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Microbiologic quality of hand creams in Pelotas, Brazil

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The use of cosmetics has been widely accepted; among them, hand creams are commonly used with the principal function of hydration. However, for the user's safety, and to ensure good-quality products, these products should follow the Good Manufacturing Practices (GMP) and a strict microbiological quality control. In this study, six samples of hand creams, two industrialized and four compounding were evaluated by microbiological methods of counting and identification of microorganisms. In the industrialized samples, there was no fungal or bacterial growth, whereas in the manipulated ones, there was a growth in 75% of the samples, but none exceeded the limits allowed by microorganisms, and they showed none pathogenic microorganisms. Through the results, it can be concluded that the origin of manufacture may influence final product quality. The results emphasize the need for a stricter control in pharmacies to prevent contamination in the production process, and to ensure safety and trustworthiness to the users of such products.

Key words: Microbiology,quality control, cosmetic Industry.

INTRODUCTION

The use of cosmetics has sharply increased in recent years both in Brazil and worldwide. By analyzing the industrial market, we observed that the national industry rose between 2003 and 2008 by 3.4%, while the cosmetic sector grew by 11.5%. Today, Brazil is already considered the third largest consumer market for cosmetics, only behind the U.S. and Japan (Leonardi, 2008).

This growth is due to the fact that nowadays both women and men are increasingly seeking for products which help them build a better personal image. Beautification, correction of imperfections and presenting a good personal image are targets of a modern society.

According to the National Health Surveillance Agency (ANVISA), the Board Resolution (RDC) number 211 of July 14th, 2005, determines that the toiletries, cosmetics and perfumes can be produced from natural or synthetic substances destined for exterior use. Cosmetics are designed to clean, perfume, hydrate, change the appearance, correct body odor, protect and keep in good appearance external features of the human body (Brasil, 2005; Ribeiro, 2010).

Among the various presentations of cosmetics, the creams used for the hands are important, since manicured hands mean concern with hygiene and welfare. Hands are one of the most vulnerable parts of

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the body, being susceptible to the effects of the environment due to being permanently without protection. In addition, they are prone to dehydration, once their backs consist of a thin and delicate skin with a small amount of sebaceous glands (Ribeiro, 2010).

The hand creams may contain moisturizing, emollient, anti-irritants, lightening, exfoliating and rejuvenating or aging preventive actives. It is the purpose of the product that is responsible for the choice of its active principles. Specific active principles must be used for the adequate purposes, being hydration and smoothing, the main reasons for seeking such formulation (Ribeiro, 2010; Kumar and Tyagi, 2014).

Moreover, cosmetic products should be prepared according to sanitary regulations, since they are substances that are used in external parts of the human body. In Brazil, ANVISA is the institution responsible for the regulation, supervision and production control, ensuring that products are safe and present good quality (Brasil, 2008).

Thus, quality control should be always performed throughout the process of cosmetics preparation. This control consists of a collection of necessary and relevant assays, which should be run during the entire production process to ensure that the medicines and cosmetics in general satisfy the quality, activity, purity, safety and efficacy desired, be such products are either processed or compound. In addition, there are the good manufacturing practices (GMP), standards, which must be followed from the beginning of the process to the final product, and guarantee the safety and quality of products offered for sale (Kumar and Tyagi, 2014; Brasil, 2008; Pinto and Kaneko, 2010; Rebello, 2004; Chorilli et al., 2007; Silva and Silva, 2011).

During quality control, physical, chemical and microbiological tests are carried out on all products that are used in the formulation (Pinto and Kaneko, 2010; Rebello, 2004). The microbial quality control of cosmetics is a quantitative assay which occurs by counting the number of microorganisms, thus evaluating the state of contamination of the product. In this counting, the presence of a limited bio burden is considered as acceptable. The absence of pathogenic microorganisms should also be evidenced. The purpose of this control was to determine the number of viable microorganisms present in formulations and perform identification of pathogenic microorganisms(Leonardi, 2008; Brasil, 2005; Pinto and Kaneko, 2010; Rebello, 2004; Andrade et al., 2005).

