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FDA 2014 survey of eye area cosmetics for microbiological safety

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Significance and Impact of the Study In the United States, cosmetic products are regulated postmarket; therefore, surveillance programmes are one of FDA's most important tools for monitoring microbiological safety of cosmetics. 'Traditional' preservatives, such as parabens and formaldehyde releasers, are perceived unfavourably by some consumers, resulting in cosmetic manufacturers increasingly using 'nontraditional' preservatives. FDA conducted an analytical survey of eye area cosmetics that claimed to be free of traditional preservatives and determined microbiological loads in tested products. This study explores the association of microbial loads with the physical and chemical characteristics of the cosmetic products, and points to the limits of preservative activity in cosmetics.

Keywords
colour cosmetics, eye area, microbiology, nontraditional, safety testing, water activity.

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Abstract

In an attempt to assess cosmetics containing 'nontraditional' preservatives, 93 eye area cosmetic products were selected based on labelled or marketed claims that these products were 'green', 'natural', 'paraben-free', 'preservative-free' or contained nontraditional preservatives (e.g. botanical extracts). Products were analysed for water activity, pH and microbiological content, which included enumeration of aerobic micro-organisms, detection of microbial growth after a 7-day enrichment and identification of microbial isolates. The survey found that 60% (56/93) of the eye area cosmetics were free of microbiological growth under test conditions, 32% (30/93) showed the presence of micro-organisms at low levels (<100 CFU per ml or g) and 8% (7/93) showed microbiological growth at higher levels (> 100 CFU per ml or g). Gram-positive bacteria such as *Bacillus* and *Staphylococcus* were the dominant genera identified in these cosmetic products, whereas Gram-negative species were relatively uncommon. The survey found a positive association between lower water activity cosmetics and the presence of micro-organisms in these products. Similarly, colour cosmetics were more likely to contain micro-organisms than noncolour cosmetics. The most represented micro-organisms in the survey were from genus *Bacillus*, suggesting that the natural raw materials are the likely source of observed microbial loads.

Introduction

Cosmetic products are complex formulations of a number of ingredients that are not intended to have a pharmacological activity in the human body. However, many of these ingredients, such as water, oils, fats, plant extracts, protein hydrolysates and colour additives, can support the growth of micro-organisms. Unimpeded microbial growth in cosmetics can lead to spoilage of the product, and if applied to the human body or eye area, can lead to serious injuries including permanent loss of vision (Wilson *et al.* 1971; Wilson and Ahearn 1977; Reid and Wood 1979; Johns and Oday 1988). To control the microbial growth in cosmetic products, manufacturers often include preservatives that inhibit bacterial and fungal growth. However, because there is a market for cosmetic products, including eye area cosmetics that are formulated without traditional preservatives such as parabens and isothiazolinones, the FDA decided to evaluate the ability of nontraditional preservatives (e.g. botanical extracts, organic

acids, glyceryl caprylate) or hurdle technology to control the growth of micro-organisms. Although cosmetic products are not expected to be sterile, the total number of aerobic micro-organisms per gram must be within certain limits (≤500 CFU per g or ml for eye area products), and they must be completely free of high-virulence microbial pathogens (Bacteriological Analytical Manual (BAM) Chapter 23), because exposure to micro-organisms from cosmetic products is a safety concern and poses a risk to consumers.

In the United States, cosmetic products do not require premarket approval; therefore, postmarket surveillance is one of the most important tools that FDA uses to monitor the safety of cosmetic products. It is the cosmetic manufacturers' responsibility to ensure microbiological safety of their products and ingredients. Because of the large number of cosmetic products on the market and the limited amount of available resources, FDA has focused its surveillance programme on higher risk products. In the case of cosmetics, these products include eye area cosmetics without preservatives or with nontraditional preservatives.

In 2014, FDA initiated an analytical survey of eye area cosmetics labelled as containing nontraditional preservatives or no preservatives, as defined on the FDA 'Fiscal Year 2014 Cosmetics Survey', with the purpose of determining microbiological load in these products prior to consumer use. The survey also aimed to investigate the association of microbial loads with the physical and chemical characteristics of the product formulations including pH and water activity. Here, we summarize and discuss the microbiological findings of this survey.

Results and discussion

Quantification and identification of micro-organisms in eye area cosmetics

FDA laboratories conducted microbiological analyses of 93 eye area products, such as mascaras, eyebrow liners, make-up removers, eye creams, shadows, liners, eye pencils and gels (Table 1).

