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MICROBIAL CONTAMINATION OF SOME COSMETICS AND PERSONAL CARE ITEMS IN EGYPT

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ABSTRACT

Out of 140 cosmetic samples collected from Cosmetic lab. National Organization for Drugs Control and Research (NODCAR), Egypt, and screened for their contamination with microorganisms, only 32 samples (22.68%) were contaminated with bacteria or fungi or both. Maximum bacterial counts were observed in shampoo samples compared to other tested cosmetic samples, followed by gel, solution, cream and oil samples. Total bacterial, yeast and mold counts in shampoo, gel, solution, cream and oil samples were varied. Results also revealed that shampoo-9, shampoo-131 and cos.gel-68 were contaminated with E. coli. While, shampoo-3, shampoo-13, shampoo-129 and cos.sol-28 samples were contaminated with Staphylococcus aureus. Shampoo-130 and shampoo-134 samples were contaminated with Pseudomonas aeruginosa. On the contrary, the detection of Salmonella sp. in tested cosmetic samples was negative.

Keywords: Cosmetic, total bacteria, yeast and mold, *E. coli, Staph. aureus, P. aeruginosa, Salmonella*

INTRODUCTION

Cosmetics are products which people use to enhance and care for their outward appearance. Cosmeceuticals a term which underlines the sometimes close relationship between some cosmetics and

pharmaceutical products. Cosmetic product means any substance or preparation intended for placing in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or principally to cleaning them, perfuming them or protecting them in order to keep them in good condition, change their appearance or correct body odors (European Communities, 1979).

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. A wide range of preservatives has been developed to combat the contamination from the latter source. Maintaining a careful balance between protection against microbial contamination and limiting the health risks of preservatives has been constituting the art of preservation (Martin, 1997).

Nasser (2008) investigated 75 cosmetic samples collected from different localities in Saudi Arabia for the presence of mesophilic and thermophilc fungi. Results indicated that the cosmetic samples were contaminated with 13 and 24 species of 6 genera of mesophilc fungi and 11 genera of themophilic fungi, respectively. In addition, Aspergillus was the most common genus while Aspergillus terreus was the most common species at 28°C and 45°C. Moreover, the highest total count of fungi was obtained from samples of lip cosmetic products, while the lowest total counts were detected in eye cosmetic products.

Some physical properties of cosmetic products such as water content and pH level play an essential role on growth, multiplication and survival of microorganisms in cosmetics products. Also, the efficiency of preservation system is negatively or positively affected by water content and pH level in cosmetic products.

Detection studies of pathogens in 49 cosmetic samples show that, 36.7% of tested cosmetic samples were contaminated with pathogenic bacteria and E. coli, Pseudomonas spp. and Bacillus spp. were the most frequently recovered pathogens (Okeke and Lamikanra, 2001).

Ferrarese et al. (2003) reported that Pseudomonas aeruginosa and Enterobacter gergovia isolated from a cosmetic production plant showed increased resistance against parabens and formaldehyde-releasing preservatives.

Microbial contamination prior to use, in use, and after use in 91 different cosmetic samples was investigated in an Italian study (Campana et al., 2006). None of the tested samples investigated was contaminated prior to use, but 6 tested samples became contaminated during use, primarily with Staphylococcus spp. Furthermore, all of the contaminated samples were bath products.

Aim of this work to study microbiological analysis of different cosmetic samples to determine the total bacterial , yeast and mold counts. Detection of pathogens in the selected cosmetic samples which are contaminated with the highest numbers of total microbial counts.

MATERIALS AND METHODS

One hundred and forty local manufactured cosmetic samples (shampoo, solutions, hair gels, hair oils and skin creams) were collected from Cosmetic Lab, National Organization for Drugs Control and Research (NODCAR) and stored at 4°C for determination of total bacterial counts beside yeast and mould counts as well as detection of some pathogens (Staphylococcus aureus, Escherichia coli, Salmonella spp. and Pseudomonas aeruginosa).

