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Research Article

UNEXPECTED CONTAMINATION OF BRAND NEW (NON-USED) COSMETIC PRODUCTS BY PATHOGENIC MICROORGANISMS

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Abstract

Recently, concern about cosmetics microbial contamination has increased. This is due to their direct impact on human health and economical loss. This study aims to investigate microbial contamination of three brand new cosmetic products such as Eyeliner, Eyeshadow and mascara, and determine the total bacterial and fungal counts in each product. Microbial culture and identification were used to evaluate the microbial contents of particular cosmetics products. In this study, out of 92 cosmetic products were evaluated in which 29.3 % and 21.7 % were found to be contaminated with bacteria and fungi respectively. The isolated contaminants bacteria include *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Escherichia coli*. with bacterial loads between 10^3 and 10^4 CFU per ml. The fungal contamination includes *Candida albicans*, *Penicillium* spp., and *Aspergillus fumigatus* were ranging in number from 10^3 - 10^4 CFU per ml. Mascara was the most contaminated product with bacteria (30 %), followed by Eyeshadow (25 %) and Eyeliner (23.3 %). Moreover, mascara was the most contaminated product with fungi (26.4 %), followed by eyeliner (20 %) and eyeshadow (15.6 %). *Staphylococcus aureus* and *Candida albicans* were isolated from all the cosmetic products. Microbial contamination was detected in the non-used cosmetic products in which pathogenic microorganisms have occurred, which may pose a potential risk to the health of users.

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Key words: Cosmetics, Bacterial contamination, Fungal contamination and Pathogenic microorganisms.

1. Introduction

The definition of cosmetics by Cosmetic Act, Drug and Federal Food as the material that is applied for beautifying, promoting attractiveness, cleansing and human body appearance alter (Butler, 2000). It was found that microorganisms can grow and multiply in cosmetic care products (Fujital and Onyerad, 2005).

In industry knowledge, it is common that microorganisms may grow on some types of cosmetic products (Swinwood and Wilson, 1990). The probability of contamination of cosmetics products can occur during the production process, which leads to a serious concern for manufactured, public and government (Kim *et al*., 2020). In addition to the contamination of the cosmetics when used individually or shared with others (Michalek *et al*., 2019).

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Cosmetics provide a good environment for the survival of different types of microorganisms. Due to the presence of water and nutrients (Kim *et al*., 2020). Starting material used in cosmetics must be in a low level of the microbial count, lower than 10 Colony Forming Unit/g (CFU/g). Moreover, aerobic microorganisms total viable count presents in the cosmetics applied around the eye area needs to be not higher than 10^2 CFU per ml according to the EU guides (Aslam *et al*., 2017). It was found that microorganisms if exceed the average specified by health care organizations, can cause several diseases related to the skin such as eczema, scabies, acne, dyschromia and many other diseases (Mahe *et al*., 2003).

Microorganisms, such *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa Salmonella* spp., and *Clostridium* spp. should be avoided when cosmetics produced (Nf, 2016). Studies found that cosmetics can be contaminated with different microorganisms including *Candida* species, *Escherichia coli* and *Staphylococcus aureus* (Onurdağ *et al*., 2010; Dadashi and Dehghanzadeh, 2016; Eldesoukey *et al*., 2016). It was found that the microorganisms such *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella* spp., can be used as an indicator for microbial contamination of the cosmetics products (Jimenez, 2001).

Fungal contamination was detected in cosmetics, which lead to infection particularly with *Penicillium* species, *Candida albicans* and *Aspergillus fumigatus* (Lundov *et al*., 2009; Babalola and Eze, 2015). For instant, mascara can be contaminated with fungi, the contamination is less frequent compared with bacterial contamination. It can lead to a serious infection in those who wear contact lenses or immune-compromised people (Draelos, 2001; Esteva, 2006; Hassan *et al*., 2008). This study was setup to discover the contamination of some new cosmetic eye makeup products of a different brand that were purchased from different markets in Iraq.

