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Microbial contamination of some cosmetic preparations in Egypt

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The present study aims to verify, elucidate and screen microbial contamination in different cosmetic samples. The samples were collected from various cosmetic brands commonly brought to the laboratory of microbiology, the National Organization for Drugs Control and Research (NODCAR) for quality assessment. Bacterial and fungal-microbial contamination was found in 32 samples (22.68%) out of 140. The maximum bacterial counts were found in shampoo samples compared to other tested cosmetic samples, followed by gel, solution, cream and oil samples. The microbial counts varied regarding cosmetic type, liquid, solid, or powder. The shampoo-9, shampoo-131, and cos-gel-68 were found to be contaminated with *Escherichia coli*, whereas the shampoo-3, shampoo-13, shampoo-129 and cos-sol-28 samples were contaminated with *Staphylococcus aureus*. However, *Pseudomonas aeruginosa* was detected in the samples of shampo-130 and 134. From health point of view the cosmetic products have to be free from any pathogenic microorganisms.

Key words: cosmetic, bio-contamination, bacteria, molds.

Introduction

Cosmetics are products which people use to enhance and care for their outward appearance. Cosmetic product means any substance or preparation intended for placing in contact with the various external parts of the human body/ or with the teeth and the mucous membranes of the oral cavity (European Communities, 1979, Högskolan Halmstad, 2014).

The microbial contamination of personal care products may already occur in the course of production, through raw materials, ingredients, and during handling, or through repeated use by the consumer. A wide range of preservatives has been developed to combat the contamination from the repeated use by the consumer (NakiSiviri *et al.*, 2006). Nowadays, maintaining a balance between protection against microbial contamination and limiting the health risks of preservatives has constituted the art of preservation (Wu *et al.*, 2010, Taher, 2011, Pyrek, 2013).

In Saudi Arabia, Nasser (2008) investigated the microbial

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contamination in 75 cosmetic samples. He found that the highest fungal counts in lip cosmetic products, and the samples were contaminated with 13 and 24 species belonging to 6 and 2 genera of mesophilic and thermophilic fungi, respectively, with *Aspergillus* was the common fungal genus, and ~ 36.7% of the tested samples were contaminated with *E. coli*, however *Pseudomonas* and *Bacillus* were frequently detected (Okeke and Lamikanra, 2001). *Pseudomonas aeruginosa* and *Enterobacter gergovia* were isolated from a cosmetic production facility, and these organisms showed increase resistance against parabens and formaldehyde-releasing preservatives (Ferrarese *et al*., 2003).

Microbial contamination prior to use, in use, and after use in 91 cosmetic samples was investigated in Italy (Campana *et al.*, 2006). The results showed that none of the samples were contaminated prior to use, however ~ 6 samples were contaminated during use, with *Staphylococcus spp*., and the all of samples were contaminated after use. The present study aims to verify and elucidate microbiological analysis of different cosmetic samples, in order to determine total bacterial and fungal counts, and to detect pathogenic microorganisms in the tested-selected cosmetic products.

Material and methods

One hundred and forty (140) local manufactured cosmetic samples including: shampoo, solutions, hair gels, hair oils, and skin creams were collected from cosmetic laboratory at the NODCAR. The samples were stored at 4°C until microbial analysis was performed.

The total bacterial and fungal (mold and yeast) counts as well as pathogenic bacteria, "*Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp*. and *Pseudomonas aeruginosa*" were investigated using different non-selective and selective media (Sigma-Aldrich, USA). Ten grams/ml of each cosmetic sample were aseptically suspended with 100 ml of sterile soybean-casein digest broth medium, in presence of tween 80, and shaken well for 15 min, at the room temperature. Aliquots, 0.5 ml, of the original samples and their serial dilutions up to 10^{-2} were spread-plated, in duplicate, onto the surface of soybean-casein digest agar medium for isolation of bacteria, and Soburaud's dextrose agar medium for isolation of fungi.

