Investigation of preservative efficacy and microbiological content of some cosmetics found on the market

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Abstract: In this study, microbial content and preservative efficacy of various cosmetic products, which are produced and sold in markets of our country, were investigated. Microbial content and preservative efficacies of products were investigated according to United States Pharmacopeia (USP) method. Microorganism counts of out 14 of 93 cosmetic products were recovered in the range between 1.5×10^2-5.5×10^5 cfu/ml. *Staphylococcus aureus* was the most common contaminant identified in samples (from six different products) and was followed by *Burkholderia cepacia* (from four different products). Gram negative organisms, including *Pseudomonas aeruginosa* and a yeast *Candida krusei,* were also isolated from samples. *Escherichia coli* and *Salmonella sp*. were not recovered from any of samples. Preservative efficacies of fourteen out of ninety-three products did not meet the general efficacy of antimicrobial preservation criteria of the USP. Among these fourteen products, degradation and color change by *Aspergillus niger* was observed in one of samples. According to results, it was observed that pathogen and potential pathogen microorganisms can be found in unused cosmetic products and also preservatives may be ineffective in preventing them. Thus, in order to prevent the contamination that can occur during production, manufacturers are required to manufacture products in compliance with wholesome manufacturing practices and, considering consumer health, it is necessary to add an effective preservative as determined by regulations.

Keywords: Cosmetic, microbial contamination, preservative, preservative efficacy.

INTRODUCTION

The field of cosmetics and microbiology had not come into contact much before the 1930s and cosmetic microbiology became more important in 1940s (Curry *et al*., 2006). The first contamination of cosmetics was reported in 1946 by several cases of neonatal death from talcum powder containing *Clostridium tetani* (Baird, 1998). Since 1960s, opportunist organisms, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas sp., Serratia sp*. and *Enterobacter sp.*, have been isolated from cosmetic products to a certain extent (Baird, 1998; Geis, 2006).

Cosmetic products are not accepted to be sterile but they must be free of pathogenic microorganisms (like *Staphylococcus aureus*, *Escherichia coli*, *P. aeruginosa*) and the total aerobic microbial count must be low (Steinberg, 2006; USP, 2003). The presence of pathogenic organisms and high levels of microorganisms in cosmetic products can cause spoilage (physical deterioration of the product) and can be a health hazard for consumers (Behravan *et al*., 2005; Campana *et al*., 2006). Cosmetic products, used in our country, are occasionally found to be contaminated and they have been reported at times. For example, Çarıkçı *et al*. studied 127 used and unused cosmetic products and they reported *P. aeruginosa, K. pneumoniae, Pseudomonas putida* etc. in unused samples and Boynukara *et al.* isolated *Aspergillus niger* from an imported commercial skin cream (Çarıkçı *et al*., 2008, Boynukara *et al.*, 2002). As shown, microbial contamination is still one of the major causes for product recalls. Therefore, it is important to improve the preservative system in order to prevent the growth of contaminating microorganisms during manufacturing, storage and use by consumers (Farrington *et al*., 1994). According to Cosmetic Regulations in our country applied to these marketed products, safety evaluations have to be made by manufacturing companies for consumer's health.

To this end, microbial content (the type and incidence of microorganisms) and the antimicrobial preservation efficacy of various cosmetic products, which are manufactured and sold in markets of our country, were investigated in our study.

MATERIALS AND METHODS

Cosmetic Products

Ninety-three commercially available cosmetic products (6 shampoos, 5 shower gels, 10 shaving products, 7 moisturizing products, 9 depilatories, 5 liquid soaps, 5 antiperspirants, 7 baby care products, 7 face care products, 11 make-up removers, 4 mouthwashes, 6 toothpastes, 5 sunscreen creams and 2 feminine deodorants) were collected and employed in the study. Samples were bought from markets and analyzed as soon as possible upon their arrival.

