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Isolation and molecular identification of some pathological strains from the make-up of school students in Anbar province

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Abstract:
This investigation conducted for isolation and molecular identification of some pathological strains from the make-up of school students in Anbar province. In 2022, researchers at the University of Baghdad, Iraq, gathered 120 samples of cosmetics used by female students. There was a total of 18–24-year-old women. Two separate samples of each face cream and lipstick were gathered using sterile cotton swabs and then sent to the lab to be cultured in enriched and selective medium. Then these were cultured on primary media, then were identified by using API kits. Molecular study was done for confirmation of different isolates in this study by using primers. a total of 65 samples (54.2%) were culturable, and 65 unique bacterial isolates were obtained from cosmetics. Additionally, 84.6% of the bacterial isolates were from the face cream, whereas just 15.4% were from the lipstick. *Staphylococcus aureus* (70% lipstick and 45.5% face cream) were the most often isolated germs. *Staphylococcus aureus* dominated Gram-positive bacterial isolates, whereas Escherichia coli dominated Gram-negative bacterial isolates. These bacteria were identified by using PCR which showed compatible results 100% with bacteriological way.
In conclusion, *Staphylococcus aureus* was shown to be most bacteria that isolated from lip sticks and face cream.
Keywords: Face Cream, Lip sticks, Anbar, Female, bacteria

Introduction:

Everyone in the world uses cosmetics to improve their personal hygiene and appearance. Although it is not required that cosmetics be completely sterile during use or even in the unopened state, microbial contamination of cosmetics may lead to a number of infections (1).
It is crucial to ensure that cosmetic products and their raw materials are produced in accordance with Good Manufacturing Practises, ISO, and FDA guidelines to avoid causing harm to consumers' skin due to microbial contamination (2).
Cosmetics shall not be contaminated at a rate more than 10^3 colony-forming units per gramme or millilitre for use anywhere other than the eyes; for use around the eyes, on mucous membranes, or on children less than three, the limit is 10^2 CFU/g or ml. The FDA and the ISO both recommend these specifications (3,4,5).
In addition, ISO 17516:2014 specifies that not a single viable cell of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, or Candida albicans may be found in a volume of 1 ml or a weight of 1 gramme of the product (6).

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There have been reports in the scientific literature of commercially accessible items becoming contaminated with microorganisms. Lipsticks have been shown to harbour a wide variety of bacteria and yeasts, including *Pseudomonas monteilii*, *Pseudomonas fulva*, *Citrobacter freundii*, *Staphylococcus spp.*, *Staphylococcus aureus*, as well as Candida spp. (2,7,8,9). Powders have been shown to harbour germs such as Pseudomonas aeruginosa and Salmonella, (2,10,11). *E. coli*, Klebsiella, Bacillus, Pseudomonas, Enterobacter, Staphylococcus, Enterococcus, *Staphylococcus aureus*, Micrococcus, Streptococcus pyogenes, as well as *Enterobacter aerogenes* have all been found in creams (2,8,10,12,13,14,15). Another research found that lip products including lip glosses and lipsticks were a breeding ground for bacteria such *Staphylococcus aureus*, *Escherichia hermannii*, Bacillus cereus, as well as Enterobacter species. *Buttiauxella agrestis* was also found in the research, despite the fact that it has never been isolated from beauty items previously. A sample of hair relaxer included it (16).

Products contaminated with bacteria pose a health risk to consumers. Conjunctivitis and allergies are examples of moderate forms; systemic keratitis, a blood infection, and systemic inflammation are examples of severe forms (17). Cosmetics contaminated with germs have even been linked to at least one fatality (18). Multiple studies found that Staphylococcus was the most prevalent skin pathogen among microorganisms (19,20,21). Conjunctivitis, impetigo, and Staphylococcus aureus have all been linked in research (22). Twenty-three women were surveyed and found to have high levels of Staphylococcus epidermidis infection, indicative of bacterial blepharitis. This was kept well away from the crease of their eyes and their eye makeup (23).

This investigation conducted for isolation and molecular identification of some pathological strains from the make-up of school students in Anbar province.

Materials and Methods:

In 2022, researchers at the University of Baghdad, Iraq, gathered 120 samples of cosmetics used by female students. There was a total of 18–24-year-old women. Two separate samples of each face cream and lipstick were gathered using sterile cotton swabs and then sent to the lab to be cultured in enriched and selective medium.

Before anything else, we mixed 1 gramme from both face cream and lip stick with 10 millilitres of sterile distilled water for 2 minutes. Up to a dilution factor of 10^6 was achieved by serially diluting the combined liquid (24). The suspected bacterial count in the samples was determined using the conventional plate count technique (25). Then, one millilitre of each diluted sample was added to twenty millilitres of sterile nutritional agar in a Petri plate. After a diluted sample was gently stirred into the nutrient agar and allowed to harden, it was placed in an aerobic incubation at 37 degrees Celsius for 18 to 24 hours.

