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TYPES OF BACTERIA FOUND IN COSMETICS USED BY FEMALE COLLEGE STUDENTS IN KUWAIT

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ABSTRACT: *The purpose of this study is to determine the presence of bacteria and other microorganisms in cosmetic samples, brought in by female students of the College of Health Sciences, and to identify these bacteria by microbiological analysis. A total of 100 expired, commercial cosmetic products, were provided by college students. They include: Mascaras, lipsticks, lip-glosses, eye shadows, eye-liners, foundation creams, body creams and talk powders. The products were tested for the presence of microorganisms by using general, enrichments and selective media. Microscopic examination and biochemical analysis (Catalase test and API test) were then performed for isolated pure cultures. 22% of the expired cosmetic products were contaminated to various degrees. Two types of yeasts were isolated: Candida Albicans, found in compact powder with a contamination rate of 20%, eye shadows, 6.6% and foundation creams, 10%. Rhodotorula yeast was isolated from eye liner with a contamination rate of 6.6%. In addition, three species of staphylococci were isolated: S. xylosus, S. warneri and S. schleiferi. Other bacterial species, like Micrococcus, Streptococcus and Bacillus species, were also isolated. These species are present normally on human skin as a normal micro-flora However, some of these microorganisms, known as opportunistic pathogens, may cause skin infection and irritation, especially, when sharing these products with others, as the micro-flora may vary from one person to another.*

KEY WORDS: microorganism, contamination, cosmetic, products, expired**.**

INTRODUCTION

Cosmetics are defined as " any material that can be rubbed, poured, sprinkled, sprayed, or otherwise applied to the human body for beautifying, cleansing, promoting attractiveness

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or altering appearance" [1]. Cosmetics include different products such as foundations, compact powders, lipsticks, eye liners, eye shadows and brushes.

Cosmetics have been used by different cultures and races throughout the entire world, and has become a part of women's life, therefore, the use of cosmetics are considered a frequent routine tool to make women beautiful, presentable, attractive and self-confident. Nevertheless, the amount of consumption of cosmetics has increased rapidly with the increase of population [2]. College students are considered an important group of cosmetic consumers; thus, it is during adolescence females become more concerned with their appearance [3]. Many students use cosmetics unaware of the health dangers they are exposed to, when using contaminated products [4]. Cosmetic products are non- sterile, and most of them provide an optimal medium for microbial contaminants [5, 6, 7]. The composition of cosmetic products with the warm and humid climatic conditions may encourage the growth of many bacteria and molds, which could lead to biodegradation of the product, and increase the risk of infection to the users [8].

Microorganisms, when contaminating cosmetics, can cause spoilage to the product or may cause serious health risk for consumers [9]. This may happen for many reasons like mishandling the product during manufacturing which can cause defects in the preservative capacities of makeup, or due to the poor quality of raw materials used in makeup [10]. Using the same product by many applicants increases the level of contamination. Inadequate storage conditions of makeup can also determine the growth of bacteria, leading to microbial contamination [11]. Moreover, the use of old makeup makes the preservatives in the products lose their effectiveness, making the product a suitable place for microbial growth [12]. Many students share their applicators and makeup with friends and family, increasing the numbers of pathogens that poses a serious health threat to these applicants, especially those who have a low immune system and are already ill or are in a weakened state [13, 14]. Some students keep using their makeup until it’s finished although the expiry date is overdue, which results in hazardous effects to health [15, 16].

In one study, old foundation brushes, lip gloss and lipstick were contaminated with *Enterococcus faecalis*, which is a deadly strain of bacteria, causing neonatal meningitis and septicemia [17]. In another study, 91 cosmetic samples were examined for microbial contamination, in three steps: before using the product, while using the product, and after using these cosmetic products. The results showed that none of the samples were contaminated before using them, however, 6 samples were contaminated, with

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Staphylococcus spp. while using them, and all the other samples were contaminated after using these cosmetics [9]. In addition, some bacteria and molds produce toxins which can cause allergic reactions and skin irritations [18]. *Bacillus cereus* bacteria were found in the lip-gloss, causing a health risk and when applied on the lips, it can be ingested and can stimulate gastro- enteric infections [19]. These bacteria released toxins into the crack lip tissue causing break down of that tissue.