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Microbiological analysis

All the collected cosmetic products were analyzed to detect the presence of total bacterial and fungal count. The microbial species *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella sp.* were investigated, as suggested in United States Pharmacopeia (USP, 2003). Each sample was weighed aseptically and diluted in pH 7 phosphate buffer, Tryptic Soy Broth (Difco) and Fluid Lactose Medium (Difco). Serial dilutions of the samples were homogenized in phosphate buffer and spread on duplicate Tryptic Soy Agar (Difco) and Sabouraud Dextrose Agar (Difco). Plates were incubated at 37°C 48h and 25°C 5-7 days. At the end of the incubation, emergent colonies were counted and the numbers of colony forming units (CFU/ml) were determined. Tryptic Soy Broth and Fluid Lactose Medium were incubated at 37° C 24h. After incubation, samples were spread on Mannitol Salt Agar (Oxoid), Baird-Parker Agar (Difco), Cetrimide Agar (Difco), MacConkey Agar (Difco), Eozine Methylene Blue Agar (Difco), Fluid Tetrathionate Medium (Difco), Xylose-Lysine-Deoxycholate Agar (Difco) and Bismuth Sulfite Agar (Difco) to determine the presence of specific microorganisms according to pharmacopeia. Plates were incubated at 37°C and identification was performed after microscopic examination and biochemical identification.

Challenge test for preservative efficacy

The efficacy of antimicrobial preservation of cosmetic products was investigated as suggested by USP. *P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739, *S. aureus* ATCC 6538, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 were used in this study. Freshly grown bacteria and yeast were harvested in sterile tryptone sodium chloride and prepared with $1x10^8$ cfu/ml inoculums (measured by spectrophotometer). The mold was harvested with sterile saline (was added Tween80) with the adjusted spore count of $1x10^8$ cfu/ml. Each sample weighed 50 grams in aseptic state and was inoculated respectively with 0.5 ml of each inoculum suspensions. All inoculated samples were shaken and incubated at 25°C for 28 days. Two gram aseptic samples were removed on days 0, 14 and 28 to the neutralizing medium. Numbers of viable microorganisms, in the inoculum suspension, were determined by the plate count. Dey-Engley Neutralizing Agar (Difco) and SDA were used on bacteria, yeast and mould, respectively. Plates were incubated at 37°C 24-48h and 25°C for 5 days. At the end of the incubation period, the number of colonies was recorded for each plate and counts were expressed as colony forming units per gram (cfu/g) were determined. The acceptance criteria for bacteria was at least the second logarithmic reduction from initial count and no increase from the 14 days count at 28 days and for yeast and mold and no increase from the initial count at 14 and 28 days (USP, 2003).

STATISTICAL ANALYSES

Chi-square goodness of fit test was performed to evaluate the statistical significance in bacterial growth reduction by the challenge tests used.

RESULTS

Microbiological analysis

The microbiological contamination in 93 cosmetic products was evaluated. Microbial counts and isolates recovered from several cosmetic products were summarized in table 1. As shown, no contamination was observed in shampoos, shower gels, liquid soaps, antiperspirants, baby care products, mouthwashes, sunscreen creams and women's deodorants. 20% of shaving products (2/10), 57.1% of moisturizing products (4/7), 42.8% of face care products (3/7), 11.1% of depilatories (1/9), 9.09% of make-up removers (1/11) and 50% of toothpastes (3/6) yielded contaminated results. The counts of products from which microorganisms were

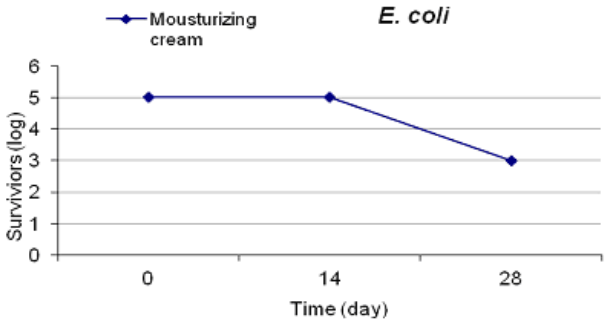
Table 1: Contaminated samples**,** total microbial counts and isolated species