Step two involved inoculating 10 ml of BHI as an enriched culture medium with 1 g of each sample of face cream and lipstick, incubating the tubes at 37°C for 18-24 hours, and then culturing the bacteria on selective and special culture media.

On primary medium, we cultivated a Loop containing each BHI-filled tube. Colony morphology, Gram staining, and biochemical testing with API 20E, API Strep, and API Staph validated the identity of the recovered bacteria.

Then these bacteria were confirmed by PCR by using specific primers as follows:

Table 1. Primers used in this study

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Name of bacteria	F	R	bp	T.
S. aureus 16s rRNA	5'-GGTCTTGCTGTCACTTATAGATGG-3'	5'-CGGAAGATTCCCTACTGCTG-3'	165	60
St. pyrogens SPY1258	5' AAAGACCGCCTTAACCACCT3'	5' TGCCAAGGTAAACTTCTAAAGCA 3'	407	58
Micrococcus spp. Pml	5'GGATCATCTATAATGAAACTG 3'	-5' CTGATAATCAACTTGGAAGTT 3'	563	52
E. coli uidA	5-CCAAAAGCCAGACAGAGT-3	5-GCACAGCACATCCCCAAAGAG-3	623	53
Proteus spp. 16s rRNA	5-GGAAACGGTGGCTAATACCGCATAAT-3	-5-GGAAACGGTGGCTAATACCGCATAAT-3	101	58
Pseudomonas spp. Gyrb222	CCT GAC CAT CCG TCG CCA CAA C	CGC AGC AGG ATG CCG ACG CC	222	60

The thermocycler programme consisted of three steps: (i) 5 minutes at 95°C for denaturation, (ii) 35 cycles of denaturation (60 sec at 94°C), annealing (60 sec at different temperature according to primer), extension (60 sec at 72°C), and final extension (5 minutes at 72°C).

Results and Discussions:

According to the data shown in Table 1, a total of 65 samples (54.2%) were culturable, and 65 unique bacterial isolates were obtained from cosmetics. Additionally, 84.6% of the bacterial isolates were from the face cream, whereas just 15.4% were from the lipstick. *Staphylococcus aureus* (70% lipstick & 45.5% face cream) were the most often isolated germs (Table 2). *Staphylococcus aureus* dominated Gram-positive bacterial isolates, whereas Escherichia coli dominated Gram-negative bacterial isolates (Table 2).

Table 2. Isolated bacteria in this study

Name of bacteria	No. of bacteria from Cream face (%)	No. of bacteria from lip stick (%)	Total
S. aureus	25 (45.5)	7 (70)	32(49.2)
St. pyogens	9 (16.4)	1(10)	10 (15.4)
Micrococcus spp.	6 (10.9)	0	6 (9.2)
E. coli	8 (14.5)	2 (20)	10 (15.4)
Proteus spp.	4 (7.3)	0	4 (6.2)
Pseudomonas spp.	3 (5.5)	0	3 (4.6)

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Total 65 (54.2%)	55 (84.6)	10 (15.4)	65 (100%)

In the course of learning how to identify bacteria from clinical samples and isolate them, most of the female trainees at the Institute have used the cosmetics in the lavatory or in the Institute's laboratories. Because different types of cosmetics contain raw materials like minerals, vitamins, as well as plant extracts that encourage the microbial growth, resulting in skin inflammation and, in rare cases, allergic reactions, eczema, and vitiligo—especially in immune-compromised individuals—most cosmetics manufacturing companies must produce safe cosmetic products but are not required to produce sterile cosmetics. Thus, certain cosmetics may include UV-blocking ingredients (26,27).

Cosmetics in Iraq may be contaminated with pathogens and skin flora due to the country's warm, acidic climate and the fact that people like to keep cosmetics in damp places like toilets, widespread use of the same lipsticks and other cosmetics among all students, and a lack of quality control. Consistent with previous research, various bacterial isolates were found to be widely obtained from cosmetic goods, including Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli (27). One hundred samples of potentially harmful bacteria were recovered from a variety of beauty shop surfaces, including face sponges, brushes, and wax, according to prior research conducted in Pakistan. Staph. aureus was present at a rate of 100% in the sponge, 100% in the brush, and 88% in the wax; Pseudomonas aeruginosa was present at a rate of 69.6% in the sponge, 81.8% in the brush, and 73.5% in the wax (28).

There was consistency between the present and prior findings, although there was variation between the bacterial species isolated from the cosmetic goods (29).

In addition to the aforementioned causes, it was also shown that students' saliva, mouth as well as nose aerosols, or unwashed hands might contaminate lipsticks and other cosmetic goods used every day in medical labs. In addition, some students apply cosmetics in the lavatories before entering the labs, spreading potentially infectious germs and fungus throughout the facilities.

