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ORIGINAL ARTICLE

MICROBIOLOGICAL PURITY ASSESSMENT OF COSMETICS USED BY ONE AND SEVERAL PERSONS AND COSMETICS AFTER THEIR EXPIRY DATE

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ABSTRACT

Background. Microbiological purity of cosmetics provides safety of users during their use, prevents physicochemical changes of a preparation, infections and diseases of the skin.

Objective. The aim of this study was to assess the level of microbiological contamination of cosmetics used by one person and by several people and cosmetics after their expiry date in relations to standards for marketed cosmetics, ensuring safety of their use.

Material and Methods. This study was conducted using 55 samples representing 19 types of cosmetics, divided into three groups: used by one person, used by several people and after the expiry date. In cosmetic samples the general numbers of aerobic mesophilic bacteria were determined with the spread plate method on tryptic-soy agar. The presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* were also checked.

Results. The number of aerobic mesophylic bacteria in the tested cosmetics ranged from the level below the method detectability to 1.3×10^7 cfu/g or ml. The presence of *Staphylococcus* spp. was found in 11 (20.0%) tested cosmetic samples and of *P. aeruginosa* in one tested preparation. Yeasts *C. albicans* were not detected, whereas contamination with fungi *Aspergillus* spp. and *Penicillium* spp. ranging from 0.5×10^1 to 1.5×10^1 cfu/g or ml was recorded in four cosmetics. The level of microbiological contamination of cosmetics used by several people was higher than that of cosmetics used by one person. Cosmetics after the expiry date showed the highest microbiological contamination.

Conclusions. The number of users of cosmetic and it expiry date exceeding influenced the level of microbial contamination of preparations.

Key words: *microbiological purity of cosmetics; cosmetics usage; overdue cosmetics; bacteria in cosmetics*

STRESZCZENIE

Wprowadzenie. Czystość mikrobiologiczna kosmetyków zapewnia bezpieczeństwo podczas ich stosowania, zapobiega zmianom fizykochemicznym preparatu oraz infekcjom i chorobom skóry.

Cel badań. Celem pracy była ocena zanieczyszczenia mikrobiologicznego kosmetyków używanych przez jedną i wiele osób oraz kosmetyków przeterminowanych w odniesieniu do norm dla kosmetyków wprowadzonych do obrotu, gwarantujących bezpieczeństwo ich stosowania.

Materiał i metody. W badaniu wykorzystano 55 próbek reprezentujących 19 typów kosmetyków, które podzielono na trzy grupy: używane przez jedną osobę, przez kilka osób oraz przeterminowane W próbkach badanych kosmetyków określano ogólną liczbę tlenowych bakterii mezofilnych metodą posiewu powierzchniowego na podłożu tryptozowo-sojowym. Sprawdzono również obecność *Staphylococcus aureus*, *Pseudomonas aeruginosa* i *Candida albicans*.

Wyniki. W badanych kosmetykach liczba tlenowych bakterii mezofilnych mieściła się w przedziale od poziomu poniżej wykrywalności metody do $1{,}3\times10^7$ j.t.k./g lub ml. *Staphylococcus* spp. wykryto w 11 (20.0%) badanych próbkach, a *P. aeruginosa* w jednej. W żadnym z badanych kosmetyków nie wykryto drożdżaków *C. albicans*, natomiast w czterech stwierdzono zanieczyszczenie pleśniami *Aspergillus* spp. i *Penicillium* spp. wahające się od 0.5×10^1 do 1.5×10^1 j.t.k./g lub ml. Poziom zanieczyszczenia mikrobiologicznego kosmetyków używanych przez kilka osób był wyższy niż używanych przez jedną osobę. Kosmetyki przeterminowane były najbardziej skażone mikrobiologicznie.

Wnioski. Liczba osób używających ten sam kosmetyk oraz przekroczenie jego terminu ważności wpływają istotnie na poziom skażenia mikrobiologicznego preparatu.

