Bacterial and Fungal Contamination in Three Brands of Cosmetic Marketed in Iraq

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Abstract

Cosmetic products must be safe for use by consumers , It is also regulated and required the legislation of countries all over the world . In this study out of 80 cosmetic products analyzed and 32.5% were found to be contaminated .Products such as mascara, lip pencil and eye pencil were analyzed . The contaminants including bacteria such as *Staphylococcus aureus* , *Staphylocoocus epidermidis* , *Pseudomonas aeruginosa* , *Escherichia coli* and *Klebsiella pneumonia* which were ranging in number from (10^3-10^4) C.F.U. /ml and fungi such as *Penicillium spp.* , *Aspergillus fumigatus* and *Candida albicans* which were ranging in number from (10^2-10^4) C.F.U /ml . The water and other nutrients present in cosmetic make them susceptible to microbial growth . Microorganisms detected in recent study considered as pathogenic to human.

Key words : Bacterial contamination , Fungal contamination,Cosmetics.

الخلاصة

مستحضرات التجميل يجب أن تكون آمنة لاستخدامها من قبل المستهلكين، وتنظم قوانين لهذا الغرض في كل بلدان العالم أيضا. في هذه الدراسة من أصل(٨٠) منتجات التجميل تم تحليلها وعثر على ٣٢.٥ ٪ منها لتكون ملوثة. المنتجات التي تم تحليلها شملت المسكرة، قلم الشفاه وقلم العين .وان الملوثات بما في ذلك البكتيريا *Staphylococcus aureus* ,*Staphylocoocus epidermidis* *Pseudomonas aeruginosa* , *Escherichia coli* and *Klebsiella pneumonia* تراوحت اعدادها بين ١٠^{٣}-١٠^{٤}. والفطريات شملت *Penicillium spp.* , *Aspergillus fumigatus* and *Candida albicans* تراوحت اعدادها بين ١٠^{٢}-١٠^{٤}. الماء والمواد الغذائية الأخرى الموجودة في مستحضرات التجميل جعلها عرضة لنمو الميكروبات . ان معظم هذه الملوثات تعتبر ممرضات خطرة على صحة الانسان .

Introduction

In daily life cosmetics are becoming very important ; they are used daily and regularly by increasing numbers of the people and the quantities consumed are increasing each year [(1)].Most cosmetics contain a lot of ingredients are good for microbial growth and the production of cosmetics is not a sterile process and at least the storage temperature is nearly optimal for microbial growth [(2)] .Cosmetics products may be contaminated during manufacturing by microorganisms existing in the environment or in the raw materials , which are mostly contain water and the later form an appropriate media for microbial growth [(3)] . The raw materials used in cosmetics products may be grouped in to categories (Table1). To avoid microbial contamination of cosmetics during use and storage , the manufacturers add preservatives to their products [(5)] . Two different problems arise when preservatives are used in cosmetics , first is that microorganisms easily contaminate the cosmetics when the amounts of antimicrobial agents are kept low for safety and economy , and second is that serious problems of skin reactions produced by antimicrobial agents are caused when their amounts are increased for preventing microbial contamination [(6)] , therefore a balance needs to be established with the preservatives of choice between killing microorganisms and not injuring the cell of the consumer who uses the product, There are several different preservatives available but the cosmetic market is dominated by a few preservatives: parabens, formaldehyde, formaldehyde releasers, and methylchloroisothiazolinone/ methylisothia-zolinone [(7)].

Table 1 : Raw materials categories .

Water
Acids , alkalis , salts .
Oils , waxes , paraffins .
Fatty acids , alcohol , esters .
Surfactants , emulsifier .
Talc , clay .
Protien , starches , botanical , gums and resin.
Humectants .
Color and pigments .
Preservatives , antioxidants and chelating agents .
Fragrances , essential oils .

Source adapted from Orth. [(4)]

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The microorganisms commonly isolated from the poorly preserved water-based products include *Klebsiella* , *Enterobacter* , *Staphylococcus* , *Bacillus species* , *Pseudomonas aeruginosa* , *Penicillium* and *Candida albicans* [8] . Dawson and Reinhard [9] survey 15 different brands of eye shadow and they recovered 67% of them were contaminated with one or more species of microorganisms representing the genera *Staphylococcus* , *Micrococcus* , *Corynebacterium Acinetobacter*, *Bacillus* and *Moraxella* . Table (2) shows some potentially pathogenic bacteria isolated from cosmetics products and some of these organisms are part of the normal human flora[10] .

