Microbiological quality of hair and skin care cosmetics manufactured in Jordan

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A B S T R A C T
A total of 57 brands of commercially available hair and skin care cosmetics manufactured in Jordan were evaluated for their microbiological quality using standard procedures. Viable microorganisms were not recovered from 56.1% of the items tested and approximately 5.3% harbored less than 10^2 CFU g^-1. Dominant bacterial isolates were Bacillus species, Pseudomonas spp., and coagulase-negative staphylococci. Further testing of different batches of the brands that were found to be contaminated with >10^5 CFU g^-1 revealed that the problem was persistent in these products. Preservative efficacy tests were carried out for the contamination-free brands using Pseudomonas aeruginosa ATCC 9027. This test demonstrated that 28.1% of the products tested were inadequately preserved. It is concluded that poor microbiological quality of the preparations investigated can be attributed to either a problem inherent in the formulation of these brands and/or poor manufacturing hygiene. It is hoped that the implementation of good manufacturing practice in the Jordan’s cosmetics industry will improve the microbiological quality of these products.

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1. Introduction

Cosmetics are formulated from a variety of chemicals in the presence of an abundant amount of water. Many of these products exhibit a pH near neutrality and are usually kept at a temperature close to or above ambient, particularly when they are used in countries with warm climatic conditions. Therefore, unless they are adequately preserved, these products provide microorganisms with environments conducive for their growth. Contaminants may gain access to cosmetics incidentally during the manufacturing process or during use by consumers. These contaminants could be pathogens, opportunistic pathogens and/or saprophytes. The consequence of such a contamination may prove to be costly in terms of health and economy (Anelich and Korsten, 1996; Álvarez-Lerma et al., 2008).

In Europe, manufacturers of cosmetic products are responsible for compliance with the principles and guidelines of Good Manufacturing Practice (GMP). These requirements were included in Directive 93/35/EEC (Anon, 1993), which was the 6th amendment of the first Cosmetics Directive agreed upon by European countries in 1976 (Anon, 1976). A recent update of this directive was issued in 2009 in order to strengthen regulatory framework for cosmetics such as in-market control, with a view to ensuring a high level of protection of human health (Anon, 2009).

The microbiology technical committee in charge of cosmetic products at the International Standardization Organization (ISO/TC 217) has come up with guidelines on GMP (Anon, 2007). These guidelines are designed to cover various quality aspects of cosmetics, including production, documentation, and specific cleaning procedures. The guidelines also cover microbiological control of raw materials, bulk and finished products, packaging material, personnel, equipment, and storage areas.

Despite all these efforts to improve the microbiological quality of cosmetics, reports of microbial contamination of commercially available products still appear in the scientific literature. Such reports have appeared in several developing countries including Iran (Behravan et al., 2005), Egypt (Abdelaziz et al., 1989), and Nigeria (Okeke and Lamikanra, 2001). In Jordan there has been just one publication concerned with this subject (Na’was and Alkofahi, 1994). Developed countries have moved a step further after the implementation of GMP regulations and have started to recall products found to be outside the microbiological limits (Wong et al., 2000; Lundov and Zachariae, 2008).

Jordan is a full member of ISO Technical Committee 217, and the Jordan Institute for Standards and Metrology has been engaged in drafting a GMP manuscript based on that adopted by the ISO cosmetics committee. The draft is now in the final stages and probably will be implemented in late 2011. The objective of this
work was to study the microbiological quality of skin and hair care products marketed in Jordan so that results would be considered as a reference against which to measure improvements in quality resulting from GMP implementation.

2. Materials and methods

A total of 57 preparations representing a variety of hair and skin care cosmetics were purchased from retail outlets in Amman; all were manufactured by Jordanian companies. Three additional samples, representing different batch numbers, of products shown to be heavily contaminated were obtained for further study. All containers were carefully examined for integrity, content discoloration, label-disclosed information, odor, and separation.

2.1. Viable microbial count

Total viable bacterial count was determined for each unit formulation purchased using the following procedure: One gram of the preparation was dispersed in an autoclaved sterilized phosphate buffer containing 0.5% Polysorbate 80 (preservative neutralizer) and then 10-fold serial dilutions were made under aseptic conditions. Pour plate technique was performed on a 1-ml aliquot taken from the appropriate dilution using soya bean casein digest (SBCD) agar. Solidified agar plates were incubated at 37 °C for 48 h before developed colonies were counted. Yeasts were counted as described for bacteria but Sabouraud dextrose agar (SDA) was used. Molds were isolated on Sabouraud dextrose agar and results were considered positive if, after incubation at 25 °C for 2 wk, fungal growth appeared on the inoculated plates. Certain bacteria can grow on SDA; thus the medium was supplemented with antibiotics (chloramphenicol and gentamycin) to inhibit bacterial growth. Colonies recovered were further confirmed as yeast by observing their resistance to antibiotics using the disc diffusion method.

