



PREVALENCE OF MICRO ORGANISMS IN COMMONLY USED COSMETICS SAMPLES IN DHAKA METROPOLIS

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ABSTRACT

Present study attempted to identify and enumerate micro organisms spoiling commonly used cosmetics samples. Among 20 brands of total 6 categories of samples studied (soap, shampoo, lotion, face wash, cream and petroleum), almost all were found to be rigorously contaminated with total viable bacteria within a range of $10^3 - 10^5$ cfu/g. Proliferation of fungal species was observed up to 10^3 cfu/g. Prevalence of *Staphylococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. was observed within a range of 10^1-10^3 cfu/g while actinomycetes were completely absent. Among the enteric bacteria, *Escherichia coli* was found completely absent from all the samples tested, however, the prevalence of *Klebsiella* sp. was noted up to 10^1 cfu/g. Such findings highlighted a great public health risk associated with skin diseases among the users and thereby specified the importance to introduce a proper guideline in maintaining good microbiological quality for such topically used healthcare products.

Keywords: Cosmetics; topical products; microbiological quality

INTRODUCTION

Cosmetics are chemical or natural preparations usually applied to human body part(s) solely with an objective of cleaning, decorating/ beautifying and protecting¹. However, since cosmetics products are basically non-sterile, most of them are prone to microbiological attack¹⁻³. Contamination of cosmetics products by several bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and some Gram negative bacteria is well known^{4,5}. Certain yeasts and molds have also been reported to degrade the microbiological quality of such products⁶. The extent of microbial contamination largely depends on the unhygienic handling of bulk ingredients during manufacturing as well as due to insufficient in-process check and defective storage or distribution⁷⁻¹⁰. ISO or FDA has some guidelines for safety product and scheduled microbiological analysis should be done to reach safety level. The level of contamination in cosmetic products with aerobic bacteria should not exceed the United States Pharmacopoeia (USP) or Food and Drug Administration (FDA) limit (non-eye area <1000 cfu/g) and if the limit exceeded, serious skin problem to the user can be encountered. Several types of diseases including scabies, acne, eczema, dyschromia and other skin diseases have been reported upon usage of cosmetics^{7,11-14}. Therefore, a regular microbiological monitoring during manufacture, packaging, storage of the cosmetics products sold in market is require in order to ensure the public health safety of consumers^{15,16}. Pharmaceutical and cosmetics industries are flourishing in Bangladesh since the last 21 years¹⁷. While the microbiological quality control of pharmaceutical products is quite validated in our country, the study of microbiological profiling of the cosmetics products is still infancy¹⁸⁻²⁰. Along these lines, present study attempted to identify and enumerate bacteria with specific pathogens and the fungal populations

reside in the commonly applied cosmetics products in Dhaka Metropolis.

MATERIALS AND METHODS

Sampling

Twenty brands of 6 categories of cosmetics (3 soap, 4 shampoo, 4 body lotion, 3 face wash, 3 cream and 3 petroleum samples) with appropriate dates of manufacturing and expiry were collected from different health-care stationary shops in Dhaka city during June 2013 - September 2013. All samples were tested to assess the bacterial and fungal load as well the presence of specific pathogenic bacteria and actinomycetes using the standard microbiological and biochemical methods²⁰⁻²².

Enumeration of total viable bacteria and fungal count

An aliquot of 0.1 ml of each suspension from the dilution 10^{-2} was spread onto nutrient agar (NA) plate for enumerating total viable count (TVC) and on Sabouraud dextrose agar (SDA) plate for the estimation of fungal load²⁰⁻²⁵. The plates were incubated at 37 °C for 48 to 72 hours, respectively.

Enumeration of specific pathogens and actinomycetes

From the dilution of 10^{-2} of each sample, 0.1 ml of suspension was spread onto MacConkey agar, mannitol salt agar (MSA), cetrimide agar, phenol red egg yolk polymyxin (MYP) agar base and Bennet agar (supplemented with nystatin) media for the enumeration of *Escherichia coli*, *Staphylococcus* sp., *Pseudomonas* sp., *Bacillus* sp. and actinomycetes, consecutively. All the plates were incubated at 37 °C for 24 hours. Presence of *E. coli* was further confirmed by the appearance of bluish-black colonies with green metallic sheen on the eosine-methylene blue (EMB) agar^{23,24}. Confirmative biochemical tests were carried out for the final identification of the isolates²⁰⁻²³.

