A Study on Cosmetic Products Marketed in Iraq: Microbiological Aspect

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Abstract

Cosmetic products contain variable amounts of nutrients that support microbial growth. Most contaminants in cosmetic products include bacteria such as *Staphylococcus, Pseudomonas, Klebsiella, Achromobacter and Alcaligenes*. Contaminated water is a likely source of organisms found in cosmetic products. Products such as shampoo, hand and body lotion, facial cleanser, and liquid soaps were analyzed. In this study, out of 60 cosmetic products analyzed, 26.4% were found to be contaminated. Most of the contamination was from bacteria and no fungal contamination was detected. The highest level of contamination occurred in shampoo.Viable bacterial were not recovered from 100%, 92.8%, 91.6% and 89.2% of showed bath soaps, facial cleanser, hand and body lotion and shampoos, respectively. Coliforms were recovered from one sample of shampoos. One isolate of shigella and pseudomonas aeruginosa was detected from two samples of shampoo.

Keywords: Microbial contamination, cosmetic

الخلاصة

تحوي مستحضرات التجميل كميات مختلفة من المواد الغذائية التي تساعد على نمو الملوثات وتشمل هذه الملوثات انواع من البكتريا مثل *Staphylococcus, pseudomonas, klebsiella, Achromobacter, Alcaligenes* . يعتبر الماء الملوث مصدر آخر للتلوث ايضاً التي تتعرض لها مستحضرات التجميل مثل الشامبوات، مستحضرات السائلة لاغراض تجميلية او طبية، المنظفات والصابون السائل. في هذه الدراسة تم اخذ حوالي ستون من هذه المستحضرات وتم فحصها مايكروبياً وقد اظهرت النتائج تقريباً ان ٢٦.٤% من هذه المستحضرات كانت ملوثة وان معظم التلوث هو بكتيري واقله هو فطري وان اعلى معدل للتلوث وجد في الشامبوات ١٠.٨% وان بكتريا الـ coliforms موجودة في احد انواع الشامبوات وايضاً وجدت بكتريا shigella و pseudomonas في نوعين آخرين من الشامبوات وهي من البكتريا المرضية (pathogenic micro organisms).

Introduction

Cosmetics are part of everyone's daily grooming routine. Most cosmetic products are based on water/oil emulsion or oil/water emulsion and contain variable amounts of nutrients that support microbial growth. The raw materials used in cosmetic products may be grouped into categories (Table 1).

Table 1 : Raw material categories

Water
Acids, alkalis, salts
Oils, waxes, paraffin
Fatty acids, alcohol, esters
Surfactants, emulsifier
Talc, clay
Protein, starches, botanicals, gums and resin
Humectants
Colour and pigments
Preservatives, antioxidants and chelating agents
Fragrances, essential oils

Source: Adapted from Orth (1989).[1]

Microbial contamination in cosmetics, toiletries and personal care products is very common and has been of great concern to the industry for many years. Bacteria, yeast and fungi are extremely diverse in their metabolic activities. The metabolic reaction of the microorganisms can cause health hazards because the metabolic products can be toxic, mutagenic.Cosmetic products need protection against microbial spoilage, first of all in order to protect the consumer against potential dangers arising from pathogens and secondly to guarantee long-term stability (shelf life) of the formulae. Preservatives play a vital role in product formulations. The selection of a germicidal is critical. In many cases, chemicals which are highly active against microbes also have similar effects against mammalian cells. Therefore, a balance needs to be established with the preservatives of choice between killing organisms and not injuring the cells of consumer who uses the product. It is important to keep monitoring the cosmetic product for contamination because an increasing number of products are recalled each year , and the majority is contaminated with potential pathogenic micro organisms.[2] More knowledge on the reasons for contamination is needed. The aim of this study is to demonstrate the microbial content of unused cosmetic products at the point of sale. The cosmetic products were manufactured in Iraq and were purchased from super market, Saloons.