Słowa kluczowe: *czystość mikrobiologiczna kosmetyków, użycie kosmetyków, kosmetyki przeterminowane, bakterie w kosmetykach*

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INTRODUCTION

From obtaining raw materials, through the technological process, to the use of the ready product by a consumer, there is a risk of microbiological contamination of a cosmetic products [17]. Avoiding primary contamination, along with the cleanness of production surfaces of devices, production hygiene, microbiological air pollution, personal hygiene of the staff and the quality of the used raw materials, ensure the quality and safety of produced cosmetics [17, 25]. During the use a cosmetic is exposed to secondary contamination, connected with the way of its use by the consumer, storage conditions, the type and size of a packaging, as well as the time of use and the number of users of the given product [17].

According to the guidelines contained in the Ordinance of the Minister of Health of 23 December 2002 [22], meeting both qualitative and quantitative requirements (Table 1) allows for authorization of the cosmetic for use. Quantitative requirements divide cosmetics into two categories. Category I refers to cosmetics for children, cosmetics intended for use in the area of eyes and on mucous membranes, and the other cosmetic products compose category II. Additional tests ale also performed for the presence of *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens* or *Burkholderia cepacia*, which may pose a potential threat to the consumer [10].

Water and materials of animal, plant and mineral origin used in production of cosmetics may cause their contamination with microorganisms from the genus *Bacillus, Clostridium, Pseudomonas, Micrococcus, Flavobacterium* and yeasts [3, 21, 23]. For this reason, cosmetics additionally must contain natural and synthetic preservatives, ensuring their microbiological purity.

The microorganisms often isolated from contaminated cosmetics in tests for microbiological purity of cosmetics are relatively anaerobic species from the genus *Staphylococcus*: *S. aureus, S. epidermidis* and *S. warneri* [6]. The most common cause of skin infections caused by the use of a cosmetic contaminated with *S. aureus* are strains MRSA (methicillin-resistant *Staphylococcus aureus*) [4]. The species *S. aureus*, being an element of natural human microflora, is responsible for purulent skin infections, such as: folliculitis, sycosis, boil, hidradenitis suppurativa and bacterial conjunctivitis. *S. aureus* may cause bullous impetigo in newborn babies (SSSS – staphylococcal scalded skin syndrome) caused by epidermolysine generated by this species. [8].

Both in lotions, soaps, shampoos and cosmetics for eye makeup the genus *Pseudomonas* is mainly represented by *P. aeruginosa* and *P. putida* [20]. *P. aeruginosa* may lead to eye infection, particularly to the ulceration and deep infection of the cornea, which results to its damage [19].

Cosmetics for body rinsing and conditioning and colour cosmetics may contain microorganisms, such as: *Serratia marcescens, Citrobacter freundii*, *P. putida*, *Enterobacter* spp. or *Klebsiella* spp. [20]. *K. pneumoniae*, *E. cloacae*, *C. freundi* and *S. marcescens* may cause pneumonia, urinary tract infections and sepsis [17, 20]. *B. anthracis* is the causing agent of anthrax, and *B. cereus* is responsible for opportunistic food poisoning and eye infections, may be found in cosmetics. *Clostridium tetani* which causes tetanus have also been isolated [8].

Eye shadows and mascaras may be contaminated *Staphylococcus* spp., *P. aeruginosa* and *K. pneumoniae*, also with the microorganisms *Micrococcus* spp., *Corynebacterium* spp., *Acinetobacter* spp., *Moraxella* spp., *Neisseria* spp. [20]. *Micrococcus* spp. are the etiological agent of infections. *Moraxella catarrhalis* and *Moraxella lacunata* are responsible for infections of the respiratory tract as well as ears and eyes [11].