Table 1: Prevalence of Pathogenic Microorganisms in Different types of Cosmetics (cfu/g)

| Sample | TVB (cfu/g) | Total fungal count | <i>E. coli</i> (cfu/g) | <i>Klebsiella</i> sp. (cfu/g) | <i>Staphylococcus</i> sp. (cfu/g) | <i>Bacillus</i> sp. | <i>Actinomycetes</i> | <i>Pseudomonas</i> Sp. |
|--------------------|--------------------|--------------------|------------------------|-------------------------------|-----------------------------------|---------------------|----------------------|------------------------|
| Soap | | | | | | | | |
| Lux | 2.3×10^5 | 2.1×10^3 | 0 | 5.4×10^1 | 2.6×10^1 | 0 | 0 | 1.6×10^1 |
| Dettol | 2.1×10^3 | 3.2×10^2 | 0 | 4.6×10^1 | 7.6×10^1 | 0 | 0 | 0 |
| Wheel (Detergent) | 2.2×10^4 | 4.5×10^2 | 0 | 5.6×10^1 | 1.6×10^1 | 0 | 0 | 6.1×10^1 |
| Shampoo | | | | | | | | |
| Dove | 1.1×10^3 | 7.7×10^2 | 0 | 2.4×10^1 | 6.2×10^1 | 0 | 0 | 0 |
| Head and Shoulder | 2.8×10^4 | 2.5×10^2 | 0 | 0 | 1.0×10^2 | 0 | 0 | 0 |
| Sunsilk | 1.8×10^5 | 2.2×10^2 | 0 | 1.8×10^1 | 7.2×10^2 | 0 | 0 | 3.3×10^1 |
| All Clear | 7.4×10^3 | 7.2×10^2 | 0 | 1.6×10^1 | 9.68×10^2 | 0 | 0 | 1.5×10^1 |
| Body lotion | | | | | | | | |
| Johnson | 1.8×10^3 | 5.0×10^2 | 0 | 1.5×10^1 | 2.7×10^1 | 1.0×10^1 | 0 | 1.2×10^1 |
| Meril | 5.5×10^3 | 5.7×10^2 | 0 | 0 | 1.4×10^1 | 5.5×10^1 | 0 | 2.5×10^2 |
| Nevia | 2.6×10^3 | 5.1×10^1 | 0 | 1.5×10^1 | 6.7×10^1 | 2.0×10^1 | 0 | 3.2×10^1 |
| Ponds | 1.9×10^3 | 3.5×10^2 | 0 | 2.5×10^1 | 2.7×10^2 | 5.3×10^1 | 0 | 7.9×10^2 |
| Face wash | | | | | | | | |
| Garnier | 6.91×10^3 | 2.50×10^2 | 0 | 0 | 0 | 2.5×10^1 | 0 | 0 |
| Johnson | 1.10×10^4 | 4.69×10^2 | 0 | 0 | 2.56×10^2 | 2.00×10^2 | 0 | 2.96×10^2 |
| Ponds | 1.40×10^5 | 1.06×10^2 | 0 | 2.00×10^1 | 5.14×10^2 | 5.00×10^2 | 0 | 8.26×10^2 |
| Cream | | | | | | | | |
| FairandLovely | 5.95×10^5 | 0 | 0 | 5.00×10^1 | 5.20×10^2 | 4.50×10^2 | 0 | 1.50×10^2 |
| Johnson | 8.28×10^3 | 0 | | 1.50×10^1 | 2.00×10^1 | 3.00×10^2 | 0 | 1.65×10^1 |
| Ponds | 8.94×10^4 | 2.24×10^2 | 0 | 1.29×10^1 | 5.30×10^2 | 2.00×10^2 | 0 | 0 |
| Petroleum | | | | | | | | |
| Vaseline | 1.5×10^3 | 3.0×10^1 | 0 | 0 | 1.8×10^2 | 0 | 0 | 0 |
| Meril | 1.3×10^5 | 1.5×10^2 | 0 | 0 | 2.5×10^2 | 5.5×10^2 | 0 | 1.2×10^2 |
| Tibet | 1.2×10^4 | 1.6×10^2 | 0 | 0 | 5.4×10^3 | 0 | 0 | 3.0×10^1 |