Table 2: Potentially pathogenic bacteria isolated from cosmetic preparations

Acinetobacter calcoaceticus	*Escherichia coli*	*Providencia rettgeri*
Citrobacter diversus	*Hafnia alvei*	*Providentia 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 gergovia		

The united state pharmacopeia (USP) specifies 4 bacterial indicators for cosmetics contamination include : *Salmonella spp.* , *Staphylococcus aureus* , *Pseudomonas aeruginosa* and *Escherichia coli* , the European pharmacopeia (EP) specifies these same 4 bacterial indicators including an additional requirements for ascertaining the different levels of *Enterobacteriaceae* [11].The addition of organic material greatly increases the chances of growth and deposits or turbidity due to algae , mold , bacteria or yeast in a range of poorly preserved pharmacopeia solutions . Emulsions can become thin , separate , decolorize or change color and become visibly heterogeneous owing the hydrolysis of the oil phase or change in pH of the aqueous phase [7] . Contaminants may be seen as sediments , turbidity and pigments such as the red prodigiosin of *Serratia marcenscens* and greenish pigments of *Pseudomonas* and these pigments may alter the products appearance [12], Wilson and Ahearn[13] have demonstrated that cosmetics may serve as a possible in transmission and persistence of microorganisms in clinical eye infections . Similarly , others have reported serious infection and even death resulting from direct or indirect exposure to other microbiologically – contaminated cosmetics including mouth wash , hand cream and mascara [14] .The aim of this study is to demonstrate the microbial content of unused products at the point of the sale . The cosmetic products were manufactured in Iraq and were purchased from super markets .

Materials and Methods

Samples

Cosmetic products used in this study including 3 brands of (25) mascara , (30) lip pencil and (25) eye pencil ; all brands were purchased from different factories of Iraqi supermarkets, the period of sampling was 3 months ranging from April to July 2010. Firstly the visible changes were observed like pigments , turbidity and presence of sediments .after that the pH number for each brand were detected by using pH meter .

Aerobic Plate Count

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 micropipette , one ml of the product was disintegrated in tryptic soy broth (9 ml) according to B.P.2010 using a flask shaker and suitable serial dilutions in tryptic soy broth were prepared . One ml sample of each dilution was poured in a sterile petridish and then 15 ml of sterile tryptic soy agar was poured on the samples , the plates were gently swirled in a round movements to allow a good mixing of the agar with the sample , then the plates were allowed to solidify on aleveled surface ,Triplicates plates for each sample were used and incubated at (35°C-37°C) for 24-28 hr. for bacteria . Sabouraud dextrose agar was used instead of tryptic soy agar for the detection of fungi . The prepared plates were incubated at 25°C for 5-7 days. After incubation the number of colonies was counted by estimating the total

count of the growing bacteria and fungi and then the mean of three plates were calculating . A laboratory control count was performed using negative control blank (tryptic soy broth without product) and with positive control (contaminated product).

Detection for specific microorganisms

The detection for specific microorganisms such as *Escherichia coli* , *Pseudomonas aeruginosa* , *Salmonella spp*. And *Staphylococcus aureus* was performed following procedure under isolation and identification test for specified microorganisms (British pharmacopeia BP2010) [14] as shown in (Table 3) , when results showed the presence of any of these microorganisms , appropriate biochemical test were performed , the detection of fungi were depending on morphology of colonies on Sabouraud dextrose agar and the direct microscopic examination by lactophenol aniline blue – stained method[15] .

Table 3 : Isolation and Identification tests for specified microorganisms (BP.2010)

Organism	Enrichment	Primary test	Secondary test	Confirmation
Enterobaceriaceae	Lactose broth 35-37 °C For 24-48 hr.	EEB-Mossel 35-37 °C For 24-48 hr.	VRBGLA 35-37 °C.	Growth of Gram negatives .
Escherichia coli	As above.	MacConkey broth 43-45 °C for 18-24 hr.	MacConkey agar 43-45 °C for 18-24 hr.	Indole at 43.5- 44.5 °C Biochemical.
Salmonella spp.	As above for 5-24 hr.	TBBG broth 42-43 °C for 18-24hr. then subculture on :DCA.XLDA or BGA 35-37 °C for 24-48 hr.	TSI agar 35-37 °C for 18-24 hr.	Biochemical Serological
Pseudomonas aeruginosa	Saline peptone 35-37 °C for 2-5 hr.	Casein digest broth 35-37 °C for 24-48 hr.	Cetrimide agar 35-37 °C for 24-48 hr.	Oxidase test
Staphylococcus aureus	As for *Pseudomonas aeruginosa* above	As for *Pseudomonas aeruginosa* above	Baird-Parker 35-37°C for 24-48 hr.	Coagulase , Catalaes , DNase test

EEB-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 ; TSI: triple sugar iron agar ; DNase : deoxyribonuclease test .

