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Microbiological study of used cosmetic products: highlighting possible impact on consumer health

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Running title; microbial contamination of used cosmetic products

Abstract

Aims; to investigate the nature and extent of microbial contamination in five categories of used cosmetic products (lipstick, lipgloss, eyeliners, mascaras and beauty blenders) and highlight the potential risk posed to consumers in the UK.

Methods and Results; used products were donated and microbial contents were determined by microbial culture and identification. 79-90% of all used products were contaminated with bacteria, with bacterial loads ranging between 10^2 to 10^3 cfu/ml, beauty blenders contained an average load of >10^6 cfu/ml. Presence of *Staphylococcus aureus*, *Escherichia coli* and *Citrobacter freundii* were detected. *Enterobacteriaceae* and fungi were detected in all product types, and were prevalent in beauty blenders (26.58% and 56.96% respectively). 93% of beauty blenders had not been cleaned and 64% had been dropped on the floor and continued to be used.

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Conclusions; significant levels of microbial contamination occur during use of cosmetic products and presence of pathogenic organisms poses a potential risk to health.

Significance and Impact of the study; the nature and high level of contamination in used cosmetic products indicates that greater user awareness and education is required. Manufacturers should ensure that product expiry dates are prominently displayed and consumers can identify the symbols used on product packaging.

Key words

Microbial contamination, E.coli, Fungi, Staphylococci

Introduction

Cosmetics are defined by the Federal Food, Drug, and Cosmetic Act as ‘articles intended to be rubbed, poured, sprinkled, or sprayed on or introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering the appearance, and articles intended for use as a component of any such articles; except that such a term shall not include soap’ (Butler, 2000).

A recent report identified the EU as having the largest cosmetic market, worth €78.6 billion in 2018. The largest national markets for cosmetics and personal care products within Europe are Germany (€13.8 billion), France (€11.4 billion) and the UK (€10,9 billion), stressing the importance of consumer safety and awareness regarding cosmetics (Cosmetics Europe - The Personal Care Association, 2019). The demand for imported products in the UK highlights the concern that products imported from non-EU markets only have to comply with the legislation of their own jurisdiction.

The European Cosmetics Regulation ensures cosmetics are made to a high standard to ensure consumer safety. Testing must be carried out to assess the physical and chemical characteristics and stability of the product, as well as to ascertain the microbiological and toxicological profile (Ec.europa.eu, 2019). US legislation states that ‘cosmetics are not expected to be totally free of microorganisms when first used or to remain free during consumer use’ and that ‘cosmetics are not required to be sterile, but microbial contamination can pose a health hazard’ (Food and Drug Administration, 2018). EU guidance states that cosmetics applied around the eye area should have a total viable count for aerobic microorganisms no higher than 10^2 CFU/ml. All other cosmetics should have a total viable count for aerobic microorganisms no higher than 10^3 CFU/ml. Potentially pathogenic species such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* must not be detectable in 1ml of a cosmetic product applied around the eye area or in 0.1ml of other products. EU guidance also states that the occurrence of *Escherichia coli* and other *Enterobacteriaceae* is not acceptable in cosmetic products (Scientific Committee on Consumer Safety, 2016).

Expiry dates of cosmetic products are dictated by the length of time the preservatives formulated in the product are capable of controlling contamination. If the expiry date is printed on the packaging, it is displayed in the form of a symbol resembling an opened pot with either 3M, 6M, 12M, 18M, 24M, or 36M printed in the middle, corresponding to the number of months the product can be used. A recent study showed that 97.9% of participants reported that they use make-up after the expiration date, with mascara the most frequently mentioned product. The most common contaminants were *Staphylococcus aureus*, found in 79% of samples, and *Pseudomonas aeruginosa*, found in 13% of products (Giacomel *et al*., 2013).

Cosmetics with high water content are at a risk of supporting microbial growth following in-use contamination (Lundov *et al*., 2009 and Birteksöz-Tan, Tüysüz, and Ötük, 2013). Use of makeup exposes the products to the commensal microflora found on the skin, and it is possible that a low level of contamination may occur at product manufacturing level.

Studies have isolated *Salmonella*, *Staphylococcus aureus*, *Staphylococcus epidermis, Escherichia coli* and *Pseudomonas aeruginosa* from mascaras, eyeliner, and face powder, whilst also identifying the link between *Staphylococcus aureus* and conditions such as conjunctivitis and impetigo (Abdelaziz *et al*., 1989 and Eldesoukey *et al*., 2016). Lip glosses and lipsticks have been identified as vectors for prohibited and pathogenic species such as *Escherichia hermannii*, *Staphylococcus aureus*, *Bacillus cereus*, and *Enterobacter* species (Babalola and Eze, 2015).

