermatitis. Manufacturers do not communicate to the public when their cosmetics are returned due to excessive contamination. Furthermore, it is unknown whether consumers are aware that their products are contaminated or they just throw them away because of obvious changes, for example in odor. The in-use contamination happens often without noticeable changes in the product making it impossible to know if the products are contaminated[12].

The production of cosmetics is regulated by the *Agência Nacional de Vigilância Sanitária* and, according to the legislation, one of the technical requirements necessary for the sale of cosmetics is a microbiological analysis. Manufacturers must carry out all physical-chemical and microbiological tests of raw materials and finished products[13]. The Brazilian Pharmacopoeia brings the microbiological specifications for products used topically where the *Staphylococcus aureus* and *Pseudomonas aeruginosa* must be absent, and a maximum of 2 x 102 UFC/g of bacteria and 2 x 101 UFC/g of fungi is accepted[14].

The lipstick contains wax, oils, antioxidants, and emollients. Microorganisms grow better in aqueous media rather than oily media and the survival of microorganisms is directly associated with the minimum necessary level of water activity[15]. Therefore, the composition of the lipstick does not favor microbial growth.

The shared use of makeup can lead to a high microbial contamination attributed, in the majority of cases, to contact with the skin, mucosa or environment. Lipstick is a popular cosmetic and prior to its purchase it is common for the consumer to sample it to evaluate texture and color. Lipsticks exposed in pharmacies and markets are used repeatedly by different clients, and this habit can lead to an excessive number of microorganisms on the product, including pathogens. Our hypothesis is that the lipsticks available as testers have a high microbiological load. The microbiological contamination of cosmetics can bring risks to consumer health, therefore the present work sought to verify the microbiological quality of lipsticks available to the consumer for trial in pharmacies.

METHODOLOGY

This work is an experimental study. The analyses were performed in the Biological Medicines Control Laboratory of University of Passo Fundo (Passo Fundo -RS).

Samples

Thirty samples of lipsticks of different manufactures were collected randomly, from pharmacy displays in the northern region of Rio Grande do Sul (RS) (fifteen samples were collected in Casca, five in Ciríaco and ten in Passo Fundo) in sterile plastic bags with the aid of a sterile scalpel.

Total Viable Microbial Count

0.5 g of each lipstick sample was solubilized in 4.5 mL of sterile isopropyl myristate with the aid of a tube shaker (Biomatic) for 3 minutes and subjected to an ultrasound bath (Unique/ USC 2850-A) for 15 minutes. Thereafter, 1:10, 1:100, 1:1000 and 1:10000 dilutions were prepared using the same diluent. 0.5 mL of the dilutions were pipetted and added into Petri dishes containing approximately 15 mL of Soy-Casein Agar for bacterial count or Sabouraud Dextrose Agar for mold and yeast count. The dilutions were added by the Pour plate method. The incubation conditions were 5 days at 35°C ± 1°C for bacteria (oven Biopar®) and 7 days at 25°C ± 1°C for fungi (oven De Leo®). The growths were evaluated daily. The total count of colony forming units (CFU) per g was calculated (14). As a negative control, a plate with only culture medium was used.

Research of the pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*

0.3 g of each sample was placed in enrichment broth (5% peptone, 1.5% yeast extract, 1.5% meat extract, 3.5% NaCl, 1% dextrose, 3.68% dibasic potassium phosphate, 1.32% monobasic potassium phosphate, 1% tween and water) for 48 hours at 35°C. Then, the tubes were placed in an ultrasonic bath for 20 minutes. After incubation, the broths were spread with a platinum handle over the surface of Sal-Mannitol Agar for S. aureus research and Cetrimide Agar for P. aeruginosa research. The Petri dishes were incubated at 35°C for 48 hours.

Colonies of S. aureus in Agar Sal-Mannitol present smooth consistency surrounded by a zone of yellow color. To confirm the identity of the suspect colony, the microorganism was submitted to Gram staining, catalase and coagulase assay[16].

Search and identification of filamentous fungi

The identification of the filamentous fungi was based on the macroscopic and microscopic characteristics of the microorganism previously isolated in dishes of Sabouraud Dextrose Agar. The macroscopic characteristics of growth (obverse and reverse) were analyzed. The microculture technique was performed for microscopic analysis (Olympus / CH20BIMF1100, 400x magnification)[17].