The purpose of this study was to determine the presence of bacteria and other microorganisms in cosmetic samples brought in by female college students and identify these bacteria by microbiological analysis.

Materials and Methods

A total of 100 commercial cosmetic products, provided by college students, consist of fifteen from each of the following items: Mascaras, eye liners, and eye- shadows; twenty lipsticks, ten lip gloss, ten body creams, ten foundation creams and five compact powders. All these cosmetic products were used by the students over twelve months. All items were examined for production and expiration date. The outer surface of each sample was swabbed and cleaned with 70% ethanol, to minimize any external contamination of the products prior to microbiological analysis.

Microbiological analysis

All items were cultured on sterile Nutrient Agar plates. The samples were then grown on selective media, such as MacConkey Agar and Mannitol Salt agar. Blood agar was used as an enrichment media and to detect the hemolytic activity of bacterial cultures. The inoculated cultures were incubated for 24-48 hours at 37C°.

Sabouraud Dextrose Agar (SDA) is another selective medium primarily used for the isolation of dermatophytes and other fungi. The acidic pH of this medium (pH about 5.0) inhibits the growth of bacteria but permits the growth of yeasts and most filamentous fungi. Antibacterial agents can also be added to augment the antibacterial effect.

Cultural characteristics of the isolated colonies include form, color, size, elevation, and margin of the isolated colonies.

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Microscopic examination

Gram staining method was applied for bacterial isolates, to differentiate between two groups of bacteria, Gram-positive and Gram-negative bacteria. In addition, Spore Staining was also performed, to differentiate between spore forming from none Spore forming bacteria.

Bacterial count

Viable count was performed to see the number of living bacteria and to calculate the colony forming unit (CFU) of each isolate. It is carried out by surface spread technique under sterile conditions, by adding 0.5 g of cosmetics to nine ml nutrient broth followed by making serial dilutions [20]. A 0.1ml of each diluted sample was poured on Nutrient Agar plates followed by spreading the dilated sample onto the surface of these plates with a glass spreader. The cultures were then incubated for 24-48 hours at 37C°.

Biochemical analysis

Catalase Test was used to identify catalase-producing bacterial isolates. The bacterial respiratory enzyme (Catalase) acts as a catalyst in the breakdown of hydrogen peroxide into water and oxygen. Coagulase test was used to identify *Staphylococcus aureus* which is able to produce the enzyme coagulase that clots blood plasma.API- Test (Analytical Profile Index), are commercial miniaturized biochemical test panels that cover a significant number of clinically important groups of bacteria, as well as food- and water-associated microorganisms. The following API rapid tests were used: API for *Staphylococcus* Identification, API for *Streptococcus* Identification.

Results were compared with API data base of reference strains (apiweb from "Biomerieux"); the isolated microbial strains were identified according to the species level.

Fungi Identification

Fungi were grown on both nutrient agar and Sabouraud Dextrose Agar (SDA). They were identified on the bases of cultural characteristics, followed by microscopic identification for further confirmation of these isolated fungi.

RESULTS

From 100 cosmetic product samples, used for more than a year by college students, a total of 22 cosmetic products (22%) were contaminated with different types of bacteria and fungi table 1.

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Table 1: Type and number of tested cosmetic products, and the contaminated percentage in these products

No. of Samples	Type of Product	Number of products	No. of contaminated products	Percentage of contamination / product
1	Mascara	15	1	6.6%
2	Eye liner	15	5	33.3%
3	Eye- shadow	15	7	46.6%
4	lipstick	20	2	10%
5	Lip gloss	10	2	20%
6	Body cream	10	1	10%
7	Foundation cream	10	2	20%
8	Compact powder	5	2	40%
Total		100	22	22%

Two kinds of yeasts were isolated: Candida Albicans, found in compact powder (with a contamination rate of 20%), eye shadow (6.6%) and foundation cream (10%). Rhodotorula isolated from eye liner with a contamination rate of 6.6%. Identification of these isolates based on growth characteristics as shown in (Table 2).