TVB total viable bacteria

Table 2: Confirmative biochemical tests for the isolates

| Assumed Organism | TSI | | | H ₂ S reaction | Indole test | MR test | VP test | Citrate test | Motility | Oxidase test |
|---------------------------|-------|------|-----|---------------------------|-------------|---------|---------|--------------|----------|--------------|
| | slant | Butt | gas | | | | | | | |
| <i>Klebsiella</i> sp. | Y | Y | - | - | - | - | - | - | - | + |
| <i>Pseudomonas</i> sp. | A | A | - | - | - | - | - | + | - | - |
| <i>Staphylococcus</i> sp. | Y | R | + | + | - | + | - | + | + | - |
| <i>Bacillus</i> sp. | R | Y | - | - | - | - | + | - | - | - |

TSI Triple Sugar Iron Test, Y Yellow (Acid), R Red (Alkaline), MR Methyl red, VP Voges-Proskauer

RESULTS AND DISCUSSION

Cosmetics are not supposed to be sterile as they contain nutrients which support the growth of variety of microorganisms²⁶⁻²⁷. However, cosmetics products must have to be free from pathogens and total aerobic bacterial load should be low which could not impair skin and mucous membrane defense mechanisms²⁶. As the occurrence of skin diseases is much more frequent in developing countries due to the unhygienic dense environment, improper sanitation, and the usage of microbiologically contaminated water²⁸⁻³⁰, maintenance of good microbiological quality in cosmetic products is important. It is therefore necessary to carry out microbiological analysis of the raw materials and final products of cosmetics for obtaining products with good microbiological quality. Present study was attempted to analyze various cosmetic products available in Bangladesh for estimating the actual scenario. Of the 6 categories of samples studied, all the samples exhibited higher load of total viable bacteria up to 10^5 cfu/g (Table 1). The fungal load was estimated within the range of $10^1 - 10^3$ cfu/g, while in two brands of cream samples, no fungal population was observed (Table 1). Among the pathogenic bacteria which were biochemically identified (Tables 1 and 2), *Staphylococcus* spp. were found to be present in almost all samples except Garnier face wash in the average of 10^2 cfu/g. *Bacillus* sp.,

which were absent in soap and shampoo samples, were detected in other samples within a range of 10^1 to 10^2 cfu/g. *Klebsiella* sp. and *Pseudomonas* sp. were encountered in 70 % of the samples. *E. coli* and actinomycetes were totally absent in all the samples (Table 1). Total bacterial and pathogenic bacterial count were found to be higher in present study than those found previously^{1,5,36}. As stated earlier, Raw materials, unhygienic handling and environmental condition may responsible for high growth in products^{20,31-35}. Some chemicals such as lipid, polysaccharide, protein, alcohol, glucoside etc. of cosmetics, storage temperature, product pH, availability of O₂ and poor activity of preservatives also can facilitate the growth of microbes^{27,36}. Presence of *Bacillus* sp. might responsible for unpleasant smell and spoilage of cosmetics products^{6,37}.

CONCLUSION

With a previous throughput on the high prevalence of contaminating microorganisms in topical products sold in Dhaka Metropolis, current study further unveiled a huge number of microorganisms in the commonly used cosmetics products. A significant number of total viable bacteria and fungi brought suggestive evidence on the detrimental impact on public health of using such products. The regulatory bodies controlling the operation as well as the distribution of

these products among the health-care stores should strictly deal with microbiological maintenance during manufacturing, packaging and storage of the cosmetics products. Presented data sufficiently indicates such urgency not only in local perspective but also for the other developing countries where skin- and other superficial diseases are not unlikely due to the usage of a variety of cosmetics. More cautions must be imposed on the maintenance of hygienic manufacturing condition, proper handling of all ingredients and finally on their storage and distribution among the consumers.

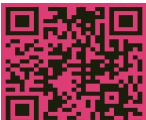
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