Detection of Pathogenic bacteria

Ten gram of cosmetic sample were aseptically suspended in 100 ml of lactose broth medium, and shaken well for 15 min at room temperature, and incubated for 24h at 37 ±2°C. After incubation, loopfuls of the original suspensions were streaked on MacConkey, Levine eosin – methylene blue, and triple sugar iron tubes agar media to detect *Escherichia coli*, and on plates containing bismuth brilliant green, xylose – lysine desoxycolate, and

triple sugar – iron tubes agar media to detect *Salmonella spp*. Moreover loopfuls of suspensions were streaked on plates of Vogel-Johnson, mannitol–salt and Baird-Parker agar media for detection of *Staphylococcus spp*. The conformation tests for detection of *Staphylococcus aureus* were done using blood agar medium and coagulant test. The plates of cetrimide agar medium and *Pesudomonas* isolation agar media were used to detect *Pseudomonas aeruginosa*.

The fungal plates were incubated at 28 °C for 5–7, and the bacterial plates were incubated at 37 °C for 48 hrs. The growing colonies were counted and the mean count was calculated, and the concentration was expressed as colony forming units per gram (CFU/g) of products. The bacterial isolates were picked up, purified and subcultured for further identification. The bacterial isolates were identified using Gram stain, oxidation fermentation, oxidase, and catalase tests described in the Bergey's Manual of Systematic Bacteriology (Sneath *et al*., 2000).

The microbiological results of cosmetic samples were compared with the Egyptian standard methods of cosmetic test (method 4636/2008), as the total bacterial counts should be ≤100 CFU/g or ml, the total fungal counts should be ≤ 10 cfu/g or ml, and cosmetic samples have to be free of any pathogenic bacteria.

Results and Discussion

Overall microbial counts

In the last years there is a growing concern with bio-contamination of cosmetics and toiletries. Many studies have been carried out to evaluate microbial contamination in cosmetic products, as a consequence of the "age of consumerism" (Sharpell and Manowitz, 1983). Not only that the absolute number of consumers was increasing but the market of personal care products was growing faster than the population (Houlton, 1998). The hygienic requirement of the products is grown concomitantly with the consumption (Berejka, 2004, NakiSiviri *et al.*, 2006).

In the present study, only 31 (22.14%) samples out of 140 samples were contaminated with bacteria or fungi or both. Table 1 shows that the maximum bacterial counts are found in shampoo samples compared to other cosmetic samples, followed by gel, solution, cream and oil samples.

The total bacterial counts in shampoo, gel, solution, cream, and oil samples varied from 3×10^4 - 14×10^5; 1.13×10^2 - 2×10^5; 1.23×10^2 - 1.8×10^4; 46 - 98; and 27 -34 CFU/g, respectively. However, the total yeast and mold achieved the greatest counts in oil samples, followed by gel, solution, cream samples (Table 1). The greatest microbial counts were found in shampoo-3; Shampoo -129; shampoo-13; shampoo-134; shampoo -9, cosmetic gel-68, shampoo-131, shampoo-130 and cos-sol 28 samples (Table

1).

The moisture contents of shampoo, gel, solution and cream samples varied from 78.2 - 89.6, 74.9 - 90.95; 74.9– 96; and 71.4 - 83.5%, respectively (Table 2). It could be concluded that the percentages of moisture contents play important role in increasing/ or decreasing the bacterial and fungal counts in cosmetic samples.

The results in the present study agree with those found by (Flores *et al.* 1997) who isolated several species of bacteria- resistant to paraben compounds. Behravan *et al***.** **(**2005) found that 27% of 47 tested cosmetic samples were uncontaminated with bacteria and fungi, and the total bacterial counts ranged from 10^2-10^6 CFU/g. Yeast and mold were detected in some tested cosmetic products **(**Anelich and Korsten, 1996; Flores et al., 1997).

