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Original Article

Evaluation of preservative efficacies of some unused cosmetic products

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ABSTRACT

Background and Aims: Cosmetics must be free of pathogenic microorganisms, and the total aerobic microbial count needs to be within acceptable limits.
Methods: In this study, preservative efficacies of ten commercially available cosmetic products were investigated against *Pseudomonas aeruginosa*, *Pseudomonas putida, Stenotrophomonas maltophilia* and *Burkholderia cepacia*, which were isolated from contaminated cosmetic products.
Results: According to our results, all products preservatives did not comply with the United States Pharmacopeia (USP) method recommended antimicrobial preservative activity criteria against at least one studied bacteria.
Conclusion: Consequently, according to our results, preservatives of unused cosmetic products can be ineffective against bacteria, especially bacteria isolated from cosmetics.

Keywords: Cosmetic, preservative efficacy, preservative

INTRODUCTION

According to the Turkish Cosmetic Regulation, a cosmetic product means any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly of cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours (Cosmetic Law, 2005). Cosmetics, like any product containing water and organic/inorganic compounds, are exposed to microbial contamination under appropriate conditions (Halla et al., 2018). Although sterility is not a requirement in cosmetics, they must be free of pathogenic microorganisms, and the total aerobic microbial count needs to be within acceptable limits (Mugoyela & Mwambete, 2010). Pathogenic organisms or high levels of saprophyte microorganisms in cosmetic products lead to spoilage, which is of great importance to the industry and also causes serious health risks for consumers (Campana, Scesa, Patrone, Vittoria, & Baffone, 2006).

Studies have shown that many bacteria and fungi can be found as contaminants in cosmetics. Among them, pathogenic Gram-negative bacteria *Pseudomonas sp., Stenotrophomonas maltophilia* and *Burkholderia cepacia* are the most common contaminants found in cosmetics, which are infectors of wounds and burns, and also cause pneumonia, especially in immunosuppressive patients. These bacteria are commonly isolated nosocomial pathogens, and *Pseudomonas sp.* are also responsible for a variety of infectious diseases affecting the eyes and surrounding tissues (corneal ulcer, bacterial keratitis) and may cause loss of sight (Brannan, 2006; Neza & Centini, 2016).

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Therefore, in order to prevent the microbial contamination, which is the biological and physicochemical deterioration of a cosmetic product, manufacturers need to use chemical preservatives with known antimicrobial properties (Sutton, 2006). Several preservatives are used to ensure the microbiological quality, consumer safety and organoleptic properties of cosmetic products (Orús, Gomez-Perez, Leranoz, & Berlanga, 2015). In our country, according to the Cosmetic Law (Law No: 5324) and Regulations/Appendixes, a list of allowed preservatives in cosmetic products with maximum concentrations in ready-for-use preparations are listed, and manufacturers have to comply with these (Cosmetic Law, 2005).

Commonly used preservatives in cosmetics are formaldehyde releasers, isothiazolinones, organic acids and alcohols. An ideal preservative should be effective in low concentrations against a wide variety of microorganisms, non-toxic and compatible with other ingredients (Geis, 2006). Due to alkyl esters of p-hydroxybenzoic acid, although parabens are excellent preservatives with antimicrobial activity, side effects on the endocrine and the reproductive systems have limited their use in cosmetics (Darbre et al., 2002; Kizhedath, Wilkinson, & Glassey, 2019).

In this study, we aimed to investigate the antimicrobial preservation efficacy of various commercially available cosmetic products against *Pseudomonas aeruginosa, Pseudomonas putida, S. maltophilia* and *B. cepacia* which are involved in recurrent contamination in cosmetic products containing preservatives.

MATERIAL AND METHODS

Cosmetic products

Ten commercially available cosmetic products (4 liquid soaps, 3 shampoos and 3 make-up removers) were collected and employed in the study. Samples were bought from markets and analyzed as soon as possible upon their arrival.

Preservatives of the cosmetic products

Three of the liquid soaps contained Dimethyl dimethylol hydantoin (DMDM hydantoin), methylisothiazolinone and methylchloroisothiazolinone and one liquid soap contained methylisothiazolinone and methylchloroisothiazolinone, sodium benzoate and potassium sorbate. Although all three shampoos contained sodium benzoate, one of them also contained phenoxyethanol, and additionally, one of them also contained potassium sorbate, DMDM hydantoin, methylisothiazolinone and sodium salicylate. Two make-up removers contained polyaminopropyl biguanide, and one contained phenoxyethanol.

Neutralizer

The neutralizer used in this study contained lecithin, Polysorbate 80, sodium thiosulfate pentahydrate, L-Histidine, proteose peptone, sodium chloride, $Na_2HPO_4.12\ H_2O$ and KH_2PO_4.