. All media of this study were obtained from Difco® Company except blood agar media (Atlas, 2004).

Microbiological data of cosmetic samples were compared with Egyptian standard methods of cosmetic test methods 4636/2008, where total bacterial counts are less than 100 cfu/g or ml. and total yeast and mold are less than 10 cfu/g or ml. while cosmetic samples have to be free from pathogenic bacteria.

Total microbial counts

Ten grams or ml of each cosmetic sample were aseptically suspended with 100 ml of sterile soybean–casein digest broth medium (in presence of tween80,0.1%, as an emulsifier) in conical flask (250 ml) and sharked vigorously for 15 min on rotary shaker (200 rpm) at room temperature as 10^{-1} dilutions and made dilutions 10^{-2} to 10^{-3} by using 5 ml of diluents in 45 ml. Fifteen ml of soybean–casein digest agar medium (for isolation of bacteria) and Sabouraud's dextrose agar medium (for isolation of yeast and molds) were separately transferred into sterile plates (9 ml in diameter) previously inoculated with 1ml of the prepared suspension. The inoculated plates of soybean– casein digest and Sabouraud's dextrose

were incubated at 37 ±2 °C and 28 ±2 °C, respectively. Each assay was performed in duplicate.

The plates were observed after 24 and 72 h for bacteria as well as yeast and molds, respectively. Plates containing 30–300 colonies were counted and average number of colonies per plate from appropriate dilution was calculated as well as the number of colony forming units per gram or ml of sample were determined considering dilution factor.

Detection of pathogenic bacteria

From pathogenic bacteria that should not be allowed to be found in cosmetic products are Staphylococcus aureus, Salmonella spp. and Pseudomonas aeruginosa. Escherichia coli is an opportunistic pathogen and was detected as bioindicator of samples contamination with human wastes as well as contamination with pathogenic bacteria.

Detection of Escherichia coli and Salmonella

Ten gram or ml of each cosmetic sample were aseptically suspended with 100 ml of lactose sterile broth medium in conical flask (250ml), shake vigorously for 15 min on rotary shaker (200 rpm) at room temperature and incubated for 24 h at 37 ±2°C. After incubation, loopfuls of inoculated flask were streaked on sterile plates of MacConkey, Levine eosin–methylene blue and triple sugar– iron tubes agar media for detection of Escherichia coli. Also, loopfuls of inoculated flasks were streaked on sterile plates of bismuth sulphate, brilliant green, xylose–lysine desoxycolate and triple sugar– iron tubes agar media for Salmonella spp. detection.

All the cosmetic samples positive for Escherichia coli and Salmonella pp. contamination were confirmed using Gram staining

(Trevino, 2002).

Detection of Staphylococcus aureus and Pseudomonas aeruginosa

Ten grams or ml of each cosmetic sample were aseptically suspended with 100 ml of sterile soybean–casein digest broth medium in conical flask (250ml), shaked vigorously for 15 min on rotary shaker (200 rpm) at room temperature and incubated for 24 h at 37 ±2°C. After incubation, loopfuls of inoculated flasks were streaked on sterile plates of Vogel– Johnson, mannitol–salt and Barid–Parker agar media and incubated for 24 h at 37 ±2°C for detection of Staphylococcus sp. The conformation test for detection of Staphylococcus aureus in cosmetic samples were done on blood agar medium and coagulant test. Also, loopfuls of inoculated flasks were streaked on sterile plates of cetrimide agar medium, Pseudomonas agar media for examination of fluorescein and pyocyanin and incubated for 24 h at 37 ±2°C for detection of Pseudomonas aeruginosa.

Water content of cosmetic samples were determined using a Karl Fisher Instrument which previously calibrated by absolute methanol. While pH of cosmetic samples was determined using a pH Meter Hanna–Instruments.

