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Research Article

Evaluating the Bacterial Contamination in Used Cosmetic Products: A Potential Threat to Consumer's Health

Rakhshanda Abbasi, Shaista Bano, Sarfraz Ali Tunio*, Nazir Ahmed Brohi, and Aasma Siddiqui

Institute of Microbiology, University of Sindh, Jamshoro, Pakistan

Abstract: Cosmetic products tend to be prone to microbial contamination as they contain growth factors, essential minerals, organic and inorganic compounds which provide favourable conditions for microbial growth. The aim of the present study is to evaluate the bacterial contamination in used cosmetic products at Hyderabad. A total of 22 samples of used cosmetic products belonging to Mascaras and Eyeliners were collected from beauty salons and homes, which were either used by a single person or used in sharing with other family members. The isolation and identification of contaminating bacteria was carried out on the basis of cultural, morphological, and biochemical characteristics. Out of 22 cosmetic samples 72.73% (n = 16) demonstrated bacterial growth, while 27.27% (n = 6) samples had no bacterial growth. A total of n = 41 bacterial isolates were recovered from used cosmetic samples. The majority of bacteria belonged to Gram-positive 82.93% (n = 34) while 7.32% (n = 03) were Gram-negative bacteria. Moreover, 9.76% (n = 04) samples showed the growth of mixed cultures. Bacteriological profiling revealed that *Bacillus* spp. were dominant with 63.41% (n = 26), followed by *Micrococcus* spp. 7.32% (n = 03), Coagulase-negative Staphylococci 7.32% (n = 03), *Staphylococcus aureus* 4.88% (n = 02), while *Proteus mirabilis* 2.44% (n = 01), *Citrobacter* spp. 2.44% (n = 01), *E. coli* 2.44% (n = 01). Our results have shown that, the shared cosmetics whether used at homes or in salons were more prone to bacterial contamination than non-shared home-based single users, suggesting that sharing increases their susceptibility to bacterial contamination, which can spread bacteria and cause skin infections.

Keywords: Bacterial Contamination, Cosmetics, Eye Liners, Mascara, Salon.

1. INTRODUCTION

Cosmetic products are being used worldwide because physical appearance is really vital for us these days [1]. A few active ingredients, stabilizers, additives, mineral pigments, colors, and fragrances are found in cosmetic items. Certain compound in cosmetics have the potential to negatively impact human health and aggravate long-term medical conditions [2]. It has been reported that these chemicals have the potential to enter the body through the skin, food, or inhaling [3]. In general, cosmetics fall into three categories: make-up (lipstick, eye makeup, etc.), rinse-off (shower gel, shampoo, toothpaste, liquid soap, etc.), and leave-on (facial cream, hand cream, antiperspirant, sunscreen, etc.) [4]. Conversely, there are three types for eye cosmetics: Eyeshadow, mascara, and eyeliner, these are applied to the eyes to accentuate their attractiveness [5]. Mascara has higher than allowable levels of bacteria than other goods. Furthermore, prolonged use of these items might constitute a health risk to humans, hence it has been suggested that the quality of these products be regulated and the consumers be warned to exercise caution while using inexpensive products [6]. The majority of females acquire eye infections each year from using mascara and eyeliner, which can result in temporary or permanent blindness [6]. The pigmented eye makeup items are called eyeshadows [7]. Furthermore, applying makeup on the eyelid can contaminate the container of the cosmetic product and increase the risk of eye infection and allergic response (redness and irritation) [8].

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* Corresponding Author: Sarfraz Ali Tunio <sarfraz.tunio@usindh.edu.pk>

After in-use contamination, microbial growth is supported by the high proportion of water content in cosmetics [9]. Long-term cosmetic product usage habits have an impact on human health as well as the state of the skin and hair. Because their usage habits have a major influence on the other effects of the cosmetic product as well as the incidence or non-occurrence of adverse reactions [10]. Cosmetics are susceptible to skin microbiota upon application, and there is also a possibility of minor contamination during production. Previously published literature has demonstrated the presence of many bacterial isolates including *S. aureus, S. epidermidis, Salmonella* spp., and *E. coli* in makeup items such as face powders, mascaras, and eyeliners. Additionally, a correlation between *S. aureus* and ailments including impetigo and conjunctivitis has been demonstrated [11]. When using personal care and cosmetic products, consumers should select items that won't be harmful to their health and aim to form good habits [12]. Cosmetic items provide an ideal environment for the growth of harmful bacteria in beauty salons. This might pose a risk to the health of the customer. Health risks can vary according to the services provided, the tools, makeup, and applicators utilized. Additionally, sharing equipment and cosmetics can lead to skin and eye infections, particularly in salons where sharing is prevalent [13]. The present study aimed to explore the bacterial contamination in the used cosmetic products from salon and home based usage at Hyderabad, Sindh. Two main categories of cosmetic products mascaras and eyeliners were included for bacteriological analysis.