Validation of neutralizer

The validation method for neutralizing the antimicrobial properties of a product must meet two criteria, neutralizer efficacy (NE) and neutralizer toxicity (NT). One mL of the test sample (test group) or peptone (peptone group) was added to a tube containing 9 mL of neutralizing broth. A third tube, containing 10 mL sterile saline (viability control) and a fourth tube containing one ml test sample and 9 ml saline solution (dilution control) were also prepared. Each of these solutions was inoculated with $1x10^4$ colony forming units/ml (cfu) of the challenge organisms, and incubated for 10 minutes on the benchtop at ambient temperature. Recovery of all organisms was determined by the plate count on tryptic soy agar (TSA, Difco Laboratories), and the plates were incubated at 30-35°C for three days. NT was determined by comparing the recovery of the microorganisms in the peptone group and the viability group. NE was determined by comparing the number of the recovered microorganisms in the test group and peptone group, and also by dilution control and viability control. An effective and non-toxic neutralizer was defined by NE and NT ratios of > or=0.70 (USP, 2006).

Challenge test for preservative efficacy

The efficacy of antimicrobial preservation of cosmetic products was investigated as suggested by USP. *P. putida, S. maltophilia* and *B. cepacia* which were isolated from contaminated cosmetic products from moisturizing cream, toothpaste and face care cream, respectively, and a standard strain *P. aeruginosa* ATCC 9027, were used in this study (Birteksoz, Tuysuz, & Otuk,2013). Freshly grown bacteria were harvested in sterile tryptone sodium chloride, and prepared with $1x10^8$ cfu/ml inoculums. 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. Aseptic samples were removed on days 0, 14 and 28 to the efficient and nontoxic neutralizing medium which is verified above. Numbers of viable microorganisms, in the inoculum suspension, were determined by the plate count by using Dey-Engley Neutralizing Agar (Difco Laboratories), and the plates were incubated at 37°C 24-48h. At the end of the incubation period, the number of colonies was recorded for each plate, and counts were expressed as cfu/g. The acceptance criteria were at least the second logarithmic reduction from initial count and no increase from the 14 days' count at 28 days (USP, 2006).

RESULTS

Validation of neutralizer

According to NE and NT ratios of > or=0.70, the neutralizer used in this study was found efficient and non-toxic to studied microorganisms. Validation results were shown at Table 1.

Table 1. NE and NT rations of the neutralizer

	NT rations	NE rations	
P. aeruginosa ATCC 9027	0.9	0.8	0.75
P. putida	0.92	0.88	0.77
S. maltophilia	0.74	0.9	0.78
B. cepacia	0.8	0.92	0.83

Antimicrobial efficacy test

The results were evaluated according to USP, 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. Ten products were studied, and it was detected that none of the products preservative complied with the USP recommendations for antimicrobial preservative activity criteria against at least one studied bacterium. Preservatives of one liquid soap, two shampoos and all the make-up removers were found to be ineffective against *P. aeruginosa* and three liquid soaps, all the shampoos and one make-up remover were found ineffective against *P. putida*. It was detected that the preservatives of three shampoos against *B. cepacia* and the preservatives of two liquid soaps, one shampoo and two make-up removers were found ineffective against *S. maltophilia* (Figure 1a-d).