Beauty blenders are recent additions to the cosmetic market and are applicators rather than a cosmetic product. They are small, synthetic sponges used to blend liquid products such as foundation and concealer into the skin, therefore have regular contact with the hands and face. A recent article revealed that in 2016 more than 6.5 million beauty blenders had been sold (Shah, 2016). Improper use of cosmetic products leading to their contamination could allow bacteria and fungi to grow, thus posing a risk to the consumer.

The aim of this study was to investigate the nature and extent of microbial contamination in four in-use cosmetic products (lipstick, lipgloss, eyeliners, mascaras) and beauty blenders and highlight the potential risk posed to consumers in the UK.

Materials and methods

A total of 467 products from the five makeup categories were donated by product users from the UK, in response to advertisement on social media. This comprised of lipstick (96), eyeliner (92), mascara (93), lip-gloss (107) and beauty blenders (79). Information regarding duration of use of each product was collected where possible from an associated questionnaire. No personal identifiable data was collected on product users.

For lipstick and lipgloss products 1g of material was placed in a sterile universal with 9ml of phosphate buffered saline (PBS) containing ten 2.5mm – 3.5mm glass beads. The used applicator tips from the beauty blender and eyeliner product were cut and placed in 10ml of PBS. The mascara wands were removed from the product tubes and placed in 10ml of PBS. Each universal was mixed by vortex for 1 minute and then left to stand for 20 minutes. A 10-fold serial dilution series was performed to reach a final dilution of 10^{-4}. 100µl of each solution was inoculated onto the surface of a Nutrient agar (NA), Mannitol Salt agar (MSA), Sorbitol MacConkey agar (SMAC), Sabouraud Dextrose agar (SAB), and Violet Red Bile Glucose Agar (VRBGA). SMAC and VRBG agar were incubated for 24 hours at 37^{o}C. MSA, whereas NA and SAB were incubated for 48 hours at 37^{o} C. The number of colonies were counted to calculate CFU/ml and colony morphology was observed and recorded. Colonies were characterised using biochemical tests including catalase, oxidase and Gram-staining. The identities of selected colonies representative of the populations obtained for each product type were confirmed using MALDI-TOF MS analysis.

Statistical Analysis

Differences in microbial loads were analysed using a Kruskal-Wallis one-way analysis of variance and Dunn's multiple comparisons test in GraphPad Prism version 7.0.

Results

Table 1 shows the in-use contamination rates of the five product types based upon the counts determined on NA, VRBGA, MSA and SDA. The NA plates showed that approximately 70-90% of all used products were contaminated with bacteria. Using selective bacterial agar the majority of contaminants were staphylococci/micrococci. *Enterobacteriaceae* were also detected in all product types, with particularly high prevalence in the beauty blenders (26.58%). Fungal contamination was observed at various rates across the product types with the highest rate in the beauty blenders (56.96%).

Figure 1 shows the microbial loads within each product group determined on growth medium. 1a) shows total bacterial load determined on NA, most used products contained bacterial loads between 10^2 to 10^3 cfu/ml, whereas beauty blenders contained an average load of $>10^6$ cfu/ml. A one way ANOVA confirmed that beauty blenders were significantly more contaminated than any of the other products tested (p=<0.003). 1b) shows *Enterobacteriaceae* load determined on VRBG agar; lipgloss displayed the highest load whereas lipstick revealed the lowest load. A one way ANOVA confirmed that lip-gloss was significantly more contaminated than any of the other products tested (p=<0.025). Lipstick was significantly less contaminated than beauty blenders (p=0.0229). 1c) demonstrates bacterial load determined on MSA, the only significant differences in rates of contamination were detected between beauty blenders and lipstick (p=0.0184) and beauty blenders compared to mascara (0.0375). 1d) Levels of fungal contamination across all product types were approximately 10^2 cfu/ml and no significant differences were observed in levels of fungal contamination across the products tested (p=>0.05).

Table 2 shows the identity of selected organisms taken from 25 products and confirmed by MALDI-TOF analysis**.** A total of 48 organisms were identified. Results revealed the presence of expected skin organisms, however opportunistic pathogens and *Enterobacteriaceae* were also detected.

Figure 2 shows microbial load versus duration of product use (months) for individual products where accurate dates were available. Logarithmic scales have been used for the microbial loads on lipgloss and beauty blenders to accommodate the wide numerical ranges obtained for these products. There was no correlation between duration of product use and level of contamination for any of the product types as determined by non-parametric Spearman correlation.

