Original article

Isolation and Identification of Microorganisms associated with high-quality and low-quality cosmetics from different brands in Mecca region -Saudi Arabia

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ABSTRACT

Cosmetic products contain several components that are ideal for microbial growth, they exposed to contamination by pathogenic bacteria and fungi, and this may cause health risks such as skin and eye infections. In this investigation, 50 samples were obtained from various shops in Mecca region, Saudi Arabia. Collected samples include high-quality and low-quality brands of makeup. Results show that most cosmetics are contaminated with microorganisms. Bacterial and fungal isolates were identified by morphological and microscopic techniques, and confirmed by molecular methods: (16s rRNA) for bacterial isolates and (18s rRNA) for fungal isolates associated with cosmetics. In low-quality cosmetics, frequency of microbial growth is higher and more diverse than high-quality cosmetics. It has been observed the most contaminated product was in lip gloss and it follows by the lipstick. The most predominant species of bacteria are *Staphylococcus aureus* (27 %), *E. coli* (27 %), which follows by *Streptococcus pneumonia* (18 %), *Staphylococcus epidermis* (17 %), *Bacilli subtilis* (12 %), and *Pseudomonas aeruginosa* (5 %). *Aspergillus* sp is the most predominant fungi (57 %), which is followed by *Penicillium sp.* (29 %) and *Rhizopus* sp. (14 %). In high quality brands, the frequency of microbial growth was the highest in mascara, lip-gloss. The most predominant species of bacteria is *Staphylococcus aureus* (41 %), which follows by *Bacilli subtilis* and *Pseudomonas aeruginosa* and *E. coli* (17 %). *Streptococcus pneumonia* is the less dominant (5 %). There is no growth on media of fungi. Due to the large number of cosmetics brands in Mecca region and for consumer safety, this study is prepared.

1. Introduction

Cosmetics are synthetic or natural substances for use on various regions of the human body skin including (lips – eyes) (Oliveira et al., 2020). Cosmetics are produced in large numbers of brands, with different prices and quality. They are used regardless of whether they are safe or unsafe. They may have been contaminated during manufacture or storage. The majority of cosmetics are not sterile and are derived from non-sterile basic materials (Choubey and Godbole, 2017). Most cosmetic products contain several components that are favorable for microbial growth since they are not often manufactured in a media that is sanitized against microbial growth. Items could be contaminated in the factory (Siegert et al., 2005). Bacteria, notably *Enterobacteria* and *Staphylococci* are the most studied microbes polluting composition (Bashir and Lambert, 2020; Dashen et al., 2011). Pathogenic bacteria found in beauty products include *Pseudomonas aeruginosa* and

Staphylococcus aureus (Sreeparna et al., 2017; Detmer et al., 2010). The most common bacteria that cause human diseases, such as skin infections, were *Staphylococcus epidermidis* and *Staphylococcus aureus*. Pathogenic filamentous fungi are of particular interest since they are linked to opportunistic infections as well as mycotoxin toxicity (Peraica et al., 1999; Hayleeyesus and Manaye, 2014).

Raw materials for cosmetics may be polluted in the first place. Additionally, water used in non-sanitized production may be contaminated. Such circumstances provide an ideal setting for microbial growth. Makeup can have negative effects on women's health if not handled properly. Cosmetic contamination can cause some kinds of infections ranging from mild to severe. There are two key reasons for needing conservatives: to prevent goods from being spoiled by microbes and to prevent disease caused by microbes (Nasir and Qasim, 2020). Cosmetics also contain a wide range of chemicals such as fragrances, preservatives, pigments and other substances known as skin sensitizers which are

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Table 1
Group (1): Cosmetics products - low quality brands.

Sample number	Cosmetic category	Number of samples collected	Expiry date	Country of region
1	Lipsticks	4	9/2024	China
2			9/2024	China
3			–	China
4			–	China (P.R.C)
5	Lip-gloss	5	7/2026	China
6			5/2024	China
7			4/2025	China
8			4/2026	China
9			4/2025	China
10	Powders	3	9/2024	China
11			8/2022	China
12			After used (30 month)	England
13	Plusher	2	12/2023	China
14			After used (24 months)	Italy
15	Mascara	3	After used (6 months)	China
16			5/2026	China
17			9/2026	China
18	Foundation	3	After used (12 months)	France
19			After used (12 months)	USA
20			After used (12 months)	France
21	Concealer	3	12/2023	China
22			–	–
23			7/2024	China
24	Eye pencil	2	4/2025	China
25			5/2025	China
26	Eye liner	2	6/2026	China
27	Eye Shadow	3	10/2026	China
28			3/2024	China

Table 2
Group (2): Cosmetics products high-quality brands.

Sample number	Cosmetic category	Number of Collected samples	Expiry date	Country of region
29	Lipsticks	3	10/2023	France
30			09/2023	Italy
31			11/2023	USA
32	Lip-gloss	3	2/2023	UK
33			11/2024	France
34			10/2023	USA
35	Powders	3	08/2024	Italy
36			09/2024	USA
37			11/2023	UK
38	Plusher	2	08/2024	France
39			09/2024	USA
40	Mascara	3	12/2023	France
41			11/2024	USA
42			01/2024	Italy
43	Foundation	3	09/2023	France
44			07/2024	USA
45			03/2024	Hong Kong
46	Concealer	1	09/2023	Poland
47	Eye pencil	2	08/2024	Uk
48			03/2024	France
49	Eye Shadow	2	08/2024	
				Germany
50			02/2024	

potentially harmful on general health (Panico et al., 2019). Microbial contamination of cosmetic products is a serious concern for the business, and it has the potential to be a major cause of both economic and product losses. Cosmetics are prone to microbial growth due to the presence of nutrients and water. Microorganisms are frequently the cause of organoleptic changes such as changes in color, viscosity, and disagreeable odors (Becks and Lorenzoni 1995; Behravan et al., 2005).

Furthermore, in rare situations, contaminated bacteria or their activities may cause human health concerns such as allergic contact dermatitis, skin irritation, and infection, particularly in the mouth, eyes,

Table 3
Cosmetics samples products with microbial contamination in low-quality makeup.

Cosmetics Category	Number of samples	Media type			
		Nutrient Agar	**Blood Agar**	**MacConkey Agar**	**Potato dextrose Agar**
Lipsticks	1	*S.aureus*	*S.aureus*	*Pseudomonas aeruginosa*	–
	2	–	–	*Pseudomonas aeruginosa*	–
	3	–	*Streptococcus pneumonia*	*Pseudomonas aeruginosa*	+ *Aspergillus*
	4	–	–	–	–
Lip-gloss	5	*Staphylococcus epidermidis*	–	–	–
	6	*E. coli*	*Staphylococcus epidermidis*	–	–
	7	*Staphylococcus aureus*	*Streptococcus pneumonia*	–	–
	8	*Staphylococcus aureus*	*Staphylococcus aureus*	–	–
	9	–	*Staphylococcus aureus*	–	+ *Aspergillus*
Powders	10	*Staphylococcus epidermidis*	–	–	+ *Penicillium* sp
	11	*Streptococcus pneumonia*	*Staphylococcus aureus*	*Escherichia coli*	–
	12	–	–	–	–
Plusher	13	*Staphylococcus aureus*	*Staphylococcus aureus*	*Escherichia coli*	+ *Aspergillus*
	14	–	*Staphylococcus aureus*	*Escherichia coli*	–
Mascara	15	–	–	–	–
	16	–	–	–	–
	17	–	–	–	–
Foundation	18	–	Bacilli (G +)	–	+*Rhizopus*
	19	–	–	–	–
	20	*Staphylococcus epidermidis*	–	*E. coli*	+ *Aspergillus*
Concealer	21	*E. coli*	–	*E.coli*	–
	22	–	–	–	–
	23	*Streptococcus pneumonia*	–	–	–
Eye pencil	24	–	–	–	–
	25	–	–	*E. coli*	+ *Penicillium* sp
Eye liner	26	*Staphylococcus aureus*	*Staphylococcus aureus*	–	–
Eye Shadow	27	*Streptococcus pneumonia*	–	–	–
	28	*Staphylococcus aureus*	–	–	–

No growth (−).

Table 4
Microorganisms in the samples from cosmetics products in low-quality makeup.

Cosmetics category	Bacterial isolates	Fungal isolates
Lipsticks	*Staphylococcus aureus., Streptococcus pneumonia, Bacillus subtilis. Pseudomonas aeruginosa*	*Aspergillus sp*
Lip-gloss	*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Streptococcus pneumonia*	*Aspergillus sp*
Powders	*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli.*	*Penicillium sp*
Plusher	*Staphylococcus aureus, Escherichia coli.*	*Aspergillus* sp.
Mascara	–	–
Foundation	*Bacilli subtilis, Staphylococcus epidermidis, Escherichia coli.*	*Aspergillus sp, Rhizopus sp* .
Concealer	*Streptococcus pneumonia, Escherichia coli*	–
Eye pencil	*Escherichia coli.*	*Penicillium sp*
Eye liner	*Staphylococcus aureus*	–
Eye Shadow	*Staphylococcus aureus, Streptococcus pneumonia*	–

or wounds (Orús et al., 2015). Cosmetics are exposed to contamination by microorganisms and may cause health risks such as skin and eye infections. In fact, a lot of microbes can enter the product via hand and mouth (Siegert, 2013; Dadashi and Dehghanzadeh, 2016). Occurrence of microbes is caused by two major factors: The first is a result of improper application of cosmetics or a failure to follow adequate hygiene procedures. Even though the products contain preservatives, this enables the deposit of skin-derived detritus, which favors the development and spread of microorganisms. The dispersed particles in indoor air, which include dust, bacteria, and spores, are the second and most significant cause of microbial proliferation in cosmetics (Oliveira et al., 2020).

