Bacteriological Profile and Preservative Capacity of Commercial Creams and Lotions

Summera Rafiq1*, Shameem Iqbal2, Shaik Jasmine Shahina3

1Head & Assistant Professor, Dept of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai-600018, India
2Dept of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai-600018, India
3Assistant Professor, Dept of Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai-600018, India

Abstract

The 15 commercially available cosmetic formulations including creams and lotions were examined in order to verify the degree of possible microbiological contamination. The TVC (Total Viable Count) in unused creams and lotions was ranged between $3 \times 10^4$ to $321 \times 10^5$ CFU/ml. While in used creams it was found to be between $23 \times 10^6$ to $539 \times 10^6$ CFU/ml. The most commonly isolated bacteria were found to be Staphylococcus aureus followed by Staphylococcus epidermidis, Bacillus spp., Klebsiella pneumoniae, E.coli and Pseudomonas aeruginosa. There was reduction of 99% in the number of bacterial counts in 6 cosmetic creams and lotions as per the Cosmetics, Toiletries, and Fragrance Association (CTFA) methods which were done to detect the efficacy of the preservatives in cosmetics. The other 9 samples had reduced bacterial counts on 7th day but this was followed by the rapid increase of bacterial counts on 14th, 21st and 28th days showing that they were inadequately preserved. Of the 15 cosmetic products which were evaluated by Rapid Challenge Test (RCT), 3 products displayed high CFU values of greater than $10^7$ CFU/ml for P. aeruginosa and P. aeruginosa ATCC 27853 after 1st and 7th day in three different dilutions therefore they were considered as high contamination risk products. While the remaining 6 products were observed to have rapid decline in bacterial counts after 1st and 7th day in three different dilutions making them medium–low contamination risk products.

1 Introduction

Cosmetics have become one of the essential needs of an urban lifestyle as they are used extensively by millions of people across the globe with increasing numbers each year1. Most cosmetics contain a lot of ingredients, which are good for microbial growth and the production and the storage of cosmetics temperature is nearly optimal for microbial growth2. Cosmetics products may be contaminated during manufacturing by microorganisms existing in the environment or from the raw materials, which mostly contain water and the later form an appropriate media for microbial growth3. These contaminants could be pathogens, opportunistic pathogens or saprophytes. The consequence of such a contamination may prove to be costly in terms of health and economy4,5. The objectives of the present study were to screen for the presence of pathogenic bacteria from used and unused creams and lotions and evaluating the efficacy of its preservatives.

2 Materials and Methods

A total of 15 commercially available cosmetic, including creams and lotions were examined in order to verify the degree of possible microbiological contamination. A set of creams and lotions were given to healthy volunteers to be used by them, and those creams were evaluated for their bacteriological quality after 14 days. Total viable counts were performed with Modified Latheen Agar (MLA) and Modified Latheen Broth (MLB) respectively. The American Society for Testing Materials (ASTM) is the Standard Test Method for Preservatives in Water Containing Cosmetics has recommended the use of MLA for bacteriological analysis. The Modified Latheen Agar and Modified Latheen Broth contained peptone and beef extract which provide the carbon and nitrogen sources necessary for the growth of the bacteria. The media had bisulfite which enhanced the growth and neutralised the preservatives added in cosmetics4,7,8,9. The pH of the media was maintained by the addition of Sodium
Chloride. It has 5\% Polysorbate which helps to dissolve or emulsify materials with a high oil or lipid content, and it also neutralises the preservatives in cosmetics.

2.1 Total viable Count

TVC was done for all the cosmetics creams and lotions. 1gm of each sample was measured aseptically, and it was serially diluted from $10^{-1}$ to $10^{-6}$ CFU/ml in MLB. 0.1ml from $10^{-4}$, $10^{-5}$ and $10^{-6}$ using spread plate method on to MLA and the plates were incubated at 37 \degree C for 48hrs. The test was performed in duplicates. TVC was done for both used and unused creams and lotions.

