Original Article

Microbial quality survey of sunscreen products in Iranian market

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Abstract

Background: Microbial contamination of cosmetic products is very crucial because of their daily use and direct contact with the skin. These products are at high risk for microbial contamination from various sources such as environment, consumer's hands, body sweat and during the time of manufacturing. Therefore, this study aimed to investigate the microbial quality of sunscreens products, manufactured in or imported to or formulated in local pharmacies in Iran.

Materials and Methods: The microbial quality were determined in three different levels; the intact product (at the time of purchase) and after three and after six months of opening it. Total Aerobic Viable Count (TAVC) and the presence of coliforms, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, molds, and yeasts were studied.

Results: At the time of purchase, 40, 73.3 and 43.3 percentage of Iranian made, imported and pharmacy formulated sunscreens were contaminated with at least one of the objectionable microorganisms, respectively. After three months of opening it, 36.6, 70 and 46.6 percentage of Iranian made, imported and pharmacy formulated sunscreens were contaminated with at least one of the objectionable microorganisms, respectively. The percentages of contaminated samples were 36.6, 70 and 50 after six months of opening for Iranian made, imported and pharmacy formulated sunscreens, respectively.

Conclusion: Microbial contamination of these sunscreens products is a potential health risk for consumers. It seems that it is necessary to inspect and monitor the products during the manufacturing and shelf life period. It is highly recommended to control and regulate cosmetic products by health organizations to ensure the quality and safety of this kind of products.

Key Words: Cosmeceuticals, microbial contamination, microbial quality, sunscreen products

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Received: 17.05.2013, **Accepted:** 03.09.2013

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.139534

INTRODUCTION

Skin is the outer protective layer of the body and it is delicate, sensitive and highly vulnerable. Skin plays a key role in protecting the body against pathogens because of its barrier role.[1] Protection of the skin from prolonged and continuous exposure to the direct sunlight is highly recommended to prevent wrinkles and skin cancers.[1,2] Using of appropriate sunscreens is the most common way of skin protection. Sunscreens

How to cite this article: Haftbaradaran B, Abedi D, Jalali M, Bagherinejad MR. Microbial quality survey of sunscreen products in Iranian market. Adv Biomed Res 2014;3:180.

are not sterile products.[3,4] However, they must not be contaminated with objectionable microorganisms. In addition, the number of non-pathogenic microorganisms should also be in limited range.[4-6]

Microbial contamination is one of the main reasons of cosmeceuticals products recalls.[7-9] Frequency of use, applying method and storage conditions could highly affect the risk of microbial contamination of the products.[7,9,10] Microbial contaminants may originate during the manufacturing processes, particularly from the raw materials, and/or during the use of the products by the consumer. Since a product is opened, it may further contaminate by consumer hands and/or environment.[3] Contamination of cosmetic products may directly affect the human health as a result of formation of harmful microbial metabolites and spoilage of the products.[5] Therefore, microbial preservation of cosmetics is of essential to ensure the consumers safety and maintenance of the hygienic level of the products.[3,11] The microbial quality of a sunscreen is determined by the relevant standards. These standards in Iran are based on standard no. 3978 of the Institute of Standards and Industrial Research of Iran (ISIRI) as presented in Table 1.

Only a few studies have been conducted on the microbial quality of sunscreens and there are very limited data available in Iran. Therefore, the present study was performed to determine the microbial quality of sunscreens products available in the market in Isfahan, Iran.

MATERIALS AND METHODS

Samples

Totally 90 samples included the sunscreen products manufactured in Iran (30 samples), imported from overseas (30 samples) or formulated in local pharmacies (30 samples) were randomly collected. The samples transferred to the Food Microbiology Laboratory, Isfahan University of Medical Sciences, in portable insulated cold-boxes and analyzed on the time of arrival. The samples were collected during 2010-2011 from three production batches of manufacturers. The samples were analyzed at three different levels including after purchasing (intact product), three months and six months after opening. Microbial analysis of the products was mostly conducted according to the method recommended by Institute of Standards and Industrial Research of Iran (ISIRI).