Product Type	Counts (cfu/ml)	Isolated Microorganism(s)
Shaving cream	$<1 \times 10^1$	*S. aureus*
Shaving cream	$<1 \times 10^1$	*S. aureus*
Moisturizing cream	$<1 \times 10^1$	*S. aureus*
Moisturizing cream	7.88×10^4	*Alcaligenes xylosoxyidas, Burkholderia cepacia*
Moisturizing cream	1.4×10^5	*Stenotrophomonas maltophilia, Pseudomonas putida*
Moisturizing cream	5.5×10^5	*Candida krusei*
Face care cream	$<1 \times 10^1$	*S. aureus*
Face care cream	2.3×10^4	*Citrobacter freundii, B. cepacia, Burkholderia gladioli*
Face care cream	1.18×10^5	*S. aureus, Enterobacter cloacae, B.cepacia, Raoultella planticola,*
Depilatory cream	$<1 \times 10^1$	*S. aureus*
Eye make-up remover	1.5×10^2	*Gram positive bacilli*
Toothpaste	3.56×10^3	*Pseudomonas aeruginosa, B.cepacia*
Toothpaste	4.56×10^4	*Pseudomonas fluorescens, S. maltophilia, Aeromonas sp*
Toothpaste	6.8×10^2	*Gram positive bacilli*

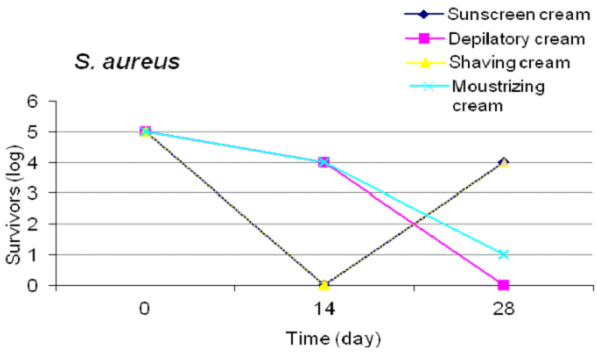
recovered ranged between 1.5×10^2-5.5×10^5 cfu/ml. The highest bacterial contamination (1.4×10^5 cfu/ml) was observed in a moisturizing product and the lowest (1.5×10^2 cfu/ml) in an eye make-up remover. S.aureus was the most frequently isolated contaminant from products. It was isolated from two shaving creams, one moisturizing cream, two face care creams, and one depilatory cream. *Burkholderia cepacia* was isolated from one moisturizing cream, two face care creams and one toothpaste. Gram negative organisms, including *P. aeruginosa,* and a yeast, *Candida krusei,* were isolated from samples as well. *E. coli* and *Salmonella sp*. were not recovered from any of samples.

Antimicrobial efficacy test

Results were evaluated according to USP in a fashion with no less than 2 log reductions from the initial count in 14 days and no more from the 14 days count in 28 days for bacteria and no more from the initial calculated count at 14 and 28 days for yeast and molds. 93 products were studied and it was detected that the preservatives in 14 products did not comply with the USP recommended antimicrobial preservative activity criteria. 4 of these 14 products were the ones to yield the highest microbial counts and their preservative activity results could not be graphed. Preservative systems of 84 products from the 93 studied products were found to be effective against bacteria ($p<0.001$). It was detected preservative systems of 5 products were ineffective. Preservatives of one sunscreen cream, one depilatory cream, one shaving cream and one moisturizing cream were found to be ineffective against S.aureus; moisturizing cream's preservative systems were found ineffective against *E. coli* (figs. 1 a-b). Preservative systems of 84 products out of the 93 studied products were found effective against fungi ($p<0.001$). It was detected preservatives of 5 products were ineffective. Among these products, one face care cream and two sunscreen cream's preservatives were found ineffective against *C. albicans* and one face care cream and sunscreen cream's preservatives were found ineffective against *A. niger* (figs. 1 c-d) Preservative system of neither of the products was found ineffective against *P. aeruginosa*. Among these fourteen products, degradation and color change was observed by *A. niger* in one of samples.