2. Materials and Methods

Makeup Samples

A total of 92 cosmetic products were used in this study. These products consist of five brands (Table 1), include 34 Eyeliner, 32 eye shadow and 34 mascara. All the products were collected from 10 local beauty salons in the province. Sample collection was performed during the period from 2020 to 2021. All the samples were kept at room temperature before being analysed.

Microorganisms

Seven different microorganisms were detected, these samples consist of four bacterial isolates and three fungal isolates. These microorganisms include *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Candida albicans, Penicillium* spp. and *Aspergillus fumigatus*. Pure cultures were obtained from isolates.

Culture media

Tryptic soy agar was used to isolate, maintained and purified the bacterial contaminants. While Sabouraud dextrose agar was used to isolate and maintained fungal contaminants. Other types of media were used for further bacterial identification such as Mannitol Salt Agar (MSA), MacConkey Agar and Cetrimide Agar.

Evaluation of microbial contaminations associated with eye makeup

The collected samples were kept at room temperature and tested as soon as possible. Before samples processing, the sample containers were disinfected using an aqueous mixture of 70 % ethanol (v/v). The contents of samples were taken under a sterilized environment by using a laminar flow cabinet. The total viable aerobic bacterial count was performed according to the British pharmacopoeia 2018 in which 1 ml of the product was mixed in 9 ml of tryptic soy broth. After that, a serial dilution of samples was prepared using the same diluent. Using a sterile petri dish, 1 ml of sample dilution was added then 15 ml of tryptic soy agar was poured over the sample. After gentle mixing of the product in the petri dish, the plates were left to solidify. Each sample was prepared as

triplicate and incubated at 35 – 37 °C for 24 - 27 hours. Similarly, Sabouraud dextrose agar was used to identify fungi. However, the prepared plate was prepared as triplicate, incubated for 5 - 7 days at 25 °C and then identified. Control blank was used which is a plate without a product sample

The count of viable microbial contaminations

According to the number of colonies that appear after incubation of the plates, the mean number of triplicates multiply by sample dilution factors (10^{-2}) were calculated to produce the total number of colonies per millilitre. This calculation was performed for colonies grown on both TSA and SDA, in which the plates with colonies between 30 to 300 were selected.

Microbial identification

The isolation and identification test for bacterial contamination was performed according to the Microbiological Examination of Non-sterile Products (Britishpharmacopoia, 2018). All the isolates were identified according to the appearance of the colony, gram stain result and biochemical test. Furthermore, fungal isolation and identification were performed according to the colony morphology.

Bile tolerant Gram negative bacteria

Primarily, 1 in 10 dilutions of 1 gram of the products was used to be examined under sterile conditions as described in BP 2018. The digest broth of casein soya bean was used as a diluent in which the samples were mix and incubate at 20 - 25 °C for a short period to rest or the bacteria rather than encourage the multiplication of the organisms. The time of incubation was between 2 -5 hours. Secondly, the *Enterobacteria* enrichment broth - Mossel was inoculated with prepared samples at 30 - 35 °C for 24 - 48 hrs. Finally, sub-culture was performed using violet red bile glucose agar plates at 30 - 35 °C for 18 - 24 hrs.

Escherichia coli

To detect *Escherichia coli*, a suitable number of prepared samples was inoculated into Casein soya bean digest broth. The tested tubes were mix and then incubated at 30 - 35 °C for 18 - 24 hrs. Then, the primary test was performed by move 1 ml of casein soya bean digest broth in to 100 ml of MacConkey broth and incubate at 42 - 44 °C for 24 - 48 hrs. Moreover, a secondary test was performed by sub-culture on a plate of MacConkey agar at 30 - 35 °C for 18 - 72 hrs. The conformation result appears as a growing colony of Gram negative bacteria. Furthermore, the Indole test was used at 43.5 - 44.5 °C.