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Microbiological contaminants of cosmetics

The most frequent contaminants of cosmetic products include *Pseudomonas*, *Klebsiella, Achromobacier* and *Alcaligenes*[3]

Baird[4] surveyed 147 unused cosmetic products purchased in England. He recovered viable bacteria from 99 of the 147 products (Table 2). Gram-negative rods were isolated from 6.1% of the products.

Table 2 : Gram-negative rods in cosmetics.

Contaminant	Product	No. of organisms per ml/g
Pseudomonas aeruginosa	Lanolin hand cream	$1.2x10^3$
P. Maltophilia	Mascara	$7.0 x10^3$
P. pseudoalkaligenes	Cleansing milk	$3.1 x10^3$
P. pseudoalkaligenes	Hair cream	$1.9 x10^3$
P. fluorescens	Hair oil	$4.0 x10^3$
P. putida	Cleansing jelly	$2.5 x10^3$
Moraxella osloensis	Moisture cream	$1.3 x10^3$
Enterobocter cloacas	Dental cream	$2.3 x10^3$
Klebsiella aerogenes	Dental powder	$3.4 x10^3$
K. oxytoca	Dental powder	-
Erwina herbicola	Dental powder	-
Enterobocter cloacae	Dental powder	-

Source Adapted from Baird (1974).

A number of surveys[1] reported the incidence of contaminants in unused cosmetic products[5]. The clinical and pharmaceutical significance of contamination for cosmetics has been reviewed by[6]. Certain products, notably aqueous preparations were more susceptible to contamination than others.

Overall, these findings indicated that under the existing manufacturing conditions, some forms of contamination in the final product appeared to be inevitable. Table (3) shows some potentially pathogenic bacteria isolated from cosmetic products and some of these organisms[7] are part of the normal human flora.

Table 3 : Potentially pathogenic bacteria isolated from cosmetic preparations

Acinetobacter calcoaceticus	*Escherichia coli*	*Providencia rettgeri*
Citrobacter diversus	*Hafnia alvei*	*Providencia stuartii*
Citrobacter freundii	*Klebsiella oxytoca*	*Pseudomonas cepacia*
Clostridium spp.	*Klebsiella pneumonia*	*Pseudomonas fluorescens*
Enterobacter aerogenes	*Morganella morganii*	*Serratia liquefaciens*
Enterobacter agglomerans	*Proteus mirabilis*	*Staphylococcus aureus*
Enterobacter cloacea	*Proteus vulgaris*	*Staphylococcus epidermidis*
Enterobacter gergoviae		
Enterobacter sakazakii		

Source: Adapted from Norman (1984).

Hazards associated with microbial contamination

Infection from non-Sterile Products

The roles of many organisms in cosmetic preparations were studied[8] by Brunch (1972). He concluded that most are objectionable for application to damaged epithelium while others are opportunistic pathogens depending on the species, the site and the health of the recipient. Contaminants in cosmetic products include bacteria such as *Staphylococcus*, *Pseudomonas* and *other opportunistic bacteria*. Contamination of talc with *Closlridium tetani*, infection of neonates with *Pseudomonas aeruginosa* from contaminated cleansing solution and scalp infection from diluted stored shampoo leading to fatality in a granulocytopenia patient are some examples. The eye is particularty vulnerable to infection. The loss of eye sight after the use, during intraocular operation, of saline solution contaminated with Pseudomonas aeruginosa[9], and severe eye disorders caused

by use of a cortisone ointment contaminated with the same organism are examples of the serious dangers posed by contaminated preparations.

Spoilage

A spoiled product is one that has been rendered unfit for its intended use. Mouldiness, colour change, fronting and packaging that bulge, leak or explode as a result of gas production are obvious effects of gross contamination and lead to chemical and physical changes in the products. Discovery by the customer of a mould colony is not likely to encourage further purchase of the particular brand.Recent reviews of the spoilage aspects of microbial contamination of medicines and cosmetics have been made by[6]. Mixed flora introduced into the product by whatever means are often extremely versatile in their metabolic activity and can adapt to a broad range of environmental conditions. All cases of natural organic compounds are susceptible to degradation by one organism or another and synthetic material may also be attacked. Products such as shampoos, which contain surfactants, are particularly susceptible to contamination by water-borne gram-negative bacteria, which may cause at the very minimum, a visible loss of lathering activity. Active ingredients may also be rendered ineffective.

