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ORIGINAL ARTICLE / ÖZGÜN MAKALE

INVESTIGATION OF THE MICROBIOLOGICAL CONTAMINATION OF USED LIPSTICKS AND MASCARAS

KULLANILMIŞ RUJ VE MASKARALARDA MİKROBİYOLOJİK KONTAMİNASYONUNUN ARAŞTIRILMASI

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ABSTRACT

Objective: *Carbon and nitrogen sources, minerals, growth factors in the composition of cosmetic products, and environmental conditions such as convenient acidic environment and humidity constitute the suitable environment for microbial growth. Microbiological contamination of these products is important in terms of both posing a risk to consumer health and causing economic losses due to changes occurring in the product. This study aimed to examine the lipstick and mascara samples used by consumers in terms of microbiological contamination.*

Material and Method: *Thirty lipsticks and thirty mascaras used by consumers were investigated for microbiological contamination. The samples were evaluated according to the microbiological limits specified in the European Standard EN ISO 17516. Contaminant bacteria were identified by VITEK® 2 Compact (bioMérieux, France).*

Result and Discussion: *According to the results obtained, it was determined that only one of the tested mascara samples and four of the lipstick samples did not comply with the specified microbiological limit values. Staphylococcus sciuri was the contaminant bacterium determined in the mascara sample. In addition, Kocuria kristinae, Streptococcus mitis, Streptococcus oralis, Streptococcus alactolyticus, Rothia mucilaginosa, Rothia dentocariosa were detected in lipstick samples. All the bacteria detected as contaminants are Gram-positive, most of which are the members of the mouth, throat, respiratory, and skin microbiota. The contamination may have been caused by the inappropriate use of consumers, such as using the same product by more than one person, contamination of saliva, and not paying attention to hand hygiene.*

Keywords: *Cosmetics, EN ISO 17516, microbial contamination, microbiological quality control, safety testing*

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ÖZ

Amaç: *Kozmetik ürünlerin içeriğinde yer alan karbon ve azot kaynakları, mineraller, büyüme faktörleri ve asidik ortam, nem gibi uygun ortam koşulları mikrobiyal üreme için elverişli koşulların oluşmasını sağlar. Bu ürünlerin mikrobiyolojik kontaminasyonu hem tüketici sağlığı açısından risk oluşturması hem de üründe meydana gelebilecek değişiklikler nedeniyle ekonomik kayıplara neden olması açısından önem taşımaktadır. Bu çalışmada tüketiciler tarafından kullanılmış ruj ve rimel örneklerinin mikrobiyolojik kontaminasyon açısından incelenmesi amaçlanmıştır.*

Gereç ve Yöntem: *Çalışmada tüketiciler tarafından kullanılmış otuz adet ruj ve otuz adet rimel örneği mikrobiyolojik kontaminasyon açısından incelenmiştir. Numuneler, Avrupa Standardı EN ISO 17516'da belirtilen mikrobiyolojik limit değerlerine göre değerlendirilmiştir. Kontaminant bakteriler VITEK® 2 Compact (bioMérieux, Fransa) otomatize sistemi ile tanımlanmıştır.*

Sonuç ve Tartışma: *Elde edilen sonuçlara göre, test edilen maskara örneklerinden yalnızca birinin, ruj örneklerinden ise dördünün belirtilen mikrobiyolojik limit değerlerine uygun olmadığı saptanmıştır. Maskara örneğinden izole edilen bakteri Staphylococcus sciuri, ruj örneklerinden izole edilen bakteriler ise Kocuria kristinae, Streptococcus mitis, Streptococcus oralis, Streptococcus alactolyticus, Rothia mucilaginosa, Rothia dentocariosa türleri olarak tanımlanmıştır. Kontaminasyona neden olan bakterilerin çoğunluğunun ağız, boğaz, solunum ve cilt mikrobiyotasının üyeleri, Gram pozitif türler olduğu görülmüştür. Test edilen kozmetik ürünlerin bulaşının, tüketicilerin uygun olmayan kullanımlarından, aynı ürünü birden fazla kişinin kullanmasından, tükürük bulaşından, el hijyenine dikkat edilmemesinden kaynaklanmış olabileceği düşünülmüştür.*

Anahtar Kelimeler: *EN ISO 17516, güvenlik testi, kozmetikler, mikrobiyal kontaminasyon, mikrobiyolojik kalite kontrolü*

INTRODUCTION

Looking good and well-groomed has been important for most people since ancient times. Although the perception of beauty differs according to the conditions of the time and the lifestyles of the societies, people have always cared about being adorned, looking good and well-groomed, and allotted time and budget for this [1]. The first findings on the usage of cosmetics belong to ancient Egyptians. The Ebers Papyrus mentioned that the Egyptians gave importance to their hair and facial appearance and cared about their physical appearance [2,3]. The Council of European Union regulation defines a *cosmetic product* as "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 to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition, or correcting body odors". Cosmetic products must be delivered to the public effectively, safely, and high-quality manner. These products must be of a nature that will not harm human health when used under conditions that can be foreseen by the manufacturer or in accordance with the presentation, labeling, or the information provided by the manufacturer [4-6].