Cosmetics need to be clean and healthy by adding preservative substances both to prevent contamination and financial loss. The additives used in the products should not be harmful to customers because the majority of the beautifying agents were used in delicate areas (Siya et al., 2019). Additionally, particularly in humid conditions, poor air quality has a significant impact on the growth of microbes in cosmetics

(Mohammed et al., 2021). Lack of attention to proper manufacturing practices, insufficient preservation systems, insufficient microbiological test procedures, and/or insufficient microbial limits for finished products are thought to be the causes of contamination (El-Bazza et al., 2009).

Therefore, a good cosmetic product will contain a good preservative system that will last for a given period of time indicated by expiry dates. Expiry date on cosmetics indicates that after the given date the product may be altered and is risky to use (Nuzhath, 2014). These preservatives are different ingredients added to cosmetics, some of them are synthetic organic compounds like petrolatum chemicals such as Parabens, Formaldehyde, Paraformaldehyde, Mercury, Butylated Hydroxy anisole (BHA), Butylated Hydroxytoluene (BHT), PEGs, cocamide DEA and toluene (EWG, 2020). Other ingredients are natural like water and oils (Halla et al., 2018).

Under the proper physicochemical circumstances all products including cosmetics containing water and inorganic/organic compounds are exposed to microbial contamination in order to ensure
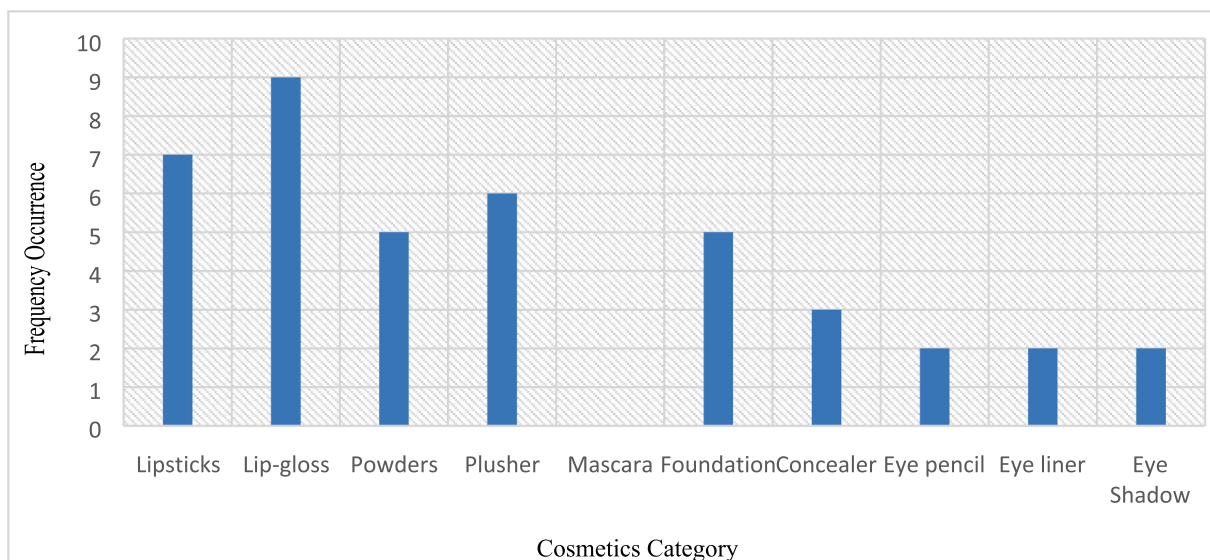
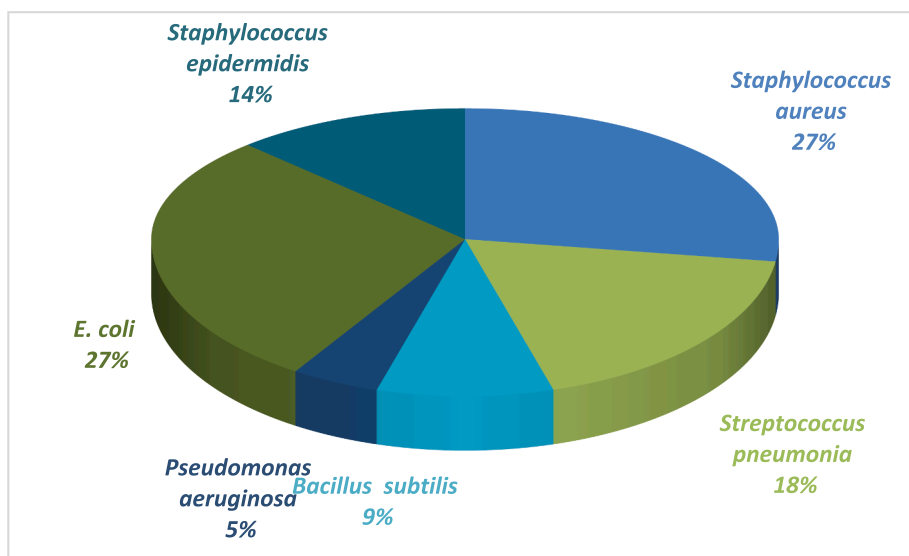**Chart 1.** Frequency of microbial growth in low-quality makeup.

Chart 2. Percentage of bacterial growth in low-quality makeup.

microbiological safety of cosmetics products. This inhibition should have a broad range of activity effectiveness and should last for a longer period of time than the cosmetic product itself, which would be similar to the projected shelf-life plus the consumption time. As a result, all possible sources of contamination must be identified and monitored (Choubey and Godbole, 2017).

Generally, microbial contamination of cosmetics can be one of these reasons (1) raw material evaluation and control, (2) manufacturing process, (3) final product delivery and finally, (4) consumer use to prevent microbial contamination without causing the product's properties (Halla et al., 2018). Due to the large number of cosmetics brands in Mecca region and for consumer safety, the study's primary goals are to: a) assess the level of microbial contamination in high-quality and low-quality cosmetics, b) isolation and identification of microorganisms from unused different brands of cosmetics.

2. Materials and methods

2.1. Samples collection

A total of 50 unused cosmetic samples were collected from different shops in Mecca region, it is situated in the western part of KSA, at a longitude of 39.826168 East and a latitude of 21.422510 North. Collected samples were divided into two groups regarding to their quality; Low- quality brands (Table 1) and High -quality brands (Table 2), the products were separated by number, from 1 to 28 for low quality products: (4) lipsticks, (5) lip-glosses, (5) powders, (3) mascara, (3) liquid foundation, (3) concealers, (2) eye- pencils, (1) eye-liner, (3) eye shadows. Samples from 29 to 50 are high quality products as a follow: (3) lipsticks, (3) lip-glosses, (3) powders, (3) mascara, (3) liquid foundation, (1) concealers, (2) eye- pencils, and (3) eye shadows. Samples were put in sterile plastic bags and transferred to the lab. Each category of cosmetics was numbered and labeled with information like brand, expiration date and country of origin.
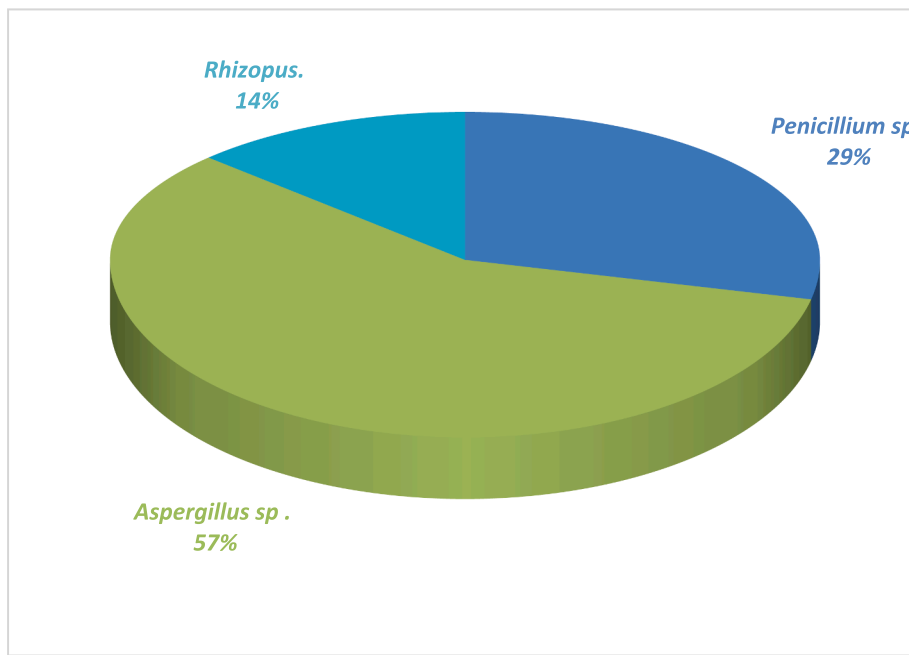
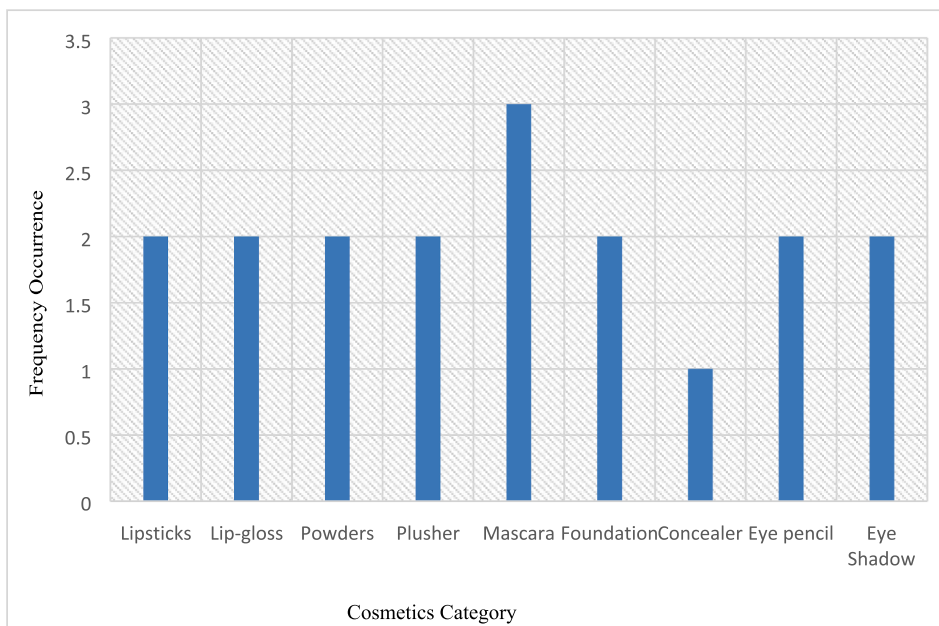**Chart 3.** Percentage of fungal growth in low-quality makeup.