The fungi most frequently isolated from hand creams and lotions are: *C. albicans*, *Aspergillus niger* and *Aspergillus fumigatus* as well as *Penicilium* spp, and mostly yeasts are isolated from mascaras and eye shadows. Not only do fungi lower the quality of cosmetic products, but they can induce infections of the skin and mucous membranes, as well as hair and nails [17].

The aim of this study was to assess the microbiological contamination of cosmetics used by one person and by several people and cosmetics after their expiry date in relation to the standards for marketed cosmetics that ensure the safety of their use. Such studies are very important. The obtained results helped assess the potential health threat for people who use cosmetics both within their expiry date and thereafter. This research also gave the answer to the question whether the use of cosmetics by several people increases the risk of it contamination and increases the threat for its users.

MATERIALS AND METHODS

In quantitative and qualitative tests of microbiological purity of cosmetics, 55 samples of cosmetics were used representing 19 types of cosmetics (Table 2), which were divided into three groups: (1) cosmetics used by one person before the expiry date, (2) cosmetics used by several people before the expiry date and (3) cosmetics used after the expiry date. The same categories of cosmetics were tested in each group of cosmetic preparations. Expiry periods and the number of people used tested cosmetics are presented in Table 2.

Cosmetics used in the study were conditioning products intended to personal hygiene and beautifying cosmetics, including preparations in aqueous and non-aqueous form. Colour cosmetics were both dry cosmetics and sticks and suspensions. Conditioning preparations in which microbiological purity was determined were characterized by oil-water character, as in the case of emulsions, and a high content of water in suspensions, fluids and milks. These were cosmetics for hair washing

and conditioning, peeling, body lotions, face creams, intimate hygiene washes and cleansing milks. The study also involved cosmetics intended for children, which included shower gels and skin conditioning creams. Cosmetics of I category accounted for 49% of the tested cosmetic products, and the other cosmetics 51%.

and the presence of yeasts from the genus *Candida* was evaluated, and then incubation was prolonged to 5 days under the same thermal conditions, to determine the occurrence and numbers of moulds. Identification of the grown fungi in respect of the genus was carried out by the macro-and microscopic assessment of grown colonies based on the mycological atlas [12].

Table 1. Microbiological requirements for cosmetic products [9]

Microbiological indices	Number of microorganisms	
	Cosmetics of category I	Cosmetics of category II
General number of aerobic mesophylic microorganisms (bacteria and moulds)	>100 cfu/g or ml	>1000 cfu/g or ml
Staphylococcus aureus	Absent in 0.1 g/ml	Absent in 0.1 g/ml
Pseudomonas aeruginosa	Absent in 0.1 g/ml	Absent in 0.1 g/ml
Candida albicans	Absent in 0.1 g/ml	Absent in 0.1 g/ml

The spread plate method was used to assess the microbiological quality of cosmetics. Three series of dilutions were performed for each tested cosmetic. Samples with a weight of 1 g or 1 ml were mixed with the neutralizer (buffered solution of sodium chloride with peptone with pH 7.0) in a ratio of 1:10 and a series of decimal dilutions in 0.9% NaCl (Avantor) were made to a level of 10^{-4}. In hydrophobic cosmetics an addition of 0.1% (m/v) polysorbate 80 was applied.

Tryptic Soy Agar (TSA) (Beton Dickinson) was used to determine the total number of aerobic bacteria. Each of the dilutions was inoculated on two Petri plates with TSA, transferring 0.1 ml of prepared dilution and spreading it throughout the agar surface. Cultures were incubated for one day at 37˚C, and then the number of grown colonies were counted on each medium and the number of bacteria was determined, expressed in cfu/g or cfu/ml.

Microbiological quality tests of cosmetics were carried out by introduction of a sample (0.1 g or 0.1 ml) into 200 µl of a neutralizer, thorough mixing and inoculation of the whole volume on the culture media suitable for the tested microorganisms. In the case of hydrophobic cosmetics, an addition of 0.1% (m/v) polysorbate 80 was applied. For positive samples, grown colonies were counted and their number was calculated for 1 g or 1 ml of the tested cosmetic.