Carbon and nitrogen sources, minerals, growth factors, and water-based mixtures in the composition of cosmetic products constitute a suitable environment for microbial growth. Microbiological contamination of cosmetic products can originate from raw materials or can occur during manufacturing, packaging steps of the cosmetic product, or during its use by the consumer. Microbiological contamination is important in terms of both posing a risk to consumer health and causing economic losses due to changes in the product. Contaminant microorganisms can metabolize the raw materials in the product with their various hydrolytic enzymes, causing some properties to change and the structure to deteriorate. For example, odor and gas formation, viscosity and colour changes, taste changes, turbidity, precipitation, and membrane formation can be listed as changes in the contaminated product. The skin and mucous membranes act as a physical barrier that prevents the penetration of microorganisms. The damage of these structures and the deterioration of their integrity for various reasons facilitate the entry of microorganisms into the body. Contaminated product use may cause infection in the disintegrated tissue, as well as endotoxin and metabolites produced by microorganisms, which may cause wear, irritation, or allergies in the body [4,6-11].

Microorganisms that frequently cause contamination in cosmetic products are reported as; *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeroginosa*, *Enterobacter* sp., *Candida albicans* and *Aspergillus* sp. [10,12-14].

Cosmetic products that must be manufactured in accordance with Good Manufacture Practice (GMP) rules are included in the group of non-sterile pharmaceutical products. After the cosmetic product is opened, its microbial safety must be ensured, and its quality and properties must be preserved until the expiration date. Preservatives can be added to cosmetic products at risk of contamination, with a broad spectrum of action, without allergic, toxic, and irritating effects on the consumer, which can protect the product in a way that is equivalent to the expected shelf life plus the usage time [15-17].

European Standard EN ISO 17516 has specified *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans* as the microorganisms that should not be present in 1 g/ml of cosmetics. Limits of total aerobic mesophilic microorganisms found in cosmetics are also mentioned. In this standard, the total aerobic mesophilic microorganisms (bacteria-yeast-mould) allowed to be present in lipsticks and mascaras is stated as "$\leq 1x10^3$ CFU per g or ml" [6,18]. This study aimed to examine the lipstick and mascara samples used by consumers in terms of microbiological contamination.

MATERIAL AND METHOD

Sampling

In this study, 30 lipstick and 30 mascara samples belonging to various trademarks and used by consumers were investigated for microbiological contamination. Before opening, the surfaces of the sample containers were disinfected with an aqueous mixture of 70% ethanol (v/v) [19,20]. Then one g of lipstick sample was weighed and dispersed in a nine ml sterile phosphate buffer solution containing 0.1% polysorbate 80 and mixed with a vortex until the sample dissolved. After the applicators of the mascara samples were removed, one ml sterile phosphate buffer solution containing 0.1% polysorbate 80 was added to it. Then the applicator was placed under aseptic conditions and mixed with a vortex. The first dilution was prepared by transferring one ml of sample into a nine ml phosphate buffer solution containing 0.1% polysorbate 80. These suspensions were the 10^{-1} dilutions. In addition, 10^{-1} to 10^{-3} dilutions of samples were prepared for enumerations of aerobic mesophilic bacteria and yeast/mold [19-21].

Enumeration of Aerobic Mesophilic Microorganism

Enumeration of aerobic mesophilic microorganisms was performed using the pour plate method. First, one ml of each sample was plated in a sterile petri dish in duplicate and pour plated with 20 ml of Tryptone Glucose Extract Agar (TGEA) (Merck, Darmstadt, Germany) for enumeration of the aerobic mesophilic bacteria. Then, the plates were incubated at 32.5 ± 2.5˚C for 3 to 5 days. After incubation, the number of CFUs per ml or per g of the product was calculated.

Enumeration of Yeast and Mold

Enumeration of yeast and mold were performed using the pour plate method. First, one ml of each sample was plated in a sterile petri dish in duplicate and pour plated with 20 ml of Sabouraud Dextrose Agar (SDA) (Merck, Darmstadt, Germany) for enumeration of the yeast and mold. Then, the plates were incubated at 22.5 ± 2.5˚C for 5 to 7 days. After incubation, the number of CFUs per ml or per g of the product was calculated.

Detection of *Escherichia coli*

One ml of each sample was inoculated onto MacConkey Agar (MCA) (Merck, Darmstadt, Germany) in duplicate. The plates were incubated at 30-35˚C for 18 to 72 h. After incubation, the plates were observed for the growth of brick red colonies with a surrounding zone of precipitated bile on the MCA, indicating the presence of *Escherichia coli* [19,22].