Chart 4. Frequency of bacterial growth in high-quality makeup.

2.2. Media for growing microorganisms associated with cosmetics

Three different media were used to isolate pathogenic bacteria. These media are: (1) Nutrient agar, NA is medium used for growing bacteria. The chemical composition of this medium is (g/L): NaCl (5.0), yeast extract (2.0), beef extract (1.0), peptone (5.0), and agar (15.0), pH 7.4; (2) MacConkey agar is a differentiated and selective medium used for growing gram-negative bacteria. It containing of (g/l): peptone (17), agar (15), toluene (0.03), protease peptone (3), NaCl (5), methyl violet (0.001), pH 7.1; (3) Blood agar is also a differentiated and selective medium used for growing bacteria. The chemical composition (g/l): blood agar base (950) that contains NaCl (5.0 g), pantone (10.0), agar (15.0 g), beef heart (3.0), cornstarch (1.0 g), and sheep blood (50.0), pH 7.4.

For fungi, Potato Dextrose agar is used for growing fungi and yeast. The chemical composition is (g/ liter): Potato infusion (200), Dextrose (20), and agar (20).

2.3. Isolation of microorganisms

To isolate microorganisms we follow procedures specified in Chapter 23 of the FDA's Bacteriological Analytical Manual (BAM) for isolating bacteria and fungi procedure associate with cosmetics (https://www.fda.gov/food/laboratory-methods-food/bam-chapter-23-methods-cosmet

Table 5
Microbial contamination in the samples from unused cosmetics products in high-quality makeup.

Cosmetics category	Number of samples	Media type			
		Nutrient Agar	Blood Agar	MacConkey	Potato dextrose Agar
Lipsticks	29	Staphylococcus aureus	–	–	–
	30	–	–	–	–
	31	Staphylococcus aureus	–	–	–
Lip-gloss	32	Staphylococcus aureus	+ Bacillus sp (G +)	–	–
	33	–	–	–	–
	34	–	–	–	–
Powders	35	–	Staphylococcus aureus	–	–
	36	–	–	–	–
	37	Staphylococcus aureus	–	–	–
Plusher	38	–	Staphylococcus aureus	–	–
	39	–	–	Escherichia coli	
Mascara	40	Staphylococcus epidermidis	–	–	–
	41	–	–	Pseudomonas aeruginosa	
	42	–	Streptococcus pneumonia	–	–
Foundation	43	Staphylococcus aureus	–	–	–
	44	–	–	–	–
	45	Staphylococcus aureus	–	–	–
Concealer	46	–	+ Bacillus sp (G +)	–	–
Eye pencil	47	–	–	Pseudomonas aeruginosa	–
	48	–	–	Pseudomonas aeruginosa	
Eye Shadow	49	–	Staphylococcus aureus	–	–
	50	Staphylococcus aureus	–	–	–

*No growth (−).

Table 6
Bacterial contamination in the samples from unused cosmetics products in high-quality makeup.

Cosmetics category	Bacterial isolate
Lipsticks	Staphylococcus aureus
Lip-gloss	Staphylococcus aureus, Bacillus subtills
Powders	Escherichia coli
Plusher	Staphylococcus aureus, Escherichia coli
Mascara	Staphylococcus aureus, Streptococcus pneumonia
Foundation	Staphylococcus aureus
Concealer	Bacillus subtills
Eye pencil	Pseudomonas aeruginosa
Eye Shadow	Pseudomonas aeruginosa

Table 7
Average of bacterial counts (CFU /10^{-5}) in low-quality brands of collected cosmetics.

Cosmetics category	Bacterial isolates CFU (10^{-5}/ml)	Fungal isolates CFU (10^{-5}/ml)
Lipsticks	251	<10
Lip-gloss	300	<10
Powders	212	<10
Plusher	198	33
Mascara	ND	ND
Foundation	119	<10
Concealer	73	ND
Eye pencil	21	<10
Eye liner	39	ND
Eye Shadow	65	ND

*ND (not detected).

ics) (FDA, 2022). In the beginning, to clean each cosmetic, a sterile cotton swab was utilized such as mascara, lip gloss, powders, and lipstick. Cotton swab was taken and added to 10 ml of sterile saline was used to dilute the solution, which was then mixed in the vortex for 10 min. To make 10^{-1} dilutions, 1 ml of sample solution and 9 ml of saline

solution were added to a fresh test tube. Dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were created. For liquid product like foundation and concealer 1 ml transfer to10ml of sterile saline to make a dilute solution,
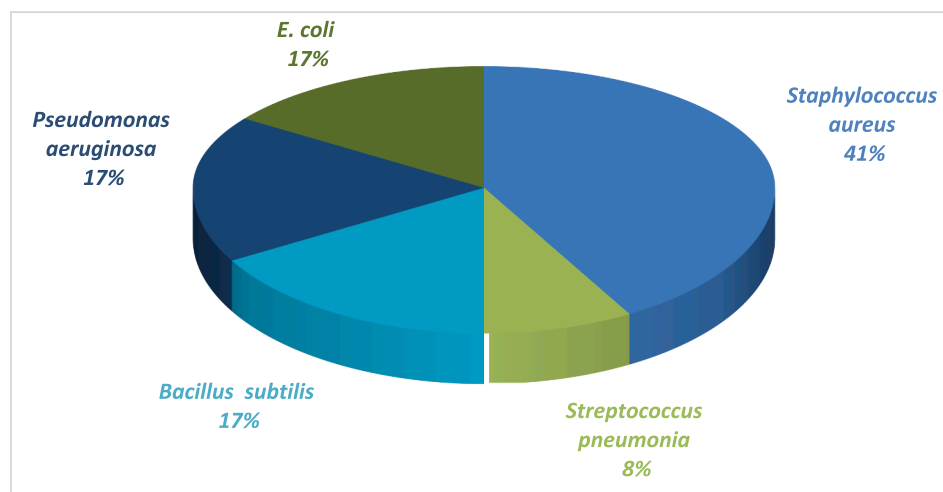

Chart 5. Percentage of bacterial growth in high-quality makeup.

Table 8
Average of bacterial counts (CFU /10^{-5}) in high-quality brands of collected cosmetics.

Cosmetics category	Bacterial isolates CFU 10^{-5}/ml
Lipsticks	89
Lip-gloss	110
Powders	77
Plusher	95
Mascara	300
Foundation	103
Concealer	<10
Eye pencil	33
Eye liner	57
Eye Shadow	68

and the same serial dilution procedure was followed until concentrtion10^{-5}. The culture dishes were incubated at 37 °C for 48 h. Most culture media showed bacterial colony development after incubation, for fungi the culture dishes were incubated at 25 °C for one week after inoculation. The total number of colonies was determined as (CFU ml^{-1}) after the incubation time. The colonies were then sub cultured on nutrient agar dishes. Each colony underwent this technique three times to confirm the purity of each isolated bacteria.

2.4. Identification of microorganisms associated with cosmetics

2.4.1. Bacteria

Bacteria were identified based on morphological traits as reported in the 8th edition of Bergey's Manual of Determinative Bacteriology (Bergey and Holt, 2000). Bacteria identified by describing colonies growing on different plates, Gram staining and shapes under the microscope (Noor et al.,2020). Oxidases, catalase, and the analytical profile index (API-20E kit) are further biochemical tests. The kits contain up to 20 biochemical tests, gelatinase, arginine Di hydrolase, â-galactosidase enzyme, Voges-Proskauer test, citrate test, lysine decarboxylase, urea hydrolysis, ornithine decarboxylase, H$_2$S production, indole test, tryptophan deaminase, as well as sugars (amygdalin, mannose, rhamnose, sorbitol, inositol, sucrose, arabinose, glucose, and melibiose) fermentation.

2.4.2. Fungi

Colonies of fungi were identified based on their microscopic and macroscopic appearance, reference to manuals of Barnett and Hunter (1972). Fungal filaments were stained by lactophenol.

