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**Original Article**

# Assessment of Microbial Contamination and Metabolite Exposure in Cosmetic Products Used in Women's Beauty Salons

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**Abstract**

**Background:** An important route of microbial transmission is the shared use of these products in beauty salons. We aimed to investigate level of contamination with microorganisms and their metabolites in shared-use cosmetics in several women's beauty salons.

**Methods:** Bacterial and fungal strains from 320 opened/used cosmetic samples were identified according to the Iranian standards for microbial quality of cosmetic products in Golestan Province (North of Iran) during Jul-Sep 2021. In order to assess production of toxins and protease by the predominant bacterial and fungal isolates, multiplex-polymerase chain reaction and the Lowry protein assay were performed, respectively.

**Results:** Microbial contamination was detected in 180 samples (56.5%), and the highest and lowest rates of microbial contamination were related to skin products (63.88%) and eye beauty products (20%), respectively. The highest level of *S. aureus* contamination (> 4,000 colony-forming units/g) was found in toner and face wash samples, and the highest level of *C. albicans* contamination was seen in lipstick samples (>20,000 colony-forming units/g). Only one (2%) *S. aureus* isolate produced staphylococcal enterotoxin B, while 3 out of 12 (25%) *C. albicans* isolates were able to produce protease.

**Conclusion:** The shared-used health and beauty products, face products, in the study area are heavily contaminated. Therefore, it is essential to store used cosmetics in dry and cool places, establish strict inspection regulations for cosmetic products before and after entering the market, and increase awareness of beauty salon workers regarding the appropriate use, sanitary control, and maintenance of health and beauty products.

**Keywords:** Microbial contamination; Metabolite; Health and beauty products; Cosmetics

## Introduction

The consumption of cosmetics in developing countries is increasing. Saudi Arabia, followed by Iran, is the main consumers of cosmetics in the Middle East. Although the microbial standards of cosmetics have gradually improved with strict regulatory enforcement, their contamination has been repeatedly reported. Microbial contamination of cosmetic and health products is an important issue due to their daily use and direct contact with the skin (1, 2). Factors such as the presence of growth factors (including organic and inorganic compounds), sufficient humidity, and storage temperature may provide a suitable condition for the growth of microorganisms. Other causes of microbial contamination of cosmetics are the use of contaminated raw materials in the

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production process and smuggling of counterfeit cosmetic and health products. The main reason for the widespread use of counterfeit cosmetics is their low price, and most consumers are not concerned with the quality of their ingredients.

Skin allergic reactions (e.g., redness, itching, inflammation, swelling, and blisters), alopecia, acne formation or exacerbation, liver and kidney poisoning (due to presence of aflatoxin), adrenal insufficiency, hair discoloration and damage, nail damage, respiratory allergies, and fetal harm have been reported as side effects of counterfeit cosmetic products (3-5). Contamination of cosmetic products by dangerous pathogenic microorganisms has also been reported. For example, consumption of creams contaminated with *Klebsiella pneumoniae* caused septicemia, and the use of baby powder contaminated with *Clostridium tetani* resulted in the death of four New Zealand children (6). Based on previous studies, *Pseudomonas aeruginosa, Staphylococcus aureus*, coagulase-negative staphylococci, *Escherichia coli*, bacilli, and fungi are the main contaminants of cosmetic products (7,8). Therefore, improving health awareness and hygiene practices in beauty salons may be effective in promoting public health and controlling diseases, while neglecting this issue leads to the spread of various types of fungal, bacterial and viral infections, as well as skin and hair diseases, lice, eczema, and skin allergies (9). Despite proper maintenance of hygienic conditions during the production and packaging of cosmetic products, environmental conditions and broken seal or package damage can expose the products to direct contamination with microorganisms or their metabolites. Some microorganisms isolated from clinical and non-clinical sources are capable of producing enzymes and toxic metabolites, which doubles the risk of their occurrence in cosmetic products (10, 11).

We aimed to determine the type and frequency of microorganisms as well as their metabolites in shared-use cosmetic products in several women's beauty salons in the north of Iran.

## Materials and Methods

### *Ethics approval*

This study received approval from the ethics committee of Islamic Azad University-North of Iran (ethical code: IR.IAU.CHALUS.REC. 1400.099), and written consent was obtained from owners of the beauty salons.

