DRUG ANALYTICAL RESEARCH
ISSN: 2527-2616
www.seer.ufrgs.br/dar

Microbiological evaluation of packaging obtained from cosmetics production establishments

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Cosmetics are commonly placed in plastic packaging, which has the essential function of protecting the product. However, cosmetics packaging can contribute to the total microbial load of the product, leading to product deterioration and putting the health of users at risk. The main purpose of this research was the microbiological evaluation of cosmetics packaging obtained from cosmetics production establishments, one industry and four compound pharmacies (pharmacies that sell custom-made medicines), from Vale do Taquari, Rio Grande do Sul, Brazil. Based on the results of a questionnaire applied at the moment of packaging acquisition, previous sanitization is performed for compound pharmacies packaging, with different handling procedures, whereas in the industry the process of packaging sanitization does not occur. However, this sanitization was not considered enough to guarantee the complete absence of microorganisms in the packaging, since a microbial growth was observed for 6 out of the 16 evaluated containers obtained from compound pharmacies, and for 2 out of the 4 industrial containers. Although several containers were contaminated, few CFU/mL (from 1- 4) were isolated from each sample. *Staphylococcus* bacteria were the most common type of contamination, with *S. aureus* being identified in one sample. Not performing prior sanitization or storing packaging with the lid open seems to lead to a higher microbial contamination of the packaging. Hence, both the sanitization process and the storage of packaging play an important role in guaranteeing packaging and cosmetics quality. The present investigation points for the importance of microbiological quality control of cosmetics packaging.

Keywords: Cosmetics; product packaging; bacteria; fungi; quality control.
Article received at 10/05/2023 and accepted at 12/12/2023.
https://doi.org/10.22456/2527-2616.135918

Introduction

Cosmetic products are formulations intended for use in different superficial parts of the body, aiming at good condition maintenance, skin protection, odor correction, and beauty highlight, enhancing self-esteem of its consumers (1). Cosmetics have been used since ancient times by many people around the world and, nowadays, the industry of cosmetics is considered a dynamic and growing sector, with an annual growth rate of 5.1% from 2021 to 2030 (2,3).

The cosmetics are placed in containers that are often used to attract consumers, but also have the important function of protecting the product (1,3,4). The primary packaging is the one in direct contact with the product (5). Overall, cosmetic products are stored in packaging of different materials, with plastic being the most used material, due to its high quality, flexibility, resistance to breakage, light weight, and low-cost (6). A frequent used plastic material for the cosmetics packaging is polyethylene of low density, which is a thermoplastic resin obtained by polymerization of ethylene (7). Different physical configurations of the cosmetics packaging can be found such as boxes, jars, bottles, flasks, sachets, tubes, aerosol propellants, etc. (1).

The quality of these containers should be examined regarding visible physical defects, as well as regarding the required specifications according to the Good Manufacturing Procedures for Cosmetics (8). Important tests for pharmaceutical packaging include visual inspection (cleanliness, defects), tests to identify the material, dimensional tests, physical tests, chemical tests and microbiological tests (9). Purity and stability of the packaging material should be known, in order to prevent migration of plastic and additives from the packaging to its content (3,7). Also, it is important to mention that the packaging can contribute to the total microbial load of the product (10,11). The recipients for packaging should be clean before the filling of the products (12), however there is no need for sterilization of the packaging, considering non-sterile products (11).

Although the majority of cosmetic products do possess preservative agents in its composition, those could be insufficient to prevent microbiological growth, especially in formulations with high quantity of water, or with vegetable or animal raw materials, leading to secondary contamination. The contamination of the product during manufacturing and packaging are considered the primary contamination (1).

Several bacteria, fungi and yeast can be found in cosmetics, being the most frequent *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Burkholderia cepacia*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Enterobacter gergoviae*, and *Serratia marcescens* (1). A packaging infected by microorganisms may become a problem, leading to product deterioration and also putting the health of users at risk. Pathogens, when present in cosmetic

products, may trigger a variety of health issues, such as allergies, skin infections and even severe health problems, especially if the skin is injured (1,10).

Pizzolitto and co-workers (2001) evaluated the internal surfaces of aluminum tubes (unlined and lined with epoxi resin), regarding microorganisms' adherence. Through microscopy evaluation, it was possible to verify that the packaging allowed *Streptococcus agalactiae*, *Staphylococcus aureus*, *Candida albicans*, and *Acinetobacter lwoffii* adherence (13). Fiorentino and co-workers (2008) evaluated the microbiological quality of glass and plastic containers for cosmetics and medications. For the plastic containers, *Aspergillus fumigatus* was detected. For glass containers, *Aureobasidium sp* and *Cladophialophora sp* were isolated (11). Only one bacterial colony was detected, which was from the evaluation of a glass container. A lack of scientific literature regarding the microbiological quality of packaging is observed.