2.5. Molecular Identification of bacteria 16S rRNA sequences

A) **DNA extraction:** by using protocol of Gene Jet genomic DNA purification Kit (Thermo Fisher Scientific) after harvesting up of bacterial ($2x10^9$) cells by centrifugation for 10 min at 5000 × g.
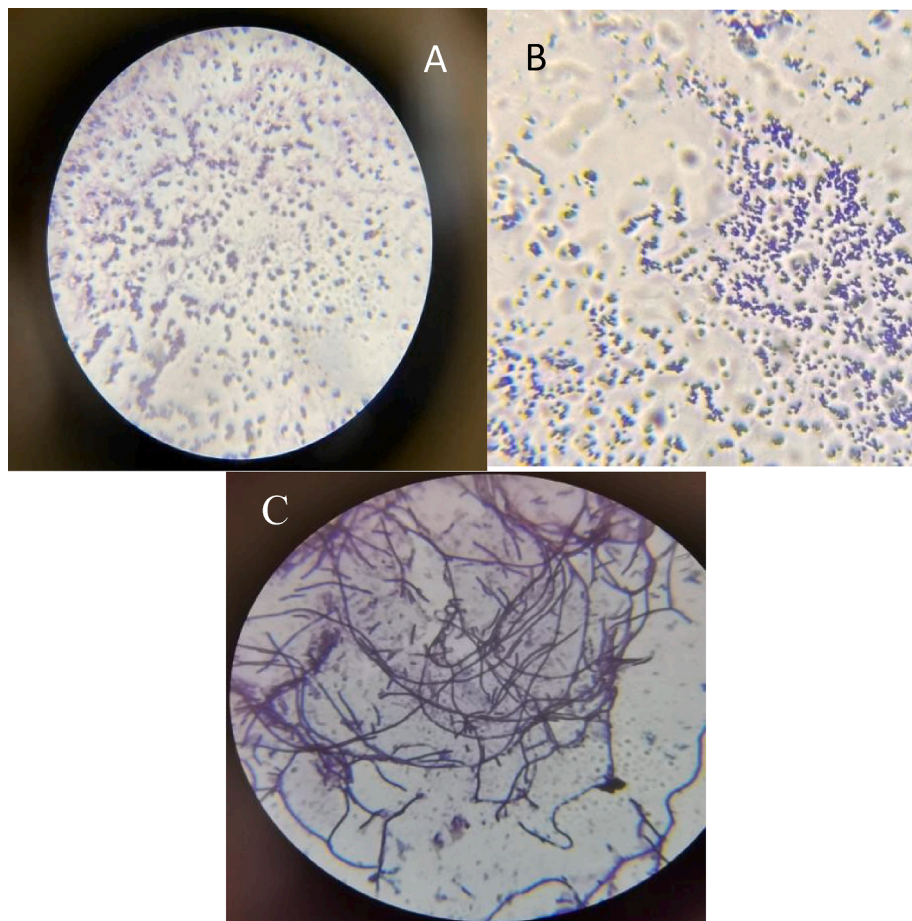
B) **PCR amplification:**

Fig. 1. Different genera of bacteria isolated from low-quality cosmetics products; (A) *Streptococci*, (B) *Staphylococci,* and *C) Bacillus* sp.
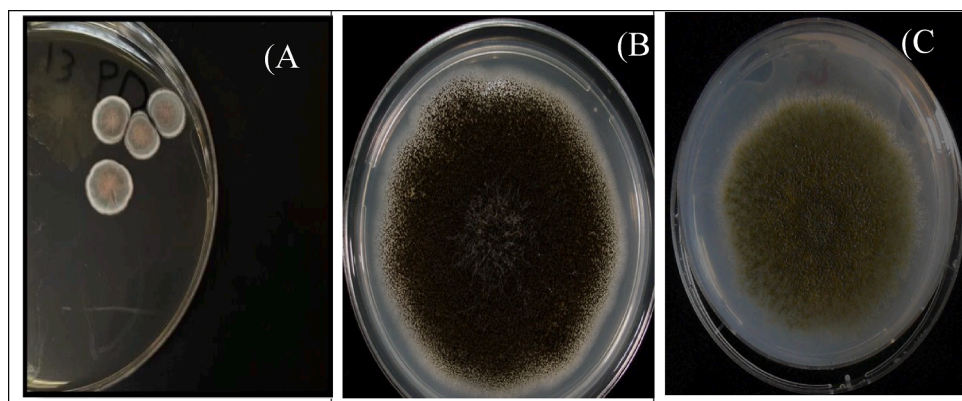
Fig. 2. Fungal cultures isolated from low-quality cosmetics products in culture medium; (A) *Penicillium* sp., (B) *Aspergillus,* and (C) *Aspegillus fumigatus.*
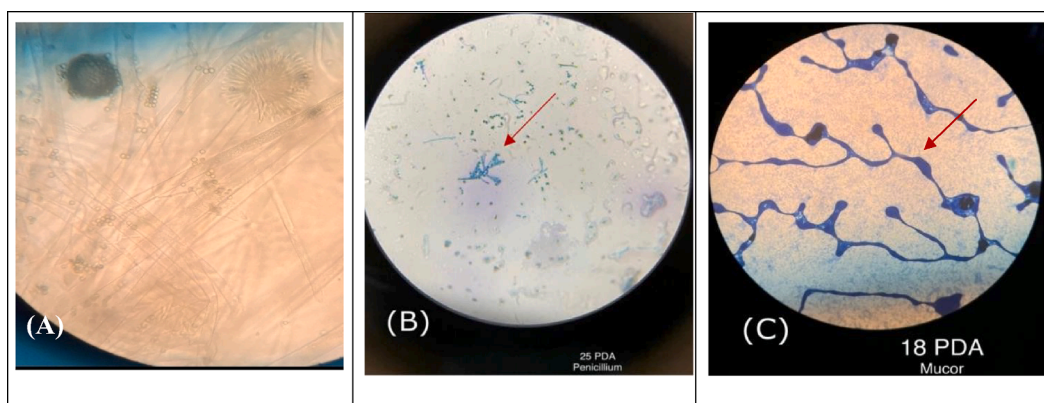

Fig. 3. Fungi isolated from low-quality cosmetic products; (A) *Aspergillus* sp., (B)*Penicillium* sp., and (C) *Rhizopus* spp. Development of zygospore and its germination.
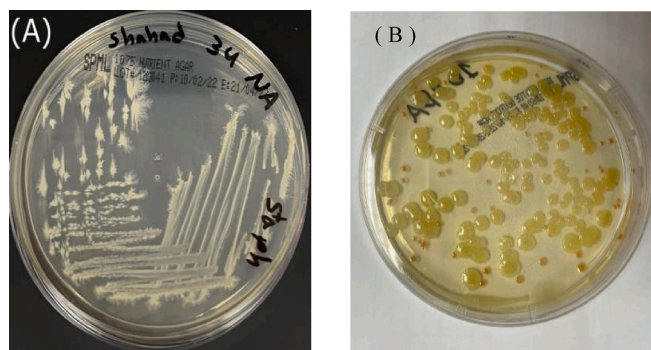

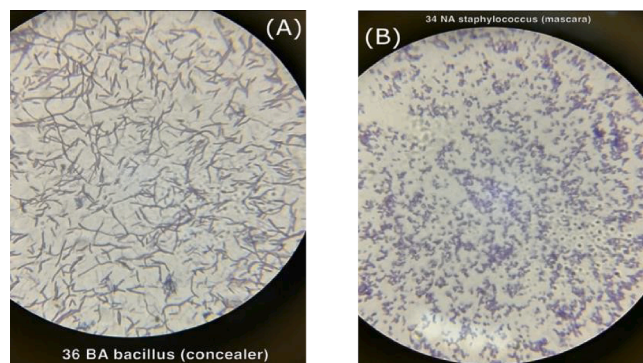**Fig. 4.** (A) *Bacillus subtilis* and *Staphylococcus aureus.*

Fig. 5. Gram positive bacteria isolated from high-quality products (A) *Bacillus* sp. and (B) *Staphylococcus* sp.

PCR amplification was done utilizing **forward primer** (5′**AGA GTT TGA TCC TGG CTC AG**'3), **reverse primer** (5′**GGT TAC CTT GTT ACG ACT T** 3) (Lane, 1991**).** PCR was performed using the recommended thermal cycling conditions as a follow: initial step for 10 min at 95 °C. Denaturation for 30 sec. at 95 °C. Annealing at 65 °C for 1 min. 35 rounds of 90-second extension at 72 °C. The extension time was 10 min. at 72 °C. Utilizing PCR products on agarose 1 %agarose gel against 1 Kb plus ladder (Thermo Fisher Scientific). BLAST analysis was then performed on the bacterial isolates' DNA sequences (Clarridge, 2004).

2.6. Molecular Identification of fungi by 18S rRNA

A) **DNA Extraction:**

Using molecular ITS regions, fungi were detected. Each fungal isolate was grown in PDA for one week at 25 °C. Each sample's DNA was

isolated using a Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research) according to the manufacturer's instructions.

B) **ITS-PCR:**

ITS4 was amplified using **forward primers (5′-TCCGTAGGT-GAACCTGCGG-3′)** and **reverse primers (5′-TCCTCCGCTTATTGA-TATGC-3′)** in PCR reactions using 20 ng genomic DNA in a total volume of 25 L. The temperature profile for PCR amplification was as follows: a 2 min warm-up at 94 °C, at 94 °C for 60 sec. 40 cycle were done, 90 sec. at 52 °C, and 2 min at 72 °C, and a final step of 7 minutes at 72 °C. The 1 % agarose gel was prepared and ran as indicated above. The fungus isolates' DNA sequences were then BLAST-ed.

Table 9
API-20E kit was used for biochemical testing of bacterial isolates.

Bacterial Isolate	Gram stain	Catalase test	Oxidase test	Citrate test	Indole test	H₂S production	Urea test	Sugar Fermentation test	Gelatin hydrolysis test
Bacillus subtilis	Gram positive	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve
E. coli	Gram Negative	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve
Staphylococcus aureus	Gram positive	+ve	- ve	+ve	-ve	-ve	+ve	+ve	+ve
Staphylococcus epidermidis	Gram positive	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve
Streptococcus pneumonia	Gram positive	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve
pseudomonas aeruginosa	Gram Negative	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve

Table 10
The matching ratio of bacterial and fungal isolates in the NCBI database.

Match ratio	Organism
99 %	*Bacillus subtilis*
99 %	*Escherichia coli*
99 %	*Staphylococcus aureus*
99 %	*Staphylococcus epidermidis*
99 %	*Streptococcus pneumonia*
99 %	*pseudomonas aeruginosa*
99 %	*Aspergillus fumigatus*
99 %	*Aspergillus niger*
99 %	*Penicillium citrinum*
99 %	*Rhizopus arrhizus*

3. Results

A Total of 50 samples of cosmetics were collected from different shops in Mecca region. Saudi Arabia. Samples were isolated on four different media (Potato dextrose agar, Blood agar, MacConkey agar, and Nutrient agar). Collected samples were divided into two groups regarding to their quality. Low - quality brands in Table 1 and high-quality brands in Table 2.

Generally, results of microbial growth are shown in Table 3 and Table 4 the bacterial out number fungi in terms of growth percentage.

Most of the bacteria that isolated were Gram- positive (Cocci and bacilli), and Gram-negative bacteria such as short bacilli. While fungal isolates were related to genera *Aspergillus*, *Penicillium* and *Rhizopus*.