### *Study design and data*

According to the Iranian standards for microbial quality of cosmetic and health products (document number W2055/00), although the raw materials used in the production of cosmetic and health products do not need to be sterile, presence of microorganisms in the products should not have a negative effect on the safety of consumers or quality of the product during the intended or expected period of use. Therefore, the microbiological limit for the final health and beauty products shall not exceed $5\times10^2$ colony-forming unit CFU/g or ml of the product for topical use. However, the acceptable microbial limit for health and cosmetic products used in the eye area and on mucous membranes is $\leq1 \times10^2$ CFU/g or ml of the product. In addition, the product must be free of *E. coli, S. aureus, P. aeruginosa,* and *Candida albicans* in 1 g or ml. In this descriptive study, samples were taken from 320 different cosmetic products (including skin, hair, eye, and face products) used in 15 beauty salons in major cities of the Golestan Province (North of Iran) including Gorgan, Bandar-e Gaz, Kordkuy, and Gonbad-e Kavus during Jul-Sep 2021.

### *Phenotypic examination for Microbial isolation*

After transferring the samples to laboratory, a 1:10 dilution was made from 1 g or 1 ml of each sample to prepare the initial suspension. The suspension was inoculated into enrichment medium and incubated at 32.5 ºC for 72 h. Then, the prepared suspension was transferred to routine bacterial and fungal culture media including cetrimide agar, Levine eosin methylene blue agar, Baird–Parker agar, and sabouraud dextrose agar

with chloramphenicol. After incubation at 37 °C for 24 h for bacteria and at 25-35 °C for 2-7 day for fungi, microorganisms were isolated and detected according to the protocols of Iran National Standards Organization (Table 1). Finally, the number of colonies in the single-plate culture method was calculated using the following formula: N=C/(V×D), where C is the number of colonies one a plate; V is the volume of inoculum to each plate; and D is the dilution factor related to the initial suspension or for the first dilution counted.

**Table 1:** Qualitative and quantitative microbiological limits for the final health and cosmetic products

| *Microorganism* | *Acceptable limit (per g/ml) for adults* | *Acceptable limit (per g/ml) for children ≤3 years/mucus membrane or eye area* | *Detection method* |
|---|---|---|---|
| Mesophilic aerobic bacteria | 5000 | 1000 | 11804 |
| *E. coli* | Negative | Negative | INSO 9933 |
| *S. aureus* | Negative | Negative | INSO 9793 |
| *P. aeruginosa* | Negative | Negative | INSO 9934 |
| *C. albicans* | Negative | Negative | INSO 9607 |
| Mold | >10 | >10 | INSO 11169 |
| Yeast | >10 | >10 | INSO 11169 |

INSO: Iran National Standards Organization

### *Molecular identification of metabolites*
### *1) Toxin identification*

After determining the dominant bacterial and fungal strains, their metabolites were tracked. Multiplex-polymerase chain reaction (M-PCR) was performed using specific primers (Takapou Zist Co., Iran) in order to evaluate presence of genes encoding five major staphylococcal enterotoxins (*sea*, *seb*, *sed*, *sec*, and *see*) in the dominant bacterial isolate (*S. aureus*) (Table 2).

**Table 2:** Characteristics of the primers used in the M-PCR test

| *Primer name* | *Sequence (5`-3`)* | *Target gene* | *Length (bp)* |
|---|---|---|---|
| SEA-f | GGTTATATCAATGTGCGGGTGG | *sea* | 102 |
| SEA-r | CGGCACTTTTTTCTCTTCGG | | |
| SEB-f | GTATGGTGGTGTAACTGAGC | *seb* | 164 |
| SEB-r | CCAAATAGTGACGAGTTAGG | | |
| SEC-f | AGATGAAGTAGTTGATGTGTATGG | *sec* | 451 |
| SEC-r | CACACTTTTAGAATCAACCG | | |
| SED-f | CCAATAATAGGAGAAAATAAAAG | *sed* | 278 |
| SED--r | ATTGGTATTTTTTTTCGTTC | | |
| SEE-f | AGGTTTTTTCACAGGTCATCC | *see* | 209 |
| SEE-r | CTTTTTTTTCTTCGGTCAATC | | |

In order to isolate genomic DNA, a suspension with a concentration equal to 3 McFarland standard was prepared in brain heart infusion broth. After 24 h of incubation at 37 °C, the suspension was centrifuged at 3,500 g for 10 min. Next, the supernatant was separated and mixed with 800 ml of lysis buffer. The mixture was incubated at 65 °C for 30 min. Next, the vials containing the lysed cells were centrifuged at 12,000 g for 5 min at 4 °C. The supernatant was transferred to a 1.5 ml microtube and mixed with chloroform-soamyl alcohol (24:1, v/v). The supernatant was aspirat-

ed, the pellet was mixed with 0.5 µl RNase, and the microtube was incubated at 37 °C for 30 min. Then, the microtube was centrifuged at 14,000 g for 10 min, and DNA was removed from the sediment and dried at room temperature. Finally, the dried DNA was dissolved in 50 µl of deionized water. The extracted DNA samples were analyzed by electrophoresis on 1% agarose gel. The reference strains *S. aureus* ATCC25923 and ATCC33591 were used as positive controls, while *S. epidermidis* was used as negative control.