Most of the cosmetic products with high water content (moisture) were at a risk of being contaminated by microorganisms, and consequently may be altered their composition or pose a health risk to the consumer **(**Steinberg, 2006, Lundov *et al*., 2009).

Table 1. Total microbial counts and physical properties of cosmetic samples

Type of cosmetic sample	CFU/g		Physical Properties	
			pH	Moisture (%)
	bacteria	fungi		
Shampoo-3	14×10^5	14.0	6.40	78.20
Shampoo-9	2.0×10^5	11.0	6.40	89.60
Shampoo-13	12×10^5	13.0	7.10	79.50
Shampoo-129	13×10^5	12.0	6.80	82.40
Shampoo-130	75×10^3	15.0	7.00	81.30
Shampoo-134	4.0×10^5	11.0	7.00	83.00
Shampoo-131	3.0×10^4	13.0	6.30	81.30
Cos.gel – 68	2.0×10^5	22.0	7.10	75.20
Cos.gel – 56	1.20×10^2	18.0	6.80	74.90
Cos.gel – 57	2.11×10^2	25.0	7.20	75.20
Cos.gel – 62	3.00×10^2	33.0	6.40	90.00
Cos.gel – 86	1.13×10^2	21.0	6.80	85.20
Cos.gel – 87	1.52×10^2	18.0	6.70	82.40
Cos.gel – 116	1.41×10^2	17.0	6.30	82.40
Cos.gel – 123	4.22×10^2	21.0	6.50	80.20
Cos-sol-28	1.80×10^4	16.0	6.70	96.00
Cos-sol-92	1.42×10^2	11.0	6.60	95.00
Cos-sol-100	1.23×10^2	14.0	6.40	95.50
Cos-sol-142	1.24×10^2	13.0	6.50	95.40
Cos. Cream-26	64.00	10.00	8.50	71.40
Cos. Cream-53	83.00	6.00	7.30	80.50
Cos. Cream-88	75.00	12.00	7.00	78.50
Cos. Cream-99	84.00	8.00	8.20	82.20
Cos. Cream-112	67.00	6.00	8.4	76.10
Cos. Cream-118	98.00	9.00	7.60	83.50
Cos. Cream-123	68.00	8.00	7.40	78.50
Cos. Cream-128	46.00	8.00	7.50	82.30
Cos. Cream-150	52.00	5.00	6.40	81.50
Cos. Skin oil–73	31.00	50.00	-	0.20
Cos. Suntan oil-79	32.00	83.00	-	0.50
Cos. Hair oil-182	34.00	34.00	-	0.30

Pathogenic bacteria

The pathogenic bacteria including: *Salmonella spp., Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli* are critical factor in food contamination, pharmaceutical and cosmetic industry, because detection of one organism, colony of the pathogen, in the final products, is enough to refuse the product license to sale (Tirumalai, 2007). *E. coli* was isolated from samples of shampoo-9, shapoo-131 and cos.gel-68 (Table 2).

Table 2: Detection of *E. coli* in the selected cosmetic samples

Cosmetic samples	Cultural and morphological characteristics				
	FOL	MAM (Brick-red colonies)	GRTSI (Yellow +gas)	GLEMB (Metallic sheen colonies)	MEG (Gram negative short rods)
Shampoo-3	ND	ND	ND	ND	ND
Shampoo-9	+	+	+	+	+
Shampoo-13	ND	ND	ND	ND	ND
Shampoo-129	ND	ND	ND	ND	ND
Shampoo-130	ND	ND	ND	ND	ND
Shampoo-131	+	+	+	+	+
Shampoo-134	ND	ND	ND	ND	ND
Shampoo-68	+	+	+	+	+
Shampoo-28	ND	ND	ND	ND	ND

Fol: fermentation of lactose, MAM: MacConkey agar medium, MEG: Microscopic examination of Gram staining. GRTSI: Growth reaction in triple sugar iron agar medium. GLEMB: Growth on Livine eosin methylene blue medium, ND: Not detected.