Analysis of the data showed 56 out of the 93 products (60%) to be free of detectable microbial growth in 5 g or ml of the product (Fig. 1a). Seven products (7/93 or 8%) displayed growth on aerobic plates (detection limit 100 CFU per g), whereas 30 products (30/93 or 32%) were positive for microbial growth only after a 7-day enrichment (detection limit 1 CFU per g; Fig. 1a). In the microbiological analysis of all the 93 surveyed products, we found almost exclusively bacterial growth, and only one mould isolate, despite the use of media specific for fungal growth. This is consistent with our observations during FDA's routine postmarket surveillance of previously examined eye area and general use cosmetics showing that fungi are not often found in cosmetic samples (Wilson *et al.* 1971; Lundov and Zachariae 2008; Neza and Centini 2016).

Representative colonies of micro-organisms from aerobic plates and enrichment growth were identified using the VITEK microbial identification system (bioMérieux, Marcy-l'Étoile, France). Of the 37 products that tested positive for microbial growth, 26 contained multiple microbial species. An unidentified species of mould was recovered in one subsample of one mascara product. From a total of 154 bacterial isolates, 137 were identified to the genus or species level, with the reminder only determined as Gram-positive ($Gram^+$) or Gram-negative ($Gram^-$). $Gram^+$ bacteria were the most represented group (148 isolates, 96%; Fig. 1b). Among the $Gram^+$ group, members of the genus *Bacillus*, or other *Bacillus*-like bacteria, were the most common (83/148 identifications or 56% of all $Gram^+$), whereas, members of the genus *Staphylococcus* were the next most common group (31/148 identifications or 21% of $Gram^+$; Fig. 1b). The prevalence of *Bacillus* and *Staphylococcus* genera is similarly observed in some cosmetic surveys in other countries (Behravan *et al.* 2005; Tan *et al.* 2013). The remaining $Gram^+$ bacteria were either not identified to a species level, or were from genera with four or fewer isolates. On a species level, the *Bacillus subtilis/amyloliquefaciens/atropheus* group was the most common (15 identifications), closely followed by *Bacillus pumilus* (13 identifications), *Staphylococcus epidermidis* (11 identifications), *Bacillus cereus* and *Bacillus lentus* (10 identifications each) and *Staphylococcus warneri* (nine identifications). These species accounted for more than one half of all identified $Gram^+$ isolates. *Bacillus* species are frequently found in soil, plant and food, and are occasionally associated with opportunistic human infections following injury or surgery (Schemmer and Driebe 1987; Ozkocaman *et al.* 2006; Yang *et al.* 2010; Long *et al.* 2014; Pitt *et al.* 2015). *Staphylococcus epidermidis* and *S. warneri,* two most common staphylococci found in the survey, are a part of normal human skin flora (Kloos and Schleifer 1975; Schleifer and Kloos 1975), and are sporadically associated with posttraumatic endophthalmitis and various nosocomial infections (Pinna *et al.* 1999; Otto 2009; Widerstrom *et al.* 2012; Long *et al.* 2014). $Gram^-$ bacteria (six identifications) were from genera *Pantoea*, *Aeromonas* and *Sphingomonas*. We did not find micro-organisms whose occurrence would be of particular health concern in the analysed cosmetic products, such as *Pseudomonas aeruginosa* or *Staphylococcus aureus,* which have previously been found in eye area cosmetics and linked to serious eye infections (Wilson *et al.* 1971; Wilson and Ahearn 1977).

Table 1 Cosmetic products analysed for the presence of micro-organisms, and measured pH and water activity (a_w) values