2. MATERIALS AND METHODS

2.1. Collection of Used Cosmetic Products

The present study was carried out at Hyderabad city, Pakistan, to assess the bacterial contamination in used cosmetic products. A total of 22 used cosmetic product samples of different brands were collected from the different beauty salons, homes and used testers of these products displayed at vendors shops. The cosmetic products belonged to two main categories, which were further subdivided into three sub-categories based on the usage patterns of consumers such as individually used or used in sharing with others (Table 1). Each sample of the used cosmetic product was collected from consumers at salon or homes. The surfaces of all samples were cleansed with an aqueous mixture of 70% ethanol (v/v) prior to transportation to the research laboratory at the Institute of Microbiology, University of Sindh, Jamshoro in a sealed bag. All samples were then processed for bacteriological analysis according to FDA's Bacteriological Analytical Manual: Microbiological Methods for Cosmetics [14].

2.2. Processing of Used Cosmetic Samples

The cosmetic samples were processed at room temperature and neither incubated nor frozen before or after analysis, unless otherwise stated. All samples were processed aseptically in a laminar flow safety cabinet and labelled properly with the appropriate code according to the category and subcategory as mentioned elsewhere. All samples were initially examined for the physical characteristics including consistency (normal/thick/dried), smell (pungent/normal), wether date of expiry present/not present, and the name of manufacturer. For culturing the samples, different diagnostic media were used that included MacConkey's Agar, Blood Agar, Nutrient Agar and Mannitol Salt Agar.

2.3. Enrichment of Cosmetic Samples

With the help of sterile swabs, appropriate amounts (1 gm) of each of the samples of mascara and

Table 1. Category and subcategory-wise samples of cosmetic products.

Categories of samples	Sub-categories				
	Home-based (Single user / Non-shared) (*n* =)	Home-based (Multiple user/ Shared) (*n* =)	Salon-based (Multiple user/ Shared) (*n* =)	Total (*n* =)	Grand Total (*n* =)
Mascaras	4	5	5	14	22
Eyeliners	2	3	3	8	

eyeliners were transferred to sterilized and labeled test tubes containing 5 ml sterile nutrient broth. The broth tubes inoculated with cosmetic samples were then incubated at 37 °C for 24 hours. The next day, all tubes were observed for bacterial growth (turbidity). Samples that showed no turbidity/ visible growth were further incubated at 37 °C for 48 to 72 hours before labelling them as non-contaminated products.

2.4. Isolation and Identification of Bacteria from Used Cosmetic Products

2.4.1. Spread plate method for isolation of pure culture of bacteria

All cosmetic samples yielding positive growth by showing the turbidity in enrichment broth culture were serially diluted by preparing up to 10^{-3} dilutions [15]. Different volumes such as 50 µl, 100 µl and 150 µl inoculum were spread in duplicate on nutrient agar and Mannitol Salt Agar (MSA) plates using sterilized glass spreader for obtaining discrete bacterial colonies. After absorption of the inoculum, plates were incubated at 37 °C for 24 to 48 hours. On the following day, morphologically distinct and isolated colonies were selected and streaked on fresh nutrient agar plates, MSA and MacConkey's Agar plates to obtain pure cultures of each of the distinct colonies [16].

2.4.2. Morphological and biochemical characterization of isolates

Bacterial identification was carried out using conventional microbiological methods including, morphological characteristics by Gram staining and microscopic analysis. Cultivation on different diagnostic and selective media, and biochemical characterization using catalase test, coagulase test, patterns of hemolysis on blood agar, Citrate utilization test, TSI, and Sulfide Indole Motility (SIM) medium (Oxoid, UK) were also performed, according to Chessbrough [17].