Table 3 and Chart 1 show the frequency of microbial growth in low quality makeup. It has been observed the most contaminated product was in lip gloss and it follows by the lipstick. It found also, the less growth were in Eye pencil, Eye liner and Eye shadow, and there's no growth in mascara. The bacterial isolates from low- quality cosmetics were *Staphylococcus aureus*, *S. epidermis*, *Streptococcus pneumonia*, *Bacillus subtilis*, and *E. coli*. The fungal isolates were, *Rhizopus* sp., *Penicillium* sp., and *Aspergillus* Sp. (Table 4).

The percentages and diversity of bacterial growth is shown in (Chart 2). The most predominant genus of bacteria is *Staphylococcus aureus and E. coli* (27 %), which follows by *Streptococcus pneumonia* (18 %), *Staphylococcus epidermis* (17 %), *Bacilli subtilis* (12 %), and *Pseudomonas aeruginosa* (5 %). In Chart 3 *Aspergillus* sp. is the most predominant fungi (57 %), which follows by *Penicillium* (29 %) and *Rhizopus* (14 %). The highest fungal growth rate was in lip-gloss that isolated from the low-quality cosmetics. In Table 4, high quality cosmetics show less growth of bacteria then low-quality, while there was no growth at PDA. Chart 4 shows the frequency of microbial growth was the highest in mascara, lip-gloss which follows in order by Eye Shadow, Lipsticks, Powders, Plusher, and Concealer. There is no growth of bacterial species in Foundation and
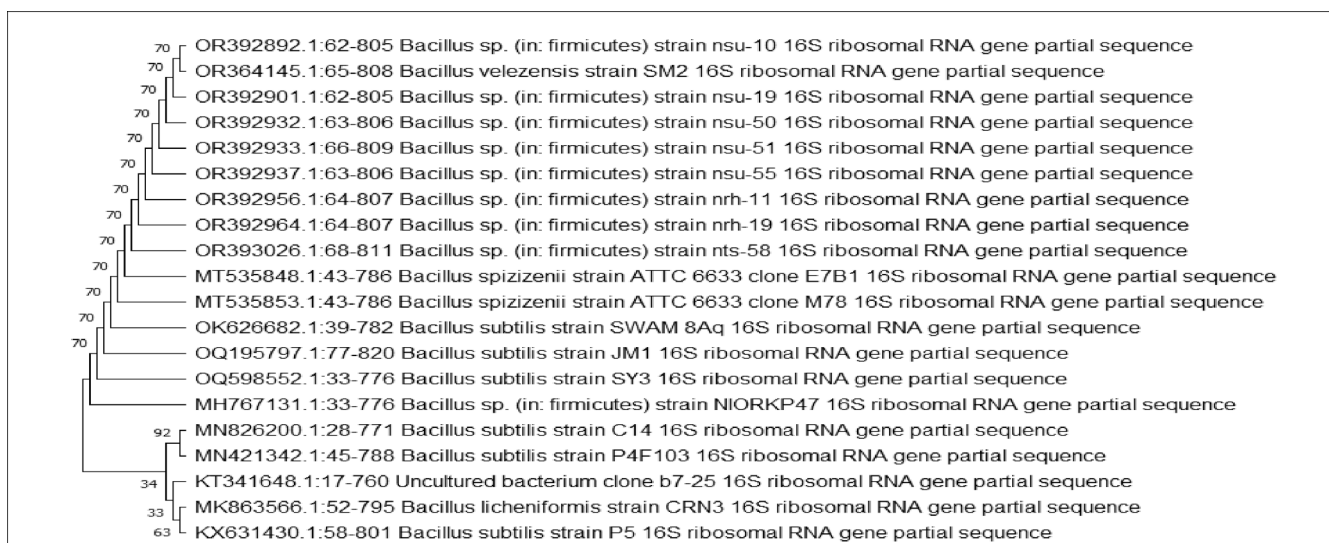


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       70 ┌ OR392892.1:62-805 Bacillus sp. (in: firmicutes) strain nsu-10 16S ribosomal RNA gene partial sequence
     70 ├── OR364145.1:65-808 Bacillus velezensis strain SM2 16S ribosomal RNA gene partial sequence
       70 └ OR392901.1:62-805 Bacillus sp. (in: firmicutes) strain nsu-19 16S ribosomal RNA gene partial sequence
     70 ── OR392932.1:63-806 Bacillus sp. (in: firmicutes) strain nsu-50 16S ribosomal RNA gene partial sequence
   70 ──── OR392933.1:66-809 Bacillus sp. (in: firmicutes) strain nsu-51 16S ribosomal RNA gene partial sequence
  70 ───── OR392937.1:63-806 Bacillus sp. (in: firmicutes) strain nsu-55 16S ribosomal RNA gene partial sequence
  70 ────── OR392956.1:64-807 Bacillus sp. (in: firmicutes) strain nrh-11 16S ribosomal RNA gene partial sequence
 70 ─────── OR392964.1:64-807 Bacillus sp. (in: firmicutes) strain nrh-19 16S ribosomal RNA gene partial sequence
 70 ──────── OR393026.1:68-811 Bacillus sp. (in: firmicutes) strain nts-58 16S ribosomal RNA gene partial sequence
 70 ───────── MT535848.1:43-786 Bacillus spizizenii strain ATTC 6633 clone E7B1 16S ribosomal RNA gene partial sequence
 70 ────────── MT535853.1:43-786 Bacillus spizizenii strain ATTC 6633 clone M78 16S ribosomal RNA gene partial sequence
 70 ─────────── OK626682.1:39-782 Bacillus subtilis strain SWAM 8Aq 16S ribosomal RNA gene partial sequence
 70 ──────────── OQ195797.1:77-820 Bacillus subtilis strain JM1 16S ribosomal RNA gene partial sequence
                OQ598552.1:33-776 Bacillus subtilis strain SY3 16S ribosomal RNA gene partial sequence
                MH767131.1:33-776 Bacillus sp. (in: firmicutes) strain NIORKP47 16S ribosomal RNA gene partial sequence
           92 ┌ MN826200.1:28-771 Bacillus subtilis strain C14 16S ribosomal RNA gene partial sequence
              └ MN421342.1:45-788 Bacillus subtilis strain P4F103 16S ribosomal RNA gene partial sequence
        34 ┌── KT341648.1:17-760 Uncultured bacterium clone b7-25 16S ribosomal RNA gene partial sequence
        33 ├── MK863566.1:52-795 Bacillus licheniformis strain CRN3 16S ribosomal RNA gene partial sequence
        63 └── KX631430.1:58-801 Bacillus subtilis strain P5 16S ribosomal RNA gene partial sequence
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Fig. 6. Phylogenetic tree of bacterial strain *B. subtills* isolated from cosmetic product based on 16s rDNA sequence.
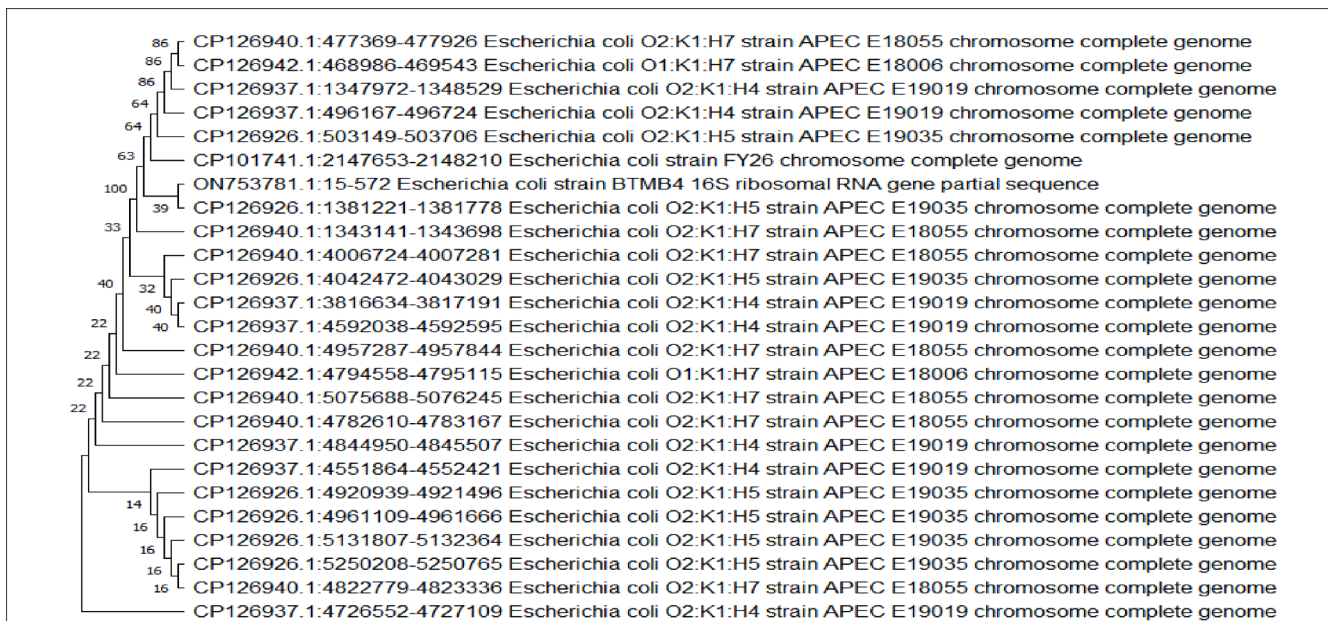
Fig. 7. Phylogenetic tree of bacterial strain *E. coli* isolated from cosmetic product based on 16s rDNA sequence.
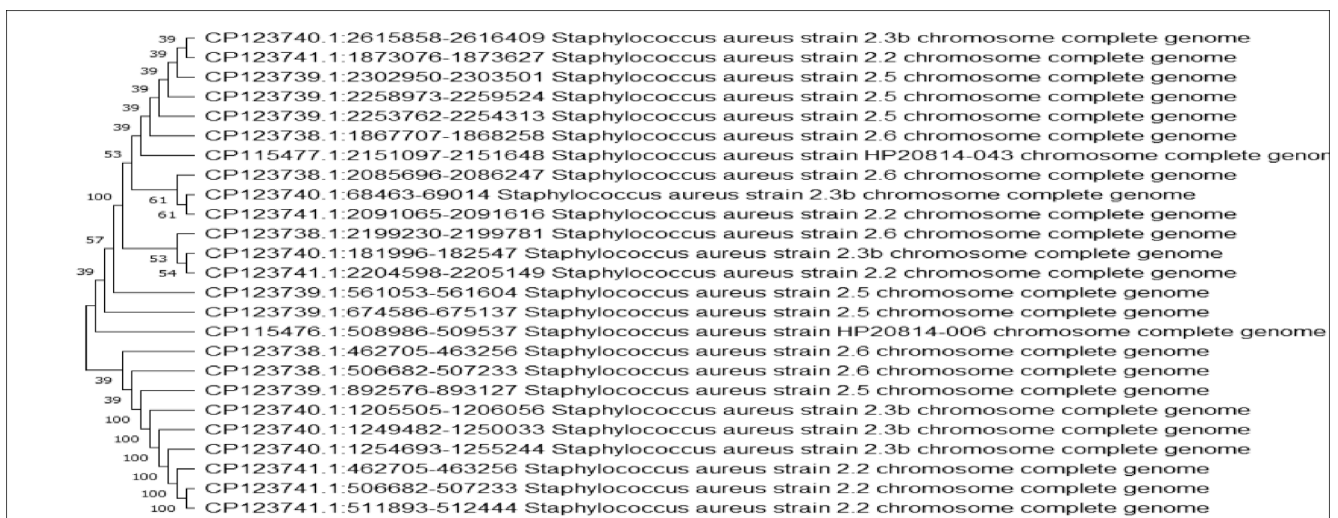