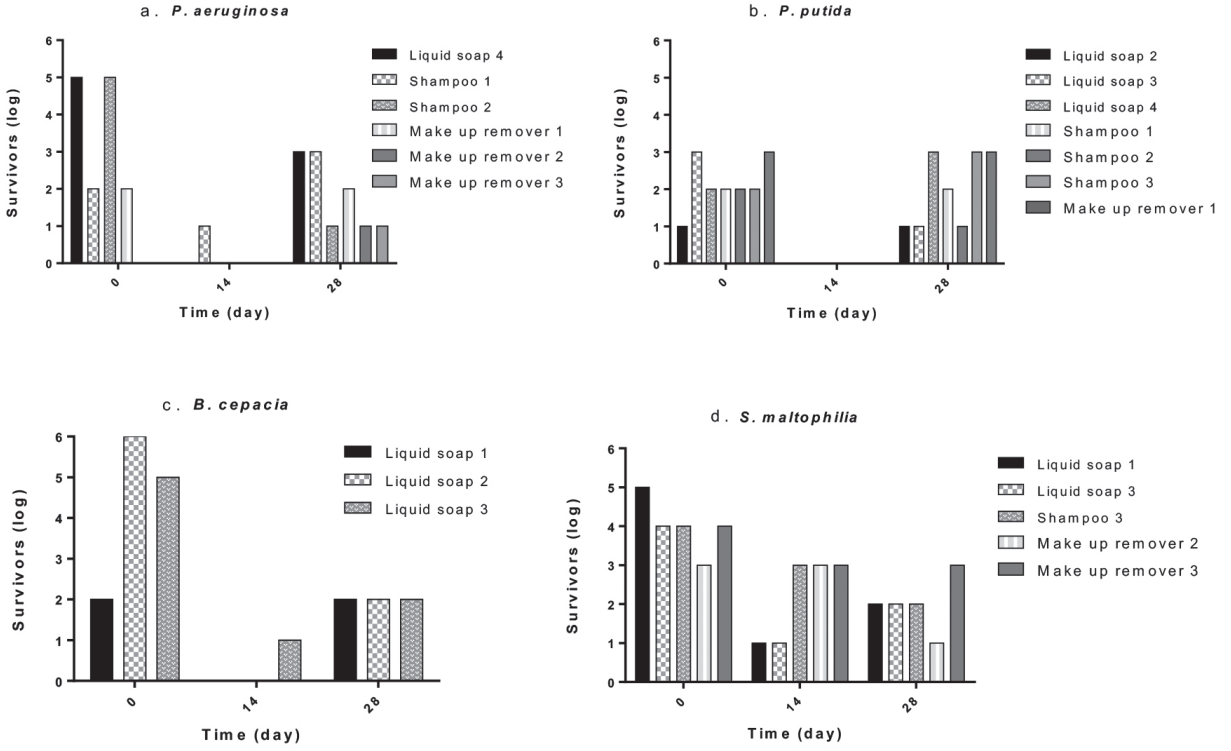

Figure 1. Microorganisms survival in cosmetic products. (a) *P. aeruginosa*, (b) *P. putida* (c) *B. cepacia*, (d) *S. maltophilia*.

DISCUSSION

Since consumer health is the primary concern, product quality can be improved and the risk of microbiological contamination can be prevented by using preservatives (Geis, 2006). In this study, we investigated the preservative efficacies of ten commercially available and unopened cosmetic products. According to our results, all the products' preservatives did not comply with the USP recommended antimicrobial preservative activity criteria against at least one of the studied bacteria.

The preservatives of a liquid soap, methylisothiazolinone and methylchloroisothiazolinone and sodium benzoate and potassium sorbate, preservatives of two shampoos, phenoxyethanol, sodium benzoate, potassium sorbate, DMDM hydantoin, methylisothiazolinone and sodium salicylate and the preservatives of make-up removers, polyaminopropyl biguanide and phenoxyethanol were found ineffective against *P. aeruginosa*. DMDM hydantoin, methylisothiazolinone, methylchloroisothiazolinone, sodium benzoate and potassium sorbate which were the preservatives of three liquid soaps, DMDM hydantoin, methylisothiazolinone, potassium sorbate, sodium salicylate, phenoxyethanol and sodium benzoate, preservatives of three shampoos and phenoxyethanol, preservative of a make-up remover were found ineffective against *P. putida*.

Pseudomonas sp. such as *P. aeruginosa* and *P. putida* are frequently found in contaminated cosmetics. In contaminated ophthalmic preparations, *P. aeruginosa* is also responsible for serious eye infections (corneal ulcer, bacterial keratitis), and even loss of vision (Birteksoz Tan et al.,2013; Brannan, 2006; Neza & Centini, 2016; Yossa et al., 2018). Furthermore, Hopfer et al. reported infections and one death due to a *P. aeruginosa* contaminated shampoo used by immunosuppressed patients (Geis, 2006).

An opportunistic pathogen, *B. cepacia,* is also a frequent contaminant in cosmetics, and it is one of the causes of product recalls (Alvarez-Lerma et al., 2008; Birteksoz Tan et al.; 2013, Jimenez, Smalls, Jimenez, & Smalls, 2000). Three shampoos' preservatives DMDM hydantoin, methylisothiazolinone and methylchloroisothiazolinone were found ineffective against *B. cepacia* in our study.

One of the important causes of nosocomial infections, *S. maltophilia*, can be found in used cosmetics such as shampoos or body lotions (Birteksoz Tan et al., 2013; Brannan & Dille, 1990).

In our study, the preservatives of two liquid soaps, DMDM hydantoin, methylisothiazolinone, methylchloroisothiazolinone, the preservatives of a shampoo, sodium benzoate and the preservatives of two make up removers, polyaminopropyl biguanide, were found ineffective against *S. maltophilia***.**

Phenoxyethanol is one of the most commonly used preservatives in personal care formulations, but it was found ineffective against both *P. aeruginosa* and *P. putida*. Similar to our results, Flores et al. (1997) found several microorganisms which were isolated from contaminated cosmetic products, resistant to phenoxyethanol.

Although among the formaldehyde releasers, DMDM hydantoin, and among the isothiazolinones, methylisothiazolinone and methylchloroisothiazolinone, are some of the most common and effective preservatives, they were found ineffective against at least one of the studied bacteria, in our study.

Because the development of resistance to preservatives is not a new phenomenon and it is a problem with serious economic and health consequences, new researches should focus on alternative or new preservatives (Chapman, 1998).

Consequently, according to our results, it has been showed that preservatives of unused cosmetic products can be ineffective against bacteria, especially bacteria isolated from cosmetics. Preservatives should be added to cosmetic products as determined by regulations, and in accordance with toxic dose limits, for consumer's health, and they should also be investigated for their effectiveness against the most isolated bacteria.

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