Detection of *Staphylococcus aureus*

One ml of each sample was inoculated onto Baird Parker Agar (Merck, Darmstadt, Germany) in duplicate. The plates were incubated at 30-35˚C for 18 to 72 h. After incubation, the plates were observed for the growth of black, shiny colonies surrounded by clear zones [19,22].

Detection of *Pseudomonas aeruginosa*

One ml of each sample was inoculated onto Cetrimide Agar (Merck, Darmstadt, Germany) in duplicate. The plates were incubated at 30-35˚C for 18 to 72 h. After incubation, the plates were observed for the growth of yellow to green colonies [19,22].

Detection of *Candida albicans*

One ml of each sample was inoculated onto SDA in duplicate. The plates were incubated at 25˚C for 5 to 7 days. After incubation, the plates were observed for the growth of white to beige colonies [19,22].

Identification of the Microorganisms

Gram staining and microscopic examination were firstly performed. Then, isolated microorganisms were identified at the species level using the VITEK® 2 Compact (bioMérieux, France) [8].

RESULT AND DISCUSSION

In this study, the number of aerobic mesophilic bacteria was found to be $3.6x10^3$ CFU/ml in only one of the tested mascara samples. No growth was observed in any of the mascara samples except this one. Considering the lipstick samples, the total number of aerobic mesophilic bacteria in only five samples was calculated as $2x10^2$ CFU/ml, $1.1x10^3$ CFU/ml, $1.2x10^3$ CFU/ml, $1.5x10^3$ CFU/ml, and $3x10^3$ CFU/ml, respectively. No growth was observed in the lipstick samples other than these five samples. According to the results, it was determined that only one of the tested mascara samples and four of the lipstick samples did not comply with the specified microbiological limit values stated in European Standard EN ISO 17516. No growth was observed in any lipstick and mascara samples inoculated in SDA medium for enumeration of yeast and mold. The presence of *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans* was not detected in any of the tested lipstick and mascara samples. *Staphylococcus sciuri* was the contaminant bacterium determined in the mascara sample. Furthermore, *Kocuria kristinae*, *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus alactolyticus*, *Rothia mucilaginosa*, and *Rothia dentocariosa* were detected in lipstick samples (Table 1).

Organic and inorganic substances in the formulations of cosmetic products create suitable environments for the growth of microorganisms. Today, the tendency toward natural products with organic content rather than inorganic chemical products and the fact that these natural environments with plant and animal content create more favorable environments for the growth of microorganisms draws attention to the importance of contamination [6-8]. Contamination of cosmetic products with microorganisms was first noticed in New Zealand in 1946, with infant deaths caused by talcum powder contaminated with *Clostridium tetani* [23]. In the following years, many studies have shown that cosmetics can be contaminated with different microorganisms from various sources [8,10,13,14,24, 25].

In a study conducted in the United Kingdom, used lip gloss (107), lipstick (96), mascara (93), and eyeliner (92) samples were evaluated for the presence of microbiological contamination. In the lip glosses *Staphylococcus saprophyticus* (2), *Staphylococcus capitis* (2), *Staphylococcus haemolyticus* (1), *Staphylococcus cohnii* (1), *Sporosarcina pasteurii* (1), *Micrococcus luteus* (1), *Bacillus litoralis* (1), *Pseudomonas monteilii* (3), *Pseudomonas fulva* (2), *Pseudomonas putida* (1), *Lactobacillus* (1), *Citrobacter freundii* (1), *Candida glabrata* (1); in lipsticks *P. fulva* (2), *P. monteilii* (1), *C. freundii* (1); in eyeliners *Cryptococcus diffluens* (1), *Micrococcus luteus* (1), *Burkholderia vietnamiensis* (1), *Bacillus muralis* (1), *Stapylococcus hominis* (2), *S. haemolyticus* (2), *S. saprophyticus* (1), *S. capitis* (2), *E. coli* (2), *Arthrobacter roseus* (1); in mascaras *Pluralibacter gergoviae* (2), *S. saprophyticus* (1) were identified as contaminant microorganisms [8].

