

Microbiological Quality Assessment of Some Brands of Cosmetic Creams Sold Within Alkhoms City, Libya

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Abstract: Creams are external preparations, are both liable to microbial contaminations either in the course of their preparation, transportation and/or accidentally, during use by the consumers which may lead to their spoilage. A total of sixty three (63) samples representing three different brands of cosmetic creams. Concerning skin moisturizing creams, samples included 18 containers of skin moisturizing creams, 27 containers of foundation creams and 18 containers of bleaching creams were subjected to microbiological examination. *Pseudomonas aeruginosae* and *Staphylococcus epidermidis* were recovered from 33.3 % and 55.5% of the tested Novo face cream respectively. However 22.2% of Nivea soft cream samples were contaminated with *Staphylococcus epidermidis*. No coliform bacteria were recovered from all samples under test. Mould (*Alternaria* spp.) were isolated from only one sample of Novo cream (11.1%) and 11.1% of Nivea soft cream was contaminated with yeast (*Candida* spp.). A total of 27 samples representing three different brands of foundation creams were tested for their total aerobic bacterial, coliform and fungal counts. *Staphylococcus aureus* was isolated from Jadore, Max-touch and Helena foundation creams at rates of 33.3%, 33.3% and 66.6% respectively. Two types of moulds were isolated from the tested foundation creams; which are *Rhizopus* spp. and *Aspergillus* spp. From 33.3% of Helena foundation cream tested samples *Rhizopus* species were isolated; while *Aspergillus* spp. detected in 22.8% of Max-touch foundation cream. All tested samples of Jadore foundation cream were free from mould contamination. Yeasts were absent in all foundation cream tested samples. A total of 18 samples representing two brands of bleaching creams were tested for their total aerobic bacterial, coliform and fungal counts. The isolated bacterial species from the contaminated sample were *Bacillus subtilis*. No viable aerobic bacteria were recovered from 61.1% of the whole tested samples. Also there is no coliforms or yeasts. Concerning fungal contamination, 5.5% of the inspected samples contain 10,000 CFU/ g. (*Penicillium* spp.), while 33.3% had fungal count less than 1000 CFU/ g. (*Fusarium* spp.). All creams samples were also examined for anaerobic bacteria and the results revealed that, *Clostridium perfringens* was detected in 11.1% of the tested cosmetic creams. Only one sample of tested face cream of Nivea soft cream brand, one sample of Jadore foundation cream brand, one sample of Fair and Lovely bleaching cream brand and one sample of tested Shirley bleaching cream brand were found to contain *Clostridium perfringens* at rates of more than 1000 CFU/ g of the positive samples, except in case of Shirley bleaching cream brand where the rate was less than 1000 CFU/g. All other tested samples were free from anaerobic bacterial contamination.

Conclusion and Recommendation: Commercial cosmetic creams evaluated did not generally meet the standards for microbial limits as specified in official monographs. Such products can adversely affect health status of consumers as well as the stability profiles of the products. Therefore appropriate control of the many factors involved in the microbiology of the products is critical. These factors include raw material quality, hygiene and training of manufacturing personal, establishment of sanitary design and materials, application of validated cleaning and sanitization process design and control, application of general chemical/physical factors including heat, time temperature, pH, addition of specific chemical preservation and use of appropriate barrier packaging. All of these factors are effective for the control of microbiological risks in the cosmetic creams.

I. Introduction

The field of cosmetics and microbiology had not come into contact much before the 1930s and cosmetic microbiology became more important in 1940s⁽¹⁾. The first contamination of cosmetics was reported in 1946 by several cases of neonatal death from talcum powder containing *Clostridium tetani*⁽²⁾. Since 1960s, opportunist organisms, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Serratia* sp. and *Enterobacter* sp., have been isolated from cosmetic products to a certain extent⁽³⁾. Creams are external preparations, are both liable to microbial contaminations either in the course of their preparation, transportation and/or accidentally, during use by the consumers which may lead to their spoilage. Product contamination may arise from raw materials or water used in formulation. This spoilage may lead to alteration in organoleptic properties of creams which may manifest in terms of changes in color, odor and/or taste; as well as

biodegradation of active constituent of such creams. However, spoilage may result in loss in term of cost on the part of manufacturer and infection on the part of consumers of such spoilt products. The warm and rather humid climatic conditions that prevail in most tropical countries, including Libya, would tend to support the survival and growth of many microorganisms. In a situation whereby a nutritionally rich pharmaceutical/cosmetic product is severely contaminated, rapid growth and multiplication would be expected. This could lead to biodegradation of the product and hence the risk of infection to consumers of the product ⁽⁴⁾.