Table 1: Microbial properties of creams and lotions, according to the Iranian standards

Microorganisms	Acceptable microbial count (CFU*/mL)
Aerobic mesophilic bacteria	≤500
Coliforms	Negative
Pseudomonas aeruginosa	Negative
Staphylococcus aureus	Negative
Yeast and molds	Negative

*CFU: Colony forming unit

Total aerobic viable count (TAVC)

Initially, the total viable count was determined using the serial dilution and pure plate methods. At the first step, the outer surfaces of the products containers were disinfected with ethanol (70% v/v). The content of each product was mixed well before sampling then 10 g of the sample was added to 10 ml of sterile polysorbate 80. After mixing, 80 ml diluted of Fluid Casein Digest Soya Lecithin with polysorbate 20 was added to the mixture and mixed well. The prepared sample was diluted 10^{-1} time. In next step, from the diluted-sample mixture, 1 ml was transferred into two sterile petri dishes (90 mm diameter), aseptically. When high count was expected, it was plated 0.1 mL of dilution into additional two petri dishes to achieve the next level of serial dilution. To determine the total viable count, 20 ml of molten Soybean Digest Agar at 45°C was added to each petri dish aseptically. The plates were rotated to completely disperse and mix agar and dilution sample. The agar plates were allowed to solidify at room temperature then were incubated at 30°C for 48 h. After the incubation time, plates were checked for growth and count all visible colonies using a colony counter. In the case of no growth, the count was reported as <10 CFU/g.

Coliforms detection

Presence of coliforms determined based on standard no. 437 of the Iran Institute of Standard and Industrial Research (ISIRI). Ten ml of the sample was added to 10 mL of Lauryl Sulfate Tryptose Broth enriched by increasing the concentration to two-fold of recommended concentration for regular preparation. The tubes were incubated at 37°C for 48 h and the samples with no gas formation were reported as negative. In the case of gas formation, a drop of culture medium was added to Brilliant Green Lactose Bile Broth medium and incubated further at 37°C for 48 h. If the case of gas formation occurred, the sample was reported as a positive for the presence of coliforms nevertheless it was reported negative.

Pseudomonas aeruginosa and *Staphylococcus aureus* detection

To study the presence of *P. aeruginosa* and *S. aureus*, 10 ml of the prepared dilution was added to 10 ml of fluid soybean casein digest medium enriched with two-

fold higher concentration compared to recommended concentration for regular preparation of medium. The tubes were incubated 35 ± 2°C for 48 h. After incubation period, tubes with no growth were reported negative for the presence of *P. aeruginosa* and *S. aureus*. In next steps, a loop of medium rom tubes which have shown growth was transferred to Cetrimide Agar, a selective media for *P. aeruginosa* identification and a loop also is transferred to Baird Parker Agar which is selective for *S. aureus*. After incubation at 35 ± 2°C for 72 h, the plates were checked. In the case of growth on these selective mediums, the sample was reported positive for the presence of related microorganism.

Yeast and mold detection

Sabouraud Dextrose Broth and Sabouraud Dextrose Agar were used to study the contamination of samples with yeast and mold. Ten mL of prepared dilution was added to a tube containing Sabouraud Dextrose broth enriched with two-fold higher concentration compared to recommended concentration for regular preparation of medium. The tubes were incubated at 25°C for 3-5 days. Tubes with no turbidity after incubation time were reported as negative. In the case of turbidity, a loop of culture was transferred on to a Sabouraud Dextrose Agar plate and the plate was incubated at 25°C for 3-5 days. Growth of any colony on the surface of the plate showed the contamination of sample with yeast or mold and the sample was reported positive for the presence of molds and yeasts.

RESULTS

Total aerobic viable count

Considering the three milestones for sample analysis and three repeats for each result, totally 270 analyses were carried out for total aerobic viable count (TAVC) study. Bacterial counts of the samples varied from 10 to 3000 CFU/g, where the majority of samples count analyses were in the range of 10 to 500 CFU/g [Table 2].

Table 2: Total aerobic viable count (TAVC) of sunscreen products available in Iranian market. Based on the Iranian standards for microbial quality of cosmetic products, TVAC should be less than 500 CFU/g. The results are from analysis of 30 samples of each type of products (totally 90 samples) and at least 3 repeats for each sample.

Test time	Type of products					
	Domestic		Imported		Formulated in pharmacy	
	*N**	*P*** (%)	*N*	*P* (%)	*N*	*P* (%)
Time of purchase	3	10	9	30	3	10
After 3 month	4	13.33	14	46.66	8	26.66
After 6 month	7	23.33	15	50	8	26.66

*N: Number of contaminated samples with more than 500 CFU/g products;
**P: Percent of contaminated samples with more than 500 CFU/g products

At the time of purchase, more than 84% of the samples showed contamination less than 500 CFU/g. The results of viable count analysis showed that after three and six months, 71.2% and 66.6% of the samples contained less than 500 CFU/g microorganisms respectively.

