

Microbial Analysis of Selected Brands of Whitening Creams

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Abstract

Background: Whitening creams are most commonly used by both men and women for beautifying and cleaning. These creams are not commonly sterile and contaminated during different stages of formulation from manufacturing to consumer. *Pseudomonas aeruginosa*, some gram negative bacteria and *Staphylococcus aureus* are involved in the contamination of cosmetic products. The use of microbial contaminated whitening cream may become health hazard to consumers. **Objective:** Microbial analysis of selected brands of whitening creams commonly used by the community of Pakistan. **Methods:** Four well-known brands of whitening creams were chosen for microbiological analysis and assigned coding as W-A, W-B, W-C and W-D respectively. Conventional culture were used for microbial analyze. Differential & Selective Media used for Identification of Microorganism and further confirmation of microbes were subjected by biochemical tests. **Results:** All four brands were observed contaminated with bacteria up to 10^6 CFU/g that finally more than the U.S.P. or F.D.A. recommended limit of less than 10^3 CFU/g for non-eye area. Growth of *Klebsiella* sp., *Staphylococcus* sp., and *Pseudomonas* sp was noted as well. Fungal population was not observed in any brand of whitening creams. *Escherichia coli* and *actinomycetes* were not present in all the samples. *Klebsiella* sp. and *Pseudomonas* sp. were detected in all brands within a range of 1. **Conclusions:** According to current microbial analysis there is higher load of total viable microbes in all samples. These contaminated products are health hazards to consumers. There should be checking during fabrication, packaging and storing of the cosmeceuticals brands by the regulatory bodies and intensive care the operation as well as the supply of cosmetics products among the health-care stores.

Keywords: Microbial Analysis, Whitening creams, Health Hazards, Skin Infections, Bacteria.

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INTRODUCTION

The use of whitening creams by both men and women are common old practice globally since from 16th century [1]. The use of whitening cream is associated with beauty in Asian countries like Korea, Japan, India China Bangladesh and Pakistan while in Africa people use it for various purposes including economic motivations, beautiful and psychological [2-6]. Skin lightening agents are also utilized for anti-aging and skin-fairness cosmetics products and gaining importance clinically and cosmetically [7]. Whitening agents consist of corticosteroids mercury-salts hydroquinone, corticosteroids lighten skin by inhibit the process of melanocyte maturation [8].

Pharmaceutical products prone to microbial contamination during manufacturing, packaging and storage conditions particularly in developing countries [9-13]. Cosmetic products, used mainly for cleaning and beautifying and protecting [13], may also undergo contamination by a variety of microbes during manufacturing process and storage same as pharmaceuticals products [14, 15].

Undeniably, in addition to pharmaceutical health care medicinal products, Cosmetics items are susceptible to microbial attack since these products are non-sterile [16,17,15] Cosmetics products are contaminated by several bacteria like *Pseudomonas aeruginosa*, some gram-negative bacteria and *Staphylococcus aureus* [16,17]. Quality of cosmetics products are also compromised by certain yeasts and molds [17].

The degree of microbial Spoilage mainly due to the unsanitary handle of bulk raw material during process of production and due to inadequate in process check and substandard storing or delivery [18-20]. The utilization of microbial contaminated cosmetics products may cause a health risk among consumers as obvious from one of the earliest and most dramatic incidences of microbial spoilage of a cosmetic. In New Zealand, the death of four babies was endorsed to the consumers of talcum powder mixed with *Clostridium tetuni* in 1946 [21], *Klebsiella pneumoniae* observed to infect hand creams causing Septicaemia in 1967[22], contamination of eye cosmetics *P. aeruginosa* [23] and finally in 1983, *Pseudomonas stutzeri* contaminate aqueous soap. After that microbiological experiments reveal that this contamination is due to unhygienic handling of ingredients during manufacturing [24-26]. In many under developed countries microbiological contamination of cosmetics have been largely reported [10, 14]. like pharmaceutical the reports on quality on cosmetics are not available[27, 28, 25].

Different cosmetics products like face washes, creams, whitening cream and soaps, all are found contaminated with bacteria and fungi. However, certain limit recommended by FDA and BP/USP for microorganism. To maintain safety of cosmetics product regulatory bodies including FDA and USP has guidelines for acceptable limits of microorganisms for microbiological analysis. According to these guidelines the degree of contamination in cosmetics brands with pathogenic bacteria should not go above the FDA and limit the United States Pharmacopoeia (USP) limit (non-eye area less than 1000 cfu/g and for eye area less than 10^2 cfu/g) otherwise there are chances of many skin problems. Eczema, acne, dyschromia and scabies are mainly skin diseases have been reported by topical application of cosmetics exceeding microbial bioburden limits [29]. In order to ensure the quality of cosmetics products as well as public health protection of consumers there should be reliable and consistent microbial monitoring throughout fabrication, covering, storing of the cosmeceuticals [30,31].