Table 2: Fungi Isolated from cosmetic samples:

Cosmetic product	Colonial Morphology of Fungi	Diagnosed Fungi and yeast	Yeast and fungal count	Percentage of contaminated products
Compact powder	Rough, filamentous border, composed of hyphae and pseudohyphae	*Candida albicans*	1.3×10^3	20%
Eye-shadow	Rough, filamentous border, composed of hyphae and pseudohyphae	*Candida albicans*	2.6×10^3	6.6%
Eye liner	Pink to coral color colonies. Colonies are yeast like, smooth, moist, mucoid, and soft. They produce budding yeasts that are round or oval	*Rhodotorula*	1.2×10^3	6.6%
Foundation cream	Rough, filamentous border, composed of hyphae and pseudohyphae	*Candida albicans*	1.5×10^4	10%

Eighteen bacterial isolates were found in mascaras, eye liners, eye shadows, lipsticks, lip-glosses, body creams, and compact powders. Eye- shadow showed the highest rate of

contamination with a 46.6%, followed by compact powder with a 40%, eyeliner 33.3%, lip-gloss and foundation cream 20%, body cream, and lipstick each 10%, finally, mascara 6.6%, show in (table 1). Other results include growth on selective media and the type of hemolysis on blood agar (table 3); bacterial counts and CFU/ml (table 4). Index 1 shows Cultural and microscopic characteristics of bacterial isolates.

Table 3: Diagnosed bacteria based on growth on selective media, the type of hemolysis on blood agar, and API tests.

Sample name	MacConkey Agar	Mannitol Salt Agar	Blood Agar	Diagnosed bacteria Based on API
Mascara3	Negative	Negative	β -hemolysis	*Bacillus spp.*
Eyeliner1	Negative	Negative	α -hemolysis	*Aerococcus viridans*
Eyeliner3	Negative	Negative	γ-hemolysis	*Kocuria varians/ rosea*
Eyeliner5	Negative	Pink growth	γ –hemolysis	*Micrococcus roseus*
Eyeliner7	Negative	Negative	γ -hemolysis	*Staph. simulans*
Eye-shadow 1	Negative	Negative	γ–hemolysis	*Kocuria varians/ rosea*
Eye-shadow 2	Negative	Negative	β -hemolysis	*Bacillus spp.*
Eye-shadow 3	Negative	Negative	γ–hemolysis	*Staph. xylosus*
Eye-shadow 4	Negative	Negative	γ-hemolysis	*Micrococcus spp.*
Eye-shadow 5	Negative	Negative	γ-hemolysis	*Micrococcus spp.*
Eye-shadow 7	Negative	Negative	γ-hemolysis	*Bacillus spp.*
Lipstick 4	Negative Negative	Negative Pink growth	γ –hemolysis γ -hemolysis	*Staph. simulans* *Micrococcus spp.*
Lip gloss 9	Trans. Negative opaque-Negative	Negative Negative	β -hemolysis β -hemolysis	*Bacillus spp.* *Staph. Schleifer*
Compact powder 2	Negative	Negative	γ –hemolysis	*Staph. Warneri*
Body Cream 4	Negative	Negative	γ -hemolysis	*Staph. simulans*
Foundation cream 1	Negative	Negative	γ-hemolysis	*Bacillus spp.*

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Table 4: Bacteria counts (C.F.U. /ml)

Sample name	Bacterial count
Mascara3	1.99×10^{3}
Eyeliner1	6.1×10^{4}
Eyeliner3	6.8×10^{4}
Eyeliner5	$1.\times10^{4}$
Eyeliner7	2.57×10^{5}
Eye-shadow 1	1.2×10^{4}
Eye-shadow 2	6.0×10^{5}
Eye-shadow 3	2.77×10^{4}
Eye-shadow 4	2.9×10^{3}
Eye-shadow 5	2.0×10^{3}
Eye-shadow 7	1.4×10^{4}
Lipstick 4	3.63×10^{5}
Lip gloss 9	7.0×10^{4}
Compact powder 2	1.38×10^{6}
Body Cream 4	1.5×10^{3}
Foundation cream	2.85×10^{6}

Biochemical analysis of isolated bacteria from cosmetic samples

Calalase test was performed for all samples tested. All isolates were catalase positive except for *Aerococcus viridans*. Coagulase test was negative for *Staphylococcus* isolates tested. API *Staphylococcus* allowed the identification of the following species (Table 3): *Micrococcus* spp., *Micrococcus roseus, Kocuria varians/ rosea , Staphylococcus Simulans, Staphylococcus xylosus, Staphylococcus schleiferi* and *Staphylococcus warneri*. API streptococcus identified one isolate as *Aerococcus viridians*.