To determine the presence of *S. aureus* we used the *Baird–Parker* medium (BTL Sp. z o.o.), and grown Staphylococci (incubation for 24 h at 37˚C) were identified based on catalase formation, coagulase-bound, the so-called clumping factor (CF), and free.

The presence of bacteria *P. aeruginosa* was detected on the cetrimide medium (PYA, Becton Dickinson). Cultures were incubated for 24 hours at 37˚C. The oxidase test was used to confirm the occurrence of *P. aeruginosa*.

Fungi in the tested cosmetics were detected on the Sabouraud medium with dextrose (Becton Dickinson). Cultures were incubated for 24 hours at room temperature

RESULTS

Of the tested cosmetics before the expiry date used by one person, the requirements concerning microbiological purity were met by: peeling, where microbiological contamination was 2×10^2 cfu/ml, and cream for children, where no aerobic mesophylic bacteria were detected (Table 3m). Contamination exceeding the maximal values was also shown in 89.0% of cosmetics used by one person. The highest level of microbiological contamination among cosmetics used by one person, exceeding the standards of microbiological purity, was recorded in hand cream, where the number of aerobic mesophylic microorganisms amounted to 2.8×10^6 cfu/g, and the lowest in eye cleansing milk – 1.5×10^3 cfu/ml (Table 3). Based on the obtained results concerning cosmetics used by several people (Table 3), it was observed that 84.0% of the tested cosmetics underwent contamination exceeding the adopted standards. Of cosmetics of I category, the lowest microbiological contamination with aerobic mesophilic bacteria was detected in cream for children, 1.5×10^3 cfu/g, and the highest in eye cleansing milk – 9.0×10^4 cfu/ml (Table 3). Based on the obtained results, it was stated that the contamination of cosmetics of II category ranged between 5×10^3 and 2.6×10^5 cfu/g or ml. The lowest level of microbiological contamination of cosmetics of II category used by several people was indicated in hair mask, and the highest in fluid (Table 3). No contamination with aerobic mesophylic microorganisms was recorded in intimate hygiene wash or peeling. Contamination of face cream did not exceed 5×10^1 cfu/g (Table 3).

In 55.0% of cosmetics used by several people, a higher microbiological contamination with aerobic mesophilic bacteria was found than in the cosmetics used by one person (Table 3).

The results of microbiological purity assessment of cosmetics after the expiry date showed the highest

Table 2. Expiry periods and the number of people using tested cosmetics

Cosmetic	Expiry period (after open) [months]	Time after expiry date [months]*	Number of people using cosmetic**
Body lotion	12	1	4
Eye shadow	24	6	2
Fluid	12	4	3
Cream for children	6	1	2
Hand cream	36	3	5
Foot cream	24	5	3
Face cream	6	2	4
Face mask	6	1	2
Hair mask	6	3	3
Eye cleansing milk	12	7	3
Toothpaste	12	1	4
Peeling	12	10	3
Intimate hygiene wash	6	1	2
Mouthwash	12	1	4
Blush	9	8	3
Shampoo	12	2	3
Lipstick	24	11	2
Mascara	6	5	3
Shower gel for children	12	2	2

* - concern tested cosmetics after expiry date
** - concern tested cosmetics used by several people