2.2 Identification of bacteria

Pathogenic bacteria were isolated by inoculating them onto different media, which includes- Mac Conkey agar, mannitol salt agar, eosin methylene blue agar and cetrimide agar. The plates were incubated at 37 \degree C for 24 to 48 hrs. The bacteria were identified as per the standard biochemical methods.

2.3 CTFA Test (Cosmetics, Toiletries, and Fragrance Association, Inc. 2001)

*S. aureus* and *E. coli* isolated from the cosmetics along with a standard strain of *S. aureus* ATCC 25923 was used to perform CTFA test. Inoculum suspension of these organisms was prepared such that they had $10^{-6}$ CFU/ml. 20gms of each test product was inoculated respectively with 0.1ml of each inoculum suspension. Samples were shaken and maintained at room temperature (RT). 1ml aliquot of test product was added into 9ml of neutralising medium leethen broth after a period of 0, 7, 14, 21 and 28 days (Nostro et al. 2002), and they were inoculated onto MLA using spread plate technique. Uninoculated sample of each preparation served as a control. The product was considered as adequately preserved when 99.9\% reduction of the initial inocula count was obtained on the 7th day of incubation and remained with no increase up to the 28th day of the experiment\textsuperscript{10}.

2.4 Rapid Challenge Test (RCT)\textsuperscript{11}

RCT was performed only for Gram-negative organisms. Twenty grams of the test products were diluted in order to obtain three final concentrations: 100\%, 90\% and 80\% of the product. It was inoculated with 0.1 ml of broth suspension containing $10^{-6}$ (CFU/ml) of Gram-negative bacteria. 1 ml was removed from each concentration of samples after 24 hrs and 7 days from challenge, and the colonies were counted\textsuperscript{12}. The interpretation was done as follows:

i) A product was considered high contamination risk if it displayed more than $10^5$ CFU/ml after 24 hrs from challenge both in the undiluted product and in the diluted product.

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ii) The product was considered a medium–low contamination risk if it showed decrease in the CFU values at the different dilutions after 24 hrs and absence of bacterial growth after 7 days from challenge.

3 Results

A total of 15 commercially available cosmetic, including creams and lotions were examined in order to verify the degree of possible microbiological contamination, and they showed varying degree of contamination. The TVC in unused creams and lotions ranged between $3 \times 10^4$ to $321 \times 10^5$ CFU/ml. While in used creams it was found to be between $23 \times 10^4$ to $539 \times 10^5$ CFU/ml. The most commonly isolated bacteria were found to be *Staphylococcus aureus* followed by *Staphylococcus epidermidis*, Bacillus spp, *Klebsiella pneumoniae*, *E.coli* and *Pseudomonas aeruginosa* (Table 1 and Figure 1).

There was a reduction of 99\% in the number of bacterial counts in 6 cosmetic creams and lotions as per the CTFA methods which were done to detect the efficacy of the preservatives in cosmetics. The other 9 samples had reduced bacterial counts on 7th day, but this was followed by the rapid increase of bacterial counts on 14th, 21st and 28th days showing that they were inadequately preserved.

Of the 15 cosmetic products which were evaluated by RCT, 3 products displayed high CFU values of greater than $10^5$ CFU/ml for *P. aeruginosa* and *P. aeruginosa* ATCC 27853 after 1st and 7th day in three different dilutions, therefore, they were considered as high contamination risk products. While the remaining 6 products were observed to have a rapid decline in bacterial counts after 1st and 7th day in three different dilutions making them medium–low contamination risked products.

4 Discussions

Cosmetics contaminated with pathogenic microorganisms not only cause spoilage of the product but also cause serious health hazards for consumers globally\textsuperscript{13,14}. Therefore it is necessary to adhere to good manufacturing practices (GMP) for better industrial standard of cosmetics.

In the present study, a total of 15 commercially available cosmetic, including creams and lotions were examined. The TVC in unused creams and lotions ranged between $3 \times 10^4$ to $321 \times 10^5$ CFU/ml. While in used creams, it was found to be between $23 \times 10^4$ to $539 \times 10^5$ CFU/ml. These findings were found to be in agreement with the studies of Okeke and Lamikanra (2001)\textsuperscript{15}.