RESULTS AND DISCUSSION

Total microbial counts

Out of 140 cosmetic samples collected from Cosmetic lab. National Organization for Drugs Control and Research (NODCAR), Egypt, only 31 (22.14%) samples were contaminated with bacteria or fungi or both. Data in Table (1) reveal that the maximum bacterial counts were found in shampoo samples compared to other cosmetic samples, followed by gel, solution, cream and oil samples. Total

bacterial counts in shampoo, gel, solution, cream and oil samples varied from $3x10^4$ to$14x10^5$; $1.13x10^2$ to $2x10^5$; 1.23×10^2 to1.8×10^4; 46 to 98 and 27 to 34 cfu /g, respectively. However, the maximum total yeast and mold counts were found in oil samples compared to other cosmetic samples, followed by gel, solution, cream and shampoo samples. Total yeast and mold counts in oil, gel, shampoo, solution and cream samples were varied from 34 to 83; 17 to 33; 11 to 15, 13 to 16 and 5 to 12 cfu/g, respectively.

Data in Table (1) showed that the moisture content of shampoo, gel, solution and cream samples were high and varied from 78.2 to 89.6, 74.9 to 90, 95 to 96 and 71.4 to 83.5%.respectively On the light of the abovementioned results, it could be concluded that, percentage of moisture content plays an important role on increasing or decreasing of bacterial or yeast and molds counts in cosmetic samples, reactively.

The current results are in agreement with those found by Flores et al. (1997) who investigated 42 different cosmetic products for contamination and they isolated several species of bacteria resistant to paraben compounds. Behravan et al. (2005) reported that only 27% out of 48 tested cosmetic samples were uncontaminated with bacteria or fungi. While, total bacterial counts of the contaminated tested cosmetic samples ranged from 10^2 to 10^6 cfu/g, with a majority of counts being at the range from 10 to 10^2 cfu/g, however, 70% of the tested samples were contaminated with less than 10^3 cfu/g.

Table (1): Total microbial counts and physical properties of cosmetic samples*

Parameters / Cosmetic samples	Total bacterial count (cfu**/ g)	Total yeast and mold counts (cfu**/ g)	physical properties	
			pH	Moisture content (%)
Shampoo-3	14×10^5	14.0	6.4	78.2
Shampoo-9	2.0×10^5	11.0	6.4	89.6
Shampoo-13	12×10^5	13.0	7.1	79.5
Shampoo-129	13×10^5	12.0	6.8	82.4
Shampoo-130	75×10^3	15.0	7.0	81.3
Shampoo-134	4.0×10^5	11.0	7.0	83.0
Shampoo-131	3.0×10^4	13.0	6.3	81.3
Cos. gel -68	2.00×10^5	22.0	7.1	75.2
Cos. gel-56	1.20×10^2	18.0	6.8	74.9
Cos. gel -57	2.11×10^2	25.0	7.2	75.2
Cos. gel -62	3.00×10^2	33.0	6.4	90.0
Cos. gel -86	1.13×10^2	21.0	6.8	85.2
Cos. gel-87	1.52×10^2	18.0	6.7	82.4
Cos. gel -116	1.41×10^2	17.0	6.3	82..4
Cos. gel -123	4.22×10^2	21.0	6.5	80.2
Cos-sol-28	1.80×10^4	16.0	6.7	96
Cos-sol-92	1.42×10^2	11.0	6.6	95
Cos-sol-100	1.23×10^2	14.0	6.4	95.5
Cos-sol-142	1.24×10^2	13.0	6.5	95.4
Cos. cream-26	64.0	10.0	8.5	71.4

Cos. cream-53	83.0	6.0	7.3	80.5
Cos. cream-88	75.0	12.0	7.0	78.5
Cos. cream -99	84.0	8.0	8.2	82.2
Cos. cream-112	67.0	6.0	8.4	76.1
Cos. cream-118	98.0	9.0	7.6	83.5
Cos. cream-123	68.0	8.0	7.4	78.5
Cos. cream-128	46.0	8.0	7.5	82.3
Cos. cream-150	52.0	5.0	6.4	81.5
Cos. skin oil-73	31.0	50.0	-	0.20
Cos. suntan oil-79	32.0	83.0	-	0.50
Cos. hair oil-182	34.0	34.0	-	0.30

* Previously protected with preservatives (parabens, phenoxyethanol or imidazolidinylurea), ** cfu: colony forming unit.