Table 3. General numbers of aerobic mesophylic bacteria in tested cosmetics

Cosmetic	Cosmetics used by one person, before the expiry date		Cosmetics used by several people, before the expiry date		Cosmetics after the expiry date	
	Bacteria number [cfu×ml^{-1}] or [cfu×g^{-1}]	Standard deviation	Bacteria number [cfu×ml^{-1}] or [cfu×g^{-1}]	Standard deviation	Bacteria number [cfu×ml^{-1}] or [cfu×g^{-1}]	Standard deviation
Body lotion	$1{,}5\times10^{4}$	$7{,}1\times10^{3}$	1×10^{4}	$5{,}7\times10^{3}$	$2{,}4\times10^{6}$	$3{,}8\times10^{5}$
Eye shadow	5×10^{3}	$7{,}1\times10^{3}$	1×10^{4}	-	$1{,}8\times10^{6}$	$2{,}6\times10^{6}$
Fluid	1×10^{4}	-	$2{,}6\times10^{5}$	$3{,}7\times10^{5}$	$1{,}1\times10^{6}$	$7{,}3\times10^{5}$
Cream for children	n.d.*	-	$1{,}5\times10^{3}$	$7{,}1\times10^{2}$	5×10^{4}	$7{,}1\times10^{4}$
Hand cream	$2{,}8\times10^{6}$	$8{,}3\times10^{5}$	$1{,}5\times10^{4}$	$2{,}1\times10^{4}$	$1{,}3\times10^{7}$	$3{,}9\times10^{6}$
Foot cream	1×10^{4}	$1{,}4\times10^{4}$	5×10^{4}	$7{,}1\times10^{4}$	$2{,}8\times10^{5}$	$1{,}5\times10^{5}$
Face cream	2×10^{4}	$1{,}4\times10^{4}$	5×10^{1}	7×10^{1}	$1{,}6\times10^{5}$	$2{,}3\times10^{5}$
Face mask	-**	-	1×10^{5}	$1{,}4\times10^{5}$	-	-
Hair mask	$1{,}3\times10^{4}$	$1{,}8\times10^{4}$	5×10^{3}	$7{,}1\times10^{3}$	$1{,}3\times10^{5}$	$1{,}8\times10^{5}$
Eye cleansing milk	$1{,}5\times10^{3}$	$2{,}1\times10^{3}$	9×10^{4}	$1{,}3\times10^{5}$	$2{,}1\times10^{5}$	$2{,}6\times10^{5}$
Toothpaste	1×10^{4}	$1{,}4\times10^{4}$	$1{,}5\times10^{4}$	$7{,}1\times10^{3}$	6×10^{4}	-
Peeling	2×10^{2}	$2{,}8\times10^{2}$	n.d.	-	$1{,}5\times10^{2}$	$2{,}1\times10^{2}$
Intimate hygiene wash	5×10^{3}	$7{,}1\times10^{3}$	n.d.	-	1×10^{6}	$4{,}8\times10^{5}$
Mouthwash	$4{,}5\times10^{4}$	$2{,}1\times10^{4}$	$1{,}8\times10^{4}$	$2{,}1\times10^{4}$	$1{,}7\times10^{4}$	$2{,}4\times10^{4}$
Blush	2×10^{3}	$2{,}8\times10^{3}$	$1{,}5\times10^{4}$	$2{,}1\times10^{4}$	$3{,}2\times10^{6}$	$2{,}9\times10^{6}$
Shampoo	1×10^{4}	$1{,}4\times10^{4}$	2×10^{4}	$2{,}8\times10^{4}$	$9{,}7\times10^{5}$	$6{,}8\times10^{5}$
Lipstick	1×10^{4}	-	$3{,}5\times10^{3}$	$2{,}1\times10^{3}$	$2{,}2\times10^{4}$	$2{,}4\times10^{4}$
Mascara	1×10^{4}	-	$1{,}5\times10^{4}$	$2{,}2\times10^{4}$	$5{,}5\times10^{3}$	5×10^{4}
Shower gel for children	1×10^{4}	$1{,}4\times10^{4}$	$2{,}1\times10^{4}$	$8{,}9\times10^{4}$	2×10^{3}	$2{,}8\times10^{3}$

* n.d.- not detected
** -- not tested/determined

level of contamination with aerobic mesophylic bacteria of all the tested samples. Of the cosmetics after the expiry date of I category, the highest level of microbiological contamination was found in eye shadow – 1.8×10^6 cfu/g, and the lowest in shower gel for children – 2.0×10^3 cfu/ml (Table 3). The number of aerobic mesophilic microorganisms in the other cosmetics after the expiry date ranged from 1.5×10^2 cfu/g in peeling, which was the only cosmetic after the expiry date that met the requirements of the Ordinance of the Minister of Health of 23 December 2002, and 1.3×10^7 cfu/g in hand cream (Table 3).