Only 6.4% of all collected samples had been cleaned, with beauty blenders being the most commonly cleaned product and none of the mascara products. Results revealed that 27.3% of products (largely eyeliner), had been applied in a bathroom. Consumers were asked whether the product had been dropped on the floor and 28.7% of products had being exposed to the floor surface. Both these unsanitary practices were observed largely in beauty blenders, with 35.6% of beauty blender samples being used or stored in a bathroom, and 64.4% having been dropped on the floor.

Discussion

Although the effects of consumer use, consumer behaviour, and ineffective preservation have been identified, there have been few publications from the UK (Dadashi and Dehghanzadeh, 2016; Eldesoukey *et al*., 2016). Therefore, the aim of this study was to determine microbial contamination of used products donated by consumers in the UK.

The results revealed varying levels of all prohibited microorganisms in the used products indicating contamination caused by the consumer whilst using the products. In many cases, organisms prohibited according to EU guidance in packaged products were present in used products (Scientific Committee on Consumer Safety, 2016). A particular concern is the presence of potentially pathogenic organisms including; *E.coli*, *C.freundii, P.aeruginosa* and *S.aureus*. Introduction of these microorganisms during application around the mouth or eyes could pose a significant threat of infection. Other studies have revealed the presence of potentially pathogenic microoganisms in cosmetic products including *Candida* species, *S. aureus* and *E.coli* (Pascher, 1982; Onurdağ, Özgen and Abbasoğlu, 2010; Dadashi and Dehghanzadeh, 2016; Eldesoukey *et al*., 2016).

We found *P.aeruginosa, P.*fulva, *P.monteilii* and *P.putida* in beauty blenders, lipstick and lipgloss. These are opportunistic pathogens capable of causing significant infections particularly in immunocompromised individuals. Although infection can be prevented, ineffective sanitary practices whilst handling products could lead to infection via cuts or abrasions in the skin during application. *Staphylococcus* species are commensal organisms found on the skin, including *Staphylococcus aureus* and *Staphylococcus epidermidis*. In the current study, *S.saprophyticus*, *S.haemolyticus*, *S.cohnii*, and *S.capitis* were identified through MALDI-TOF analysis from mascara, eyeliner and lipgloss.

S.capitis, is commonly found on the face and therefore expected as a contaminant of cosmetic products. *S.capitis* infection has been reported in endocarditis following pacemaker implantation and bacteraemia in premature infants (Wang *et al*., 1999 and Cone *et al*., 2005). *S.haemolyticus* is found in the axillae, perineum, and inguinal areas (Fischetti *et al*., 2006). Known as an emerging nosocomial pathogen, it has the ability to cause UTIs but is not known to cause skin infections (Gunn and Davis, 1989 and

Fischetti *et al*., 2006). *S.cohnii* is a nosocomial pathogen, presenting high levels of methicillin resistance. It has been known to cause bacteraemia originating from multiple sources, including pressure ulcers, UTIs and pneumonia, and has been unusually identified as the cause of fatal meningitis (Okudera *et al*., 1991). *S.saprophyticus* is commensal to the genitourinary tract but can cause urinary tract infections and acute cystitis. Although many of these organisms are commensals to the body, they should not be present in products applied to the skin, their presence indicates poor sanitary practices by the consumer.

C.freundii is a Gram-negative species belonging to *Enterobacteriaceae* family, found in water, soil, and the gastrointestinal tract. This was detected in lipstick and lipgloss products. *E.coli*, a known gut pathogen was also observed across all product types and confirmed in beauty blenders and eyeliner products by MALDI-TOF. The cross-contamination of cosmetic products with both of the organisms indicates poor consumer hygiene whilst handling and applying products.

Beauty blenders have only been recently introduced as an application product and limited information is available on how best to use or clean them. Our results have shown that these products carried the highest bacterial load during use and more than a quarter were contaminated with *Enterobacteriaceae. A.ursingii* was also detected in this product type, a Gram-negative coccobacillus known as an important opportunistic pathogen known for causing nosocomial infections. Beauty blenders could pose a significant risk to consumers, their product design allows microorganisms to accumulate. Beauty blenders can be cleaned with warm, soapy water therefore encouraging microbial proliferation if not dried.