DISCUSSION

This study showed that the expired used cosmetic products were mostly contaminated with different microorganisms. Viable count was performed for the identified microorganisms which showed contamination of more than 10^{2}cfu/g of the product according to publisher of the Danish ministry of the environment [21].

It was found that a high diversity of bacterial and fungal contamination was obtained in the shared cosmetics by college female students, and since the skin micro-flora for each person

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is distinctive, therefore it could cause harm to the other person while sharing the same product. Results showed that eye- shadow (powder like) had the highest rate of contamination with a 46.6%, compact powder showed a 40% contamination, which indicates that powders have higher levels of contamination than the other products. This may be due to the high exposure of these products to air and skin, causing that higher contamination to the powders.

Both *Candida* and *Rhodotorula* were isolated from different cosmetics. *Candida* was isolated from old (compact powder, eye shadow and foundation cream). Contamination rate reached the highest in compact powder 20%, followed by foundation cream with a 10%, and 6.6% in eye shadow. Candida is opportunistic yeast that can become pathogenic when the body immune system is weakened [22]. It causes skin lesions, dermatitis and rashes [23]. *Candida albicans* may cause cutaneous candidiasis. It often occurs in warm, moist, creased skin areas of the body, like the armpits. It has been reported in other personal toiletry studies the presence of *Candida.* [24][25]. *Rhodotorula* was found in eyeliner with a contamination rate of 6.6%. It is an ubiquitous saprophytic yeast, common in the environment, humans, plants and animals. *Rhodotorula* was isolated in a study from patients and hospital employees' hands [26].

In this study, three species of *Staphylococcus* were isolated from the used, expired cosmetics, *S. xylosus, S. warneri* and *S. schleiferi. Staphylococcus xylosus,* was isolated from eye shadow, *S. xylosus* can inhabit the skin and the mucous membrane of both mammals and birds [27, 28], and it has the ability to adapt to different environments; therefore, it is considered ubiquitous and can be found in numerous niches [29, 30, 31]. *S. xylosus* is found naturally in food, raw meat and milk; they are defined as nonpathogenic *Staphylococcus,* but some strains of *S. xylosus* are related to human and animal opportunistic infections [32, 33, 34, 35].

Staphylococcus warneri was found in compact powders; it is a commensal of the skin of animals and humans. 50% of the populations have *Staphylococcus warneri* and this species represents less than 1% of the total staphylococcal population [36]. They are common nosocomial pathogens that can cause illness and severe reactions in those with weak immune system [37]. In literature, cases of infection with *Staphylococcus warneri* were reported, such as endocarditis, osteomyelitis, septicemia, and urinary tract infection [38, 39]. .

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In this study, one strain of *Staphylococcus schleiferi* was isolated from a lip-gloss of the students. In 1988 Freney *et al*. was the first to report *Staphylococcus schleiferi* as a new coagulase negative species [40]. It was then considered to be a part of the skin flora [41], especially the preaxillary skin [42, 43]. It has also been reported in adults that have wound infections, when in contact with dogs [40, 44, 45, 46]. *Staphylococcus simulans,* is a *Coagulase*-negative staphylococci, found in eyeliner, body cream and lipstick of the used expired products. *Staphylococcus simulans* are mostly animal pathogens that cause infections in horses, cows, goats, and sheep. It may cause infections to humans, when in touch with these animals. *S. simulans* has been implicated in native valve endocarditis, osteoarticular infections, and diabetic osteitis, [47, 48, 49] with diabetes and prosthetic joints identified as additional risk factors.