3. RESULTS

3.1. Bacteriological Analysis of Cosmetic Products

Bacteriological analysis of the used cosmetic products demonstrated that out of 22 samples 72.73% (n = 16) were positive for bacterial growth thus considered as contaminated, while 27.27% (n = 06) samples were negative for bacterial growth. Overall, n = 41 bacterial isolates were isolated from the used cosmetic samples. The majority of bacterial isolates belonged to Gram-positive 82.93% (n = 34), whereas 7.32% (n = 03) showed the growth of Gram-negative bacteria, and 9.76% (n = 04) yeilded growth of mixed culture.

3.2. Distribution of Bacterial Isolates of Mascara and Eyeliner Cosmetic Samples

Overall distribution of isolated bacterial strains demonstrated that among Gram-positive bacteria *Bacillus* spp. 63.41% (n = 26), were highly prevalent followed by *Micrococcus* spp. 7.32% (n = 03), CoNS 7.32% (n = 03) and *S. aureus* 4.88% (n = 02), whereas Gram-negative rods accounted 7.32% (n = 03), which included *E. coli* (n = 01), *Proteus mirabilis* (n = 01), and *Citrobacter* spp. (n = 01). The remaining 9.76% (n = 04) samples showed the growth of mixed cultures (Figure 1).

3.3. Analysis of Hemolytic Activity in Bacteria Isolated from Cosmetic Samples

In order to determine hemolysis potential, all bacterial isolates recovered from used cosmetic products were cultured on blood agar to assess their potential for hemolysis of RBCs (Figure 2). As shown in Table 2, the majority of isolates (53.66%) were β-hemolytic, while 26.83% were α-hemolytic, and 19.51% of the bacterial isolates displayed

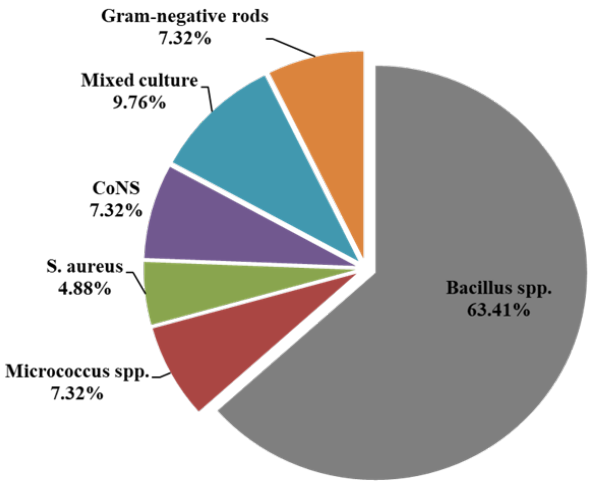

Fig. 1. Pie chart showing the overall distribution of bacteria isolated from used cosmetic products.

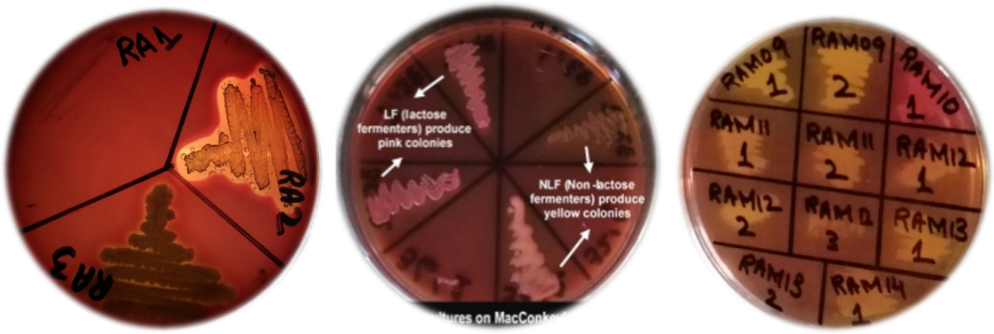

Fig. 2. Pure cultures and hemolytic analysis of bacterial isolates of cosmetic products.

Table 2. Analysis of haemolytic activity of isolated bacteria.