### *Enzyme identification*

In order to isolate protease from the dominant fungal strain (*Candida*), first, 5 ml of yeast broth culture were centrifuged at 2,500 g for 20 min. Then, 1 ml of the supernatant containing the enzyme was mixed with 1 ml of substrate (pH 11) and placed in Bain-Marie at 40 °C for 10 min. Next, 2 ml of 0.4 M trichloroacetic acid solution were added, and the tube was centrifuged at 12,000 g for 10 min. Next, 1 ml of the supernatant was mixed with 5 ml of 0.4 M sodium carbonate solution and 1 ml of Folin-Ciocalteu's phenol reagent (0.1 M), and the tube was placed in a water bath at 40 °C for 20 min in the dark. Finally, the optical density of the solution at 660 nm was read, and the enzyme activity was determined by plotting a standard curve for the amount of L-tyrosine (µg). According to the definition, one unit of alkaline protease activity is the amount of enzyme that can release 1 µg of L-tyrosine from casein in 10 min and at 40 °C.

In order to investigate effects of different pH values on the activity of alkaline protease, first, yeast extract–peptone–glycerol (Merck, Germany) broth was prepared with different pH values (5, 6, 7, 8, 9, and 10). After inoculating the yeast suspension, the cultures were incubated in a shaking incubator for 72 h at 30 °C and with an aeration speed of 100 rpm. Next, a sample was taken from each medium, and the alkaline protease activity was evaluated using the Lowry protein assay (12, 13).

### *Statistical analyses*

Data were analyzed with the SPSS software (ver. 23, IBM Corp., Armonk, NY, USA) using the Chi-square test and analysis of variance. Graphs were drawn with the Microsoft Excel software (2010). Confidence intervals (CI) were calculated using the Stata MP 14 (Stata Corp LP, USA). All analyzes were carried out at significance level of 0.05.

## Results

Out of 320 samples collected from cosmetic and health products, microbial contamination was detected in 180 samples (56.25%). The most common bacterial and fungal contaminants were *S. aureus* (28.5%) and *C. albicans* (11%), respectively (Fig. 1). Among saprophytes, 38 (62.30%) *Mucor*, 12 (19.67%) *Penicillium*, and 11 (18.02%) *Rhizopus* were detected. The most and least prevalent microbial strains were isolated from skin (63.88%) and eye makeup (20%) products, respectively (Tables 3 and 4). About 70% of the cosmetics and 37% of health products in the beauty salons were contaminated with bacteria. In addition, hair products were less contaminated by bacteria and more contaminated with fungi compared to cosmetic products. The number of fungi in cosmetic products ranged between 10 and $320\times10^4$ CFU/g. The number of bacterial isolates ranged between 3 and $250\times10^3$ CFU/g. The highest level of *S. aureus* (>4,000 CFU/g) was found in toner and face wash samples, while the highest level of *C. albicans* was present in lipsticks (>20,000 CFU/g). In terms of metabolic tracking, only one of 51 *S. aureus* isolates (2%) produced staphylococcal enterotoxin B (SEB). The presence of 120 bp, 164 bp, 451 bp, 378 bp, and 209 bp fragments confirmed presence of the sea, seb, sec, sed, and see genes, respectively. Out of 12 *C. albicans* isolates, 3 isolates (25%) were able to produce protease. The activity of alkaline protease was highest at pH of 8 (420 U/ml) and lowest at pH of 5 (290 U/ml) (Fig. 2).