Fig. 8. Phylogenetic tree of bacterial strain *S. aureus* isolated from cosmetic product based on 16s rDNA sequence.

Eye pencil.

Tables 5, 6 and Chart 5 show the most predominant genus of bacteria is *Staphylococcus aureus* with 41 %, which follows by *Bacilli subtilis* and *Pseudomonas aeruginosa* and *E. coli* with 17 %. *Streptococcus pneumonia* is the less dominant with 5 %.

Most of the bacteria that isolated were Gram-positive (cocci and bacilli), and in Gram-negative bacterial isolates (*Bacilli*, and short bacilli). Table 7 shows the average of the total counts of colony in low quality brands as (CFU/ ml). In low quality brands, the lip gloss and lipstick recorded high numbers than other products for bacterial isolates with (300X10^{-5} and 251 $\times$ 10^{-5}) while fungal isolates recoded low percentage number less than 10 colonies in most low-quality brands except plusher. Table 8 shows the average of the total counts of colony in high quality brands which indicates the decrease of microbial population compared to low quality brands, mascara recoded the highest numbers with (300 $\times$ 10^{-5}) while no fungal growth observed in all products.

According to morphological and microscopic characters, bacteria were identified based on the reaction of gram stain and the arrangement of bacteria. Gram positive bacteria that isolated from low-quality makeup are shown in Fig. 1, Fig. 2, Fig. 3. While fungi isolates were identified by morphological cultures and microscopic detection by observing mycelium and spores' arrangement on pores or sporangium or vesicles. Fungal cultures are shown in Fig. 4, Fig. 5.

3.1. Identification of bacteria by biochemical tests

Bacteria were identified using the biochemical assays described in Table 9. and the findings were Bergey's handbook compared, which showed the following microbes: *Escherichia coli, Streptococcus pneumonia, Bacillus subtilis, Staphylococcus aureus,* pseudomonas *aeruginosa, and Staphylococcus epidermidis.*

3.2. Bacterial and fungi molecular identification

The resultant sequences of bacteria's 16 srRNA and fungi's ITS were aligned in the NCBI database, and matched sequences were supported
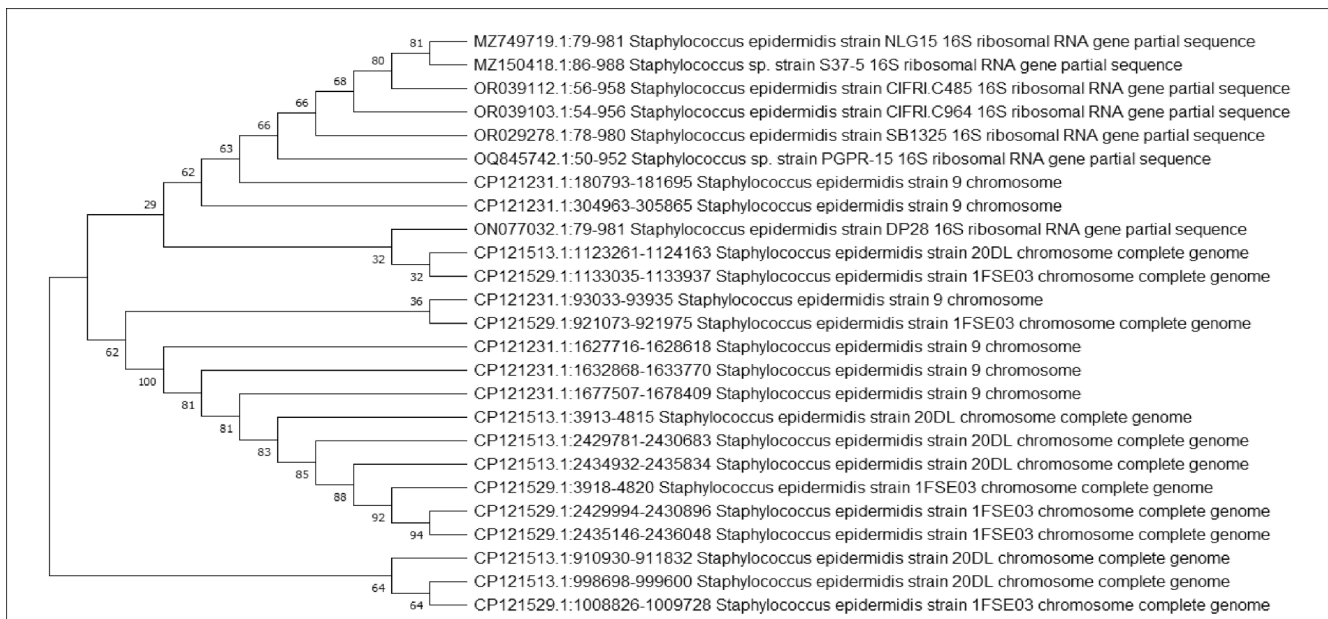
Fig. 9. Phylogenetic tree of bacterial strain *S. epidermis* isolated from cosmetic product based on 16s rDNA sequence.
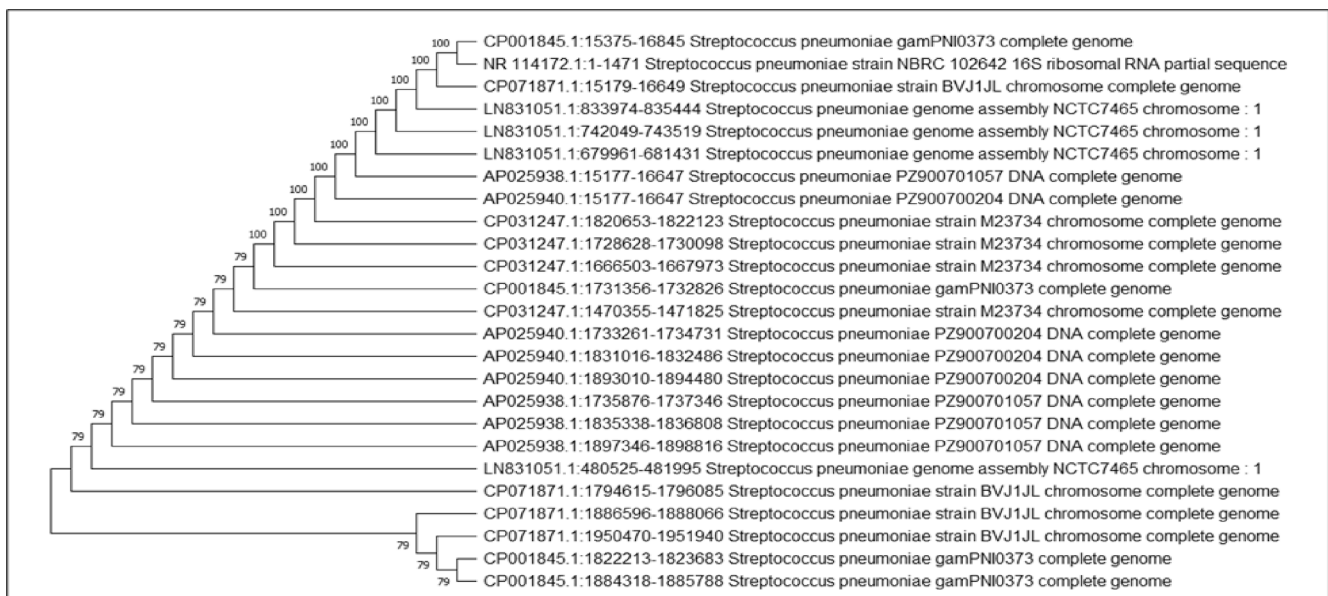

Fig. 10. Phylogenetic tree of bacterial strain *S. pnenmoniae* isolated from cosmetic product based on 16s rDNA sequence.

and identified, by earlier biochemical studies. Based on the matching ratio of sequencing of bacterial and fungal isolates, as shown in Table 10 below, Phylogenetic tress for each isolate is shown in Figs. 6–15.