Pseudomonas aeruginosa

To detect *Pseudomonas aeruginosa*, a suitable number of prepared samples was inoculated into Casein soya bean digest broth at 30 - 35 °C for 18 - 24 hrs. Sub-culture performed by growing bacteria on Cetrimide agar and incubate at 30 - 35 °C for 18 - 72 hrs. Furthermore, the Oxidase test was used to confirm the growth of Pseudomonas *aeruginosa.*

Staphylococcus aureus

As mentioned in *Pseudomonas aeruginosa*, a suitable number of prepared samples was inoculated into Casein soya bean digest broth. The tested tubes were mix and then incubated at 30 - 35 °C for 18 - 24 hrs. Then, sub-culture is performed by the cultivation of the detected bacteria Mannitol salt agar and incubate at 30 - 35 °C for 18 - 72 hrs. The appearance of yellow/white colonies surrounded by a yellow zone was confirmed by different biochemical tests such as coagulase, catalase and deoxyribonuclease test.

Identification of the Fungal isolates

The fungal isolates such as *Candida albicans*, *Aspergillus fumigatus* was grown on Sabouraud's dextrose broth at 30 - 35 °C for 3 - 5 days. The incubated mixture was sub-culture on a plate of Sabouraud's dextrose agar at 30 - 35 °C for 24 - 48 hrs. Finally, the white colonies were confirmed by colonies morphology and microscopical examination using special stains such as lactophenol aniline blue.

3. Results

A total of 92 cosmetic products, collected during the period of study were tested for the presence of bacterial contaminations (Table - 1). Mascara was the most contaminated product in which the percentage of contamination was 30 % followed by Eyeshadow and Eyeliner in which the percentage of contamination by bacteria were 25 % and 23.3 % respectively. *Staphylococcus aureus*, was the most common bacteria present in all cosmetic products.

Table - 1: The total number and the percentage of bacterial contamination of the cosmetic products

Cosmetics products	No. of products	No. of contaminated products (%)	Name of bacteria	Bacterial count
Eyeliner	34	0	-*Escherichia coli*, -*Pseudomonas aeruginosa*, -*Staphylococcus aureus*,	2.7×10^4 C.F.U./ml 1.9×10^4 C.F.U./ml 13×10^4 C.F.U./ml
Eyeshadow	28	7 (25)	-*Escherichia coli* - *Staphylococcus aureus*,	3×10^3 C.F.U./ml 14×10^4 C.F.U./ml
Mascara	30	9 (30)	-*Staphylococcus aureus*, , -*Staphylococcus epidermidis*, -*Pseudomonas aeruginosa*	14×10^4 C.F.U./ml 7.3×10^3 C.F.U./ml 17×10^3 C.F.U./ml
Total	**92**	**27 (29.3)**		

Table - 2: The total number and the percentage of fungal contamination of the cosmetic products

Cosmetics products	No. of products	No. of contaminated products (%)	Fungi	The count of isolated yeasts and fungi
Eyeliner	34	7 (20)	- *Candida albicans*	4.3×10^4 C.F.U./ml
Eyeshadow	28	5 (15.6)	- *Candida albicans* - *Penicillium* spp.	3×10^4 C.F.U./ml 2×10^3 C.F.U./ml
Mascara	30	8 (26.4)	- *Aspergillus fumigatus*, - *Candida albicans* - *Penicillium* spp.	1.3×10^4 C.F.U./ml 2×10^3 C.F.U./ml 10×10^3 C.F.U./ml
Total	**92**	**20 (21.7)**		

Regarding fungal contamination of the cosmetic products, mascara was the most contaminated product (26.4 %), followed by eyeliner and eyeshadow in which the percentage of contamination were 20 % and 15.6 % respectively (Table - 2). Mascara was found to be highly contaminated with *Penicillium* spp. followed by *Candida albicans* and *Aspergillus fumigatus*. However, *Candida albicans* was isolated from all the cosmetic products.

The data of combined contamination of the cosmetic product with both bacteria and fungi showed that eyeliner was the most contaminated product (14.7 %) followed by eyeshadow and mascara in which the percentage of contamination with both bacteria and fungi were 10.7 % and 10 % respectively (Table - 3). *Candida albicans* was the most common fungi isolated from the products in addition to contamination with bacteria such as *Staphylococcus aureus* and *Escherichia coli*.