The objective of this study: is to assess the microbial quality of some selected brands of commonly used cosmetic creams with different dates of production in Alkhoms markets and to recommend the possibility of some health risk to consumers.

II. Materials And Methods

Sample collection:

A total of sixty three (63) samples representing three different brands of cosmetic creams. Samples included 18 containers of skin moisturizing creams, 27 containers of foundation creams and 18 containers of bleaching creams. Examined samples were selected at random from unbroken containers obtained from different stores located in Alkhoms city, Libya. All tested items were having manufacture or expiration dates and the batch numbers were recorded.

Media used:

Nutrient agar, Blood agar and Mannitol salt agar, Peptone water and Tryptone yeast extract agar were used in the isolation and of determination of the bacterial load of the sample. Sabouraud dextrose agar was used for the isolation and enumeration of yeasts and molds. The media were reconstituted and sterilized according to the direction of the manufacturer. (all obtained from Oxoid).

Bacteriological counts of the Cosmetic Powders:

In order to assess the degree of contamination, 1g of material was dispersed in 4 ml sterile Ringer solution containing 0.25% tween 80. Appropriate dilutions were made in the same dispersing vehicle and 0.5 ml was plated out on the appropriate solid medium using the surface viable method. All the plates were incubated at 37 °C for 24-48 hours. Emergent colonies were counted after the necessary incubation. All operations were carried out in duplicates. Results were expressed as colony forming unit per gram (CFU/g).

Yeasts and Moulds Count of the Cosmetic Powders:

One ml of the last two dilutions mentioned in prepared above were inoculated on Sabouraud dextrose agar plates using spread plate method. The plates were then incubated at 25 °C for 2-3 days. Colonies were counted after three days. Results of colony count was expressed as yeasts and moulds counts per gram.

Identification of Bacterial Isolates:

All bacterial isolates were identified based on their Gram reaction and biochemical tests, as described by U.S.FDA manual online ⁽⁵⁾.

Identification of fungal Isolates:

All fungal isolates were identified based on their macroscopic and microscopical appearance with reference to standard manual ⁽⁶⁾.

III. Results

Microbial contents of different batches and containers of cosmetic creams:

a) 1-Microbial contents of different batches and containers of skin moisturizing creams:

A total of 18 samples representing two brands of face creams were tested for their total aerobic bacterial, coliform and fungal counts. The samples were also qualitatively examined for the presence of some potential pathogens. The results are summarized in tables (1&2). It has been found that 22.2% of the tested samples contained up to 100 CFU of aerobic bacteria/g., while 16.7 % had a bacterial count ranged from 100-1000 CFU/ g. Also 16.7% of the tested samples were heavily contaminated (contained more than 1000 CFU/ g. The remainder 44.4% of the tested face cream samples was free from any viable bacteria.

Pseudomonas aeruginosae and *Staphylococcus epidermidis* were recovered from 33.3 % and 55.5% of the tested Novo face cream respectively. However 22.2% of Nivea soft cream samples were contaminated with *Staphylococcus epidermidis*. No coliform bacteria were recovered from all samples under test.

On the other hand, 11.1% of those samples were contaminated with 100 CFU/g of fungi. The other 88.9% of these face creams have no fungal contamination.

Mould (*Alternaria* spp.) were isolated from only one sample of Novo cream (11.1%) and 11.1% of Nivea soft cream was contaminated with yeast (*Candida* spp.) Tables (-).

b) Microbial contents of different batches and containers of foundation creams:

A total of 27 samples representing three different brands of foundation creams were tested for their total aerobic bacterial, coliform and fungal counts. The samples were also qualitatively examined for the presence of some potential pathogens. The results are summarized in tables (3, 4 & 5).

The results showed that, 11.1% of the tested samples contained up to 100 CFU of aerobic bacteria/g. Also a similar percentage of foundation cream samples contained aerobic bacterial count ranged from 100 to 1000 CFU/g., while 22.2 % were heavily contaminated (contained more than 10000 CFU/ g.). Viable aerobic bacterial count were not recovered from 55.5% of the tested foundation cream samples. All samples tested gave zero coliform count.

Staphylococcus aureus was isolated from Jadore, Max-touch and Helena foundation creams at rates of 33.3%, 33.3% and 66.6% respectively.