The presence of bacteria of the genus *Staphylococcus* was indicated in 11 (20.0%) examined samples of cosmetics. They included 6 (10.9%) cosmetics used by several people and 5 (9.1%) cosmetics after the expiry date. In the above cosmetic products the lowest contamination caused by *Staphylococcus* spp. was detected in the blush after the expiry date and its contamination amounted to 5.0×10 cfu/g (Table 4). The highest contamination by these cocci, which amounted to 1.0×10^2 cfu/g, was found in the eye shadow after the expiry date (Table 4). *S. aureus* occurred only in the lipstick after the expiry date, and other species of this genus *Staphylococcus* were present in the other cosmetic products.

Qualitative studies of microbiological purity for detecting *P. aeruginosa*, show their presence in the intimate hygiene wash used by one person. The studied cosmetic contained 2.1×10^2 cfu/ml (Table 4).

Table 4 - Presence of microorganisms in tested cosmetics

Cosmetic	Cosmetics used by one person, before the expiry date			Cosmetics used by several people, before the expiry date			Cosmetics after the expiry date		
	Number of microorganisms [cfu×ml^{-1}] or [cfu×g^{-1}]			Number of microorganisms [cfu×ml^{-1}] or [cfu×g^{-1}]			Number of microorganisms [cfu×ml^{-1}] or [cfu×g^{-1}]		
	Staphylococcus spp.	*P. aeruginosa*	Fungi	*Staphylococcus* spp.	*P. aeruginosa*	Fungi	*Staphylococcus* spp.	*P. aeruginosa*	Fungi
Body lotion	n.d.*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Eye shadow	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$1,0\times10^2$ ($1,4\times10^2$)	n.d.	n.d.
Fluid	n.d.	n.d.	n.d.	$1,5\times10^1$ ($2,1\times10^1$)	n.d.	n.d.	n.d.	n.d.	n.d.
Cream for children	n.d.	n.d.	n.d.	n.w.	n.d.	n.d.	$1,5*10^1$ ($0,7\times10^1$)	n.d.	n.d.
Hand cream	n.d.	n.d.	$1,5\times10^1$ ($2,1\times10^1$)	$1,0\times10^1$ ($0,1\times10^1$)	n.d.	n.d.	n.d.	n.d.	n.d.
Foot cream	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Face cream	n.d.	n.d.	n.d.	$1,5\times10^1$ ($2,1\times10^1$)	n.d.	n.d.	n.d.	n.d.	n.d.
Face mask	-	-	-	$6,5\times10^1$ ($6,3\times10^1$)	n.d.	n.d.	-	-	-
Hair mask	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$1,5\times10^1$ ($0,7\times10^1$)
Eye cleansing milk	n.d.	n.d.	n.d.	$1,5\times10^1$ ($2,1\times10^1$)	n.d.	n.d.	n.d.	n.d.	n.d.
Toothpaste	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$2,5*10^1$ ($2,1\times10^1$)	n.d.	n.d.
Peeling	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Intimate hygiene wash	n.d.	$2,1\times10^2$ ($5,6\times10^1$)**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Mouthwash	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$0,5\times10^1$ ($0,7\times10^1$)
Blush	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	$0,5\times10^1$ ($0,7\times10^1$)	n.d.	$0,5\times10^1$ ($0,1\times10^1$)
Shampoo	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipstick	n.d.	n.d.	n.d.	$6,0\times10^1$ ($8,4\times10^1$)	n.d.	n.d.	$2,5\times10^1$ ($0,3\times10^1$)	n.d.	n.d.
Mascara	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Shower gel for children	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* n.d.- not detected, **- standard deviation