The results in the present study agree with Ashour *et al.* (1989) who found that ~ 5.56% of the tested cosmetic samples were contaminated with *E. coli*. Okeke and Lamikanra (2001**)** reported that contamination rates of cosmetics products with *E. coli* in North America and Europe varied from 2 to 43%. Behravan *et al.* (2005) found that, the contamination of tested cosmetic samples with *E. coli* attained to 13% compared to other Gram negative bacteria which attained to 8%.

Table 3 reveals that bacteria isolated from shapoo-3, shampoo-13, shampoo-129 and cos.sol-28 samples are contaminated with *Staphylococcus aureus*. The results are in agreement with Baird (1984) who investigated 232 cosmetic samples, and found that only 53 (23%) of total tested cosmetic samples were contaminated with *staphylococcus aureus.*

Table 3. Detection of *Staphylococcus aureus* in selected cosmetic samples

Cosmetic samples	Cultural and morphological characteristics				
	VJAM (Black colonies with yellow zone	MAM (Yellow colonies, with yellow zone)	MEG (Gram-positive cocci in clusters)	CAT	BAM (β-hymosissp)
Shampoo-3	+	+	+	+	+
Shampoo-9	ND	ND	ND	ND	ND
Shampoo-13	+	+	+	+	+
Shampoo-129	+	+	+	+	+
Shampoo-130	ND	ND	ND	ND	ND
Shampoo-134	ND	ND	ND	ND	ND
Shampoo-131	ND	ND	ND	ND	ND
Cos.gel-68	ND	ND	ND	ND	ND
Cos.sol-28	+	+	+	+	+

VJAM: Vogel-Johnson agar medium, MSAM: Mannito salt medium. BPAM: Baird-Parkar agar medium, BAM: Blood agar medium, CAT: Co-agulation test. MEG: Microscopic examination of Gram staining, ND: Not detected.

Pseudomonas aeruginosa was isolated samples of shampoo-130 and shampoo-134 (Table4). The results in the present study are in agreement with those reported by (Baird, 1984), who found that only 53 (23%) of total tested cosmetic samples were contaminated with *Pseudomonas* species and Pseudomonas was detected in three tested groups of cosmetic samples. Anelich and Korsten (1996) found that 30% out of 58 samples were contaminated with *Pseudomonas sp,* and *Staphylococcus aureus*, *and Pseudomonas aeruginosa* was frequently isolated from the contaminated cosmetic products.

Table 4. Detection of *Pseudomonas aeruginosa* in selected cosmetic samples

Cosmetic samples	Cultural and morphological characteristics			
	CAM (Greenish fluorescence colonies under UV light)	PAMF (Colorless to yellowish colonies under UV light)	PAMP (Colorless to greenish fluorescence colonies under UV light)	MEG (Gram negative long rods)
Shampoo-3	ND	ND	ND	ND
Shampoo-9	ND	ND	ND	ND
Shampoo-13	ND	ND	ND	ND
Shampoo-129	ND	ND	ND	ND
Shampoo-130	+	+	+	+
Shampoo-134	+	+	+	+
Shampoo-131	ND	ND	ND	ND
Cos.gel-68	ND	ND	ND	ND
Cos.sol-28	ND	ND	ND	ND

CAM: Cetrimide agar medium, PAMF: Pseudomonas agar medium for detection of Fluorescein. PAMP: Pseudomonas agar medium for detection of pyocyanin, MEG: Microscopic examination of Gram staining, ND: Not detected.

In the present study, all the tested cosmetic samples were free of *Salmonella* species, as the presence of *Salmonella* in the cosmetic products may be rarely. The current results agree with those obtained by many investigators (Behravan *et al.*, 2005; Lundov *et al.*, 2009) who found *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* the common pathogenic bacteria in cosmetic samples.