The most commonly isolated bacteria were found to be *Staphylococcus aureus* followed by *Staphylococcus epidermidis*, Bacillus spp, *Klebsiella pneumoniae*, *E. coli* and *Pseudomonas*
Microorganisms are ubiquitous in nature and are common contaminant of many substances including cosmetics. The common ingredients used in preparation of cosmetics include sugar, starch, protein, amino acids, organic acids, alcohols and lipids which favours microbial growth. In addition, water is a fundamental requirement for any microorganisms to multiply and contaminate the cosmetics products; thus untreated or non sterile water can enhance microbial growth leading to contamination of cosmetics products. The 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. Personnel who may come in contact with water during the manufacturing process can also contaminate the products.

Table 1: Prevalence of bacteria in unused and used cosmetic creams and lotions

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Initial Examination in unused creams</th>
<th>Final Examination in used creams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total products tested (n = 15)</td>
<td>Percentage of isolation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total products tested (n = 15)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>8</td>
<td>53%</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>5</td>
<td>33%</td>
</tr>
<tr>
<td>Bacillus spp</td>
<td>11</td>
<td>73%</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>4</td>
<td>27%</td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
<td>33%</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>2</td>
<td>13%</td>
</tr>
</tbody>
</table>
contact with the product during production may also contribute to product contamination with gram-positive cocci, including species of Staphylococcus and Micrococcus.

Good Manufacturing Practices (GMP) has to be followed using suitable and ample preservatives to prevent microbial contamination of cosmetics. Preservatives are chemicals substances that are supplemented to eliminate the growth of microorganisms and to preserve the product. The esters of para-hydroxybenzoic acid (parabens), formaldehyde releasers, isothiazolinones, organic acids and organic alcohols are the commonly used cosmetic preservative agents. Additional to well-known cosmetic preservatives, products also include other antimicrobial components, such as alcohol, chelating agents, phenolic antioxidants, plant-derived essential oils and extracts and fragrance ingredients.

While testing for the efficacy of preservative systems, a combination of methylparaben and propylparaben was found to have good antibacterial effect except against P. aeruginosa due to its evolving multidrug resistance. In the present study, there was a reduction of 99% in the number of bacterial counts in 6 cosmetic creams and lotions as per the CTFA methods which were done to detect the efficacy of the preservatives in cosmetics. The other 9 samples had reduced bacterial counts on 7th day, but this was followed by the rapid increase of bacterial counts on 14th, 21st and 28th days showing that they were inadequately preserved. In RCT evaluation of 15 cosmetic products, 3 products displayed high CFU values of greater than 10^5 CFU/ml for P. aeruginosa and P. aeruginosa ATCC 27853 after 1st and 7th day in three different dilutions, therefore, they were considered as high contamination risk products. While the remaining 6 products were observed to have a rapid decline in bacterial counts after 1st and 7th day in three different dilutions making them medium–low contamination risked products.

5 Conclusions

Cosmetics creams and lotions could be contaminated due to raw materials used and during the manufacturing processes. The most commonly isolated bacteria in the present study were found to be Staphylococcus aureus followed by Staphylococcus epidermidis, Bacillus spp, Klebsiella pneumoniae, E.coli and Pseudomonas aeruginosa. This contamination could be avoided if suitable measures are taken by manufacturers to implement hygienic manufacturing conditions and in applying quality assurance protocols. The efficiencies of the preservative agents in the creams were tested using standard methods, which demonstrated few creams to be contaminated in spite of additives in the creams. There was a reduction of 99% in the number of bacterial counts in 6 cosmetic creams and lotions as per the CTFA methods. Of the 15 cosmetic products which were evaluated by RCT, 3 products were

Rafiq et al. Bacteriological Profile of Commercial Creams and Lotions considered as high contamination risk products. The remaining 6 products were found to be medium–low contamination risked products.

6 Conflicts of interest

None

7 Author's contributions

SR and SJS carried out the literature review, collected the data and drafted the manuscript. SI carried out the bacteriological analysis of creams and lotions. SR and SJS scrutinized the results and data. All authors have read and approved the final manuscript.

8 References