Objectionable microorganisms

The results of tests for identification of coliforms, *P. aeruginosa*, *S. aureus* and molds and yeasts are summarized in Table 3. At the time of purchase 27%, 15.5%, 27% and 12.2% of the samples showed contamination with *S. aureus*, *P. aeruginosa*, yeasts and molds respectively. Contamination with *S. aureus*, *P. aeruginosa* and yeasts and molds were increased after three months of opening the products (31.1%, 17.7% and 14.4% respectively). In the case of coliforms, contamination of the products is reduced to 7.7% from 27%. Results showed that after 6 months, contaminated products with *S. aureus* were 28.8% and 18.8%, 8.8% and 15.5% of products showed contamination with *P. aeruginosa*, coliforms and yeasts and molds, repectively.

In this study, 40% of the Iranian sunscreens, 73.3% of the imported products, and 43.3% of the formulated products at the time of purchase contained at least to one of the objectionable microorganisms. After passing three months of opening the products, contamination is reduced to 36.6% and 70% for Iranian and imported products respectively but formulated sunscreens show more contamination rate when compared to the time of purchase. After six months this rate increased to 50%. This makes sense because of limitation in the shelf life and expiry date of the products formulated in pharmacies.

DISCUSSION

Microbial contamination of cosmetics during the manufacturing processes was a major problem during the 1960's and early 1970's. Today, significant progress has been made in the cosmetic industry and more rigorous microbiological control measures are developed and applied.[3] On the other hand, the level of microbiological quality should be maintained in the cosmetic products during their use, in spite of the contamination imposed by the users, through the using of an appropriate preservative system in the products (Linter and Genet 1998).[12,13] Although, some cosmetics products which contained more than 10% ethanol, propylene glycol, glycerol, or cosmetics in self-pressurized containers had self-preserving system.[3] According to European Union (EU) legislation, cosmetic products must not contain more than 1000 CFU/g

Table 3: Microbial quality of sunscreens products collected from Iranian market. Based on Iranian standards of microbial quality of cosmetic products, the products have to not contain coliforms, *S. aureus, P. aeruginosa*, mold and yeast. The microbial quality of samples was analyzed at three milestones; at the time of purchase, three and six months after opening the product. The results are from analysis of 30 samples from each type of products and at least 3 repeats for each sample.

Type of products	Test time	Coliforms positive		*S. aureus* positive		*P. aeruginosa* positive		Mold and yeast positive	
		*N**	*P***(%)	*N*	*P* (%)	*N*	*P* (%)	*N*	*P* (%)
Domestic	Time of purchase	0	0	6	20	5	16.66	2	6.66
	After 3 month	2	6.66	7	23.33	5	16.66	2	6.66
	After 6 month	3	10	5	16.66	5	16.66	2	6.66
Imported	Time of purchase	0	0	12	40	8	26.66	5	16.66
	After 3 month	2	6.66	13	43.33	9	30	5	16.66
	After 6 month	2	6.6;6	12	40	10	33.33	6	20
Formulated in pharmacy	Time of purchase	3	10	7	23.33	1	3.33	4	13.33
	After 3 month	3	10	8	26.66	2	6.66	6	20
	After 6 month	3	10	9	30	2	6.66	6	20

*N: Number of contaminated samples with objectionable microorganism; **P: Percent of contaminated samples with objectionable microorganism

of microorganisms and *S. aureus*, *P. aeruginosa* and *Candida albicans* must not be detectable in 0.5 g of a product.[14] FDA stated it is not necessary for cosmetic products to be sterile, however, they must not be contaminated with pathogenic microorganisms and the density of non-pathogenic microorganisms should also be low. In addition, cosmetics should remain in this condition during the period of usage by the consumers [15]

Based on the FDA guidelines, cosmetic products must be completely free of high-virulence microbial pathogens, and the total count of aerobic microorganisms per gram must be low. There are no widely acceptable standard for total microbial counts. Based on temporary guidelines the total count should not be greater than 500 CFU/g for an eye-area product and for non-eye area products, counts should not be greater than 1000 CFU/g.[5,15]

According to No. 3978 of the Institute of Standards and Industrial Research of Iran (ISIRI), cosmetic products must not be contained more than 500 CFU/g TAVC and *S. aureus*, *P. aeruginosa*, *coliform, yeast and molds* must not be detectable in these product.

A cream with good preservative capacity is one that is capable of inhibiting immediate post-production contaminants, as well as subsequent low inocula of in-use contaminants, and thereby maintains acceptable low levels of microorganisms in the preparation.