Two types of moulds were isolated from the tested foundation creams; which are *Rhizopus* spp. and *Aspergillus* spp. From 33.3% of Helena foundation cream tested samples *Rhizopus* spp. were isolated; while *Aspergillus* spp. detected in 22.8% of Max-touch foundation cream. All tested samples of Jadore foundation cream were free from mould contamination. Yeasts were absent in all foundation cream tested samples.

c) Microbial contents of different batches and containers of bleaching creams:

A total of 18 samples representing two brands of bleaching creams were tested for their total aerobic bacterial, coliform and fungal counts. The samples were also qualitatively examined for the presence of some potential pathogens. The results are summarized in tables (6&7).

The results revealed that, 16.6% of the examined samples contained less than 1000 CFU of aerobic bacteria/g, while 16.6% of samples under examination contained aerobic bacterial count ranged from 1000 to 10000 CFU/g. Furthermore, 5.5 % were heavily contaminated (contained more than 10000 CFU/ g.). The isolated bacterial species from the contaminated sample were *Bacillus subtilis*. No viable aerobic bacteria were recovered from 61.1% of the whole tested samples. Also there is no coliforms or yeasts.

Concerning fungal contamination, 5.5% of the inspected samples contain 10,000 CFU/ g. (*Penicillium* spp.), while 33.3% had fungal count less than 1000 CFU/ g. (*Fussarium* spp.).

d) Anaerobic microbial contents of different batches and containers of cosmetic creams:

Clostridium perfringens was detected in 11.1% of the tested cosmetic creams. Only one sample of tested face cream of Nivea soft cream brand, one sample of Jadore foundation cream brand, one sample of Fair and Lovely bleaching cream brand and one sample of tested Shirley bleaching cream brand were found to contain *Clostridium perfringens* at rates of more than 1000 CFU/ g of the positive samples, except in case of Shirley bleaching cream brand where the rate was less than 1000 CFU/g. All other tested samples were free from anaerobic bacterial contamination (Table 8).

Table (1): Aerobic microbial contents of different batches and containers of Novo face cream.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	2.0 X 10 ³	<i>P. aeruginosae</i>	1.0 X 10 ²	<i>Candida</i> spp	-
	2	2.0 X 10 ³	<i>P. aeruginosae</i>	0	-	-
	3	5.0 X 10 ²	<i>P. aeruginosae</i>	0	-	-
B	1	8.0 X 10 ²	<i>S. epidermidis</i>	0	-	-
	2	4.0 X 10 ²	<i>S. epidermidis</i>	0	-	-
	3	1.0 X 10 ³	<i>S. epidermidis</i>	0	-	-
C	1	2.0 X 10	<i>S. epidermidis</i>	0	-	-
	2	0	-	0	-	-
	3	1.0 X 10 ²	<i>S. epidermidis</i>	0	-	-

- 1- *P. aeruginosae* = *Pseudomonas aeruginosae*.
- 2- *S. epidermidis* = *Staphylococcus epidermidis*
- 3- No coliform detected.

Table (2): Aerobic microbial contents of different batches and containers of Nivea Soft face cream.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	1.0 X 10 ²	S. epidermidis	1.0 X 10 ²	-	Alternaria spp.
	2	1.0 X 10 ²	S. epidermidis	0	-	-
	3	0	-	0	-	-
B	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-

- 1- S. epidermidis = Staphylococcus epidermidis
- 2- No coliform detected.

Table (3): Aerobic microbial contents of different batches and containers of Max-Touch foundation cream.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
B	1	1.0 X 10 ²	S. epidermidis	3.0 X 10 ³	-	Asperg. spp.
	2	1.0 X 10 ²	S. epidermidis	2.0 X 10 ³	-	Asperg. spp.
	3	5.0 X 10	S. epidermidis	0	-	-
C	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-

- 1- S. epidermidis = Staphylococcus epidermidis
- 2- Asperg. spp. = Aspergillus. spp.
- 3- No coliform detected.

Table (4): Aerobic microbial contents of different batches and containers of Helena foundation cream.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	6.0 X 10 ³	S. epidermidis	1.0 X 10 ²	-	Rhizopus spp.
	2	5.0 X 10 ³	S. epidermidis	1.0 X 10 ²	-	Rhizopus spp.
	3	8.0 X 10 ³	S. epidermidis	1.0 X 10 ²	-	Rhizopus spp.
B	1	3.0 X 10 ³	S. epidermidis	0	-	-
	2	3.0 X 10 ³	S. epidermidis	0	-	-
	3	4.0 X 10 ³	S. epidermidis	0	-	-
C	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-

- 1- S. epidermidis = Staphylococcus epidermidis
- 2- No coliform detected.
- 3-

Table (5) Aerobic microbial contents of different batches and containers of Jadore foundation cream.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	3.0 X 10 ²	S. epidermidis	0	-	-
	2	4.0 X 10 ²	S. epidermidis	0	-	-
	3	5.0 X 10 ²	S. epidermidis	0	-	-
B	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-

- 1- S. epidermidis = Staphylococcus epidermidis
- 2- No coliform detected.