2.2. Enrichment culture and identification of isolates

Aliquots of 1 g of the preparation under investigation were aseptically transferred into a flask containing 50 ml SBCD broth supplemented with 0.5% Polysorbate 80. After incubation at 37 °C for 48 h, a loop full of the flask content was streaked onto SBCD agar (supplemented with 0.5% Polysorbate 80) and plates were then incubated at 37 °C for 48 h. Developed colonies were obtained in pure culture and identified to species level using the diagnostic tables given by Barrow and Feltham (1993). Identification tests included: coagulase, catalase, urease, Gram reaction, shape, gelatin liquefaction, spore formation, sugar fermentation, and other conventional tests. Isolation of Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus was performed as described in ISO technical publications (Anon, 2006a,b,c).

2.3. Preservative efficacy evaluation

Products found to be free of microbial contamination were challenged with P. aeruginosa ATCC 9027 to give a count of 106 CFU g⁻¹ of the respective brand. One gram of the sample was then diluted with 9 ml phosphate buffer amended with 0.5% Polysorbate 80 before a 10-fold serial dilution using similar buffer was made. All dilutions were kept at room temperature for 40 min to allow for neutralization to occur. Recovery of at least 50% of the inoculated bacteria on SBCD agar supplemented with 0.5% Polysorbate 80 indicated the effectiveness of the neutralization procedure.

2.4. Effectiveness of neutralization

Twenty g-aliquots of the products that were found to be free of microbial contamination were challenged with P. aeruginosa ATCC 9027 to give a count of 106 CFU g⁻¹ of the respective brand. One gram of the sample was then diluted with 9 ml phosphate buffer amended with 0.5% Polysorbate 80 before a 10-fold serial dilution using similar buffer was made. All dilutions were kept at room temperature for 40 min to allow for neutralization to occur. Recovery of at least 50% of the inoculated bacteria on SBCD agar supplemented with 0.5% Polysorbate 80 indicated the effectiveness of the neutralization procedure.

3. Results

Purchased items were inspected for physical appearance; all were neat and un-tampered with. A visual check revealed that there was no separation of phases in the case of creams or emulsions nor was there any sedimentation or discoloration in the case of shampoos or the other products. Only five out of 16 shampoo preparations disclosed the type of preservative used in their formulation. Three manufacturers indicated that their products were preserved with cocamidopropyl betaine (a foaming ampholytic surfactant and not a preservative) and the other two were preserved with isothiazolinones. All other preparations in the various product categories either indicated use of parabens or did not specify the name of the preservative.

Results of microbial count in relation to product types are illustrated in Table 1. It is evident from the figures presented that when contamination was detected, microbial count was in most cases well above the acceptable limit. It was calculated that 88% of the contaminated products contained microbial counts in excess of 10⁸ CFU g⁻¹. Table 2 demonstrates types of contaminants in relation to the product category from which they were isolated. P. aeruginosa and Bacillus spp. were the most commonly isolated Gram-negative and positive bacteria, respectively. It is worth noting that coagulase-negative staphylococci and Micrococcus species were only isolated from products that harbored >10⁴ CFU g⁻¹.

<table>
<thead>
<tr>
<th>Product type</th>
<th>Number of brands</th>
<th>No of samples contaminated (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;10⁵</td>
</tr>
<tr>
<td>Shampoo</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Hair conditioner</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Hair styling gel</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Hair groom</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Hair repair emulsion</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Body lotion</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Hand &amp; body cream</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Peeling cream</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total No (%)</td>
<td>57 (100)</td>
<td>3 (5.3)</td>
</tr>
</tbody>
</table>
Testing was performed on three more replicates for each of the brands that were found to be heavily contaminated and those replicates were with different batch numbers than those used in the initial experiment. Further samples included three shampoos, two hair conditioners, and two hair repair emulsions, as well as four hand and body creams. Results showed that these additional samples were again heavily contaminated and observations were very much similar to those given in Table 1; therefore data are not shown.