Eye area product characteristics, by category	Eye cream	Eye gel	Eye liner	Mascara	Make-up remover	Eye shadow	Eyebrow liner pencil	Eye liner pencil	All products
Number of products	26	5	9	18	5	16	7	7	93
Presence of micro-organisms									
No microbial growth detected	19 (73%)	5 (100%)	8 (89%)	8 (44%)	5 (100%)	5 (31%)	3 (43%)	3 (43%)	56 (60%)
Bacterial growth on aerobic plates, no. (%)	1 (4%)	0 (0%)	0 (0%)	2 (11%)	0 (0%)	1 (6%)	1 (14%)	2 (29%)	7 (7·5%)
Bacterial growth only after enrichment, no. (%)	6 (23%)	0 (0%)	1 (11%)	8 (44%)	0 (0%)	10 (63%)	3 (43%)	2 (29%)	30 (32%)
Physical characteristics									
Products tested for pH, no. (%)	19 (73%)	5 (100%)	3 (33%)	12 (67%)	4 (80%)	9 (56%)	5 (71%)	2 (29%)	59 (63%)
Average pH (±SD)	5·3 (0·9)	5·8 (1·0)	6·9 (0·5)	6·3 (1·2)	6·4 (0·5)	6·4 (0·8)	5·2 (0·4)	5·5 (0·4)	5·9 (1·1)
Products tested for a_w, no. (%)	19 (73%)	5 (100%)	3 (33%)	13 (72%)	4 (80%)	8 (50%)	5 (71%)	3 (43%)	60 (65%)
Average a_w (±SD)	0·97 (0·02)	0·98 (0·01)	1·00 (0·01)	0·96 (0·04)	0·82 (0·25)	0·70 (0·22)	0·54 (0·26)	0·35 (0·12)	0·85 (0·23)

Similarly, FDA's CAEMS (CFSAN Adverse Events Management System) database did not indicate occurrence of adverse events resulting from the use of the surveyed cosmetics.

Comparison of microbial isolates between APC and enrichment

To understand which bacterial types were present in very small *vs* larger quantities in cosmetic products, we investigated the distribution of bacterial types found on APC plates compared with the distribution found in cultures only after enrichment. Because the sample size for the APC group was small, we examined the distribution of the two largest groups of micro-organisms in our survey, $Gram^+$ rods and $Gram^+$ cocci. Contingency table analysis which examines the association between two variables, showed that, at least in this sample population, $Gram^+$ rods were more likely to be found growing only after enrichment than $Gram^+$ cocci. $Gram^+$ cocci were more likely to give detectable colony counts on APC plates than $Gram^+$ rods (Fig. 2a, Fisher's exact test, odds ratio (OR) = 2·3 with 95% Confidence Interval (CI) = 1·13–4·86 and $P = 0·02$). Similar contingency table analyses showed the tendency of *Bacillus* species ($Gram^+$ rods) to be associated with the samples displaying enrichment-only growth, and *Staphylococcus* species ($Gram^+$ cocci) to be associated with the samples that grew on enumeration plates (Fig. 2b, Fisher's exact test, OR = 2·8 with 95% CI = 1·18–6·76 and $P = 0·02$).

Association between colour cosmetics and microbial load

The two groups of products tested in this survey were (1) colour cosmetics (products that impart colours to users) such as mascaras, eye and eyebrow pencils, eye shadows, and liners, and (2) noncolour cosmetics such as eye creams, eye gels and make-up removers. Among colour cosmetics (57 total), similar number of products (30 products, 53%) showed microbial growth compared to those with no detectable contamination (27 products, 47%). Among noncolour products (36 total), 7 products (19%) showed microbial growth, whereas 29 (81%) had no detectable growth. To determine whether microbial growth was significantly more common in colour *vs* noncolour cosmetics, we performed contingency table analysis using Fisher's exact test, which revealed that colour cosmetics are associated with a significantly greater likelihood of finding microbial growth than noncolour cosmetics (Fig. 2c, Fisher's exact test, OR = 4·6 with 95% CI = 1·74–12·21 and $P = 0·002$). This may suggest that the pigment or pigment-associated ingredients are conducive to increased microbial load at any time during or prior to the manufacturing process. This association of microbial growth with pigment or pigment-associated ingredients together with the observation that the most represented micro-organisms in our cosmetic survey were bacilli, common soil, plant and food bacteria, suggests that the natural raw materials are a likely source of observed microbial loads (Brannan and Geis 2009; Mpuchane *et al.* 2010; Di Maiuta and Schwarzentruber 2011).

Association between water activity, pH and microbial growth

All bacteria and fungi require water and most require an environment that is close to a neutral pH (pH = 5–9) for their growth (Gale and Epps 1942; Scott 1953; Lambert