Table 1. Microbiological Limits Test results of the mascara and lipstick samples and identified microbial contaminants

		Mascara / n* (30)		Lipstick / n* (30)	
Microbiological Limits Test Results	**Aerobic Mesophilic Microorganism**	n (1)	$3.6x10^3$ CFU/ml	n (5)	$2x10^2$ CFU/ml $1.1x10^3$ CFU/ml $1.2x10^3$ CFU/ml $1.5x10^3$ CFU/ml $3x10^3$ CFU/ml
	Yeast and Mold	No growth		No growth	
	Escherichia coli	No growth		No growth	
	Pseudomonas aeruginosa	No growth		No growth	
	Staphylococcus aureus	No growth		No growth	
	Candida albicans	No growth		No growth	
Microorganisms Identified		*Staphylococcus sciuri*		*Kocuria kristinae* *Streptococcus mitis* *Streptococcus oralis* *Streptococcus alactolyticus* *Rothia mucilaginosa* *Rothia dentocariosa*	

*** n:** number of samples

Expiration dates of cosmetic products are determined by the length of time the preservatives formulated in the product are capable of controlling contamination [8]. In a study, three different cosmetic product groups were evaluated regarding microbiological contamination: (i) the expiration date was not passed and was used by only one person, (ii) the expiration date was not passed, and it was used by more than one person, and (iii) it was used after the expiration date. The level of contamination of cosmetic products used by more than one person was found to be higher than those used by only one person. Furthermore, it was observed that the highest contamination was in the products that continued to be used after the expiration date. The contaminant microorganisms were identified as *P. aeruginosa*, *Staphylococcus* sp., *Penicillium* sp. and *Aspergillus* sp. [26].

Studies indicate that cosmetic products are often contaminated during use by the consumer. The most important causes of contamination can be listed as the use of products without considering the expiration date, the use by more than one person, the insertion of fingers or contaminated objects into them, wetting them with saliva, and being in contact with air [8,27]. A study reported that 97.9% of consumers continue to use cosmetic products even if their expiration date has passed. Although the expiration date has passed, mascara is the most used cosmetic product. *S. aureus* (79%) was reported as mascara's most identified contaminant, followed by *P. aeruginosa* (13%) [28]. It was observed that only one of the mascara samples tested in our study did not comply with the microbiological limit values reported by the European Standard EN ISO 17516. Contaminant bacteria isolated from the mascara sample was identified as *Staphylococcus sciuri*. *S. sciuri*, coagulase-negative staphylococci, is mainly found in animals but also common in soil, water, and plants. Although the incidence is rare, *S. sciuri* has been associated with severe infections in humans, such as peritonitis, endocarditis, wound infections, and septic shock [29]. It is thought that the contamination in the tested mascara sample may originate from pets or the environment due to the user does not pay attention to the hygiene rules.

In a study, it was reported that corneal ulcers caused by *P. aeruginosa* develop via scratches on the cornea due to the use of mascara [30]. In another study, Reid and Wood indicated that a corneal ulcer developed in a 47-year-old woman due to using mascara contaminated with *P. aeruginosa* [31].

Cosmetic products with high water content are more suitable for microbial growth in case of contamination [7,9,10]. Dadashi and Dehghanzadeh reported that the contaminated mascaras have more bacterial diversity due to their aqueous-based formulation [32].

Lipsticks contain oils, wax, antioxidants, and emollients. Oily environments are less conducive to microbial growth than aqueous media. Therefore, lipstick's composition does not support microbial growth [33]. Jung et al. indicated that the contaminant bacteria in lipsticks are generally Gram-positive, facultative anaerobe opportunistic pathogens, and they identified the contaminant bacteria as *S. aureus*, *S. salivarius*, and *S. epidermidis*. Most microorganisms in the microbiota are opportunistic pathogens, which are essential because they can cause significant infections, particularly in immunocompromised individuals. Sharing cosmetics with different people may pose a health risk since each person's microbiota is unique and could harm others [8,32,34]. In our study, we isolated the *Kocuria kristinae*, *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus alactolyticus*, *Rothia mucilaginosa*, and *Rothia dentocariosa* from lipstick samples. All these bacteria were Gram-positive and members of the mouth, throat, respiratory, and skin microbiota. Therefore, it is thought that the contamination may have been caused by the inappropriate use of consumers, such as the use of the same product by more than one person, contamination of saliva, and not paying attention to hand hygiene.

Our findings showed that the contaminant bacteria are Gram-positive, most of which are the mouth, throat, respiratory, and skin flora members. Therefore, it is thought that the contamination may have been caused by the inappropriate use of consumers, such as the use of the same product by more than one person, contamination of saliva, and not paying attention to hand hygiene. In order to prevent microbial contamination, cosmetic products should be used by paying attention to hygiene rules, not sharing with others, storing them under appropriate conditions, and paying attention to the labels, which usually express the product's life after opening.

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AUTHOR CONTRIBUTIONS

Concept: E.K.Y., M.E.; Design: E.K.Y., M.E.; Control: M.E.; Sources: E.K.Y., M.E.; Materials: E.K.Y., Data Collection and/or Processing: E.K.Y., M.E.; Analysis and/or Interpretation: E.K.Y., M.E.; Literature Review: E.K.Y., M.E.; Manuscript Writing: E.K.Y., M.E.; Critical Review: E.K.Y., M.E.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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