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A Study on the Conformity of Microbiological Criteria for Hand and Face Creams Sold in Market

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Abstract: Cosmetic products, which hold an important place in human life, are products that do not have to be sterile in terms of microbiology. However, these products can be contaminated with microorganisms due to various reasons. For this reason, the microbiological suitability of cosmetic products is very important for consumer health. In this study, the suitability of hand and facial creams used for cosmetic purposes, which are not opened at all, and belonging to different firms, to microbiological criteria have been investigated. For this purpose, a total of 30 cream creams including 24 hand creams and 6 hand and face creams were used. The number of total microorganisms in creams was determined by classical counting method, and selective media were sown. Accordingly, it has been determined that 3 of the 30 cream samples contain more than 1000 microorganisms, which is the permitted value in the cosmetic regulation. There have also been no cases of the presence of pathogenic microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, which are not desired to be present in cosmetics at all. However, there were many microorganisms with or without various pathogens in 16 of the 24 handcreams and 5 of the 6 hands and facecreams. It has also been observed that some of the creams may contain many pathogenic and opportunistic pathogenic microorganisms. For this reason, it is suggested to manufacture in facilities suitable for good manufacturing conditions in order to prevent contamination in unused products, to protect consumer's health, to increase product quality and reliability in microbiological terms. In addition, from the point of view of public health, inspections of these products and microbial risk analyzes should be done absolutely.

Keywords: Cometics, Microorganism, Microbiological criteria

Introductıon

Since ancient times, people have always desired to look beautiful, be well-groomed and feel attractive. For that reason, the use of cosmetics has increasingly continued from the early ages until today, depending on the development of humankind. It is known that in Ancient Egypt, Cleopatra used to take a bath in milk to whiten and soften her skin. It is also known that French women rub grape pulp on their faces while making wine, and Hungarian women use tomato for that purpose while making tomato paste (Sağlam, 2010).

The consequences of archeological studies have been revealed and show that people have been ornamenting and changing their bodies in different ways for half a million years. Generally, making changes to, coloring and ornamenting human body, is in the nature of humankind, and it is sociological and social impulses that drive people accordingly. In archaeological excavations, sharpened red ochre sticks, thought to be used for coloring the lips, were found. About 5000 years ago, wealthy Egyptian women were using henna, hair dye and various pomades to distinguish themselves from lower classes. Also, it is mentioned in several sources that the Egyptians used to bath quite often and apply perfumed creams and oils to their bodies (Curry et al., 2006).

According to Turkish Cosmetics Legislation by the Ministry of Health, published in the Official Gazette on March 24, 2005, "Cosmetics are all preparations and materials including hair dyes and lighteners that are prepared to be applied to several outer parts of human body such as epidermis, nails, hair, body hair, lips and genital organs, mouth and teeth or mucosa, and of which main or secondary purpose is to care for such parts by cleaning, perfuming and protecting, changing the appearance and correcting bad odors" (Official Gazette of the Republic of Turkey, 2005). Creams are cosmetic products that are applied externally to various parts of the body and have different functional tasks. These cosmetics can be used as products that have protective, cleansing, moisturizing and healing properties and can change the external appearance of skin. Thanks to these properties, creams have become products that are needed in every aspect of human life in many areas like preventing diaper rash, postponing aging effects as well as hair and skin care. Therefore, the cosmetic industry has allocated the largest part of its market share to creams (Milton, 2004). Cosmetics do not have sterility obligation. However, cosmetic products may create a media suitable for the reproduction of microorganisms due to the various substances contained.

Thus, cosmetic products should be protected against microorganism contamination (Altan, 2010). Cosmetic products contain various minerals, vitamins, amino-acids, carbohydrates, sugar alcohols, fatty acids, proteins, fatty alcohols, steroids, peptides, glycosides, raw vegetable materials and water, which are highly used in products. Such substances contained in cosmetic products create media suitable for the development of microorganisms (Baird, 2004).