4. Discussion

The majority of the isolated microorganisms in this investigation were pathogens found in unused cosmetics. Even though these products are new brands and have never been used, the risk of microbiological infection was considerable. The results showed that both high-quality and low-quality cosmetic samples were contaminated with fungus and germs. Gram positive cocci such *Staphylococci, Streptococci*, and *Bacilli* were among the isolated microorganisms. Moreover, short bacilli are preferred by gramme negative bacteria. While isolates of fungi from the

Rhizopus, Penicillium, and *Aspergillus* genera.

In general, high-quality brands exhibit less contamination than low-quality ones; this may be related to the products' ingredients, the way preservatives are applied, the circumstances of transit and storage. The most prevalent genus was *Staphylococci*, which was discovered in both high- and low-quality samples with a frequency of (41 %) both. Numerous research has established that *Staphylococcus* sp. is present in various cosmetic items. Some of these strains are dangerous and bring on skin conditions like desquamate and acne (Dadashi & Dehghanzadeh, 2016) and (El-Tablawy, 2009). Additionally, a study by Noor et al., 2020 discovered that *staphylococci* were the most typical species isolated from a variety of cosmetic tests.

Another potentially hazardous organism was identified during this examination, called *Bacillus*. *Bacillus* causes localized necrotizing
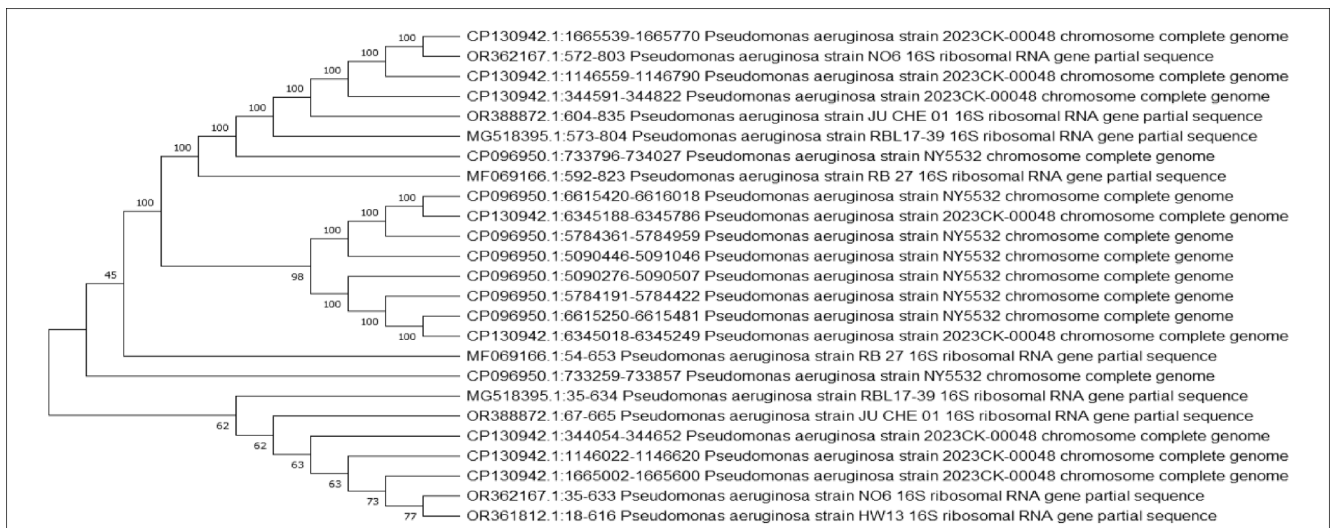
Fig. 11. Phylogenetic tree of bacterial strain *P. aeruginosa* isolated from cosmetic product based on 16s rDNA sequence.

Fig. 12. Phylogenetic tree of fungal strain *A. fumigatus* isolated from cosmetic product based on 18s rRNA sequence.

cellulitis in the skin, making it risky to use eye cosmetics that have *Bacillus* contamination (Dadashi & Dehghanzadeh, 2016). According to a 2019 study by Bashir and Lambert, cosmetic regulations expressly state that products shouldn't contain pathogenicity's organisms. Between 70 and 90 percent of the products were contaminated with germs. Fungi also are pathogenic organisms, in low quality cosmetics items *Aspergillus, Rhizopus* and *Penicillium* were recovered. *Penicillium* sp. was the most abundant organism in other research, appearing in 75 % of samples in the study of (De oliveira et al., 2020). Due to their involvement in the synthesis of mycotoxins, particularly ochratoxin A and citrinin, *Penicillium* represent a risk to human health. Additionally, both mycotoxins are extremely nephrotoxic at micromolar doses (Geisen et al., 2018). Filamentous fungus can utilize the humidity of the environment to assure growth on the surfaces of cosmetics with low quantities of water, such foundation, qualifying these goods as semi-aqueous preparations. This implies that moisture promotes the growth of

filamentous fungus. Despite the fact that there was a lot of water in those preparations (Oliveira et al., 2020).

In Saudi Arabia, Nasser (2008) investigated the microbial contamination in 75 cosmetic samples. She discovered that lip cosmetic products had the highest fungal counts, and the samples were infected with 13 and 24 mesophilic and thermophilic fungus species from 2 and 6 genera, respectively. The most common fungal genus was *Aspergillus*, and 36.7 % of the analyzed samples were contaminated with *E. coli*, while *Bacillus* and *Pseudomonas* were commonly observed (Okeke and Lamikanra, 2001). From a cosmetic manufacturing facility, *Pseudomonas aeruginosa* and *Enterobacter gergovia* were identified, and these microorganisms demonstrated increased resistance to formaldehyde-releasing preservatives and parabens (Ferrarese et al., 2003). The risk of infection from these germs, which can infect the lips or eyes, is very high (Bashir and Lambert, 2020; Burleson and Martinez-Vaz, 2011).

On the other hand, because nature can encourage microbial growth

Fig. 13. Phylogenetic tree of fungal strain *A.niger* isolated from cosmetic product based on 18s rRNA sequence.
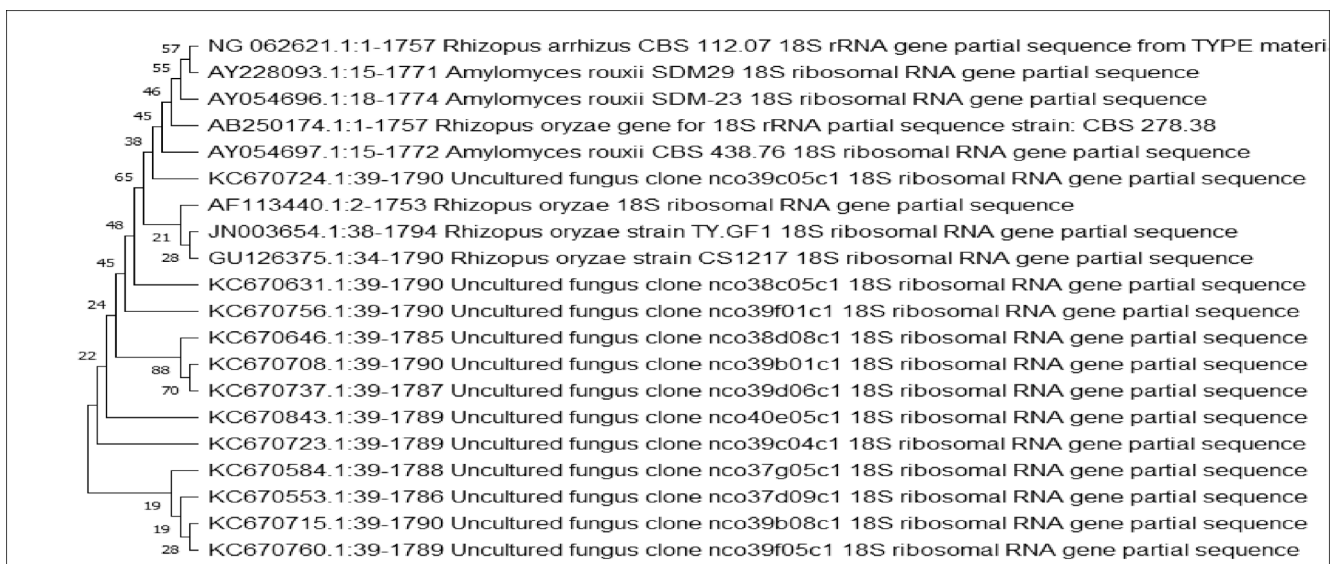

Fig. 14. Phylogenetic tree of fungal strain *R. arrhizus* isolated from cosmetic product based on 18s rRNA sequence.

in cosmetics, which can lead to infection, these products are more likely to be contaminated by microorganisms than those that don't contain water. (Halla et al., 2018 & Skowron et al., 2017). Water that hasn't been sterilized or treated can encourage bacterial growth, which can contaminate cosmetics. Additionally, cosmetics can serve as a nutritious environment for the growth of microorganisms. (Alwan, 2018). Microbial contamination, according to (Choubey & Godbole, 2017) can occur during the manufacturing process, in raw materials, during consumer usage of the product, or as a result of dust in retail marketplaces. After a product has been opened, the consumer's hands or the surroundings may contaminate it further.

Due to the creation of hazardous bacteria metabolites and product degradation, cosmetic product contamination can directly affect human health. Therefore, maintaining the sanitary standards of the product and ensuring consumer safety depend on the microbiological preservation of

cosmetics (Choubey & Godbole, 2017). It is generally known that microorganisms have the capacity to multiply and proliferate in cosmetic products. Cosmetics include a variety of components, including sugar, vitamins, proteins, oils, and water, all of which are necessary for the growth of microbes (Ahmida et al., 2018). According to (Wilson et al., 1975), climatic variables like temperature variation and moisture from humidity promote microbial growth in cosmetic goods.

(Ahmida et al., 2018) recommended Keep cosmetics in a dry, cool environment to prevent spoiling, shield consumers from infections, and lengthen the shelf life of your items. A good preservative system, as described by (Nuzhath, 2014 will prevent quick postproduction contamination, resulting in acceptable low levels of microorganisms in the preparation and so making it safe for consumers, which in this case is ineffective. Cosmetics that have been contaminated by microbes run the risk of breaking down and costing consumers money. As a result, it's

Fig. 15. Phylogenetic tree of fungal strain *Penicillium citrinum* isolated from cosmetic product based on 18s rRNA sequence.

crucial to guarantee that cosmetic goods are bacteria free, and the preservatives used in them should be able to kill any potential microbes (Siya et al., 2019).