In view of the above, this study aimed at evaluating the microbiological condition of cosmetics packaging obtained from industry and compound pharmacies, relating the results to the acquisition, cleaning and storage data obtained from companies.

Experimental section

Materials

NaCl was obtained from Vetec, Ethanol 96° GL from Synth, Plate Count Agar (PCA), Soybean Casein Broth and Mannitol Salt Agar from Oxoid, Cetrimide Agar and MacConkey Agar from Acumedia. The reagents for Gram staining (violet crystal, fuchsin and lugol's solution) were obtained from LaborClin, while hydrogen peroxide was purchased from Dinâmica.

Packaging acquisition

The companies included in this study were identified by letters, being A, B, C and D compound pharmacies and E being a cosmetic industry, all located in Vale do Taquari, Rio Grande do Sul, Brazil (cities: Arroio do Meio, Lajeado, Teutônia and Venâncio Aires). Wide-mouth plastic jars of different capacities that were considered ready for the filling step were acquired from these establishments. From each company, four containers were acquired, reaching a total of 20 units. The packaging was packed in a closed plastic bag for transportation from the companies to the laboratory for analysis. They were open or closed in such bags according to the establishment storage procedures (A, B, and C closed jars, and D and E opened jars, without the lid). A questionnaire was applied to the technician responsible for the company when the containers were collected in order to verify the company protocols for sanitizing and storage of the cosmetics packaging. This project was approved by the Ethics Committee of UNIVATES (project number 1.972.941).

Sample collection for microbiological analysis

First, the external sanitization of all containers was performed with 70% alcohol, and then a visual analysis of the inside part of the packaging was performed. From each company, two containers were evaluated by rinsing the packaging with sterile saline solution (0.9% NaCl, 10mL) and two were evaluated by using a sterile swab previously immersed in sterile saline solution (0.9% NaCl). The containers were properly identified by letters (A to E), according to their origin, followed by a number (1 to 4) and their respective collection method (R - rinsing or S - swab). For both sampling techniques, care was taken so that the internal parts of the packaging (that have contact with the formulation) were evaluated. The containers were sampled exactly as received, according to the storage of the establishments (A, B, and C closed jars, and D and E opened jars, without the lid). After the sampling procedures, the rinsing solution (100 µl) or the used swab were applied on the surfaces of petri dishes containing the non-selective PCA. All tests were performed in the laminar flow cabin, under aseptic conditions, using sterilized materials in order to prevent contamination, and the petri dishes were incubated at 37°C for 5 days in order to verify the development of bacterial and fungal colonies (14).

Evaluation of colony-forming units (CFU) in non-selective medium

After 5 days of incubation, the plates were removed from the incubator and the colonies formed were circled and counted manually, with the naked eye. Such colonies were evaluated by the gram staining and regarding the presence of pathogens as described below.

Gram staining

The Gram staining technique was used to assist in the identification of possibly pathogenic colonies, and it was performed by preparing glass slides with the colonies detected in the PCA plates. The slides were colored and observed under a microscope, with an immersion objective.

Evaluation of pathogens in selective media

The colonies detected in PCA plates were inoculated in tubes containing sterile Soybean Casein Broth for subsequent confirmation on selective agar. The tubes were then incubated at 37°C for 2 days. After such period, a sample was seeded using the streak method on the selective agars (Manitol Salt, Cetrimide and MacConkey agars) for identification of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* whose presence are not allowed in cosmetics (5). The plates were incubated at 37°C for 5 days. To confirm the presence of *Staphylococcus aureus*, the biochemical test of Catalase, which consists of adding a drop of 3% hydrogen peroxide to the colony of the microorganism, was performed, with subsequent formation of bubbles in case of a positive test.

Results and Discussion

Questionnaire results and visual observation of the packaging

The results obtained from the application of the questionnaire to the responsible technicians can be seen in Table 1. It can be observed that 60% of the establishments (B, C, D) perform quality control tests upon receiving the packaging. Also, 100% of the establishments receive the packaging with a report and qualify the suppliers at the time of purchase. The quality control of primary

Table 1 – Questionnaire applied to the companies regarding their cosmetics packaging