Glycerol, which is used in creams and lotions, is a substance that can be metabolized by various microorganisms. This compound can be metabolized by the species of *Bacillus*, *Staphylococcus* and *Micrococcus* (Flores et al., 1997). In recent years, natural products are in high demand in cosmetic industry like in other industries. Owing to natural raw materials used in such products, reproduction potential of the microorganisms within cosmetic products has also increased. Cosmetic products face the possibility of contamination with various microorganisms in media or in raw materials, starting with the water that is used in manufacturing phase. Employees, packaging material and all kinds of devices used during and after the manufacturing are among the sources that also might cause contamination besides water. In addition to the risk of contamination in products during manufacturing, such risk may also occur after manufacturing due to poor storage conditions. Furthermore, this risk may also occur during consumer use (Russell, 1996; Clegg and Perry, 1996).

Microbiological quality of cosmetic products is highly important for consumer health. The best way to prevent microbial contamination in cosmetic products is to manufacture in accordance with hygienic rules that lowers the risk of contamination. Starting with the water and raw materials that are used in the manufacturing, manufacturing sites, tools and equipment used in the manufacturing, ventilation systems, employees and methods during the use of products should be in compliance with certain standards that are fit for purpose in order to reach the desired quality in the production. Requirements to ensure such conditions have been published by World Health Organization under Good Manufacturing Practices. In Turkey, these requirements have been published by the General Directorate for Pharmaceuticals and Pharmacy of the Ministry of Health under Cosmetic Good Manufacturing Practices Guideline. Pursuant to this guideline, the conformity of each phase in cosmetic manufacturing with hygienic conditions should be inspected. Hygiene should be observed around all of the manufacturing facility including the building, installations and equipment and in addition, the raw materials, product compounds, unpackaged and finished products should also be stored in accordance with hygienic

conditions. Air and water systems within the manufacturing facility should be in compliance with sanitation. Equipment used in the manufacturing should be cleaned in line with design and usage requirements and disinfected at regular intervals. Moreover, employees should observe personal hygiene rules and perform manufacturing in accordance with work instructions. Again, according to this guideline, all potential contamination sources should be detected and measures should be taken to eliminate such sources (Mulhall et al., 2006; Anonymous, 2005). Undesired changes may occur in color, odor, viscosity and performance of cosmetic products due to microorganism contamination, and the reasons for such changes might be deterioration by the microorganisms in the product compounds and microorganism-related metabolites in the products (Sivri, 2005).

Microorganisms in contaminated cosmetic products may infect the skin and furthermore, the infections developed in the user as a result of contamination might cause significant damage in soft and hard tissues. Additionally, endotoxin and other metabolites produced by microorganisms may cause abrasion, irritation and allergy in the skin (Lachapelle and Gour, 1982).

In the "Guideline on Microbiological Controls of Cosmetic Products" that was prepared based on the Cosmetic Regulation published by Turkish Medicines and Medical Devices Agency (TİTCK) of the Ministry of Health of the Republic of Turkey in 2005, the maximum allowable microorganism count in 1 g or 1 mL of cosmetic products was established as 100 microorganisms in cosmetics for children below the age three, 100 microorganisms in cosmetics for use in eye contour and mucous membranes, and 1000 microorganisms for other cosmetic products. Also again, according to Turkish Medicines and Medical Devices Agency, pathogenic microorganisms that are hazardous for human health such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* should be definitely absent in cosmetic products (TİTCK, Ministry of Health of the Republic of Turkey, 2005). The fact that cosmetic products can be contaminated by microorganisms was first realized by infant deaths associated with the use of talcum powder contaminated with *Clostridium tetani* in New Zealand in 1946 (Tremewan, 1946). A study by Professor Kallings in Sweden in 1969 revealed for the first time that cosmetic products could be contaminated (Curry et al., 2006). In the 1970s, the users developed various infections because of cosmetic products contaminated with the species of *Pseudomonas aeruginosa* and *Klebsiella*, and as a result of the studies, it was found that the situation originated from the microorganisms and microbiological analyses should have been conducted on cosmetic products (Baird, 1998). Therefore, contaminations in creams can have great significance in terms of human health. Several types and brands of hand and face creams are manufactured and are available in the market for public use. However, it is controversial whether the manufacturers are in accordance with the requirements of good manufacturing practices in the Cosmetic Regulation of the Ministry of Health of the Republic of Turkey and abide by the hygiene requirements or not. This study aims to determine whether hand and face creams available in the market are in compliance with the microbiological criteria.