Bacteria	α- hemolytic % (n=)	β- hemolytic % (*n*=)	γ- hemolytic % (n=)
Bacillus spp.	24.39 (10)	36.59 (15)	2.44 (01)
Micrococcus spp.	0 (0)	0 (0)	7.32 (03)
S. aureus	0 (0)	4.88 (02)	0 (0)
CoNS	0 (0)	0 (0)	7.32 (03)
Mixed culture	0 (0)	7.32 (03)	2.44 (01)
Gram-negative rods	2.44 (01)	4.88 (02)	0 (0)
Total	26.83 (11)	53.66 (22)	19.51 (08)

γ-hemolysis. The majority of *Bacillus* spp. (36.59%) were β-hemolytic, whereas all *S. aureus* displayed β-hemolysis on blood agar plates. Among the mixed culture (7.32%) were β-hemolytic, and 2.44% were γ- hemolytic in nature. The hemolytic profiling of Gram-negative bacteria demonstrated that (4.88%) were β-hemolytic and 2.44% were α-hemolytic. On ther otherhand both α-hemolytic and β-hemolytic activity was absent in CoNS and *Micrococcus* spp., thus were considered as γ- hemolytic bacteria.

3.4. Sub-category wise Bacterial Load Analysis of Eyeliner Samples

Eight Eyeliner samples demonstrated the growth of only Gram-positive bacteria as no Gram-negative bacteria was recovered out of *n* = 13 positive samples. Based on usage patterns of eye liner samples, data demonstrate that eyeliner samples 37.5% collected from beauty salons were found contaminated, whereas 12.5% of the home-based multiple user samples yielded bacterial growth and 25% failed to show any bacterial growth. Moreover, all home-based single user (non-shared) samples (25%) were negative for bacterial growth (Table 3). Two types of bacteria belonging to *Bacillus* spp. (*n* = 11) and *S. aureus* (*n* = 02) were recovered from eyeliner cosmetic products.

3.5. Sub-category wise Bacterial Load Analysis of Mascara Samples

Out of 14 mascara samples, 85.71% (*n* = 12) were found contaminated and 14.29% (*n* = 2) were negative for bacterial growth. Based on usage patterns, the samples were categorized as single user

Table 3. Sub-category-wise distribution and bacterial contamination in eyeliner samples.

Sub-categories	Total sample % (n=)	Contaminated samples % (n=)	Non-contaminated samples % (n=)
Salon Products (shared)	37.5 (03)	37.5 (03)	0 (0)
Homebased single user (non-shared)	25 (02)	0 (0)	25 (02)
Homebased multiple user (Shared)	37.5 (03)	12.5 (01)	25 (02)
Total	**100 (08)**	**50 (04)**	**50 (04)**

and shared at either home or beauty salons. Among the beauty salons and home-based multiple users samples 35.71% (*n* = 5/5) and 14.29% (*n* = 2/4) were found contaminated, respectively; whereas 14.29% (*n* = 2/4) of the home-based signle usage samples did not yield any bacterial growth (Table 4). The mascara sample's bacterial load analysis demonstrated contamination of seven different types of bacteria, including *Bacillus* spp. (*n* = 15), followed by *Micrococcus* spp. (*n* = 03) and CoNS (*n* = 03). While the Gram-negative rods were the least prevalent bacteria in Mascara samples with one isolate of each of the *E. coli*, *Proteus mirabilis.* and *Citrobacter* spp. and mixed culture growth (*n* = 04).

4. DISCUSSION

The present study reports the bacterial contamination in different used cosmetic products, that were further sub-divided based on the usage patterns by the consumers. Although, a number of research studies have been carried out on the consequences of consumer behavior, use, and inadequate preservation from different countries, there are a few studies on bacterial contamination of used cosmetic products published from Pakistan, particularily from Sindh province [18, 19]. Despite the fact that the cosmetic products are prepared under strictly controlled conditions, which inhibit the growth and proliferation of microorganism during usage and increase their shelf life, depending upon the effectiveness of the preservatives [20]. The current study has therefore evaluated the bacterial contamination in used cosmetic products at Hyderabad, Sindh. The data of current study showed that the makeup items yeilded bacterial growth in the used products, indicating contamination from the customers during the usage of the product. Our finding are supported by published studies that have reported the contamination in used cosmetic produtcs [21, 22]. The current study has demonstrated that majority of cosmetic samples were contaminated with either one or more than one bacterial isolates. Gram-positive bacteria were highly prevalent in used cosmetic products as compared to Gram-negative bacteria. Gram-positive isolates included *S. aureus*, CoNS, *Bacillus* spp. and *Micrococcus* spp., while Gram-negative bacteria consisted *Proteus mirabilis*, *Citrobacter* spp*.,* and *E. coli*. The major concern is the presence of bacterial pathogens including *E. coli*, *Proteus mirabilis, Citrobacter* spp., and *S. aureus* in used cosmetics products [21].