Many products are imported from other countries and are not obliged to provide expiry information on product packaging. Although EU guidance has incorporated the use of symbols to indicate expiry dates (Scientific Committee on Consumer Safety, 2016), there are no regulations or requirements under current U.S. laws that require cosmetic manufacturers to print expiration dates on the labels of cosmetic products. However, manufacturers have the responsibility to determine shelf life for products as part of their responsibility to substantiate product safety (Food and Drug Administration, 2019).

Sharing makeup products and makeup testers found on beauty counters, may also provide a route for contamination and infection. Commonly testers are not cleaned regularly, and are left exposed to the environment and to passing customers who are allowed to touch and try the product. Sharing makeup greatly increases the level of contamination seen in testers due to the increased use and pressure on cosmetic preservatives, and this can include contamination by bacteria, fungi and yeast (Dadashi and Dehghanzadeh, 2016).

All cosmetic products are manufactured under strictly controlled conditions to control microbial content and proliferation during use. The lifetime of the product is limited by the protection afforded by the preservation system and this information is provided on the label of the product. Most products have an expiry date of 3-12 months, providing the user has not had an infection such as conjunctivitis. However, our study shows in practical terms products are used beyond the expiration date of the products and considerable levels of microbial contamination have been identified across a range of cosmetic products.

The microbial levels and frequency of contamination in used products revealed in this study suggests that further detailed investigations should be carried out to ensure better user compliance. Future studies should incorporate effective preservative utilization steps and anaerobic culture should be included. This study has not considered the effect on microbial contamination of sharing of cosmetic products or factors such as procedures used to clean beauty blenders between use.

Cosmetic regulations clearly state that products should not contain pathogenic organisms. 70-90% of all used products were contaminated with bacteria. The presence of organisms including *S.aureus*, *E.coli* and *C.freundii* in used products may be introduced through ineffective hand hygiene. Information derived from our questionnaires showed that 93% of beauty blenders had not been cleaned and 64% had been dropped on the floor and continued to be used. *Enterobacteriaceae* were also detected in all product types, with particularly high numbers observed in the beauty blenders (26.58%). Fungal contamination was observed across the product types with the highest rate detected in the beauty blenders (56.96%). Clearly further advice and education is needed on the use and maintenance of these products to avoid self-contamination with potentially harmful microorganisms. Such potential hazards would be increased where products are used by multiple individuals such as in beauty salons. We are not aware of any regulations or discussion groups addressing contamination issues of beauty blenders, which are applicators used to apply cosmetics rather than a cosmetic product incorporating a preservation system. Manufacturers also need to ensure that product expiry dates are prominently displayed and consumers can identify the symbols used on product packaging.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Table 1: In-use contamination rate of products (% of used products found to be contaminated). Four selective agars were used to determine the presence of organisms in the cosmetic product categories tested.

Table 2: Identification by MALDI-TOF MS. The identities of 48 isolates were determined. The number of times the isolates were detected in each product type is listed in parenthesis.

Figure 1 a-d: the microbial loads within each product group determined on selective growth medium. *Enterobacteriaceae* levels were detected using SMAC and VRBG agar. Error bars show standard error.

Figure 2 a-e: microbial load plotted against the age of products

Table 1: In-use contamination rate of products (% of used products found to be contaminated)

	Eyeliner n=92	Lipstick n=96	Mascara n=93	Beauty blenders n=79	Lipgloss n=107
Nutrient Agar	80.43	76.04	79.57	92.41	71.96
Violet Red Bile Glucose Agar	9.78	5.21	7.53	26.58	9.35
Mannitol Salt Agar	77.17	56.25	59.14	72.15	55.14
Sabouraud Dextrose Agar	28.26	36.46	17.20	56.96	10.28

Table 2: Identification by MALDI-TOF MS

Cosmetic product type	MALDI-TOF Identification of microorganisms	
Beauty Blenders	*Escherichia coli (2)* *Citrobacter freundii (2)* *Acinetobacter ursingii (2)*	*Pseudomonas monteilii (2)* *Pseudomonas aeruginosa (2)*
Mascara	*Pluralibacter gergoviae (2)* *Staphylococcus saprophyticus (1)*	
Lipstick	*Pseudomonas fulva (2)* *Pseudomonas monteilii (1)* *Citrobacter freundii (1)*	
Lip gloss	*Staphylococcus haemolyticus (1)* *Staphylococcus cohnii (1)* *Staphylococcus saprophyticus (2)* *Staphylococcus capitis (2)* *Staphylococcus pasteurii (1)* *Micrococcus luteus (1)* *Bacillus litoralis (1)*	*Pseudomonas putida (1)* *Pseudomonas monteilii (3)* *Pseudomonas fulva (2)* *Lactobacillus (1)* *Citrobacter freundii (1)* *Candida glabrata (1)*
Eye liner	*Cryptococcus diffluens (1)* *Micrococcus luteus (1)* *Burkholderia vietnamiensis (1)* *Bacillus muralis (1)* *Staphyloccus saprophyticus (1)* *Staphylococcus capitis (2)*	*Staphylococcus hominis (2)* *Staphylococcus haemolyticus (2)* *Escherichia coli (2)* *Arthrobacter roseus*