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.

In the present study, *Aspergillus spp.*30%, *Rhizopus spp.*(30%), *Candida spp.*(18%), *Trichoderma spp.*(15%), *Penicillium spp.*(7%) were the common isolated fungal types. Fungal isolates are in the permit range according to FDA and pharmacopeia, and they were present in different species according type of cosmetic shape, and preparation where the *Aspergillus* dominated in shampoo, *candida* in cream, and *penicillium* in other shapes.

The results in the present study relatively similar to the results were

found by (Hugbo *et. al*., 2003, Omorodion *et.al.*, 2014, Gamal *et.al.*, 2015) who found *Aspergillus fumigatus, Pencillium and Microsporium spp* in cosmetic sample. Abdelaziz *et al*. (1989) found cosmetic creams harbor high numbers of bacteria and fungi, including infectious types of *Staphylococcus epidermidis* and *Micrococcus spp*., *Enterabacter agglomerans*, *Citrobacter freundii* and *Escherichia coli*, as well as some filamentous molds**.**

The high fungal contamination of some cosmetic creams, in the present study is 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**.**

Das *et .al*. (2013) isolated *Bacillus spp.* and he considered it might responsible for unpleasant smell and spoilage of cosmetics products. How was it possible that microbiological contamination persisted as the leading cause of recalls for such a long time? The reason is partly in the ever tightening microbiological requirements on the one hand, and in the ever widening basis of raw materials, on the other hand. It is also a reflection of the fact that many cosmetic products and raw materials are susceptible to microbial contamination, and that environmental pressures cannot always be successfully withstood (Katusin-Razem *et al.,* 2003)

Cosmetics are not intended to permanently alter the physiology of the target organ, although some "health care products may contain an active substance or make medicinal claims. Microbiology of cosmetics is therefore complex due to the wide range of formulations, manufacturing procedures and conditions of consumer use. There is a wide spread exposure to potential contaminants during manufacture, particularly from raw materials (Taher, 2011). Water is the most common ingredient and possesses obvious problems, but seemingly innocuous substances such as talc can be contaminated with dangerous pathogens.

The principles of good manufacturing practice must always be followed and raw materials, particularly those of natural origin, must be tested for contamination before use and limits of acceptability established. Areas where contamination may be introduced must be identified and controlled. Due to GMP, contamination during actual production is of such a low order that modern cosmetics manufacturing plants can achieve" absence of microorganisms in almost 100% of units produced".

Manufactures also aim, wherever possible, to develop formulations which are incapable of microbial growth. The level of microbial contamination in a non-sterile product such as, cosmetics formulations, is made clear in the microbial limit standards which should be maintained in the products during their use, in spite of the inevitable contamination by the users, through the addition of a suitable preservative in the products which guarantees the control of microbial growth even before they are marketed.
Cosmetic product are used all over the world and, although aiming at the same high level of consumer protection, their regulations and requirements

are quite different from one part of the globe to another. Contaminating microorganisms in cosmetic may cause spoilage of the product and represent a serious health risk for consumers (Behravan*et al.,* 2005).

Therefore, the need to control microbiological contamination of products has been of considerable concern to manufacturer. Modern pharmaceutical, cosmetics and toiletries strive for high microbiological standards to protect their products from spoilage on the hand, and their consumers from infection, on the other hand unlike foodstuffs, which are usually kept refrigerated (or thrown away after a few days), a much longer shelf life is expected of personal care products (Katusin-Razem *et al.,* 2003).

Conclusion

The microbial contamination of personal care products may occur already in the course of production, through raw materials, ingredients and handling, or the contamination of a final product may ensue through its repeated use by the consumer. Different dangerous bacterial and fungal genera were found in cosmetic samples. Commercial cosmetic creams evaluated did not meet the standards for microbial limits. The need to control microbiological contamination of products has been of considerable concern to cosmetic manufacturer.

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