Used eye cosmetic samples of mascara demonstrated immense bacterial diversity yielding both Gram-positive and Gram-negative bacteria. The presence of pathogenic bacteria such as *E. coli*, *Citrobacter* spp., and *Proteus* spp. in the mascara raises concern about the safety and health issues of the consumers. Our results are in agreement with previous studies demonstrating presence of *Salmonella, Citrobactor and Klebsiella* spp. in cosmetic products [22]. High bacterial diversity in mascara samples may possibly be attributed to the aqueous formulation of mascara and its high potential of interacting with bacteria originating from the eyelashes surfaces and environmental exposure [23, 24]. Moreover, eye cosmetics, specifically mascaras have been demonstrated to be associated with ocular infections in consumers using contaminated products [25, 26]. Presence of potentially pathogenic bacteria may pose serious threat of infections among consumers as these products are applied on skin near to eyes and mouth area [21]. Moreover, using testers at beauty counters or stores, sharing makeup at home, and applying cosmetics in salons may also expose the customers to contamination and skin infections. It has been noted that the testers are not routinely cleaned and are also exposed to the outside air as

Table 4. Sub-category-wise bacterial load analysis of mascara samples.

Sub-categories	Total sample % (n=)	Contaminated samples % (n=)	Non-contaminated samples % (n=)
Salon Products (shared)	35.71 (05)	35.71 (05)	0 (0)
Homebased single user (non-shared)	28.57 (04)	14.29 (02)	14.29 (02)
Homebased multiple user (Shared)	35.71 (05)	35.71 (05)	0 (0)
Total	**100% (14)**	**85.71 (12)**	**14.29 (02)**

well as the consumers may touch and test them, these shared cosmetics in salons and homes are often highly contaminated [22].

Previously published studies have demonstrated the presence of *Bacillus* spp., *Staphylococcus* spp., and *E. coli* in the cosmetic samples. *Staphylococcus* spp. have been shown to cause skin infections including acne. Although *Bacillus* spp. are known to be the transient skin microflora, some of its species have been reported to cause necrotizing cellulitis of skin and severe eye infections [27]. *Staphylococcus* spp. are commensal organisms found on the skin; however, published studies have reported the clinical significance of *S. aureus* in conjunctivitis [28]. In the current study, Staphylococcal spp. were recovered from both mascara and eyeliner cosmetic products. Our results are in agreement with previously published studies in which they have shown the presence of *Staphylococcus* spp. in cosmetic products especially mascara, eyeshadow and lip-gloss [29, 30].

It has been reported that most of the cosmetic products expire within 3-12 months [21], suggesting that except the consumers having medical issues, the cosmetic product would not be the source of infection until their expiry date [21]. However, the frequent contamination of used cosmetic products may result due to using them beyond the expiry date or not stored properly under hygienic conditions. Moreover, when such expired cosmetic products containing the preservatives are applied on the skin, they may alter the skin microflora [31]. The skin microflora of every individual tend to be diverse and in some cases may be harmful for another individual, thus, sharing the same cosmetic product between many people poses a high risk of contamination and spread of skin infections. In conclusion, the high prevalence of bacterial contamination in used cosmetic products pose serious threats to consumers health especially when applied near eyes and mouth area [32] and warrants the need of conducting a comprehensive investigation to ensure better user compliance to mitigate the risk of infectious disease in consumers.

5. CONCLUSIONS

The present study investigated the bacterial contamination of used cosmetic products being used by individuals and in sharing at homes and salons. High level of bacterial contamination has been observed in shared cosmetic products as compared to those used by single consumers. Contamination of pathogenic bacteria in the used cosmetic products may pose serious threat of infections as well as other serious issues to consumer's health.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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