The challenge test was performed for the products that were not contaminated using *P. aeruginosa* ATCC 9027. Results are summarized in Table 3. This table clearly demonstrates that nine out of 32 formulations of the cosmetic brands that were of acceptable microbiological quality at the point of sale were found to be inadequately preserved. The neutralization procedure of preservatives in the products studied using Polysorbate 80 was effective as it was possible to recover more than 50% of the number of the challenge bacteria used in the test.

4. Discussion

Results of this survey revealed that at the point of sale 56% of the items tested were free of microbial contaminants and 5% harbored less than $10^2$ CFU g\(^{-1}\), giving a total of 61% of the products studied found to be in compliance with specifications. These observations are not far away from published figures, particularly those from the developing countries; Abdelaziz et al. (1989) found that the rate of microbial contamination in shampoo brands marketed in Egypt was 43% but bacterial count in these products was low and pathogens were absent. They also found that only 15% of these commercial brands contained microbial counts in excess of $10^2$ CFU g\(^{-1}\). These results are almost identical to those obtained in this investigation for the shampoo brands, as 18.5% of them were found to be contaminated with high microbial populations.

### Table 2

Types of microbial contaminants isolated from various products category.

<table>
<thead>
<tr>
<th>Product type</th>
<th>Organisms isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shampoo</td>
<td><em>Pseudomonas</em> spp., <em>E. coli</em></td>
</tr>
<tr>
<td>Hair conditioner</td>
<td><em>S. aureus</em>, Yeast, <em>Coag – ve Staphylococci</em>(^a)</td>
</tr>
<tr>
<td>Hair groom</td>
<td><em>Alcaligenes</em> spp., <em>Coag – ve Staphylococci</em>(^a)</td>
</tr>
<tr>
<td>Hair repair emulsion</td>
<td><em>Pseudomonas</em> spp., <em>Bacillus</em> spp.</td>
</tr>
<tr>
<td>Body lotion</td>
<td><em>Bacillus</em> spp., <em>Coag – ve Staphylococci</em>(^a)</td>
</tr>
<tr>
<td>Hand &amp; body cream</td>
<td><em>Bacillus</em> spp., <em>Enterobacter</em> spp., <em>Micrococcus</em> spp.</td>
</tr>
</tbody>
</table>

\(^a\) Coagulase-negative *Staphylococci*.

Okeke and Lamikanra (2001) investigated the microbiological quality of 49 skin-moisturizing creams and lotions distributed in Nigeria. They found that 41% of these products were contaminated and only 16.3% harbored contaminants in excess of $10^3$ CFU g\(^{-1}\) or ml of product examined. In Jordan, Na’was and Alkofahi (1994) tested 19 different brands of topical creams and found that microbial growth was detected in cultures from all products. High counts ($>10^4$ CFU g\(^{-1}\)) were isolated by the same authors from six (33.3%) different brands. These results are comparable with findings reported here, where 28.6% of the cream brands tested revealed high microbial count. Recently, recent reports from Nigeria and India have indicated that the microbiological quality of commercial cosmetics, particularly creams and lotions, have improved slightly over the years (Okeke and Lamikanra, 2001; Gopalakrishna et al., 2010). In developed countries, the prevalence of contaminated cosmetics on sale seems to be relatively uncommon (Campana et al., 2006). However, product recalls from the market in these countries due to microbial contaminations is still a matter of concern (Wong et al., 2000; Lundov and Zachariae, 2008).

Regarding the types of isolated contaminants, the presence of Gram-positive organisms is consistent with reports in the scientific literature (Abdelaziz et al., 1989; Ravita et al., 2009). The interesting finding in this study was that coagulase-negative *Staphylococci* and *Micrococcus* species were recovered only from products that were heavily contaminated and this is probably the first observation reported that draws attention to this phenomenon. It is worth mentioning that this finding was recorded in products of different brand categories, as shown in Table 2, and that these brands were manufactured by various companies. Therefore, this is a generic observation and not related to a single product or firm. The inability to isolate these Gram-positive organisms from cosmetics with low microbial counts or as solo contaminants may suggest that they were unable to grow or even survive in these products without the help of other microorganisms. Although this assumption requires confirmation and perhaps further investigation, it is consistent with the general consensus that Gram-negative bacteria can thrive in industrial products more than Gram-positive ones.