Figure 1 a-d: Microbial load of products

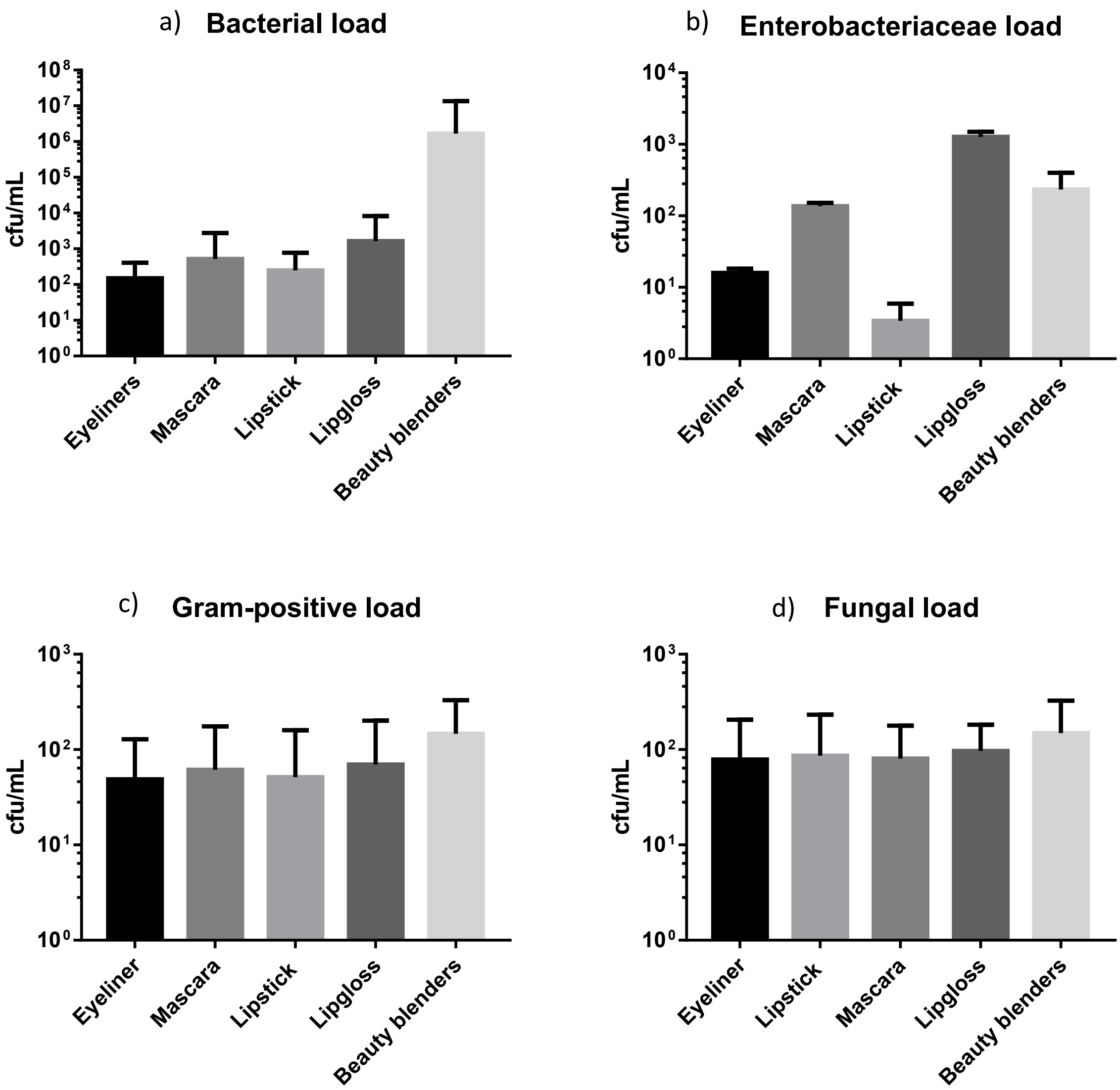

Figure 2 a-e: microbial load plotted against the age of products