RESULTS

According to the Brazilian Pharmacopeia, the acceptable microbiological limits of total viable bacteria of freshly marketed products are 2 x 10^2 CFU/g or mL; for mold and yeasts, the limit is 2 x 10^1 CFU/g or mL (14). Considering this, 54.33% of the products (16 samples) analyzed were reproved in total viable bacteria counts and 40% (12 samples) failed regarding mold and yeast counts. Although the percentage of disapproval is based on specifications required for products without use, the result shows microbiological contamination of the lipstick samples available to the consumers for trial. The absence of bacterial growth in 6 samples and fungal growth in 16 samples may be related to the fact that these samples were exposed for less time or were not used. These data were not made available to the researchers because the establishments also did not know when the lipsticks were initially made available for consumer testing.

Total viable bacteria count in the lipstick samples ranged from 0 to 1.9 x 10^5 CFU/g after incubation. The mold and yeast count ranged from 0 to 7.3 x 10^3 CFU/g after incubation (Table 1).

The sample A12 was contaminated with *S. aureus*.

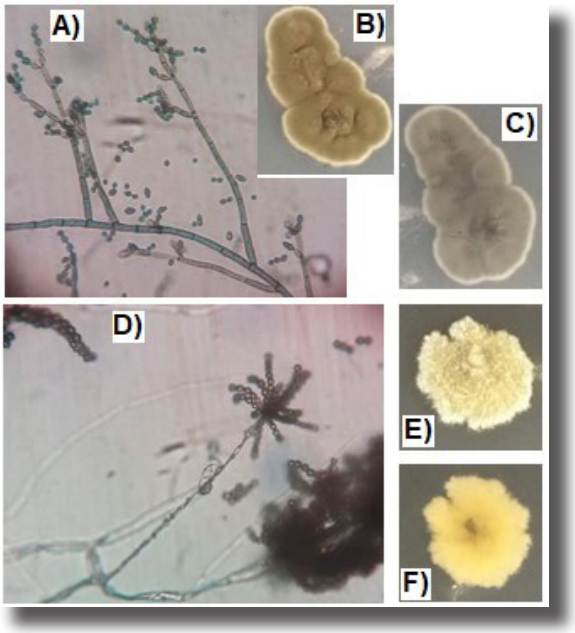
The presence of coagulase-negative Staphylococcus and *Bacillus* sp. was also detected (data not shown). The Gram-positive bacillus, sporulated, with growth after heat shock at 70°C and that reacted to catalase was considered *Bacillus* sp.[18]. None of the samples presented Pseudomonas aeruginosa, which should be also absent according to the specifications[19].

The filamentous fungi identified in the lipstick were *Cladosporium* sp. and *Aspergillus* sp. (Figure 1).

Table 1 – Total count of viable bacteria and mold and yeast colony forming units (CFU)/ g of lipsticks samples exposed in the South of Brazil pharmacies (Passo Fundo, 2017).

Sample	Total viable bacteria count (CFU/g)	Total mold and yeast count (CFU/g)
A1	1.9 x 10^5 *	7.3 x 10^1 *
A2	8.5 x 10^4 *	7.4 x 10^2 *
A3	4.0 x 10^2 *	0
A4	3.6 x 10^4 *	0
A5	0	4.7 x 10^2 *
A6	4.1 x 10^2 *	0
A7	5.0 x 10^2 *	0
A8	3.9 x 10^2 *	0
A9	0	0
A10	3.6 x 10^4 *	0
A11	2.0 x 10^4 *	7.3 x 10^3 *
A12	1.5 x 10^2	0
A13	1,0 x 10^4 *	2.7 x 10^1 *
A14	9.1 x 10^4 *	0
A15	5.0 x 10^1	1.0 x 10^1
A16	0	3.7 x 10^1 *
A17	0	0
A18	1.1 x 10^3 *	1.4 x 10^3 *
A19	5.5 x 10^1	0
A20	1.0 x 10^1	3.3 x 10^1 *
A21	4.5 x 10^2 *	3.7 x 10^1 *
A22	1.1 x 10^2	0
A23	5.4 x 10^4 *	0
A24	1.5 x 10^2	1.4 x 10^2 *
A25	5.0 x 10^3 *	0
A26	5.5 x 10^1	4.0 x 10^1 *
A27	1.4 x 10^2	2.3 x 10^1 *
A28	0	0
A29	0	1.0 x 10^1
A30	1.3x 10^5 *	0

* Samples demonstrating amount of bacterial and fungi above specified limits by Farmacopeia[14]



Legend: A) Cladosporium sp. - microscopy (400x); B) Verse and C) Reverse of Cladosporium sp. colony on Sabouraud Dextrose Ágar D) Aspergillus sp. - microscopy (400x); E) Obverse and F) Reverse of Aspergillus sp. colony on Sabouraud Dextrose Agar.

Figure 1 – Filamentous fungi isolated from lipsticks samples displayed for trial in the North of Rio Grande do Sul pharmacies (Passo Fundo, 2017).