In addition, most cosmetic products with high water content were at a risk of being contaminated by microorganisms that can alter the composition of the product or pose a health risk to the consumer (Steinberg, 2006 ;Lundov et al., 2009). Furthermore, yeast and mold were also found in contaminated cosmetic products (Baird, 1984; Anelich and korsten., 1996 ;Flores et al., 1997).

The maximum total 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 Therefore they were used in subsequent studies.

Detection of pathogens in the selected cosmetic samples

Contamination of pathogens (Salmonella spp, Staphylococcus aureus, Pseudomonas aeruginosa or E. coli) is one of the most

critical factor in food, pharmaceutical and cosmetic industrial, because detection of one colony of pathogen in the final products is enough to refuse the product license to sale (Tirumalai, 2007a&b).

Detection of E. coli in the selected cosmetic samples

Data in Table (2) reveal that, bacteria isolated from shampoo-9, shampoo-131 and cos.gel-68 samples could utilize lactose as a sole carbon source, grew and gave brick-red colonies on MacConkey agar medium, grew and gave metallic sheen colonies on Livine eosin methylene blue medium, produced acid and gas on triple sugar iron agar medium and their microscopic examination revealed that bacterial cells were Gram-positive short rod-shaped.

The current results are conformed with the keys described in Tirumalai (2007 a&b), thus, shampoo-9, shampoo-131 and cos. gel-68 samples are contaminated with E. coli. The present results are in agreement with those found by Ashour et al. (1989) who found that 5.56% of total tested cosmetic samples were contaminated with E. coli. Also, Okeke and Lamikanra (2001) reported that contamination rates of cosmetics products with E. coli in North America and Europe varied from 2 to 43%. Moreover, 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 (2): Detection of E. coli in selected cosmetic samples

Cosmetic Samples	Cultural and morphological characteristics of isolated bacteria[2]				
	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
Cos. gel –68	+	+	+	+	+
Cos–sol–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

Detection of Staphylococcus aureus in the selected cosmetic samples

Data in Table (3) reveal that bacteria isolated from shampoo-3, shampoo-13, shampoo-129 and cos.sol-28 samples could grow and give black colonies with yellow zone on Vogel-Johnson agar medium; yellow colonies with yellow zone on mannitol salt agar medium and the microscopic examination reveal that the bacterial cells were Gram-positive spherical-shaped that occur in clusters resembling grapes.

In addition, the isolated bacteria could produce coagulase enzyme and could grow on blood agar medium and gave beta-hemolytic reaction.

The current results are confirmed with the keys described by Tirumalai (2007 a&b), thus, shampoo-3, shampoo-13, shampoo-129 and cos.sol-28 samples were contaminated with Staphylococcus aureus.

The above mentioned results arein agreement with those reported by Baird (1984), who investigated 232 cosmetic samples which divided into three groups (products, products used at home, and products used at a maternity ward in a hospital) and he found that only 53 (23%) of total tested cosmetic samples were contaminated. In addition, Staphylococcus spp. and Pseudomonas spp. were detected in all the tested cosmetic samples groups.

Also, Lundov et al. (2009) reported that, cosmetic products with high water content were at a risk of being contaminated by micro-organisms that can alter the composition of the product or pose a health risk to the consumer.