Table (6) Aerobic microbial contents of different batches and containers of Fair & Lovely bleaching cream.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	4.0 X 10 ²	Bacillus subtilis	3.0 X 10	-	Fusarium spp.
	2	8.0 X 10 ²	Bacillus subtilis	6.0 X 10	-	Fusarium spp.
	3	3.0 X 10 ²	Bacillus subtilis	7.0 X 10	-	Fusarium spp.
B	1	2.0 X 10 ⁴	Bacillus subtilis	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	0	-	4.0 X 10 ²	-	Fusarium spp.
	2	0	-	5.0 X 10 ²	-	Fusarium spp.
	3	0	-	5.0 X 10 ²	-	Fusarium spp.

1- No coliform detected.

Table (7) Aerobic microbial contents of different batches and containers of Shirley bleaching cream.

Batch	Serial No.	Aerobic bacteria		Fungi		
		Total count/g.	Isolated microorganism	Total count/g.	Yeasts	Moulds
A	1	4.0 X 10 ³	Bacillus subtilis	1.0 X 10 ⁴	-	Penici. spp.
	2	7.0 X 10 ³	Bacillus subtilis	0	-	-
	3	6.0 X 10 ³	Bacillus subtilis	0	-	-
B	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-
C	1	0	-	0	-	-
	2	0	-	0	-	-
	3	0	-	0	-	-

1- Penici. spp. =Penicillium spp.

2- No coliform detected.

Table (8) : Anaerobic microbial contents of different batches and containers of cosmetic creams.

Batch	Serial No.	Anaerobic microbial contents in							
		Nivea soft cream		Jadore foundation cream		Fair and Lovely bleaching cream		Shirley bleaching cream	
		Total count/g.	Isolated M.O	Total count/g.	Isolated M.O	Total count/g.	Isolated M.O	Total count/g.	Isolated M.O
A	1	4 X 10 ³	C. perf.	0	-	0	-	0	-
	2	0	-	0	-	0	-	0	-
	3	0	-	0	-	0	-	0	-
B	1	0	-	3 X 10 ³	C. perf.	0	-	0	-
	2	0	-	0	-	0	-	0	-
	3	0	-	0	-	0	-	0	-
C	1	0	-	0	-	3 X 10 ³	C. perf.	0	-
	2	0	-	0	-	0	-	0	-
	3	0	-	0	-	0	-	3 X 10 ²	C. perf.

N.B: C. perf.=Clostridium perfringens.

IV. Discussion

Microbial contamination, from manufacturer to consumer, can be controlled by sanitary processing and using appropriate and adequate preservatives. According to European Union (EU) legislation cosmetic products must not contain more than 1,000 CFU/g cream and Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans must not be detectable in 0.5 g of the product. Results obtained in the current study approximating the results of the mentioned studies. Peter et. al., reported that the fungal contaminants of cosmetics consisted largely of Aspergillus fumigatus, Penicillium and Microsporium spp. (7 & 8). The present study showed that results of cosmetic creams were in agreement with those of .Abdelaziz et al ., they have found that cosmetic creams could harbour a high number of bacteria and fungi including hazardous type such as: Staphylococcus epidermidis and Micrococcus spp. rather than Enterobacter agglomerans, Citrobacter freundii and Escherichia coli, as well as, filamentous moulds (9). On the other hand, results of cosmetic creams were in agreement with those of Jarvis et. al., they attributed the high contamination level of such products to poor manufacturing conditions (10). Also, Baird, reported that 32.7%; of the tested creams were having great number of microorganisms. The high fungal contamination of some cosmetic creams, in our study, may be attributed to that products are often water in oil emulsions with high concentrations of solutes and lowered water activity.

These conditions are favorable for fungal growth ⁽¹¹⁾. Also a similar results obtained in a study published by, Kamal Kanta Das et al., who isolated Bacillus sp., and he considered it might responsible for unpleasant smell and spoilage of cosmetics products ⁽¹²⁾. Campana et. al., have studied 91 commercially available cosmetic products in order to verify the degree of possible microbiological contamination during their use by consumers. They have studied the intact product (at the time of purchase), the in-use products (after 14 days of use) and the ending product (post use). In all cases the contamination was found in ending products, while in one case it was observed in the in-use product. Also in the study, the preservative systems of the two tested products were studied and they have showed long lasting antimicrobial activity ⁽¹³⁾.

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