In our study, at the time of purchase 16% of the samples were contaminated by large numbers of CFU/g cream. After passing three months of opening the products, contamination is increased to 29% and after six months this rate increased to 34%, which indicates that some preservative, unable to suppress the growth of several micro-organisms.

Contamination with *Staphylococcus aureus* and *Pseudomonas aeruginosa* are of particular concern, especially in the cosmetic products.[5,11,16] Based on current guidelines in cosmeceuticals (Cosmetic, Toiletry and Fragrance Association Inc.; CTFA, Cosmetic, Toiletry and Perfumery Association Ltd.; CTPA) and the requirements for cosmetic products set by the FDA the products contained high microbial counts or contained pathogenic microorganisms would be regarded as spoiled.[17]

Antimicrobial preservatives are one of the ingredients added to cosmetics to prevent contamination and microbial growth during the shelf life and usage period.[11] The efficacy of the preservative system can be evaluated by USP antimicrobial preservative efficacy tests or the test for solid cosmetic preservative efficacy proposed by Tran, *et al*.[5]

According to Table 3, after passing three and six months of opening the products, contamination by *S.aureus*, *P. aeruginosa*, yeasts and molds were increased. In the case of coliforms, contamination of products is reduced to 8.8% from 27%. The findings show that the preservatives employed in these cosmetic products did not possibly possess adequate preservative capacity to be able to bring about acceptable low levels of microbial contamination during uses. There is therefore, a pressing need to search for compounds with such additional properties if the microbiological wholesomeness of such products is to be ensured.

The results also showed significant differences in bacterial contamination with Iranian sunscreens and imported products compared with formulated products. These formulated products have no preservative system, so these products were unable to suppress the growth of several micro-organisms during uses. Although, the addition of a suitable preservative in

the products which guarantees the control of microbial growth even before they are marketed.

At the time of purchase Iranian sunscreens and imported products are contaminated, because the shelf life is not significantly studied during the drug development.

The shelf life is mostly influenced by several factors: Exposure to light and heat, transmission of gases (including humidity), mechanical stresses, and contamination by things such as micro-organisms.

Also, poor hygiene during manufacturing can cause microbial contamination before using the sunscreens.

On the basis of our data, 40% of the Iranian sunscreens, 73.3% of the imported products, and 43.3% of the formulated products at the time of purchase were unacceptable.

In a study conducted by Campana, *et al.,* (2006), 91 cosmetics were tested to measure the rate of bacterial growth after 0, 2, 7, 14 and 28 days of use. The results showed that 6-7% of the shampoos contained bacterial contamination while no contamination was seen in the lotions. The two most common bacteria found in the shampoos were *Staphylococcus warneri* and *Staphylococcus epidermidis*. [7]

In another report, the incidence of contamination by Gram positive Bacilli, *Staphylococcus aureus* and non-*Escherichia coli* Gram-negative organisms was investigated in used and unused cosmetic creams and it was found that the contamination was higher for used cosmetic creams (54%, 38% and 8% respectively) than unused creams (38%, 25% and 0% respectively). Viable microorganisms were not recovered from 17% of the unused items whilst only 10% of the used creams did not contain viable microorganisms.[7]

In a study performed by Okeke and Lamikanra (2001), bacteria in lotions and creams used in tropical countries were tested for microbiological quality at the time of purchase and after 14 days of use by a consumer. In 49 products sample, they found many microbes before and after use; the most common were *Escherichia coli* and *Pseudomonas aeruginosa.*[7]

Because of the relative dryness of the skin, in the absence of trauma or unusual predisposing conditions, skin infections by Gram-negative bacteria are not common. However, application of cosmetic creams with high moisture content on the skin may change this situation.[5]

CONCLUSION

The findings of this study show that the majority of sunscreen products in Iranian market are contaminated and none of them pass the standards. This study shows that the products we use in our homes cannot always be trusted, and maybe after a few weeks, it is necessary to replace our cosmetic products, especially sunscreen creams. However, in this study the microbial contamination of cosmetics because of manufacturing processes or/and inadequately preservative system has been demonstrated. It is highly recommended to the local cosmetic industry to apply set of guidelines such as FDA or EMA guidelines for manufacturing and control of their products and it is necessary to carry out a routine microbiological analysis of each batch of the finished product before releasing it to the market. This necessity is even more evident if the cosmetic industry is looking for opportunities to export and compete in the international markets.

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Source of Support: Nil, **Conflict of Interest:** Nil.