The microbiological quality is defined by microbial standards described in official compendia and regulatory standards. In view of the above, to produce a cosmetic quality standard, the manufacturer must demonstrate its effectiveness, safety, acceptability and credibility through validated tests that should be performed by qualified professionals (Ribeiro, 2010; Rebello, 2004; Martinelli et al., 2005; Souza and Maciel, 2010).

Besides quality control, to ensure that the product continues satisfactorily, it is necessary to use suitable preservative formulations, since they aim at inhibiting microbial growth, preserving the product. It is important that consumers are directed to use the product, making its proper use and keeping it in appropriated storage conditions (Chorilli et al., 2007; Siqueira, 2005).

Due to the great importance of microbiological control, and considering that these cosmetics are used in an area of the body that may come in contact with the eyes, mouth and mucous membranes, and therefore, can transmit such undesirable microorganisms, besides leading to serious consequences to the consumer if there are values of fungi and bacteria above the allowed levels or even the presence of microorganisms considered pathogenic, the present study evaluated the microbiological quality of hand creams.

METHODOLOGY

Six samples of hand cream with the main function of hydration were analysed. Among these, four were from compounding pharmacies, chosen randomly among pharmacies available in the city of Pelotas. And two samples were industrialized, acquired randomly in shops in the same city.

Compounding pharmacies make their cosmetics under a prescription or request from the user, while industrial pharmacies have a stock production and national/global distribution system. From both sources, was chosen the same reagents for both products, so it was possible to compare the quality of each manufacture method.

All samples were within the expiration date. The samples are identified by letters from A to F, with A, B, C and D regarding compound pharmacies, E and F of industries.

Evaluation of organoleptic characteristics was performed on all samples before testing, where color, odor and appearance were analysed. Microbiological analyses were performed by counting total viable microorganisms and subsequent identification of pathogenic microorganisms.

Total viable count of microorganisms– plate count method

The technique of counting the total viable microorganisms was adapted according to the Brazilian Pharmacopoeia(Brasil, 2010). The adaptation was necessary because the objective of this study was to analyze the final product with its conservation proprieties.

For the dilution of samples, 10 g of each analysed cream was transferred for a volumetric flask containing 90 mL of soybean-casein broth with 0.1% sodium tetradecyl previously prepared, heated to 45°C, stirring until a homogeneous mixture was formed.

For the detection of bacteria, 5 mL of dilution of each sample was pipetted into Petri dish. After this procedure, 30 mL of soybean-casein Agar medium liquefied at 45°C was added. The agar was mixed homogeneously with the sample, mixture which was expected to solidify. The samples were incubated at 30-35°C. Observation was made daily for four days. In the samples in which bacterial growth was observed, the number of colonies was quantified using a colony counter with controlled artificial lighting.

For detecting fungi, 5 ml of dilution of each sample was pipetted into Petri plate. After, 30 ml of Sabouraud-dextrose Agar medium liquefied at 45°C was added. The agar was homogeneously mixed into the sample until it solidified. The samples were incubated at 20-

25°C. Observation was performed in a daily basis up to seven days. The number of colonies was counted in the samples which presented fungal growth using a colony counter with controlled artificial lighting.

All samples were performed in triplicate, both in the tests for bacteria and for fungi. The negative control was performed by adding 5 mL of sterile water in a Petri plate, to which was added 30 mL of soybean-casein agar liquefied at 45°C. Likewise, the negative control was performed using Sabouraud dextrose agar. In addition, we performed a control of the means employed by adding them alone on the plates.The positive controls of *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739) and *Candida albicans* (ATCC 10231) were also made.

Identification of pathogenic microorganisms

After the counting conducted by using the artificial lighting counter, colonies with different morphological characteristics were identified. In order to permit the isolated growth of the colony, they were sown in simple broth. The tube was incubated in an oven for 24 h. After the growth, Gram staining was performed for prior identification of microorganisms.