	Questions	Companies that answered yes	Companies that answered no
Receiving	Do the company perform quality control tests of the packaging?	B, C, D	A, E
	Is the packaging received with report?	A, B, C, D, E	-
	Do the company qualify the packaging suppliers?	A, B, C, D, E	-
Storage before sanitization	Is the packaging stored open, without the lid?	D, E (stored in packs)	A, B, C
	Is the packaging stored closed?	A e B (stored in packs) C (removed from packs)	D, E
	Is the storage area periodically cleaned?	A, B, C, D, E	-
	Is there temperature and humidity control in the storage area?	A, C, D	B, E
	Are there other materials stored in the same area? Which ones?	A, C (raw-materials) E (secondary packaging like cardboard boxes)	B, D
Sanitization	Do the company perform packaging sanitization before use?	A, B, C, D (70% alcohol)	E
	Do the company perform packaging drying at room temperature?	A, C, D	B, E
	Do the company perform another kind of drying?	B (cotton + 70% alcohol)	A, C, D, E
Storage after sanitization	Is the sanitized packaging re-stored?	A, B, D	C
	Is the packaging stored open, without the lid?	D (in closed drawer)	A, B
	Is the packaging stored closed?	A, B (in closed cabinet or box)	D
	Is the storage area periodically cleaned?	A, B, D	-
	Is there temperature and humidity control in the storage area?	A, B, D	-
	Are there other materials stored in the same area? Which ones?	A, B, D (equipment and materials that belong to the compounding laboratory)	-
Storage time from acquisition until use	Is the packaging stored for up to 3 months?	A, C, D	B, E

packaging, in direct contact with the product, plays an essential role, as it is an important element in controlling contamination. Through quality control it is possible to identify obstacles that interfere with the final quality of a product. Quality control guarantees to the consumer a safe product and establishes user confidence (8,11).

When the packaging is received, they are stored open in 40% of the establishments (D and E). 100% of the companies have the packaging storage area cleaned periodically, and 60% of these (A, C, and D) have temperature and humidity control of the area. Companies A, C and E use the packaging storage area for other materials such as raw materials and secondary packaging materials.

The cleaning of the packaging is one of the most important points to avoid microbiological contamination of the product that it contains. In the case of packaging for cosmetics, which are non-sterile products, the removal of residues that are contained inside the bottles must be done with some precautions, such as the quality of water used and the way it is dried (11). Therefore, according to the results obtained in the questionnaire, 80% of the companies (A, B, C, and D), representing all of the evaluated compound pharmacies, perform sanitization of the packaging before use, using 70% alcohol. It is also observed that establishment E (the only industrial establishment evaluated) does not sanitize the packaging. The large-scale production and the large number of containers stored make it unfeasible for industries to clean all of them before the filling of cosmetics. However, quality control of those containers should be performed in order to verify factors that could affect the quality of the final product, such as microbiological contamination of packaging (8). The drying of the packaging is done at room temperature for 60% of the establishments evaluated (A, C, and D), representing 3 out of the 4 establishments that perform prior sanitization. Establishment B performs drying with cotton and 70% alcohol.

Regarding the sanitization process, it is known that the packaging may or may not be sanitized in the establishments before use. Among the companies that perform the sanitization process (A, B, C, and D), companies A, B and D clean and store the containers again, while establishment C cleans the packaging but do not store them again. Considering the 3 establishments that perform sanitization and store the packaging again, company D stores the containers open while companies A and B store them closed. The companies have periodic cleaning, besides temperature and humidity control of the storage areas.

As for the storage period of the packaging at the establishments, they are stored for up to 3 months from their acquisition until their use for 60% of the places (A, C and D). It is important to consider that the packaging that will be in direct contact with the product should be compatible with the products expiration date. Hence, the quality of the packaging should be guaranteed (1,11).

In the visual analysis of the inside part of received packaging, it was observed that the 4 containers from company E were internally dirty, as observed for 2 containers from company D. As seen in the answers to the questionnaire, the containers from establishment E are not sanitized, and company D stores their containers open in a drawer, after the sanitization process, which is probably the factor responsible for the appearance of these residues inside the packaging. No residues were found in the containers obtained from establishments A, B and C.

Microbial evaluation of the packaging

From the analysis of the tests on non-selective PCA (Plate Count Agar), the growth of colonies was observed for the samples obtained from containers BS2, CR1, CS1, DR1, DR2, DS1, ES1, and ES2 (Table 2). Establishment A was the only one with no microbial growth detected from the samples of their containers.

When analyzing the results, it can be seen that, from a total of 20 evaluated containers, 8 presented microbiological contamination, being 5 samples collected by swab and 3 by rinsing. For 2 of the 5 companies evaluated (B and E), colony formation was observed only by swabbing, and not by rinsing. Therefore, considering the sample group evaluated in this research, the swab method seems more effective for collecting microorganisms from cosmetic packaging. The rinsing sampling technique is a method used for industrial routine, making it possible to cover the entire surface of the equipment. On the other hand, the swab technique is normally used for a specific area of the site where the rinsing water has no contact (15).

The fact that the packaging from establishment A did not present microbial contamination can be probably explained by the effective sanitization process that is performed by the establishment, and due to the care taken after sanitizing the packaging. One of the facts that was reported during the application of the questionnaire was that after the entire sanitization process, the packaging is stored in a closed cabinet and before use, they are again sanitized with paper towels and 70% alcohol.