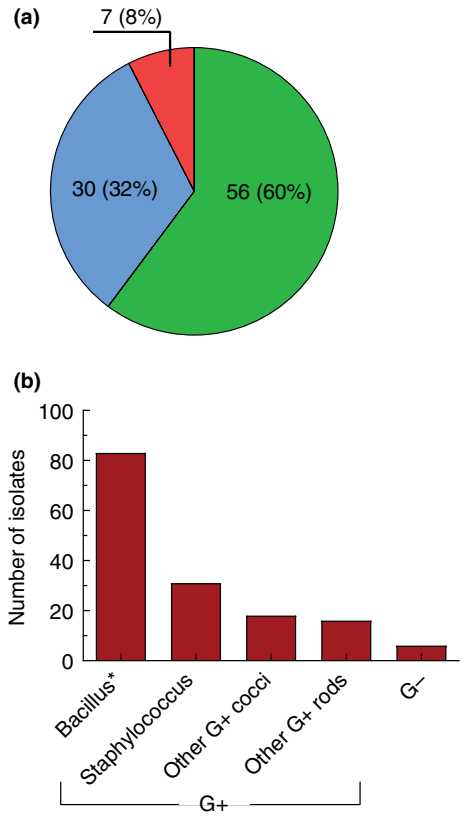

Figure 1 Microbiological load in eye area cosmetics. (a) Different levels of bacterial growth detected in 93 cosmetic products. Green (▬), no bacterial growth detected under any condition. Blue (▬), growth detected after 7-day enrichment, but not on aerobic plates. Red (▬), growth detected on both aerobic plates and in enrichment. (b) Number of bacterial isolates in all subsamples of 37 products that showed bacterial growth. *Bacillus** includes genus *Bacillus,* and *Bacillus*-like genera, *Brevibacillus*, *Paenibacillus* and *Alicyclobacillus*. [Colour figure can be viewed at wileyonlinelibrary.com]

2011). Therefore, pH and water activity (a_w) are two major factors related to microbial growth in cosmetic products, especially those that lack traditional preservatives (Orth 2010). The pH and a_w values of these cosmetic products were measured to examine the extent to which they are associated with antimicrobial preservation. Because of technical difficulties such as product dryness, viscosity and the amount of product collected during the survey, not all samples were analysed for their pH and a_w (Table 1). The pH values, measured in a total of 59 products, were slightly acidic with average values of each product category ranging between pH 5·2 and 6·9 (Table 1). Of the products analysed, eye creams tended to be more acidic (pH = 5·3) than eye liners (pH = 6·9); however, there was a considerable variation within each product group (Fig. 3a). We found no correlation between pH

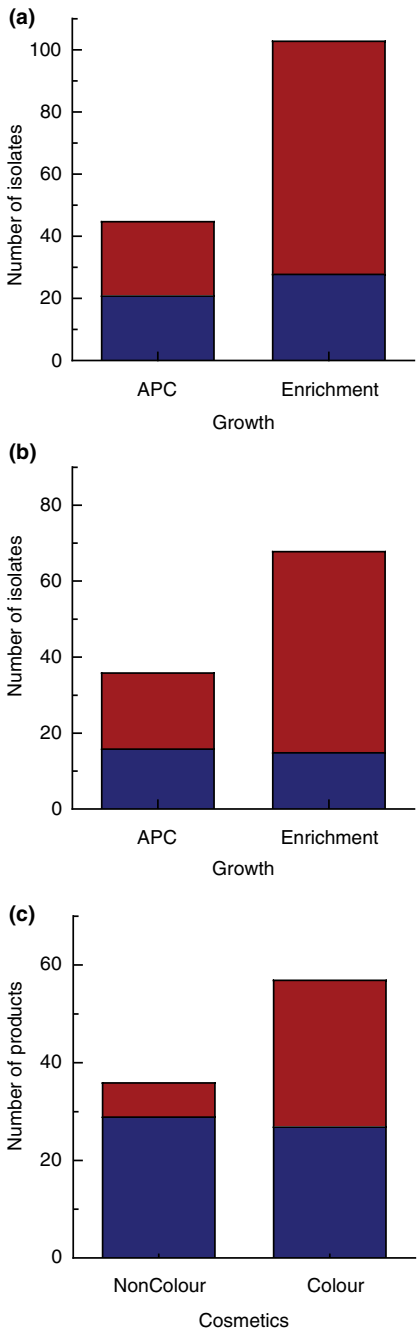

Figure 2 Bacterial groups and growth associations in the products. (a) Contingency table analysis of broad bacterial groups (Gram-positive (Gram$^+$) rods (red, ▬) and Gram$^+$ cocci (blue, ▬), and growth detection method. The presence of Gram$^+$ rods is associated with products displaying growth in the enrichment. (b) Contingency table analysis of *Bacillus* (red, ▬) and *Staphylococcus* (blue, ▬) identifications from APC and enrichment growth. (c) Contingency table analysis of noncolour and colour cosmetic products and their association with the presence of bacterial growth (red ▬), or its absence (blue ▬). [Colour figure can be viewed at wileyonlinelibrary.com]

and microbial growth (Spearman's rank correlation coefficient $r = 0{\cdot}05$, $P = 0{\cdot}7$), likely because most of the analysed products had permissible pH values for bacterial survival. Among the products evaluated in this survey, pH does not appear to have been used as part of a preservative system. In most products, growth was likely prevented by some form of preservative (especially in products with high water activity), and some might have been simply free of detectable growth even though they did not have preservatives.