Colonies with Gram-negative characteristics were plated on Mac Conkey. The ones with Gram-positive Cocci characteristics and Gram-positive bacilli were plated on blood agar. These were incubated at 30-37°C in an oven for 24 h. For identification of *S. aureus*, we used the technique of the coagulase tube. The Gram-positive bacilli were not identified because they are not described in the Brazilian Pharmacopoeia (Brasil, 2010).The identification of fungi was performed by colony morphology. In the case of colonies with characteristics of *C. albicans*, the Chromium agar test was conducted for identification.

RESULTS AND DISCUSSION

Microbial contamination of pharmaceuticals, whether processed or compound, can be derived from various origins, either due to the complex production process, the contaminated raw material or from other sources. Due to this fact, companies should always ensure that all process steps follow GMPs, and that there is staff trained for this function, since the production process is the responsibility of manufacturers (Brasil, 2008; Pinto and Kaneko, 2010; Yamamoto et al., 2004; Bazzo et al., 2006). Besides the preparation, quality control at all stages, as well as in the final product, should be serviced by companies. This control includes both physic-chemical and microbiological tests.

The microbiological quality control of cosmetic formulations should be performed mainly because the preparations consist of vehicles and active principles that constitute an ideal environment for the growth of microorganisms, since it can find in cosmetics, a source of carbohydrates, proteins, amino acids, vitamins, organic salts, water, among others (Bazzo et al., 2006). It is extremely important to evaluate water quality, since this is the main component of cosmetics and a major source of contamination (Martinelli et al., 2005).

The importance of evaluating the water is evident in the article of Andrade et al. (2005), which analysed 59 samples of water for pharmaceutical use. It was found that 44% of the samples were in disagreement with the pharmacopoeia specifications. Among them, there were samples with contamination above the limit allowed. In a sample of deionized water, for instance*E. coli*, a pathogenic microorganism was found. This evaluation confirmed that water quality can contaminate pharmaceuticals, which points out to the necessity of having a stricter quality control of such a raw material.

The microbial contamination of the product can also be influenced by the whole process of manufacturing, raw material control, equipment and facilities sanitization, material packaging, cleaning procedure, environmental and operational conditions(Andrade et al., 2005; Bazzo et al., 2006; Associação Brasileira de Cosmetologia, 2008). Therefore, cosmetics are subject to the growth of fungi and bacteria, a fact which depends on the product composition, water availability, storage temperature, hygiene in manufacturing, among others (Chorilli et al., 2007).

This study evaluated six samples of hand creams, of which two were from industrialized sources, and four were from compound sources. Among the industrial samples tested, there was no growth of microorganisms during the days of observation. The results shown in Table 1 suggest that these industries have stricter control policies and are correctly following quality control and the Good Manufacturing Practices.

The GMPs for the toiletries, cosmetics and perfumes industry were established by the ANVISA and seek compliance with the guidelines established in the technical regulation handbook of good manufacturing practices, with the goal of protecting public health(Chorilli et al., 2007).Besides the requirement of GMPs, another factor reducing the chances of contamination is that industries buy raw materials in large quantities, which avoids the fractioning of the material, and the fact that the entire production is made in closed batches (Yamamoto et al., 2004). Therefore, there are differences in the possibility of microbial contamination in cosmetics depending on the manufacturing origin.

There are also the Good Compounding Practices (GCP), these compound products should follow a stricter standard, which goes beyond what the Good Manufacturing Practices, the GMPs, prescribe. The GCPs aim to analyze all the inputs involved in the production process, and ensure that the compound products are manufactured, transported, stored and distributed securely, reducing the chances of microbial contamination and other physical and chemical changes (Medeiros et al., 2007; Marques and Moreira, 2009). In spite of the existence of such resolution, many pharmacies have not yet deployed the microbiological quality control on products that are not sterile (Medeiros et al., 2007).

In this study, from the four samples of hand cream manufactured in compounding pharmacies that were

Table 1. Growth of microbial samples in compound and processed hand creams.