The companies with the greatest number of CFU/mL collected from their containers (D and E), are companies that either stored the packaging opened after cleaning (D) or that did not perform sanitization of the packaging (E). Therefore, the handling of the packaging can probably explain the contamination found. As reported in this paper, a sanitization step does not guarantee that the packaging will be free of microorganisms and that will not contaminate the product, and also a recontamination is possible.

Cosmetic product packaging, although require no sterility, is considered a waterless environment. Therefore, the growth of only a few colonies was expected, as observed in this study. The samples presented a maximum of 4 CFU/mL. Regarding microbiological quality, the containers are frequently evaluated considering the maximum limits of CFU/mL or g for the final products (11), which are $5x10^2$ or $5x\ 10^3$(5). However, several companies establish their own microbiological limits for the packaging, which are lower than the limits for the products.

Table 2 – Results of the applied microbiology tests

Company	Sample	Number of CFU/mL detected in non-selective agar (PCA)	Microscopy	Selective agar evaluation (Salt Manitol)	Catalase test
B	BS2	2	Gram-Positive coccus	Small red colonies	Positive
C	CR1	1	Gram-Positive coccus	Big yellow colonies	Positive
	CS1	1	Gram-Positive coccus	Small red colonies	Positive
D	DR1	1	Gram-Positive coccus	Small red colonies	Positive
	DR2	1	Gram-Positive coccus	Small red colonies	Positive
	DS1	1	Gram-Positive rods	NE	NE
E	ES1	2	Gram-Positive rods	NE	NE
	ES2	4	Gram-Positive coccus (3) and Fungi (1)	Gram-Positive coccus: no growth; Fungi: NE	Gram-Positive coccus: Positive (2), negative (1); Fungi NE

NE = not evaluated

Samples BS2, CS1, CR1, DR1, DR2, and ES2 indicate the presence of bacteria of the genus *Staphylococcus*, since, according to what is observed in Table 2, the presence of Gram-positive coccus that are catalase positive can be observed. These microorganisms can be found in nature and are part of the normal skin and mucosa microbiota (1,16). Regarding the growth on selective media, there was no growth on Cetrimide and MacConkey agars, whereas for mannitol salt agar there was. On mannitol salt agar, it can be seen that there was a growth of large yellow colonies only for the CR1 sample, suggesting the presence of *Staphylococcus aureus*, a pathogen for human health, which is the cause of several diseases and can endanger the health of those who use this contaminated product. The diseases caused by *Staphylococcus aureus* can range from simple skin infections such as pimples, boils, and cellulitis to opportunistic and more serious infections that can be potentially fatal, such as pneumonia, meningitis, and sepsis. Moreover, they can also produce toxins that lead to food infections and cause vomiting, diarrhea, and abdominal pain (16). For the other *Staphylococcus* colonies observed, there was a growth of small, reddish colonies on the mannitol salt agar, indicating they were from different species other than *S. aureus*.

In samples DS1 and ES1, the presence of Gram-Positive rods was observed. Since the focus was to evaluate the presence or absence of pathogens that cannot be present in cosmetic formulations, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, the microorganism was not further identified.

For the sample ES2, the presence of a fungal colony growth was also observed. It is known that the presence of fungal colonies in packaging of cosmetic products compromises the quality of the product, since the fungus can be the cause of several pathologies (11).

Although the number of CFU/mL was low, as expected, and a discrete difference was observed comparing the companies that sanitize (A, B, C, D) and not sanitize (E) their containers before use, the investigation performed revealed that one of the packaging was contaminated with a pathogen (Company C). Such contamination could be passed on to the final product, which would not meet the requirements of the current legislation that requires the absence of pathogens, such as *Staphylococcus aureus*, in the final product. To avoid the contamination of products, internally standard operating procedures related to sanitization, storage and microbiological quality control of cosmetics packaging should be well established and validated.

Conclusions

It was observed that previous sanitization of the packaging was performed by compound pharmacies, with different handling of the packaging (storage and sanitizing procedures), whereas, for the evaluated industry, the previous sanitization process does not occur. The sanitization was not enough to guarantee the complete absence of microorganisms in the packaging; however only a few number of CFU/mL was isolated from each sample. *Staphylococcus* bacteria were the most common source of contamination found in the packaging, with *S. aureus* being identified in one sample. The swab technique was considered the most sensitive one for collecting microorganism from the packaging. The present investigation points for the importance of microbiological

quality control of cosmetics packaging, with internally well stablished and validated standard operating procedures. Based on the results obtained, in order to avoid the microbial contamination of cosmetics packaging and the presence of residues, their sanitization with alcohol 70% is indicated, followed by the storage of the jars closed, with re-sanitization right before the filling step.

Conflict of interest

None to declare.

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