Visible Effects

Contaminants may be seen as sediment, turbidity or pellicles in liquid products. On more solid preparations, coloured colonies may form. The appearance of bright yellow micrococcus colonies on a white cream is an alarming sight. This may result from use of natural ingredients such as dried egg, which can carry a large number of organisms, if improperly treated. Contamination by *Pseudomonas spp.,* which is metabolically versatile, causes colour changes. This is due to alteration in product components as a result of direct consequence of metabolism or indirectly because of alteration of parameters such as pH or oxidation-reduction. The addition of organic material greatly increases the chances of growth and deposits or turbidity due to algae, mould, bacteria or yeast in a range of poorly preserved pharmacopoeia solutions. Emulsions can become thin, separate, decolorize or change colour and become visibly heterogeneous owing to hydrolysis of the oil phase or change in pH of the aqueous phase. Products such as shampoos are particularly susceptible to contamination by Gram-negative water-borne bacteria[5]. Slimy sediments, pellicles and discoloration may occur.

Degradation of Actives Constituents

Beveridge[10] reported on the inactivation of potent drugs and antimicrobial agents by a wide variety of microorganisms. Penicillin can rapidly be destroyed by β-lactamases. Other antibiotics, preservatives and disinfectants can be metabolized. Atropine in eye drops can be destroyed by *Corynebacterium* and *pseudomonas* spp[11]. and the transformation of hydrocortisone in the dermatological cream to a therapeurically different compound by a contaminant Cladosporium herbarum. has been reported by[12].

Methodology

The objective of this study was to monitor the scenarios of Iraqian market cosmetic products. In this study, various types of cosmetic products were purchased from supermarkets, saloons. These products were then subjected to microbiological tests to detect the presence of microorganisms at the point of purchase.

Aerobic Plate Count

Sterile materials and equipments were sterilized before use and aseptic techniques were used. The caps of the products were wiped with ethanol (70%).Microbiological media were reconstituted and prepared from their dehydrated powder according to manufacturer instructions. By means of a syringe, or sterile spatula, one ml or one gram of the product was disintegrated in tryptic soy broth (9ml) according to B.P 2004 using a flask shaker and suitable serial dilations in tryptic soy broth were prepared. One-ml sample of each dilution was poured in a sterile petridish and then 15ml of sterile tryptic soy agar was poured on the samples, the plates were gently swirled in a round movement to allow a good mixing of the agar with the sample, then the plates were allowed to solidify on a leveled surface. Triplicate plates for each sample were used and incubated at (35^0C -37^0C) for two days for bacteria. Sabourand dextrose ager was used instead of tryptic soy agar-for the detection of fungi. The prepared plates were incubated at 25^0C for 5 days. After incubation the number of colonies was counted by estimating the total count of the growing bacteria and fungi then the mean of three plates was calculated.A laboratory control count was performed using negative control blank (without product) and with positive control (contaminated product). More than two colonies on the negative control plate

invalidated the test.The colonies were counted. Colony counts exceeding 1000 were considered too high to count and the product further diluted. Plates with colonies of 30-300 were selected. The microorganism content per milliliter or gram is the colony count multiplied by the appropriate dilution factor (10 or 100).

Samples

Samples used in this study included shampoos, liquid bath soaps, facial cleansers, hand and body lotions and moisturizers, which were purchased from supermarkets and saloons.Detection for specific microorganisms such as *Escherichia coli*, *staphylococcus aureus*, *pseudomonas*, and *salmonella* was performed following procedure under isolation and identification tests for specified microorganisms (B.P 2004) as shown in table (4). When results showed presence of any of these organisms, appropriate biochemical tests were performed.