No presence of fungi was detected in cosmetics used by several people. Among the tested cosmetics after the expiry date, contamination with fungi stayed within range from 0.5×10^1 cfu/g or ml in the case of the mouthwash and the blush and 1.5×10^1 cfu/g win the case of the hair mask and the hand cream used by one person (Table 4). The fungi that contaminated the tested cosmetics were *Aspergillus* spp. and *Penicillium* spp. No contamination of cosmetic preparations with the *C. albicans* was recorded.

DISCUSSION

In the cosmetics used in this study no physicochemical changes were found that could indicate microbiological contamination, such as: a change in colour, smell, change in consistence, appearance of sediment or phase separation. Similar study results are reported by *Hugbo* et al. [11], *Abu Shaqra* and *Al-Groom* [1] as well as *Mwambete* and *Simon* [15]. The results differed from the present were shown by Muhammed [14], who noted changes in colour, the appearance of sediment and cloudiness of a cosmetic batch.

The majority of cosmetics tested in the present study which were used by one person before the expiry date did not meet the requirements of the Ordinance of the Minister of Health of 23 December 2002 [22]. Preparations which contained a high percentage of water were characterized by a higher level of microbiological contamination. Similar results were presented by *Campana* et al. [6], who indicated that the microbiological contamination of preparations stays within the range from 1×10^2 to 3×10^4 cfu/ml for cosmetics for personal hygiene. No contamination of oil/water emulsions and toothpastes were found. Similar results were obtained by *Lamikanra* and *Okeke* [13], who noted higher microbiological contamination in water cosmetics.

Onurdağ et al. [18] results differ from the present results of microbiological purity of used colour cosmetics (100% contaminated samples). Only in 5 (6.9%) used make-up cosmetics *Onurdağ* et al. [18] observed contamination with aerobic mesophylic bacteria exceeding the standards of microbiological purity.

In the present study, in the group of cosmetics used by one person, rods of *P. aeruginosa* were isolated in the intimate hygiene wash. *Staphylococcus* have not been observed. In contrast to the results obtained by *Behravan* et al. [5], *Dashen* et al. [7] and *Campana* et al. [6], *Staphylococcus* spp. were the most often isolated potentially pathogenic bacteria in this group of cosmetics.

Varied microflora, specific of each person, at not following the principles of hygiene, is the cause of a higher level of microbiological contamination of cosmetics which are used by several people. Based on the present study, it can be observed that *Staphylococcus* spp., being an element of the human microflora, are the most often isolated from cosmetic preparations used by several people (10.9% of samples). This is confirmed by the results obtained by *Anelich* and *Korsten* [2], who studied 58 samples of cosmetics and proved the presence of bacteria from the genus *Staphylococcus* in 9.0% of samples. The presence of *Pseudomonas* spp. was found in 30.0% of samples, *Enterobacter* spp. - in 17.0%, and the mould *Aspergillus* spp. in 13.0% of samples [2]. In the present study, no *Pseudomonas* spp. were indicated in this group of cosmetics. *Naz* et al. [16], in turn, observed the presence of *S. aureus* in 100% of samples of make-up sponges and brushes, and *P. aeruginosa* and fungi in more than 50% of the tested cosmetics. The most of used waxes were contaminated with *S. aureus* and *P. aeruginosa*, and contamination with fungi was low.

CONCLUSIONS

Based on the obtained results, it was found that the contamination of cosmetics which are past their sell-by date or were used by more than one person is considerably higher than in cosmetics before their expiry date or used by single person. Most often isolated potentially pathogenic microorganisms are *Staphylococcus* spp., which constitute an element of the natural microflora of the human skin.

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Conflict of interest

The authors declare that they have no conflict of interest.

Financial disclosure

The authors have no financial interests related to the material.

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