(a)



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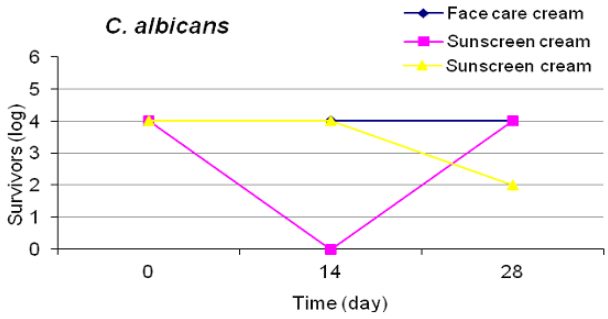
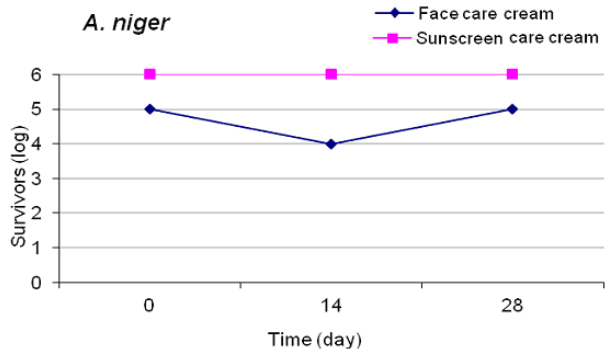


(c)



(d)

Fig. 1: Microorganisms survival in cosmetic products. (a) *E. coli*, (b) *S. aureus* (c) *C. albicans*, (d) *A. niger*.

DISCUSSIONS

In this study, we investigated 93 commercially available and unopened cosmetic products. 79 cosmetic products showed no microbiological contamination. Counts ranged between 1.5×10^2 - 5.5×10^5 cfu/ml, in which micro-organisms were recovered. The most frequently identified microorganism was *S. aureus*, which is the most common bacterial skin pathogen. Cosmetic's application is largely restricted to skin and *S. aureus*, which is a common skin organism that can cause boils, impetigo, conjunctivitis, folliculitis and food poisoning (Brannan, 2006). *P. aeruginosa,* the most frequently found contaminant in

cosmetics which infects wounds and burns and can also cause pneumonia in immunosuppressive patients, was also detected in one product (Brannan, 2006; Tenenbaum, 1967). Hopfer *et al*. reported infections and one death due to a shampoo used by immunosuppressive patients, which was contaminated with *P. aeruginosa* (Geis, 2006). Several cases of eye infections and even loss of vision were also caused by contaminated cosmetic products contaminated with *P. aeruginosa* (Tenenbaum, 1967; Reid and Wood, 1979). According to microbiological standards of cosmetic products, they must be free of high-virulence microbial pathogens like *S. aureus* and *P. aeruginosa;* however, our study reveals that these microorganisms can be found in unused cosmetic products. Neither of the samples were contaminated by *E. coli* and *Salmonella sp*.. Except these organisms, *B. cepacia, A. xylosoxyidans, S. maltophilia, P. putida, C. freundii, B. gladioli, E. cloacae, R. planticola, P. fluorescens, Aeromonas sp* and *C. krusei,* were also identified in contaminated cosmetic products. These microorganisms are also known to be opportunistic, with some of them resistant to microbial agents, and they can also cause infections to immunosuppressive patients (Öztürk, 2008; Kanj *et al*., 1997, Goldmann, 1986; Hautala *et al*., 2007). Previous studies with cosmetic products also yielded similar results. A survey on recalls of microbiologically contaminated cosmetics in Europe between 2005 to May 2008 reports 24 different cosmetic products have been contaminated with *P. aeruginosa*, *B. cepacia*, *S. aureus*, *Enterococcus sp*. etc. (Lundov and Zachariae, 2008). Okeke and Lamikanra evaluated the bacteriological quality of skin moisturizing creams and lotions that were purchased at a market (Okeke and Lamikanra, 2001). They recovered *E. coli*, *Pseudomonas sp*., *Staphylococcus sp*. and *Bacillus sp.*. Similar results were shown by Hugbo *et al.,* ten commercially available cosmetic creams and lotions were purchased and their microbiological content was evaluated (Hugbo *et al*., 2003). Investigators identified *S. aureus*, *Staphylococcus sp.* and *Bacillus sp.,* similar to previous studies. There were also outbreak investigations which resulted in the identification of these opportunistic pathogens in contaminated cosmetic products (Alvarez-Lerma *et al.,* 2008).