Sample	Bacteria(CFU/g)	Microbial growth		Microorganisms found (UFC/g)
		Fungi (UFC/g)	Microorganisms count (UFC/g)	
*A	-	1	1	Non identified
*B	21	14	35	Gram-positive Bacilli/Gram-positive Coccus
*C	-	-	-	-
*D	2	-	2	Gram-positive Bacilli
**E	-	-	-	-
**F	-	-	-	-

*Compound samples, **industrialised samples.

analysed, there was bacterial growth in two and fungal growth in one (Table 1). This may have occurred because in the compounding pharmacies, the preparation of the formulations is prescribed individually, which requires acquiring a smaller amount of raw material. This factor ends up leading to the material fractioning in the distribution company, which may create possibilities to the microbial contamination (Yamamoto et al., 2004).

It is important to highlight that raw materials often come from the distribution companies contaminated. Therefore, the quality control should start even before the production process. Besides fractioning, the procedure is individualized, which can increase the chances of contamination.

Microbial growth can also happen because pharmacies still have a less strict control than industries. This fact can as well result from contaminated water, as this is the main component of cosmetics and often ends up contaminating the products. Besides these facts, among others, there is also the training of employees, which often can contaminate the formulations accidentally or due to poor hygiene. In Table 1, we can observe the growth of microorganisms in the samples.

The microbial growth may or not cause visible modifications in formulation. Loss of stability may occur by breaking the emulsion, alteration in physicochemical characteristics, and changes in color, odor and consistency of the product. It can also undergo degradation and inactivation of active ingredients and excipients, damaging the material and causing loss of effectiveness, causing the consumer to abandon its use(Andrade et al., 2005; Yamamoto et al., 2004; Blume et al., 2007). Furthermore, contamination can lead to a high health risk, since this can occur by pathogens or saprophytes in a high number(Yamamoto et al., 2004).

During the visual inspection of products, no samples showed alterations or modifications in its organoleptic characteristics. However, in this case, there was no relation between the contaminated creams and their characteristics, which did not differentiate a contaminated from an uncontaminated cream.

Since this contamination can occur by pathogenic microorganisms, the development of microbial growth must always be identified in order to avoid the presence of these microorganisms.According to the Brazilian Pharmacopoeia (Brasil, 2010), for pharmaceutical topical non-sterile products, the presence of the following pathogens is not accepted: *Salmonella* sp., *E. coli, Pseudomonas aeruginosa, S. aureus, Serratia marcescens, Klebsiella* sp., *Pseudomonas cepacia, Pseudomonas maltophilia, Pseudomonas stutzerie*and group B *Streptococcus*. It also determines the acceptable values of non-pathogenic microorganisms and determines which pathogens should be absent in non-sterile product formulations. Cosmetics used for the area of the hands belong to the product group type I. For such products, the limit of non-pathogenic microorganisms is a maximum of 10^3CFU/g and still there should not be sign of the pathogen microorganisms like*P. aeruginosa*, *E. coli* and *S. aureus*(Brasil, 2010).

The present study was performed to identify pathogens. The results found showed colonies with morphology and microscopy of Gram-positive Coccus (Table 1). However, having the coagulase technique performed, a negative result was obtained, which led to the identification of coagulase-negative *Staphylococcus*.

The results also evidenced colonies with morphology and microscopy of Gram-positive bacilli (Table1). Nevertheless, these were not identified because they are not considered pathogenic for this type of sample according to the Brazilian Pharmacopoeia (Brasil, 2010). There was no growth among the strains of Gram-negative bacilli. To identify the fungi, the observation of colony morphology was performed. It was observed that there were filamentous structures with varying sizes and color. Thus, it was found that these microorganisms were not *C.albicans*, because according to Zaitz et al. (2010), this fungus is yeast, and is present in the culture medium as small colonies and white coloring.

The positive and negative controls behaved as expected.

No samples exceeded the limit allowed by law, and none was contaminated with pathogenic microorganisms. According to the data presented here, there was growth in 50% (3/6) of the analysed samples. However, when evaluating the manufacture origin of the products, 75% (3/4) of the compound samples were contaminated.