The topical use of skin whitening creams has been increased in last few decades. Most of the cosmetics are not sterile and they are made of non-sterile raw material. So, there is microbial content in Whitening creams which may cause contamination of products and responsible many serious health risks for consumers. Although, preservatives are added to products to control microbial growth in cosmetics products but no expiry date mention on mostly cosmetics products. So, they lose preservative activity and become risk for consumers. The objectives of this research were to ascertain the incidence of the microbial contamination of different skin fairing creams brands. The magnitude and type of microbial contaminants were also determined

MATERIALS AND METHODS

Sample's preparation and dilutions

4 leading brands of whitening creams were included in this study. These are most commonly used by people. All samples were obtained from famous selling outlet in Lahore Market with appropriate dates of manufacturing and expiry date, in January 2019. According to USP Chapter 61 Under "Microbial enumeration tests" sample prepared. For dilution Phosphate buffer saline (BPS) was used. 1g of sample was mixed with sterile Tween 80 which is not more than of its weight and warm at temperature which is not more than 40°C. Temperature sustained using water bath. 1:10, 1:100 and 1:1000 dilution of product was prepared by adding sufficient amount of pre-warmed Phosphate buffer saline.

Microbial Enumeration

Microbial cultures from sample were culture on nutrient agar plates and potato dextrose agar plate for enumerating total viable count and fungal's load estimation respectively and incubate for 24 hours at 37°C[28].

For specific pathogen and actinomycetes for enumeration of *Pseudomonas* sp., actinomycetes, *Escherichia coli*, *Staphylococcus* sp., and *Bacillus* sp. Suspension was spread on cetrimide agar, Bennet agar, MacConkey agar, mannitol agar and Phenol red agar respectively. Eosine-methylene blue (EMB) agar was used for confirmation of *E. Coli* by observation of dark center and green metallic sheen [32]. Biochemical testing was used for further identification [28].

RESULT AND DISCUSSION

Cosmetics contain favorable nutrients which support the growth of microorganism that's why they are not supposed to be sterile [18]. The prevalence of skin disease due to inadequate sanitation, the consumption if microbiological contaminated water and unhygienic environment is common in developing countries[24].

Therefore, cosmetic products should be free from pathogen and total aerobic bacterial load should be below limits as they are unable to cause any skin infections[33]. Maintenance of good quality of cosmetics product is important. To avoid the chance of skin infection microbiological analysis of finishing product is necessary to achieve good quality product. In present study different whitening cream brands commonly used by people of Pakistan were analyzed for microbial contamination for assessing the actual situation. 4 brands of local and multinational whitening creams were selected that are most commonly used by people for facial whitening.

All the brands especially locally manufactured showed higher concentration of total viable bacteria up to 10^6 cfu/g which ultimately cross the USP or FDA limit of $< 10^3$ cfu/g for non-eye area. Among the pathogenic bacteria which were confirmed by biochemically test Staphylococcus specie were present in all sample. E. coli and actinomycetes were completely absent in all the samples. Klebsiella specie and Pseudomonas sp. were detected in all brands within a range of 10^1 cfu/g. No fungal population was detected in any whitening cream sample (Table4.1).

Total bacteria and pathogenic bacteria were found higher in local brands as compared to multinational brand. It may be due to environmental condition and unhygienic handling and raw material which are responsible for microbial growth [34, 14, 35]. Sometimes storage temperature, poor activity of preservative, PH of product, availability of oxygen and chemicals such as lipids, alcohol, polysaccharides, glucoside etc. also responsible for microbial growth (Commonly and Dhaka) [36, 37].

Table-3-1: Population of Pathogenic Microorganisms in various brands of Whitening Creams (cfu/g)

Sample	TVB (cfu/g)	Pseudomonas Specie (cfu/g)	Klebshialla specie (cfu/g)	Staphylococcus specie (cfu/g)	E.coli (cfu/g)	Bacillus specie (cfu/g)	Actinomycetes (cfu/g)	Total fungal count
Tibbet	5×10^6	3.5×10^1	5.29×10^1	8.25×10^1	0.00	5.0×10^1	0.00	0.00
Ponds	1.4×10^4	2.2×10^1	1.00×10^1	1.2×10^1	0.00	4.3×10^1	0.00	0.00
Fair & lovely	6.3×10^5	3.7×10^1	1.25×10^1	4.2×10^1	0.00	5.3×10^1	0.00	0.00
Handsome	8.2×10^5	2.9×10^1	4.29×10^1	3.25×10^1	0.00	3.2×10^1	0.00	0.00

Table-3-2: Confirmatory biochemical tests for the isolation of Pathogenic Bacteria

Supposed Organisms	Indole Test	Citrate Test	H ₂ S	Motility Test	Oxidase test
Staphylococcus specie	=	+	+	+	=
Klebsiella specie	=	=	=	=	+
Pseudomonas specie	=	+	=	=	=
Bacillus specie	=	=	=	=	=

CONCLUSION

Current study showing a higher load of microorganisms in different commonly used whitening creams. The higher population of total viable bacteria brought indicative mark on the detrimental effect on consumer health of using such non sterile cosmetics and strong evidence for further research on other cosmetics products to reduce the burden of skin diseases. Many People use whitening creams unaware of potential harms due to contamination of such products. There is chance of microbial contamination and facial skin infections. So, there should be necessary regular training for manufacturing companies and improved Good Manufacturing Practice.

There should be monitoring during manufacturing, packing and storing of the cosmeceuticals by the governing bodies, monitoring the procedure and the delivery of cosmetic products among the health-care stores. Present data significantly presenting indications not only in Pakistan but also in developing countries where such non-sterile cosmetic products are commonly used. More attentions should be enforced on the maintenance of sterile manufacturing situation, appropriate management of all raw materials and lastly, their storing and delivery to health-care stores.

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