DISCUSSION

The cosmetics reached the market complying with microbiological specifications; however, this quality can be lost after contact with the consumer and/or environment. Studies report microbiological contaminations of cosmetics after use. Onurdağ, Özgen, and Duygu (2010) analyzed 73 cosmetic samples (eyelash masks, eyeshadows, bases and lipsticks), ten were contaminated, and the presence of *Candida* sp., *Staphylococcus aureus* and *Escherichia coli* was also detected. Analysis of cosmetic kits of shared use with 2802 samples (eyeshadows, lipsticks, bases and other unspecified products) obtained in commercial establishments in the United States detected contamination of 50% of cosmetics where 5% contained legally unacceptable microbial loads[21]. Moreover, 67% of 1345 eyeshadows exposed to consumers as testers for a period of 6 to 24 months were contaminated[22]. The presence of *S. aureus*, *S. epidermidis*, *S. warneri*, *Bacillus* sp., *Klebsiella pneumoniae*, *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. was detected in eyeshadow samples, eyelash masks, and eyeliners[23]. Furthermore a recent work performed microbial analysis of used lipsticks (n = 80) and found 70% growth of microorganisms, principally Gram-positive organisms (93%) such as *S. aureus*, *S. epidermidis*, *S. saprophyticu* and *Streptococci genus*[24].

Legislation requires an absence of the S. aureus in freshly produced topically used products, so sample A12 would be disapproved[14,25]. The genus Staphylococcus was present in 32 species, many of them colonize the human skin and mucous membranes. Staphylococci are classified according to the coagulase test, among the coagulase-positive strains S. aureus is highlighted. This species is the most virulent of the genus and is associated with diseases mediated by toxins (food poisoning, toxic shock syndrome, scalded skin disease) and infectious processes such as impetigo, folliculitis, boil, carbuncle, skin wound infections, bacteremia, endocarditis, pneumonia, empyema, osteomyelitis, and septic arthritis. The dissemination of this bacterium can occur through direct contact between people or exposure to contaminated fomites[18], such as the sample analyzed in this study. Other studies also reported the presence of S. aureus in cosmetics (eyelash masks, eyeshadows, bases and lipsticks[20] and other unspecified products[21]).

The rigor of the microbiological quality control required in the cosmetics production avoids that these products reach the market with the degree of contamination detected in the study. In addition, the basic

composition of the lipsticks is wax, oil, pigments, fragrance, alcohol, preservatives and antioxidants[15,26], components that do not favor microbial growth due to lipophilic characteristics, with low water content. Therefore, the presence of *S. aureus* and high microorganism counts in the samples is related to the use of the lipsticks by many people. The microbial count found in the lipsticks was high, showing that shared use of cosmetics could bring risks to the health.

Filamentous fungi could cause damage through the production of toxins or by triggering infectious processes. Mycotoxins may cause allergic reactions, cutaneous necrosis, leukopenia, immunosuppression, cancer, impaired hepatic or renal function, and neurological damage[27,28]. The genus Aspergillus found in the samples under study is one of the main producers of mycotoxins[27]. This genus is easily dispersed in the environment through its reproductive structures, it is found in soil, water, and air. Beside the mycotoxins production, it can trigger infectious processes, like the aspergillosis in immunocompromised patients. It grows fast, developing in 3 days with a white surface that becomes yellow, green, brown or black, depending on the species. The reverse could be white, gold or brown, and its texture is velvety or cottony. Its conidiophore has clusters of conidia at the tips forming a kind of swollen vesicle[17,29]. *Cladosporium* sp. is a filamentous fungus found in the environment[30] that can trigger allergic processes and subcutaneous mycotic infections. It grows in 7 days with greenish, brownish or grayish-black velvety surface, piling up and becoming slightly folded. The reverse is black. It has dark septate hypha and the brown, smooth and oval conidia[29]. Other study found *Aspergillus fumigatus*, *Penicillium* sp. and *Cladosporium* sp. in cosmetics powders[31].

CONCLUSION

The lipsticks exposed to consumer trials presented microbial contamination, and are, consequently, not safe. The hypothesis of this study was confirmed. 54.33% and 40.0% of the samples demonstrated excessive amounts of bacteria and fungi, respectively. The microorganisms S. *aureus.*, coagulase-negative *Staphylococcus*, *Bacillus* sp., *Cladosporium* sp. and *Aspergillus* sp. were detected. A practical and low-cost alternative to solve this problem is the implementation of disposable brushes for the application of the product. This could reduce the microbiological contamination since it avoids the direct contact of the product with the consumer.

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