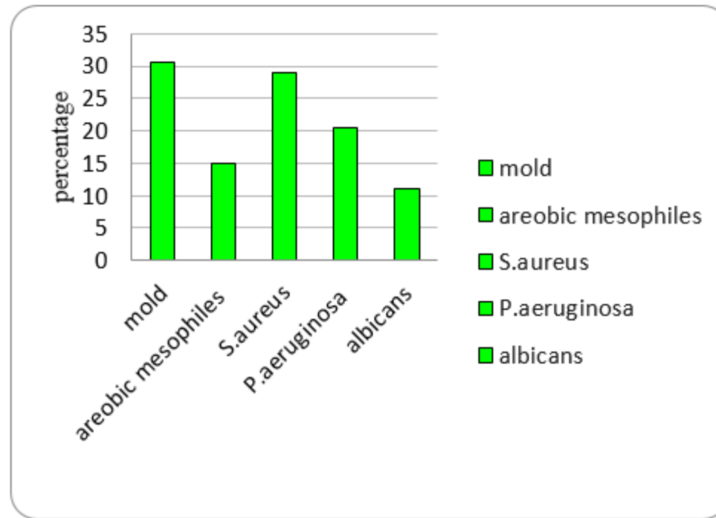


**Fig. 1:** Frequency of microbial contaminants in cosmetic products

**Table 3:** Frequency of contaminants in cosmetic products according to type of microorganism and product

| ***Product type*** | ***Country of manufacture*** | ***Microorganisms (N=180)*** | | | | |
|---|---|---|---|---|---|---|
| | | *S. aureus* (N=51) | *Candida* (N=12) | *P. aeruginosa* (N=37) | Saprophytic molds (N=61) | Mesophilic aerobic bacteria (N=19) |
| Hair cream | Iran | 5(9.80%) | - | - | 13(21.31%) | 4(21.05%) |
| Eyeliner | UAE | 3(5.88%) | - | 3(8.10%) | 1(1.63%) | 4(21.05%) |
| Face powder | Spain | 1 1.96%) | 1(8.33%) | 1(2.70%) | 5(8.19%) | - |
| Face toner | Iran | 9(17.64%) | 3(25%) | 5(13.51%) | 4(6.55%) | 1(5.26%) |
| Face wash | Iran | 13(25.49%) | 1(8.33%) | 4(10.81%) | 8(13.11%) | 1(5.26%) |
| Shampoo | Iran | 2(3.92%) | - | - | 9(14.75%) | 2(10.52%) |
| Lipstick | Iran | 11(21.56%) | 6(50%) | 11(29.72%) | 10(16.39%) | 4(21.05%) |
| Mascara | Turkey | - | 1(8.33%) | 9(24.32%) | 7(11.47%) | 2(10.52%) |
| Face cream | Iran | 7(13.72%) | - | 4(10.81%) | 4(6.55%) | 1(5.26%) |
| *P*-value | 0.12 | 0.23 | 0.17 | 0.30 | 0.33 | 0.37 |

**Table 4:** Frequency of microbial contaminants in cosmetic samples

| ***Product type*** | ***Bacteria*** | | ***P value*** | ***Fungi*** | | ***P value*** |
|---|---|---|---|---|---|---|
| | Number (%) | 95% CI | | Number (%) | 95% CI | |
| Skin care (n=115) | 73 (63.48) | 83.11-99.00 | NS | 42 (36.52) | 81.40-98.00 | NS |
| Eye makeup (n=30) | 21 (70) | 45.17-98.00 | NS | 9 (30) | 17.10-88.30 | NS |
| Hair products (n=35) | 13 (3.14) | 37.50-99.00 | NS | 22 (62.85) | 32.80-70.30 | NS |
| Total | 107 (59.44) | 91.03-99.00 | NS | 73 (40.55) | 81.22-97.00 | NS |

NS= Not statistically significant

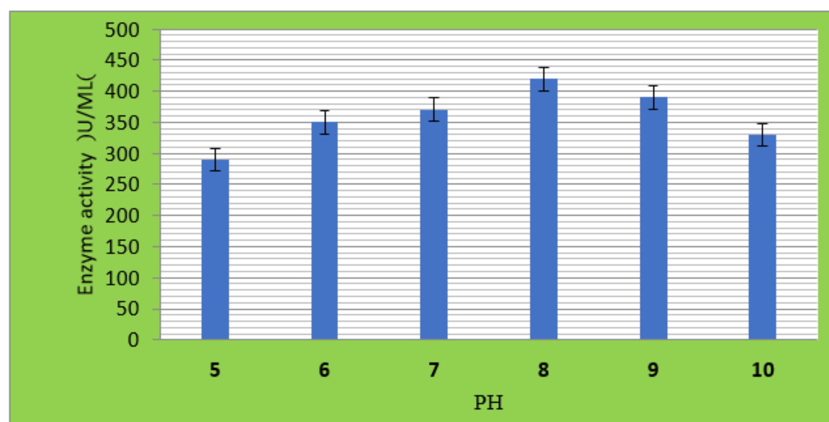


**Fig. 2:** The average enzyme activity in alkaline protease-producing C. albicans isolates at different pHs after 72 h

## Discussion

In recent years, there has been growing concern about Microbiological safety of cosmetic products. Due to the increasing demand for such products, significant research has been done on the microbial contamination of these products to maximize their safety for consumers (14,15).