Table 4 : Isolation and Identification tests for specified microorganisms (BP. 2004)

Organism	Enrichment	Primary test	Secondary test	Confirmation
Enterobacteriaceae	Lactose broth 35-37^{0}C	EEB-Mossel 35-37^{0}C For 24-48hr.	VRBGLA 35-37^{0}C	GROWTH OF Gram negatives
E. coli	As above	MacConkey broth 43-45^{0}C for 18-24hr.	MacConkey agar 43-45^{0}C for 18-24hr.	Indole at 43.5-44.5^{0}C biochemical
Salmonella spp.	As above for 5-24hr.	TBBG broth 42-43^{0}C 18-24hr. then subculture on: DCA.XLDA or BGA for 35-37^{0}C for 24-48hr.	TSI agar 35-37^{0}C for 18-24 hr.	Biochemical serological
P. aeruginosa	Saline peptone 35-37^{0}C for 2-5hr	Casein digest broth 35-37^{0}C for 24-48hr.	Cetrimide agar 35-37^{0}C for 24-48hr.	Oxidase test
Staph. aureus	As for P.aeruginosa above	As for P.aeruginosa above	Baird-Parker 35-37^{0}C for 24-48hr.	Coagulase, catalase, DNase tests

EEB-m-Mossel. Enterobacteriaceae enrichment broth-Mossel; VRBGLA, violet red bile agar with glucose and lactose; TBBG, tetrathionate bile brilliant green broth; DCA, deoxycholate citrate agar; XLDA, xylose lysine deoxycholate agar; BGA, brilliant green agar; TSI, triple sugar iron agar; DNase, deoxyribonuclease test.

Results and Discussion

Of 60 products analysed for their total aerobic bacterial, coliforms and fungal counts, 26.4% were found to be contaminated table (5). Most products were of bacterial contamination and no one were of yeast and mould. Shampoos were more suceptible to contamination than other products presumably because they contain surfactants fig (1) and table (5).Viable bacteria were not recovered from 100%, 92.8%, 91.6% and 89.2% of shower bath soaps, facial cleanser, hand and body lotion and shampoos, respectively. Table (6) shows the microbial counts (C.F.U)/gm or ml) and types found in shampoos and body lotions ,only 2%, of shampoos were heavily contaminated by aerobic spore forming bacteria (bacilli) with more than 10^4 c.fu/gm or ml. while non of the others contained such a high number of bacteria. With regard to the medium range contamination levels; 5% of shampoos showed bacterial counts ranging from 10^2 to 10^3 C.F.U/gm or ml, compared to 2% of hand and body lotions which were contaminated to same level. Coliforms were recovered from one sample of shampoos; *staphylococcus* were not recovered from any samples. One isolate of *pseudomonas aeruginosa* was also detected in a sample of shampoo. One isolate of shigella was also detected in a sample of shampoo. No fungal contamination was detected, as shown in table (6). The pH of all the tested samples was alkaline pH (8.2-9), which is well known to inhibit fungal contamination.Bacterial contamination in unused cosmetic products is common because of the environment in which the products are manufactured, packed and the ingredients them selves. Cosmetic ingredients are rich in nutrients and these provide organic substrates in the form of sugar, starch, protein, amino acid, organic acid, alcohol, amines, lipid and etc. for microbial growth. Organisms such as *Pseudomonas Putida* possesses a mixed functions oxidase enzyme that enable them to utilizes substrates that many other organisms are unable to use. The ability of microorganisms to utilize substrates depends on their survival strategies. Nutrients needed by organisms include nitrogen, sulphur, phosphorus and minerals. These materials,