Micrococcus species were isolated from the lipstick, eye liner and eye shadow. They are calalase positive and coagulase negative, they are found usually in soil, water and colonize human skin especially the face, hands and legs, in people harboring animals and plant. Occasionally, *Micrococcus species* can cause invasive disease to immune-compromised patients. *Kocuria* species, known before as *Micrococcus kristinae,* isolated from the expired eye shadow and eye liner in the current study. It has been reported to be normal flora of the skin, mucous membrane and oral cavity of human and animals [50]. It is found usually in the urinary tract of patients with urinary tract infections [51], it is also isolated from different environments and ecological niches [52]. They are mostly regarded as laboratory contaminants and usually as non-pathogenic bacteria which are seldom associated with human infections [53, 54]. The expired eyeliner was harbored with *Aerococcus viridians* (a S*treptococcus spp*), which is a gram positive, alpha- hemolytic, catalase negative. It is considered a significant human pathogen capable of causing subacute bacterial endocarditis, urinary tract infection and bacteraemia [55]. *Bacillus* species are also transient skin micro-flora; they are mostly air and soil contaminants. In this study *Bacillus* species was isolated from Mascara, eye liner, eye shadow, lipstick, lip gloss and foundation cream. Reports showed that *Bacillus subtilis* is involved in food poisoning [56]. Results showed that *Bacillus* spp. was found in mascara, eye shadow, lip gloss and foundation cream. *Bacillus cereus* a widespread organism found in the environment and is significant hazard for eye trauma, caused during application of near-eye cosmetic products. Individuals that suffered trauma to the eye or eye infections should not use near eye product, to minimize the risk of infection caused by *B. cereus* and also to

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reduce the risk of infection caused by other microorganisms that harbor non-sterile cosmetics at low numbers [57].

CONCLUSION

It can be concluded from this study that expired, used cosmetics were contaminated by different opportunistic pathogens such as, *Bacillus* species, *Streptococcus spp*., *Micrococcus species* and *Staphylococcus spp*., all these microorganisms were gram positive bacteria that are predominant contaminants in the skin flora, these isolates may cause skin infection and irritation, especially, when sharing cosmetic products with others that differ in the micro-flora from one person to another.

Recommendation

It is of great importance to elevate and improve health education programs among students about the harm behind using expired, old cosmetic products that could impair their health causing serious infections and other hazards. Proper storage of used cosmetics should be considered, to keep these cosmetic products from expiration before the expected duration time of expiration.

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Index 1: Cultural characteristics of the isolated colonies from cosmetics & Result of Microscopic examination of isolated bacteria from cosmetics

Sample name	No. of Colonies	color	Size	Form	Margin	Elevation	Gram Stain	Shape of Bacteria
Mascara3	1	creamy	Small/ Medium	irregular	undulate	Flat	G+ve	Short bacilli Spore forming
Eyeliner1	1	yellow	Pinpoint	circular	undulate	Raised	G+ve	Irregular clusters
Eyeliner3	1 1	Light yellow Red to coral	Small Small	circular circular	entire entire	Convex convex	G+ve	Short chains, tetrads. irregular clusters Yeast like
Eyeliner5	1 1	Dark yellow to orange Pink	Small Pinpoint	Circular circular	Entire/ undulate Entire	Raised convex	G+ve G+ve	Bacilli spore forming Irregular clusters
Eyeliner7	1	Pale yellow	Medium	circular	entire	Raised	G+ve	Staphylococci
Eye-shadow 1	1	yellow	Small	circular	entire	convex	G+ve	Short chains, tetrads, irregular clusters
Eye-shadow 2	1	Translucent	Small	circular	entire	convex	G+ve	Bacilli
Eye-shadow 3	1	Gray white	Small/Medium	circular	entire	Raised	G+ve	Clusters of cells.
Eye-shadow 4	1	yellow	Small	circular	entire	convex	G+ve	Staphylococci
Eye-shadow 5	1 1	pink Yellow	pinpoint pinpoint	circular circular	entire entire	convex raised	G+ve G+ve	Irregular clusters Bacilli/ short thin rods, spore forming
Eye-shadow 7	1	white	small	circular	entire	raised		*Candida albicans*
Lipstick 4	1 1	orange1 pale yellow	Small Medium	Circular Circular	entire entire	convex Raised	G+ve G+ve	Bacilli, spore forming Irregular clusters
Lip gloss 9	1 1	off-white Opaque	Small Small/Medium	Irregular Circular	Undulate Entire	Flat Raised/ convex	G+ve G+ve	Bacilli spore forming Staphylococci
Compact powder 2	1 1	white (Fungi) GrayWhite	Medium Small	Circular Circular	Entire Entire	convex Raised	- G+ve	Fungi Candida Albicans Clusters
Body Cream 4	1	Pale yellow	Medium	Circular	Entire	Raised	G+ve	Staphylococci
Foundation cream 1	1 1	White Beige White	Small/Medium Small	Irregular	Lobate	Flat	G+ve	Bacillus spore forming Candida Albicans

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