Results and Discussion

Microbial contamination of cosmetic products is a matter of a great importance to the industry and it can become a major cause of both product and economic losses [16]. Results shown in (Table4) investigated that 32.5% of (80) products were contaminated and the Mascara were more contaminated than other tested products . (Table 5) shown that all products contaminated with bacteria in varying degrees including Gram positive bacteria such as *Staphylococcus aureus* , *Staphylococcus epidermidis* and Gram negative bacteria such as *Klebsiella pneumonia* , *Pseudomonas aeruginosa* and *Escherichia coli* , the colony count of all detected bacteria ranging from (10^3- 10^4)C.F.U./ml. (Table 6) represented that all products contaminated with fungi including *Penicillium spp*. , *Aspergillus Fumigatus*.,and *Candida albicans* , the colony count of fungi were ranging from (10^2-10^4) C.F.U/ml . The pH number of all tested products ranging from (6.2-8.1) while the visible changes include color change ,pigments and sediments in some of contaminated products. Poltikin and Ahearn , Ahearn *et al* ,Bharauria and Ahearn reported that mascaras mostly contaminated by *Pseudomonas aeruginosa* , *Staphylococcus epidermidis* , *Klebsiella pneumonia* and *Candida parapislosis* [17][18][19]. Dawson and Reinhardt reported that the genera *Staphylococcus* , *Micrococcus* , *Acinetobacter* , *Bacillus Moraxella* , *Pseudomonas* are contaminated eye pencil [9] . Peter *etal* reported that the fungal contaminants of cosmetics consisted largely of *Aspergillus fumigatus* , *Pencillium* and *Microsporium spp*.[20].The acceptable microbiological limits are recommended in guidelines for a variety of cosmetics preparations , these limits are between 10^2 to 10^3 C.F.U/ml or gram for pathogenic and non pathogenic bacteria [3]. Results obtained in recent study approximating the results of the

mentioned studies . Microorganisms can grow on almost every substances existing in nature and often able to attack or even decompose them , cosmetic ingredients are rich in nutrients that provide organic substrates in the form of sugar , starch , protein , amino acids , organic acids , alcohols , lipids and etc. for microbial growth [21], addition to that ,water is a fundamental requirements for any microorganisms likely to contaminate the cosmetics products , thus untreated or non sterile water can support microbial growth leading to contamination of cosmetics products [11] , generally microorganisms of interest in raw materials or cosmetic products grow best around neutral pH 7.0 and many yeast and molds are able to tolerate acid pH conditions[7] . According to all results obtained in recent study : Most cosmetic products require the addition of preservative to prevent microbial contamination and rancidity, and cosmetic should be produced in a perfectly clean hygienic environment , equipment , instruments , storage tanks and containers should accordingly be maintained in a high standard of cleanliness.

Table 4: The total number of tested cosmetic products and the percentage of contamination in these products

Cosmetics products	Total NO. of products	NO. of contaminated product	Percentage of contaminated products
Mascara	25	10	40%
Lip pencil	30	8	26.6%
Eye pencil	25	8	32%
Total	80	26	32.5%

Table 5: The diagnosed Bacteria and their counts (C.F.U./ml)

Cosmetic products	Diagnosed bacteria	Bacterial counts
Mascara	*Klebseilla pneumonia,* *Pseudomonas aeruginosa,* *Staphylococcus aureus,* *Staphylococcus epidermidis.*	1.5×10^5 C.F.U./ml 20×10^3 C.F.U./ml 3.7×10^4 C.F.U./ml 9.1×10^3 C.F.U./ml
Lip pencil	*Escherichia coli* , *Staphylococcus aureus,* *Staphylococcus epidermidis.*	13×10^3 C.F.U./ml 15×10^4 C.F.U./ml 3.8×10^4 C.F.U./ml
Eye pencil	*Pseudomonas aeruginosa,* *Escherichia coli* , *Staphylococcus aureus.*	1.8×10^4 C.F.U./ml 2.9×10^4 C.F.U./ml 12×10^4 C.F.U./ml

Table 6: The diagnosed yeasts and fungi and their counts (C.F.U./ml)

Cosmetic products	Diagnosed yeasts and fungi	Yeasts and fungal counts
Mascara	*Candida albicans,* *Penicillium spp.* , *Aspergillus fumigatus* .	2.5×10^3 C.F.U./ml 20×10^3 C.F.U./ml 1.6×10^4 C.F.U./ml
Lip pencil	*Candida albicans*	4.8×10^4 C.F.U./ml
Eye pencil	*Candida albicans*	6.6×10^4 C.F.U./ml

Conclusion

Microbiological safety is one of the most dynamic and critical of cosmetics qualityparameters . From this study it was found that microorganisms such as *Escherichia coli* , *Klebsiella pneumonia, Pseudomonas aeruginosa* , *Staphylococcus aureus* ,*Staphylococcus epidermidis* , *Penicillium spp.* , *Aspergillus fumigatus* and *Candida albicans* were contaminated mascara ,

lip pencil and eye pencil in a varying degrees . The cosmetic industry has many compelling reasons to establish and maintain microbiological quality of its products . As these rarely produced under a sterile conditions , 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 products .

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