Table - 3: The total number and the percentage of mixed contaminations with both bacteria and fungi

Cosmetics products	No. of products	No. of contaminated products (%)	Name of Microorganisms
Eyeliner	34	5 (14.7)	- *Staphylococcus aureus,* - *Candida albicans*
Eyeshadow	28	3 (10.7)	-*Staphylococcus aureus,* - *Candida albicans*
Mascara	30	3 (10)	- *Escherichia coli* - *Candida albicans*
Total	**92**	**11 (11.9)**	

4. Discussions

In this study, microbial contamination of cosmetic products was evaluated. This is due to the importance of these products on consumer health and the industrial revolution (Washington *et al*., 2006; El-Bazza *et al*., 2009). This study showed, different types of cosmetic products can be contaminated with different type of bacteria and fungi, in which mascara was the most contaminated product with bacteria (30 %) and fungi (26.4 %). A study found cosmetics such as mascara, lipstick, eyeliner and foundation were contaminated with microorganisms. Moreover, fungal growth in the same cosmetic products is less than bacterial growth (Hassan *et al*., 2018). In this work, at least five types of bacteria were isolated such as *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* and *Escherichia coli*. *Staphylococcus* species are commensal organisms found on the skin; opportunistic pathogen such as *Pseudomonas aeruginosa* can produce a serious infections particularly in immunocompromised people (Bashir and Lambert, 2020). In this study, both microorganisms had been isolated.

Staphylococcus aureus, was the most common contaminant in all products. A similar study found that *Staphylococcus aureus, was* the most common contaminant 79 % and *Pseudomonas aeruginosa* 13 % (Orus and Leranoz, 2005). Another study found that cosmetics can be contaminated with a different types of microorganisms including *Escherichia coli* and Enterobacteriaceae (Detmer *et al*., 2010). Besides, a study found that mascara can be contaminated with *Staphylococcus aureus, Staphylococcus warneri* and *Staphylococcus epidermidis* (El-Bazza *et al*., 2009). The colony count of all detected bacteria in this study was ranging from (10^3 - 10^4) CFU per ml. Another study found that the colony count of contaminated cosmetic products ranges from 10^2 - 10^3 CFU per ml (Bashir and Lambert, 2020). The study includes cosmetic products include mascara and eyeliners. Moreover, Different type of fungal contamination was detected in the mascara compared with another type of cosmetic products. In which mascara was found to be contaminated with *Aspergillus fumigatus*, *Candida albicans* and *Penicillium* spp. (Table - 2). *Penicillium* spp. was the highest contaminant followed by *Aspergillus fumigatus* and *Candida albicans*.

Fungi colony in this study was ranging from 10^3 - 10^4 CFU per ml. A similar study found that *Penicillium* spp. is the most fungi isolated in Mascara (Wilson and Ahearn, 1977). Another study found that the colony count of fungi in contaminated cosmetic products ranging from 10^2 - 10^4 CFU per ml (Muhammed, 2011). The study found that all the cosmetic products contaminated with fungi such as *Penicillium* spp., *Candida albicans* and *Aspergillus fumigatus*.

Mixed contamination of the cosmetic products with both bacteria and fungi were observed in this study. In which the eyeliner was the most contaminated product followed by eyeshadow and mascara, the percentage of contamination with both bacteria and fungi were

14.7 %, 10.7 % and 10 % respectively. Despite mascara was the most contaminated product in bacteria and fungi, the eyeliners were the most cosmetic products that have mixed contamination. This may relate to the started material or the brand of the products, which enhanced the growth of mixed infection in particular products such as eyeliner compared to other products. However, another study found that eye shadow samples had higher contamination with bacteria and fungi followed by mascara and eyeliner samples, this may be related to the brand of cosmetic products (El-Bazza *et al*., 2009).

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CONFLICT OF INTEREST

No conflict of interest is declare by the authors.

AUTHORS' CONTRIBUTION

All the authors was collaborated on this review, literature review plane preparation for the work and manuscript editing.

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ETHICS STATEMENT

This article does not include human participants or any animals.

AVAILABILITY OF DATA

The manuscript include all the datasets that have been generated or analyzed.

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