Table (3): Detection of Staphylococcus aureus in selected cosmetic samples

Cosmetic samples	Cultural and morphological characteristics of isolated bacteria[2]				
	VJAM (Black colonies with yellow zone)	MSAM (Yellow colonies with yellow zone)	MEG (Gram-positive cocci occur in clusters)	CAT	BAM (β-hymosis sp.)
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: Mannitol salt agar medium, BPAM: Baird-Parkar agar medium, BAM: Blood agar medium, CAT: Co-agulation test, MEG: Microscopic examination of Gram staining, ND: Not detected.

Detection of Pseudomonas aeruginosa in the selected cosmetic samples

Data in Table (4) reveal that the bacteria isolated from shampoo-130 and shampoo-134 samples could grow and gave greenish fluorescence colonies on cetrimide agar medium; colorless colonies on Pseudomonas agar medium for detection of fluorescein; greenish fluorescence colonies on Pseudomonas agar medium for detection of pyocyanin and the microscopic examination reveal that the bacterial cells are Gram negative short rod-shaped. The current results were confirmed with the keys described by Tirumalai (2007a&b), thus, shampoo-130 and shampoo-134 samples were contaminated with Pseudomonas aeruginosa.

The above mentioned results are in agreement with those reported by Baird (1984), who found that only 53 (23%) of total tested cosmetic samples were contaminated. In addition, Pseudomonas sp. was detected in three tested groups of cosmetic samples.

Also, Anelich and Korsten (1996) mentioned that out of 58 cosmetic samples exported from South Africa, 30% of the total were contaminated with Pseudomonas sp. Moreover, Lundov et al. (2009) reported that, cosmetic products with high water content were at a risk of being contaminated by microorganisms that can alter the composition of the product or pose a health risk to the consumer. Pathogenic microorganisms such as Staphylococcus aureus and Pseudomonas aeruginosa are frequently found in contaminated cosmetic products.

Table (4): Detection of Pseudomonas aeruginosa in selected cosmetic samples

Cosmetic Samples	Cultural and morphological characteristics of isolated bacteria			
	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 .

Detection of Salmonella spp. in the selected cosmetic samples

Detection of Salmonella spp. in the tested cosmetic samples was negative and this means that this pathogen absent in the cosmetic products may be rarely. The current results are in agreement with those obtained by many investigators who mentioned that

Staphylococcus aureus, Pseudomonas aeruginosa and E. coli were the most pathogenic bacteria isolated from the tested cosmetic samples (Baird, 1984; Anelich and Korsten, 1996; Okeke and Lamikanra, 2001; Behravan et al., 2005 ;Lundov et al., 2009).

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التلوث الميكروبى لبعض مستحضرات التجميل والعناية الشخصية في مصر

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٢– الهيئة القومية للرقابة والبحوث الدوائية

تم تجميع ١٤٠ عينة مستحضر تجميل تم تجميعها من معمل تحليل عينات التجميل بالهيئة القومية للرقابة والبحوث الدوائية واستعراضها للتحليل لتحديد مدى تلوثها بالميكروبات، وقد أظهرت النتائج ان من بين ١٤٠ عينة، ٣٢ عينة (٢٢.٨٦%) كانت ملوثة بالبكتريا او الفطريات او الاثنان معا. اعلى عدد للبكتريا تم ملاحظته فى عينات الشامبو مقارنة بباقى العينات المختبرة، يليها عينات الجل والمحلول والكريم ثم عينات الزيوت. وقد أوضحت نتائج الكشف عن البكتريا الممرضة الى ان عينات shampoo–9و shampoo–131 وcos–gel–68 كانت ملوثة ببكتريا E.coli . فى حين shampoo–3 و shampoo–13 و shampoo–129 و cos–sol–28كانت ملوثة ببكتريا Staphylococcus aureus. بالإضافة الى ذلك فان عينات shampoo–130و shampoo–134 كانت ملوثة ببكتريا Pseudomonas aeruginosa. و على النقيض فان نتيجة الكشف عن بكتريا Salmonella لنفس العينات المختبرة كانت سلبية.