5. Conclusion

Microbial contamination of cosmetic products is a matter of great importance to the industry, and it is potentially a major cause of economic losses. Water and some ingredients support microbial growth. The results of this study indicated that all samples in high-quality and low-quality brands show bacterial and fungal growth more than the acceptable number prescribed by FDA and other legislations. The high-quality of cosmetics doesn't mean the high price only, it means selection a good raw material, offering a good storage and transport, and have preservation system which may reduce microbial growth. For consumers, we recommend sterilizing these items with a good sanitizer. Our vision is to keep searching for more studies in cosmetics microbiology discuss the microbial contamination and solutions to reduce it.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ahmida, N., Elsharaa, I.A.F., Salem, S.Y., Ahmida, N.H.S., Elshara, I.A., Elfeituri, M., Al-Ojali, S.M., Fouad, I., 2018. Microbiological detection of some facial cosmetic products collected from Benghazi-Libya markets. Int. J. Multidisc. Innovative Res. 1 (4), 54–64.

Alwan, S.K., 2018. Infection points in some cosmetics and beauty shops in Al Diwaniyah city. Biochem. Cell. Arch. 18, 1127–1132.

Barnett, H.L., Hunter, B.B., 1972. In: Illustrated Genera of Imperfect Fungi, third ed. Burgess Publishing Co., Minneapolis, p. 241.

Bashir, A., Lambert, P., 2020. Microbiological study of used cosmetic products: highlighting possible impact on consumer health. J. Appl. Microbiol. 128 (2), 598–605. https://doi.org/10.1111/jam.14479.

Becks, V.E., Lorenzoni, N.M., 1995. Pseudomonas aeruginosa outbreak in a neonatal intensive care unit: a possible link to contaminated hand lotion. Am. J. Infect. Control 23, 396–398. https://doi.org/10.1016/0196-6553(95)90272-4.

Behravan, J., Bazzaz, F., Malaekeh, P., 2000. Survey of bacteriological contamination of cosmetic creams in Iran. Int. J. Dermatol. 44 (6), 482–485. https://doi.org/10.1111/j.1365-4632.2005.01963.x.

Bergey, D.H., Holt, J.G., 2000. Bergey's manual of determinative bacteriology, ninth ed. Lippincott Williams & Wilkins, Philadelphia.

Burleson, K.M., Martinez-Vaz, B.M., 2011. Microbes in mascara hypothesis-driven research in a nonmajor biology lab. J. Microbiol. Biol. Educat. 12 (2), 166–175. https://doi.org/10.1128/jmbe.v12i2.320.

Choubey, S., Godbole, S., 2017. Methods for evaluation of microbiological safety, guidelines governing the quality and survey on microbial contamination of commercial cosmetic products-a review. World J. Pharm. Med. Res. 3 (8), 85–146.

Clarridge, J.E., 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin. Microbiol. Rev. 17 (4), 840–862. https://doi.org/10.1128/cmr.17.4.840-862.2004.

Dadashi, L., Dehghanzadeh, R., 2016. Investigating incidence of bacterial and fungal contamination in shared cosmetic kits available in the women beauty salons. Health Promotion Perspectives 6 (3), 159–163. https://doi.org/10.15171/hpp.2016.25.

Dashen, M.M., Chollom, P.F., Okechalu, J.N., Ashulee, J., Aji, M., 2011. Microbiological quality assessment of some brands of cosmetics powders sold within Jos Metropolis, Plateau State. J. Microbiol. Biotech. Res 1 (2), 101–106.

de Oliveira, J.F., Zenaide-Neto, H., de Sousa, A.C.B., Arruda, R.R.A., Vasconcelos, U., 2020. presence of filamentous fungi in powder and semi aqueous makeup. Brazilian J. Dev. 6 (7), 54029–54039. https://doi.org/10.34117/bjdv6n7-884.

Detmer, A., Jørgensen, C., Nylén, D., 2010. A guidance document on microbiological control of cosmetic products. Danish Ministry Environ. Protection Agency 1–43.

El-Bazza, Z.E., El-Tablawy, S.Y., Hashem, A.E., Nasser, H.H., 2009. Evaluation of the microbial contamination of some eye-make up products before and after use. Biohealth Sci. Bull. 1 (2), 68–75.

El-Tablawy, S.Y., 2009. Evaluation of the microbial contamination of some eye-make up products before and after use In Vitro Appraisal of Potential Association between Some Virulence Factors and Resistance to Antifungal Agents in Candida albicans Recovered from Different Clinical Specimens View project. Biohealth Science Bulti 1 (2), 68–75.

Websites: EWG, 2020: https://www.ewg.org/skindeep/products/937672-Iman_Cosmetics_Luxury_Lip_Shimmer_Impetuous_2020_formulation/.

FDA, 2022. Microbiological Safety and Cosmetics. official website of U.S. food and drug administration.

Ferrarese, L., Paglia, R., Ghirardini, A., 2003. Bacterial resistance in cosmetics industrial plant: connected problems and their solution. Ann. Microbial. 53, 477–480.

Geisen, R., Schmidt-Heydt, M., Touhami, N., Himmelsbach, A., 2018. New aspects of ochratoxin A and citrinin biosynthesis in Penicillium. Curr. Opin. Food Sci. 23, 23–31.

Halla, N., Fernandes, I.P., Heleno, S.A., Costa, P., Boucherit-Otmani, Z., Boucherit, K., Rodrigues, A.E., Ferreira, I.C.F.R., Barreiro, M.F., 2018. Cosmetics preservation: a review on present strategies. In Molecules 23 (7), 1–41. https://doi.org/10.3390/molecules23071571.

Hayleeyesus, S.F., Manaye, A.M., 2014. Microbiological quality of indoor air in university libraries. Asian Pac. J. Trop. Biomed. 4 (1), S312–S317. https://doi.org/10.12980/APJTB.4.2014C807.

Lane, D.J.,1991.16S/23S rRNA Sequencing. In: Stackebrandt, E. and Goodfellow, M., Eds., Nucleic Acid Techniques in Bacterial Systematic, John Wiley and Sons, New York, pp. 115-175.

Mohammed, A., Blebil, A., Dujaili, J., Hassan, B.A., 2021. Perception and attitude of adults toward cosmetic products amid COVID-19 pandemic in Malaysia. J. Cosmet. Dermatol. 20 (7), 1992–2000. https://doi.org/10.1111/jocd.14147.

Nasir, M., Qasim, Q., 2020. Microbiological contaminant isolation and detection in cosmetics sold in Iraq. Int. J. Pharm. Res. 12 (04), 4434–4439. https://doi.org/10.31838/ijpr/2020.12.04.606.

Noor, A.I., Rabih, W.M., Alsaedi, A.A., Al-Otaibi, M.S., Alzein, M.S., Alqireawi, Z.M., Mobarki, K.A., AlSharif, R.A., Alfaran, H.S., 2020. Isolation and identification of microorganisms in selected cosmetic products tester. Afr. J. Microbiol. Res. 14 (9), 536–540. https://doi.org/10.5897/ajmr2020.9399.

Nuzhath, F., 2014. Bacteriological evaluation and comparison of unused branded and non-branded cosmetic products available in Jazan region of Saudi Arabia. J. Microb. World 16 (2), 57–69.

Okeke, N., Lamikanra, A., 2001. Bacteriological quality of skin-moisturizing creams and lotions distributed in a tropical developing country. J. Appl. Microbiol. 91 (5), 922–928. https://doi.org/10.1046/j.1365-2672.2001.01456.x.

Orús, P., Gomez-Perez, L., Leranoz, S., Berlanga, M., 2015. Increasing antibiotic resistance in preservative-tolerant bacterial strains isolated from cosmetic products. Int. Microbiol. 18, 51–59. https://doi.org/10.2436/20.1501.01.234.

Panico, A., Serio, F., Bagordo, F., Grassi, T., Idolo, A., De Giorgi, M., Guido, M., Congedo, M., De Donno, A., 2019. Skin safety and health prevention: an overview of chemicals in cosmetic products. J. Prev. Med. Hyg. 60, 50–57. https://doi.org/10.15167/2421-4248/jpmh2019.60.1.1080.

Peraica, M., Radić, B., Luci, A., Pavlović, M., 1999. Toxic effects of mycotoxins in humans. Bull. World Health Organ. 77 (9), 754–766.

Siegert, W., 2013. Comparison of microbial challenge testing methods for cosmetics. Household and Personal Care Today 8 (2), 32–39.

Siegert, W., Schulke, A., Gombh, M., 2005. Microbiological quality management for the production of cosmetics and toiletries cosmet. Sci. Tech. 19 (3), 11–17.

Siya, K., Thomas, J., Vinod Kumar, R., Saji, A., Iype, A., Akhil, S., 2019. Lipsticks: the microbial cellar: an original study. J. Microscope Ultrastruct. 7 (4), 194–197. https://doi.org/10.4103/jmau.jmau_14_19.

Skowron, K., Jakubicz, A., Budzyńska, A., Kaczmarek, A., Grudlewska, K., Reśliński, A., Gospodarek-Komkowska, E., 2017. microbiological purity assessment of cosmetics used by one and several persons and cosmetics after their expiry date. Rocz. Panstw. Zakl. Hig. 68 (2), 191–197.

Sreeparna, S., Braj, R., Alok, A., 2017. Intracellular synthesis of gold nanoparticles using an ectomycorrhizal strain EM-1083 of laccariafraterna and its nanoanti-quorum sensing potential against *Pseudomonas aeruginosa*. Indian J. Microbiol. 57 (4), 448–460.

Wilson, L.A., Julian, A.J., Ahearn, D.G., 1975. The survival and growth of microorganisms in mascara during use. Am. J. Opthalmol. 79, 596–601.