In the present study, the highest rate of contamination (59.5%) was related to bacteria, especially Gram-positive bacteria. This could be partly associated with the skin microflora. The unique skin microflora of one person can be harmful to another person. The most prevalent bacterial isolate was *S. aureus* (28.5%), a Gram-positive pathogenic bacterium. Regardless of the contamination of the products with saprophytic molds, related to the greater resistance of molds to cosmetic preservatives, the frequency of *C. albicans* (11%), an opportunistic yeast, was highest among fungal isolates.

Previous studies have also reported microbial contamination in both new and partially used cosmetics. In 2017, a study in Iran found that 19.2% of cosmetic products were contaminated with Candida (1). In Brazil, *S. aureus*, Aspergillus, and *Cladosporium* as the major contaminants of lipsticks (16). Other studies in Brazil (17) and Iran (18) also reported *S. aureus* as the most frequently isolated microorganism from cosmetic products. However, two separate studies in Iran in 2010 (19) and 2012 (20) reported *P. aeruginosa* as the predominant bacterial strain isolated from lotions and beauty creams. The difference in the microbial contamination of the examined cosmetic products can be due to conditions and manner of their storage, type of raw materials used in the production process, transportation conditions, or the climate of area under investigation (21).

The findings of the present study showed that skin products were the most contaminated, which may be due to exposure to air or the presence of compounds such as bentonite and talc that can increase the level of contamination (21). In addition to frequent exposure to air, shared-use skin products are constantly in contact with powder puffs and facial pads that can cause more contamination.

Unlike skin and eye makeup products, hair products had lower rate of bacterial contamination. However, we detected a relatively high rate of microbial contamination (20%) in eye makeup products, mainly caused by bacteria. In a previous study in Iran (22), the highest rate of contamination was observed in eye makeup products, including mascaras (14.5%) and eyeliners (7.2%), which was higher than the rates found in our study. The difference in the contamination rate of products offered for sale in different countries may be attributed to the national regulations, cli-

mate, and several other factors such as public awareness, compliance with the principles, and the use of materials that can lower the risk of contamination in cosmetic products (11). In our study, the highest rate of contamination was related to products manufactured in Iran (78.5%) and Turkey (10.6%). A study in the United Arab Emirates reported that the products manufactured in India, the Middle East, and the United States had the highest level of microbial contamination (23).

Among the microorganisms that contaminate health and beauty products, *C. albicans* has always been considered as one of the main causes of fungal skin and mucosal infections. In this regard, in the present study, all tested products had fungal contamination, mainly caused by *C. albicans*. Many factors contribute to the increased pathogenicity of this yeast, one of which is the production of protease (24). In our study, 25% of *C. albicans* isolates were able to produce protease. In previous studies, 59.1% and 86.8% of *C. albicans* isolates were able to produce protease (25). In the present study, the highest level of alkaline protease activity was recorded at pH of 8, which is consistent with the results of a previous study in Iran (26). However, the highest level of alkaline protease activity was observed at pH of 9 (21), caused by activation of the alkaline protease-encoding gene in alkaline environment (21) or the difference in the yeast growth medium.

Unlike fungi, bacteria often prefer neutral conditions to grow and produce metabolites, including toxins. In the present study, only 1 of 51 *S. aureus* isolates (2%) from cosmetic products produced enterotoxin. The low frequency of enterotoxin producing *S. aureus* isolates compared with other studies could be related to the difference in the type and source of the sample, because enterotoxigenic strains are usually isolated from food and clinical samples (27,28). The small variety of cosmetic brands for further comparison of products from different manufacturers, the non-uniformity of the number of products sampled from the salons, and the short sampling period (summer season) are some limitations of the present study.

## Conclusion

The metabolic secretion of the isolated microorganisms from cosmetic products was negligible. However, the number of isolated microorganisms exceeded the microbial limits, and the rate of microbial contamination of health and beauty products was high, especially in face products, which could have serious health implications for consumers in the study area. Therefore, it is essential to store used cosmetics in dry and cool places, establish strict inspection regulations for cosmetic products before and after entering the market, and increase awareness of beauty salon workers regarding the appropriate use, sanitary control and maintenance of beauty and health products. Moreover, manufacturers must adhere to good manufacturing practice regulations in order to reduce the level of contamination. All raw materials used for manufacturing cosmetics must meet the standards and be properly tested for quality control and validation processes.

## Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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## Conflict of interest

The authors declare that there is no conflict of interests.

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