Materials and Methods

Cosmetic products used in the study

Our study examined 30 cream samples including 24 hand creams and six hand and face creams that were supplied from perfumery stores, supermarkets and pharmacies in various districts of Gaziantep province, used for cosmetic purposes, had unopened packages and were products of different companies.

Preparation of Samples

As cosmetic-like creams are oily and water-insoluble products, surface-active agents should be used so that microorganisms in these products pass the aqueous phase. Among the surface-active agents recommended for that purpose, Tween 80 was chosen, due to its low inhibiting effect on the microorganisms (Turakka et al., 1986; Hitchins, 2000).

Each of the 30 cream samples from different companies were numbered from 1 to 30. The creams were weighed under sterile conditions and 1 g of each sample was filled into a sterile tube. 0.5ml of Tween 80 and 8.5ml of Eugon Broth were added into samples. Afterwards, in order to facilitate dispersion, the prepared mixture was kept in shaking water bath at 45˚C until homogenization. Attention was paid to not to exceed 30 minutes so that the microorganisms present could stay alive.

Preparation of Dilutions and Counting of Total Living Microorganisms

In this study, a 1:10 dilution was obtained containing 5% Tween 80. 100 microliters of 1:10 dilution was taken considering the possibility of too many colonies to count, mixed with 900 microliters physiologically saline solution and 1:100 and 1:1000 dilutions were prepared. Before each dilution, samples were stirred in vortex device for 15 seconds. 100 microliters from each dilution were taken, poured onto Plate Count Agar (PCA), which was the ready medium, and spread on medium by using sterile T drigalski. Parallel cultures were made for dilutions. Colonies that developed in Petri dishes incubated at 37˚C for 48 hours were counted to calculate total living microorganism count.

Gram Staining

A homogeneous suspension was obtained by taking samples from each different colony type cultured in Plate Count Agar by using a loop and mixing them with a drop of physiological saline solution on microscope slide. After the physiological saline solution on the microscope slide dried, it was fixed by being singed three times. Crystal violet dye was poured onto dried preparation, then the preparation was held for one minute and washed with distilled water. Lugol's iodine was dripped onto the washed preparation and it was held for one minute. After one minute, the iodine was also washed with distilled water. The preparation was left in 96% alcohol for 30 seconds and immediately washed with distilled water. After the washing process, aqueous fuchsine was dripped onto the preparation and held for one minute. Following this period, the preparation was washed with a large amount of distilled water and was left to dry. Immersion oil was dripped under the microscope into the preparations that were washed and left for drying, and they were examined under 100x objective lens. Bacteria which appeared pink by failing to retain crystal violet were considered Gram (-), and those appeared purple by retaining the dye inside cells were considered Gram (+). Colony shapes were defined (Altan, 2010). By this means, an information on the morphologies of microorganisms was gathered.

Microorganism Isolation in the Samples

In light of this information, samples were taken from these colonies by using a loop and cultured in selective media. Selective media that were necessary to look for the presence of *E. coli, P. aeruginosa*, *S. aureus* and *C. albicans* were cultured. EMB Agar was cultured for detecting *E. coli* contamination, Cetrimide Agar was cultured for *Pseudomonas aeruginosa*, Baird-Parker Agar was cultured for *S. aureus*, Chromogenic Candida Agar was cultured for *C. albicans* yeast and Trypticase Soy Agar (TSA) was cultured to look for the presence of other bacteria types, and they were all incubated at 37°C for 24-48 hours. Sabouraud Caf Agar, a general purpose medium, was cultured for other yeasts and filamentous fungi and incubated at 25°C for 5-7 days.

Conclusion and Discussion

A microorganism was found in 16 (67%) of the 24 hand cream samples used in the research. However, no microorganism reproduction was observed in eight (33%) of the creams. A microorganism was found in five (83%) of six hand and face cream samples used in the research. And no microorganism reproduction was observed in one (17%) hand and face cream. When all cream samples were considered according to research results, a microorganism reproduction was seen in 21, i.e. 70% of 30 creams used in the study. On the other hand, no reproduced microorganisms were found in nine, i.e. 30% of 30 creams.