The isolation of Gram-negative bacteria from some of the products is of serious concern. *P. aeruginosa* is an opportunistic pathogen with spoilage potential and *E. coli* is an indicator of fecal pollution. The mere presence of any of these two organisms renders the product unsafe for use. *E. coli* was isolated from one shampoo preparation whereas *P. aeruginosa* was isolated from all contaminated products except the hair conditioners. Most legislative authorities require the product to be free from specified microorganisms such as *E. coli*, *S. aureus*, and *P. aeruginosa*. This specification does not exempt manufacturers from protecting their products against other objectionable microorganisms with possible health risks and deterioration potentials (Sutton, 2006b). It is clear from Table 2 that many brands studied were out of specifications as certain named microorganisms were detected. According to the ISO standards these organisms should be absent. Objectionable microorganisms are product-related and it is the responsibility of the manufacturing companies to establish what constitutes such a flora in a given product.

Likely sources of microbial contaminants in cosmetics at the point of sale are raw materials used in production, personnel, and the environment in which products are manufactured. Water, which is the bulk component in many cosmetic products, has long been described as the most likely source of Gram-negative bacteria, particularly *Pseudomonas* spp. (Olson, 1967). Personnel who may come in contact with the product during production may also contribute to product contamination with gram-positive cocci, including species of *Staphylococci* and *Micrococcus*. It is therefore

### Table 3

Preservative performance (as determined by challenge test) in relation to product category.

<table>
<thead>
<tr>
<th>Product type</th>
<th>Number of items</th>
<th>No. adequately preserved</th>
<th>No. poorly preserved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shampoo</td>
<td>13</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Hair conditioner</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hair styling gel</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hair groom</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hair repair emulsion</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Body lotion</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Hand &amp; body cream</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Peeling cream</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total No (%)</td>
<td>32 (100)</td>
<td>23 (71.9)</td>
<td>9 (28.1)</td>
</tr>
</tbody>
</table>
evident that most contaminants recovered can be controlled if suitable measures are taken by manufacturers to observe hygienic manufacturing conditions and apply quality assurance protocols. It is important to note that detection of Candida species was confined to hair conditioners and hair repair emulsion; these products contained quaternary ammonium compounds. These compounds can exert their conditioning effect only if the preparation is of acidic pH. Thus, the environment in these brands seems to have favored the growth of yeast, but again it should be noted that many other bacteria were also isolated from these products.

The repeated failure of replicates of certain brands to pass the microbiological test indicated that these brands suffer from inherent problems. The persistence of contamination in different batches of the same brand casts doubts about the effectiveness and compatibility of the preservative system with other ingredients used in formulations. Had the right preservatives been used, the products would have been able to inhibit contaminants.

The ISO test for the evaluation of preservative effectiveness requires the use of five different microbial cultures in order to establish the ability of the product to cope with possible microbial challenges that may get into the product during its manufacture or use by consumers. However, only P. aeruginosa was used in the preservation efficacy study described here; results presented in Table 3 showed that nine out of 32 products investigated were poorly preserved. It is probable that, had more than one organism been used, the preservation failure rate in the products investigated would have been higher. The use of one microbial culture in the challenge test should not be considered a drawback to the setup of this investigation, as it was intended to demonstrate that the contamination-free products may also contain items with poor microbiological quality, and this was definitely achieved. These findings, in addition to those given in Tables 1 and 2, suggested that a lot of improvement is needed in the various stages of cosmetics manufacture in Jordan. Special attention should be given to the production environment and the formulation process, including the choice of preservative. This should be established in the development stage as part of the microbiological stability study.

The information disclosed on the labels of the cosmetic containers studied exhibited many irregularities, and these included the names of preservative used in formulation. Therefore, not all attempts to link the rate and extent of contamination to the preservative system used in the products studied were successful. In fact, Hugbo et al. (2003) found that nine of 10 manufacturers of different cosmetic products in Nigeria gave indications of inclusion of a preservative(s) but not the type of preservative used, and only four manufacturers disclosed this information. One company did not even state whether a preservative was included at all. These observations are very similar to those reported herein, and thus the lack of information disclosure seems at present a common problem in developing countries. It is hoped that these irregularities will be rectified once the GMP regulations are enforced.

In conclusion it should be said that the microbiological quality of the brands investigated is consistent with the general quality of cosmetics manufactured and marketed in developing countries. The effect of enforcing GMP regulations in Jordan is believed to result in the production of better quality cosmetics, but this remains to be ascertained. Data presented in this paper can be used as a baseline in the evaluation of this effect.

References

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