Microbial contamination, from manufacturer to consumer, can be controlled by sanitary processing and using appropriate and adequate preservatives. In the second part of our investigation, preservative efficacies of 93 products were assayed and preservatives of 79 products were able to meet the USP criteria. Nevertheless, preservatives of 14 products were ineffective against at least one microorganism. Preservatives are chemicals that are added to the finished product to eliminate or inhibit the growth of microorganisms and preserve the product (Sasseville, 2004). Very little of the literature describing the preservative efficacy of unopened cosmetic products features this aspect. Today, the most widely used cosmetic preservatives are esters of para-hydroxybenzoic acid (parabens), formaldehyde releasers, isothiazolinones, organic acids and organic alcohols (Geis, 2006). Additional to well-known cosmetic preservatives, products also include other antimicrobial components, such as alcohol, chelating agents, phenolic antioxidants, plant-derived essential oils and extracts and fragrance ingredients (Varvaresou *et al*., 2009).

Most of the studied samples, especially four of the five antiperspirant products, methyl, ethyl, butyl, isobutyl and propyl parabens, were included. Although parabens have ideal preservative properties, some recent *in vitro* and *in vivo* studies indicated that they adversely affect endocrine and the reproductive system (Oishi, 2002; Darbre *et al*., 2002). It was also reported that using parabens in cosmetics, particularly in underarm deodorants and antiperspirants, can be associated with breast cancer (Soni *et al*., 2005). Therefore, it is required to be careful not to use parabens in underarm cosmetic products that have estrogenic activities. In the preservative efficacy test on some products (shaving products, depilatories, antiperspirants), the number of microorganisms decreased from 10^6 to zero at the beginning of the test. Decreased number of microorganisms showed that, in these products, different types of preservatives and antimicrobial substances exceed permitted levels. Consequently, products that contain large amounts of antimicrobial substances, especially in non-rinsed products (such as after shaves and antiperspirants), can damage user's epithelial cells and it is important to be careful to protect consumer health. On the other hand, during the preservative efficacy test on a face care product which showed similar results with initial and first day counts for fungi, *C.albicans* and *A.niger* counts increased in 14 and 28 day counts. This number of fungus increase showed that benzyl alcohol, methyl-chloroisothiazolinone and methylisothiazolinone, which were this product's preservatives, are ineffective against fungi. Furthermore, in a sample that was added *A. niger* at the preservative efficacy test, white, the original color, turned to green during the test period. It was interpreted that, carbohydrates and plant derived raw materials are consumed as a food source by *A. niger* and this caused degradation of the product.

Our results showed that cosmetic products, produced in our country, can be contaminated during the production process. Therefore, it is important to take precautions during production process in order to prevent infections due to microbial contamination. This case shows that it is necessary to comply with GMP standards strictly during the production. According to the results of our study, preservatives should be added to products as determined by regulations and in accordance with toxic dose limits, for consumer's health.

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