In this report, higher water content ('wet') product categories have an average a_w value that is >0·8, whereas lower water content ('dry') product categories have an average a_w value of 0·8 or less. Water activity was high for eye creams and gels, mascaras and eye liners (average $a_w \geq 0{\cdot}96$, Table 1, Fig. 3b), whereas eye shadows, eye pencils and eyebrow liner pencils had lower average water activity ($a_w \leq 0{\cdot}7$, Table 1, Fig. 3b).

Among the 67 high water content products, 20 (30%) products showed microbial growth, whereas 47 (70%) had no detectable growth (Fig 3c). Among the 26 low water content products, 17 (65%) products showed microbial growth, whereas 9 (35%) had no detectable growth (Fig. 3c). To examine association between water activity and the presence of micro-organisms in products, we performed Fisher's exact test using two variables: water content (higher or lower) and the microbial growth (presence or absence) in enrichment medium. The results indicated a positive association between the presence of micro-organisms and lower water content of products, indicating that dryer products had a higher likelihood of containing micro-organisms compared with higher water content products (Fig. 3c, OR = 4·4 with 95% CI = 1·70–11·62 and $P = 0{\cdot}002$). This seemingly counterintuitive association can be explained by the fact that naturally occurring micro-organisms are destroyed by preservative systems in aqueous environments, whereas in anhydrous environments, the presence of preservatives has no effect on the naturally occurring organisms that are present (Orth 2010). Manufacturers sometimes add preservatives to anhydrous cosmetics to suppress microbial growth when the products acquire moisture and micro-organisms during their normal use (Orth 2010). While microbes cannot grow in dry products, once water and nutrients are available (as in enrichment medium), the microbes can exit dormant state and grow, which is consistent with our observations under enrichment conditions.

Our analysis showed that the majority of cosmetic products, prior to consumer use, did not have microbial contamination, but a few products contained micro-organisms at concentrations greater than 100 CFU per g or ml. We did not find adverse events or micro-organisms whose occurrence would be of particular health concern in the