which are required for enzyme function and cellular osmoregulation are furnished as components of raw material or in water. Water is a major ingredient in many cosmetic products and it has been the source of *finished* product contamination.Malcom and Woodroffe (3) reported that the most frequent contaminants of cosmetic products are *Pseudomonas, Klebsiella, Achromobacter* and *Alcaligenes*. They observed that these genera are common residents in water and contaminated water is likely source of the organisms found in contaminated cosmetic products. Some microbes survive by forming endospores, biofilms, capsules, extracellular enzymes and by exhibiting acid tolerance.[14-18] Our result is similar or like the report of *okekre* [19], Gram negative bacilli were seen in these studies, but unlike the report of *Hugo*[20].Unlike the report of. [19], [20], salmonella spp. Was isolated in our study. And Generally, microorganisms of interest in raw materials or cosmetic products grow best around neutral pH 7.0 and many yeasts and moulds are able to tolerate acid pH conditions. Cosmetic ingredients supply nutrients for microbial growth. Therefore, cosmetics should be produced in a perfectly clean hygienic environment. Product premises, equipment, instruments, storage tanks and containers should accordingly be maintained in a high standard of cleanliness. In order to minimize contamination, cosmetic manufacturers should follow guidelines of good manufacturing practice (GMP). All starting materials should correspond to the agreed standards and be of consistently good quality[6]. Ingredient listing is an important aspect of the labeling of cosmetic products. During the Nances Pharmaceutical Control Bureau Cosmetics Seminar 2002, one of the recpureinents discussed was labelling of cosmetic products[21].

Table 5 : Shows the microbial counts (C.F.U)/gm or ml) and types found in shampoos and body lotions.

Samples	Total No. of products	Contaminated products
Shampoo	15	10.8
Hand and body lotion	15	8.4
Shower bath soaps	15	Zero
Facial cleaser	15	7.2
Total	60	26.4

Table 6 : Microbial counts (C.F.U/ml or gm) and types found in shampoos and body lotions.

Shampoo	Counts	Types diagnoized
2%	> 10^4 C.F.U/gm	Aerobic spore forming bacteria (bacilli) high level
5%	10^2-10^3 C.F.U/gm	Aerobic spore forming bacteria (bacilli) Medium level
1%	10^3-10^4 C.F.U/gm 5.0x10^2 1.0x10^3	Pseudomonas aeruginosa Shigella spp.
Hand and body lotion	13x10^3	Escherichia
	>10^4 C.F.U/gm	
2%	10^2-10^3 C.F.U/gm	Aerobic spore forming bacteria (bacilli) Medium level
1%	10^3-10^4 C.F.U/gm	

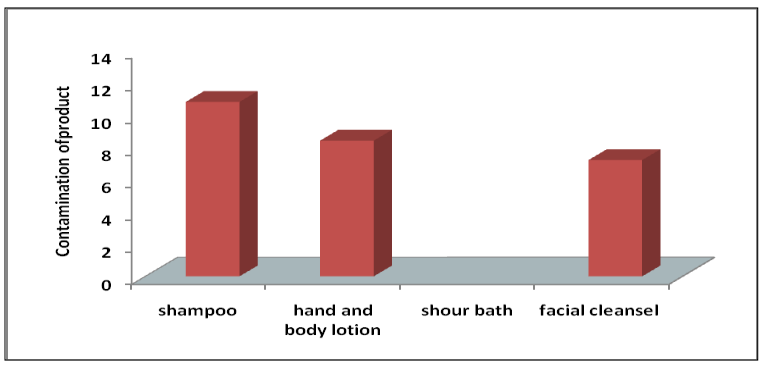

Figure 1 : Contamination based on product types.

Conclusion

From this study it was found that bacterial contmination is more likely to occur than yeast and mould contamination. Bacterial growth is favoured at neutral pH and most cosmetic products are at this range. Microorganisms such as *Psudomonas, Klebsiella, Achromobacter* and *Alcaligenes* are the most frequently reported contaminants of cosmetic products. Also, contamination is higher in shampoos than other products. This may be because they contain surfactants which are susceptible to contamination by water-borne Gram-negative bacteria. Cosmetic manufactures can prevent contamination by controlling raw materials, validating processes, instituting effective cleaning and sanitizing procedures, and training personnel. Even low contamination does not necessarily mean that the manufacturers have followed the newly adopted EU regulation.There might be a possibility that the manufactuer used excessive preservatives in the product. A further study on preservatives will be carried out to detect the preservative level in cosmetic products marketed in Iraq.

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