In two (8%) of 24 hand cream samples and one (17%) of the six hand and face cream samples examined in the study, the microorganism count was above 10^3 cfu/g, which was the limit defined by cosmetics regulation for cosmetic products. Based on the overall samples, the microorganism count was above 10^3 cfu/g in three (10%) of 30 cream samples. No non-bacterial microorganism such as yeast or fungus was found in 30 samples.

The presence of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans* which were prohibited by the cosmetic regulation was not found in any of the samples studied. However, it was observed that the examined creams were contaminated with other types of Staphylococcus.

Table 1. Count and percentages of creams in which microorganisms reproduced

	Number of the studied cream	Cream number of microorganisms reproducing	Cream number of microorganisms reproducing (%)	Cream number of microorganisms non-reproducing	Cream number of microorganisms non-reproducing (%)
Hand cream	24	16	% 67	8	% 33
Hand and face cream	6	5	% 83	1	% 17
Total	30	21	% 70	9	% 30

Table 2. Number of samples containing an undesired amount (>10^3 cfu/g) of microorganisms in creams

Sample type	Number of samples	Number of samples containing an undesired amount of microorganisms in creams (>10^3 cfu/gr)	Number of samples containing an undesired amount of microorganisms in creams (>10^3 cfu/gr) (%)
Hand cream	24	2	%8
Hand and face cream	6	1	% 17
Total	30	3	% 10

In a study by Çarıkçı et al. on cosmetic products in 2008, a microorganism level amount greater than 10^3 cfu/g was found in one (2.32%) of 43 unused cosmetic products (Çarıkçı et al., 2008). A study by Tüysüz in 2010 reported that the total bacteria count was above 10^3 cfu/g in three (6%) of 50 cosmetic products that were examined in terms of microbiological content. Tüysüz found bacteria count to be above 10^3 cfu/g in one (20%) of five facial care creams and two (40%) of five hand and body care creams (Tüysüz, 2010). Again in a study by Altan on oral and dental preparations in 2010, microorganism count was found to be above 10^3 cfu/g in one (12.5%) of eight unused samples (Altan, 2010). In Kabukçu's study on 104 unused shampoo samples in 1997, it was observed that bacteria count in 35 (33.65%) shampoo samples exceeded the limit of 10^3 cfu/g limit defined by the cosmetic regulation (Kabukçu, 1997).

In our study on creams, bacteria count above 10^3 cfu/g was found in three (10%) of 30 cream samples. This rate was determined as 17% in hand and face creams compared to 8% for hand creams. Therefore, these results obtained in our study are in parallel with the results of studies by Çarıkçı, Tüysüz, Altan and Kabukçu.

Like this current study, a study by Campana et al. on unused bath foams in 2006 also found *Staphylococcus epidermidis* (Campana et al., 2006). In their study on unused moisturizers in 1997, Flores et al. detected the species of *Bacillus megaterium, Staphylococcus epidermidis* and *Bacillus pumilus* similar to this study (Flores et al., 1997). Again similiar to this current study, Anelich and Korsten isolated *Enterobacter gergoviae* from face creams and body lotions in their study in 1996 (Anelich and Korsten, 1996). Also in Ergun's study in 1987, *Bacillus subtilis* species was isolated from three of 38 shampoo samples (Ergun, 1987). Therefore, this current study's results is in parallel with the literature.

Furthermore, as bacteria of the*Kocuriakristinae* species has not been observed in the literature on cosmetics so far, our study is unique and highly significant.
As in other studies within Turkey and abroad, this study also observed that contamination rates were high in the products, as evidenced by the trials on creams. According to these results, it can be said that the hand and face

creams that we have examined do not have sufficient microbiological quality. The detection of microbial contamination in the studied products analyses of hand and face creams, asserted that contamination could occur during manufacturing of the products. Therefore, measures to be taken during manufacturing come into prominence to prevent adverse affects that might potentially occur in the products and harm users due to microbial contamination. Manufacturers should comprehend that microbial reproduction will harm the preparation and users, and give due consideration by fulfilling necessary controls in manufacturing. This condition can only be ensured by complying with the principles of good manufacturing practices during manufacturing processes and by taking control measures at every phase. On the other side, it is obvious that healthy products can be obtained by observing hygiene rules and through the control of protective actions. In the light of this guidance, manufacturers should make a habit of conscious manufacturing

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