These results are similar to those of Chorilli et al. (2007), who analysed six samples of compound cream for the eye area. In such research, there was no product with pathogenic bacterial contamination or with the level of pathogenic microorganism above the established limits. Nevertheless, two samples were with fungi values above the permitted, suggesting that those pharmacies should follow more strictly the GMPs and GCPs to minimize these contamination values.

In another study, Blume et al. (2007) analysed five samples of pressed powder, and found that 20% of these were contaminated with microorganisms, but, in this case, contamination was above the limit set by law.

In the study of Yamamoto et al. (2004), a total of 260 samples, from raw materials, pharmaceutical products, cosmetics and herbal medicines from 25 producers were analysed. Of these, 92.3% were approved and 7.7% were rejected. Among the rejected, 5% presented microbial contamination above the established limit of both bacteria and fungi. Among the cosmetic analysed, two were contaminated with *E. coli* and *P. aeruginosa* and one with *S. aureus*, which proves that the quality control of products should be more rigorous, since such contamination can harm the health of users. For the authors, the results of this study pointed out faults in the manufacturing process, where standard operating procedures should be evaluated and preventive measures to improve product quality should be established.

Marques and Moreira (2009) conducted a similar study in which 13 samples of compound sunscreens were analysed, of which 53.84% were rejected. Among these, seven were contaminated with *S. aureus* and *P. aeruginosa.*

In addition, some samples were above the legally set limits for bacterial and fungal presence. According to the authors, this contamination was probably generated by reason of noncompliance with the good compounding practice and could be avoided or minimized through acts as washing hands, wearing gloves and clean coats and tying the hair. Another factor that may have caused the contamination may have been water or other contaminated raw materials.

Medeiros et al. (2007)analysed nine samples of compound products, including syrups and cosmetics. Among these samples, 54.5% were contaminated and/or above the specified allowed limits. This result can be derived from the lack of quality control in raw materials or the contamination which may have occurred during production.

Although, it is uncommon for a contaminated hand cream to reach the blood stream and cause a major infection under normal circumstances, the contamination can cause minor adverse reactions, like an erythema (Dupont et al., 2013). In the study of Huf et al. (2013), 200 people from the administrative staff of the Municipal Guard of Rio de Janeiro (Brazil), were analyzed and was found that 38% of the participants had adverse reactions to cosmetics use. While Hufet al. (2013) study did not investigate the cause of the reactions, microbial contamination could be a factor.

One of the major obstacles to the cosmetic health security, is the lack of an open knowledge integration system. Each finding from the cosmetic industry is still considered “internal knowledge” preventing other industries from copying their techniques and technology. This knowledge blockage is very detrimental to public health, as it makes more difficult the standardization of the process as discussed in this study (Celadon, 2014).

Given the above, in this work, it was observed that all tested samples were acceptable according to the current legislation, although there is contamination on three samples, however, below the specified threshold. This fact highlights the need for greater rigor in the production process which has to be adopted by compounding pharmacies, since there was a growth in 75% of the analysed compound samples. With the implementation of more stringent GMPs and GCPs these contaminations could be avoided. Moreover, in pharmacies in general, the use of preservatives is smaller, since samples are produced with a shelf life of about six months, which may also justify such microbial growth.

In the industrialized samples, there was no growth registered, which implies that the origin of the industrial process is more rigorous. However, in this case, the possibility of using a larger amount of preservative is not discarded, considering that the formulations are manufactured to have a shelf life of two years on average. However, the amount of preservatives has not been evaluated in this study.

Conclusion

Although, the present study has a low number of samples, the findings are fascinating: it can be concluded that 50% of the samples were contaminated, but all the contamination was within the group from a compound source (75% contamination in compound hand creams). However, the contamination was below the specified threshold and there was an absence of pathogens, being all considered as good quality and proper to use.

While the products analysed had an acceptable microbiological quality, the results imply that the manufacturing origin may have an influence in the quality of the final product, suggesting the necessity of a review in the GMP to ensure no difference between compound and industrial source.

Conflict of interests

The authors did not declare any conflict of interest.

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