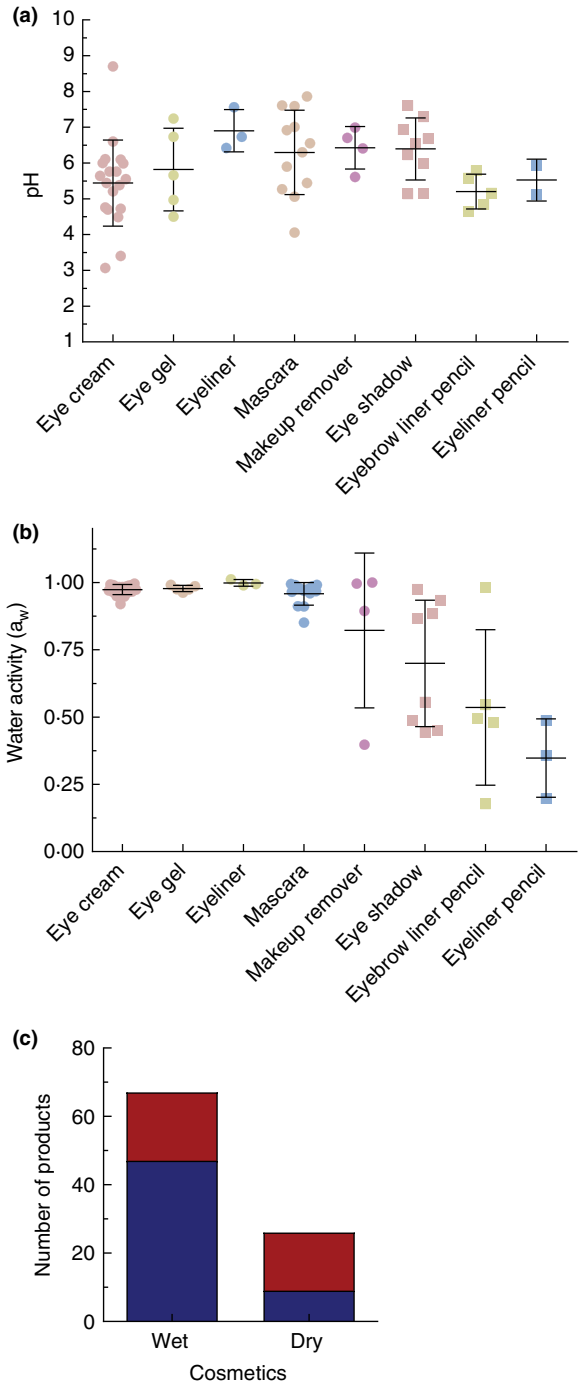

Figure 3 (a) Distribution of pH values measured in different products. Total of 59 measurements and their spread across eight product categories. (b) Distribution of 60 a_w measurements across eight product categories. a_w measurements of dry product categories tend to be spread more than those of wet product categories. (c) Contingency table analysis of wet and dry products for the presence (red ■) or absence (blue ■) of microbial growth. Wet product categories are defined as having average $a_w > 0{\cdot}8$, while dry products categories have average $a_w \leq 0{\cdot}8$ (see also Table 1). [Colour figure can be viewed at wileyonlinelibrary.com]

analysed cosmetic products. We found that lower water activity products and colour cosmetics were more likely to contain micro-organisms compared to higher water activity products and noncolour cosmetics. These findings, together with the identification of *Bacilli,* common soil, plant and food bacteria, as the most represented micro-organisms in our cosmetic survey, suggest that the natural raw materials are likely source of observed microbial loads. Clays, carbonites and other natural raw materials found in cosmetics often contain bacilli (Brannan and Geis 2009; Mpuchane *et al.* 2010; Di Maiuta and Schwarzentruber 2011). Preservatives, even if present in the lower water activity cosmetics, will not be effective against micro-organisms carried over through raw materials, and are added to prevent bacterial growth after opening the product and introducing moisture during recurrent use. In contrast to raw materials, bacterial contaminations that originate in production facilities often contain Gram$^-$ micro-organisms and are associated with plant's water system (Ferrarese *et al.* 2003). Understanding and better evaluation of sources of microbial load in raw materials and production facilities may help prevent their carry-over to cosmetic products.

Materials and methods

Sample selection

A total of 93 eye area cosmetic products were identified using the Mintel Global New Products database, and Internet searches and products were selected based on labelled or marketed claims that they contained nontraditional preservatives or no preservatives. Some terms used in selection of the products were 'natural', 'paraben-free', 'preservative-free' and 'green'. Review of labelling for ingredient information, which was provided as part of the survey, revealed that some of the analysed products contained ingredients such as phenoxyethanol, sorbate, benzoate and diazolidinyl urea that may function as preservatives. Some products contained no obvious preservatives, and some contained a variety of botanical extracts which may, or may not, function as preservatives. Products were purchased from retail locations and shipped to FDA laboratories for microbiological analysis. A total of 93 eye area products (26 eye creams, 18 mascaras, 16 eye shadows, 16 eye liners and pencils, 7 eye brow liner/pencils, 5 make-up removers and 5 eye gels) were analysed for microbial content, 59 products for pH and 60 products for water activity (a_w) (Table 1). The number of units of each product was sufficient to guarantee at least 15 g of product for analyses.

Sample analysis

The microbiological analysis was performed using BAM, Chapter 23 'Microbiological Methods for Cosmetics'. Five subsamples (usually from five retail units) of each product, each containing 1 g or 1 ml, were analysed individually. The tests included enumeration of aerobic micro-organisms (aerobic plate counts (APC)) and fungi (yeast and mould plate counts (YMPC)) and detection of microbial growth after a 7-day enrichment. The detection limit of the plate counts was 100 CFU per g or ml, and detectable growth after enrichment indicated that at least one microbial cell was present in 1 g or 1 ml of product. Microbial colonies recovered from enumeration plates and enrichment broths were identified using the VITEK microbial identification system (bioMérieux). The protocol from BAM Chapter 14 was followed to further differentiate *Bacillus cereus* group by the presence of toxin crystals and rhizoid growth.

Water activity and pH values were measured using standard procedures described in the instruments' user manuals.

Statistical analysis

Correlation and contingency table analyses using Fisher's exact test were performed with GRAPHPAD PRISM 6 software (GraphPad Software Inc., La Jolla, CA).

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Conflict of Interest

The authors declare no conflict of interest.

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