These bacteria were identified by using PCR which showed compatible results 100% with bacteriological way (Fig. 1,2,3,4,5,6)

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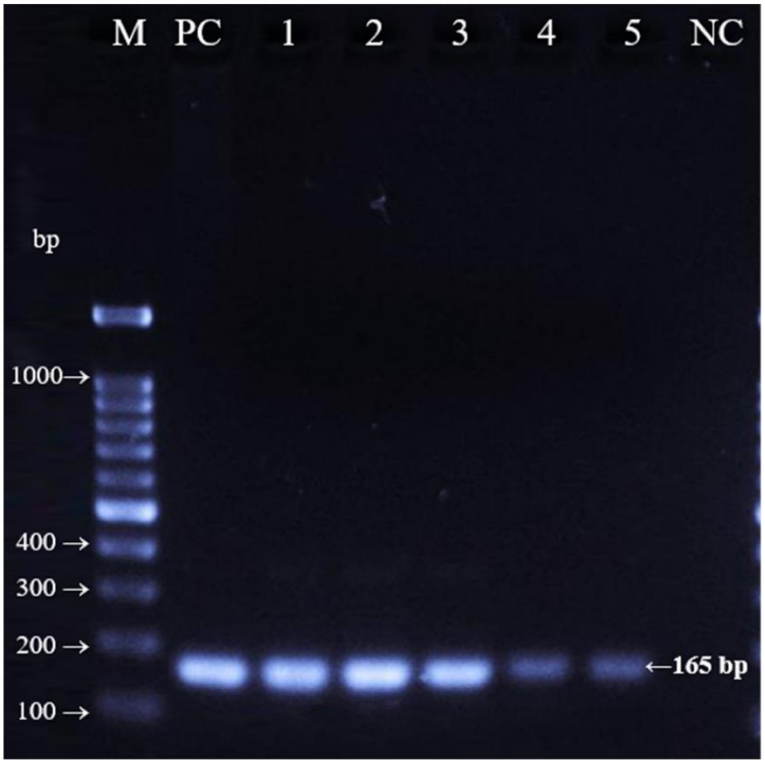

Figure 1. Electrophoresis in agarose gel reveals portion of the 165 bp for 16s rRNA gene

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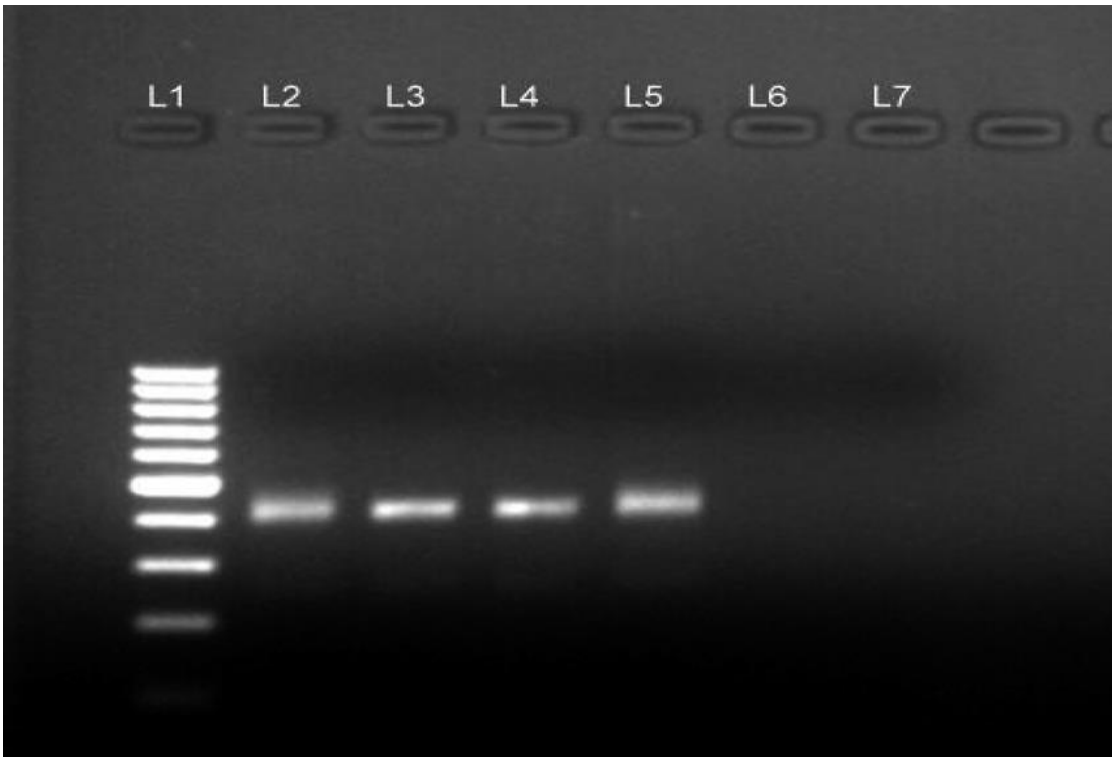

Figure 2. Electrophoresis in agarose gel reveals portion of the 407 bp for SPY1258 gene

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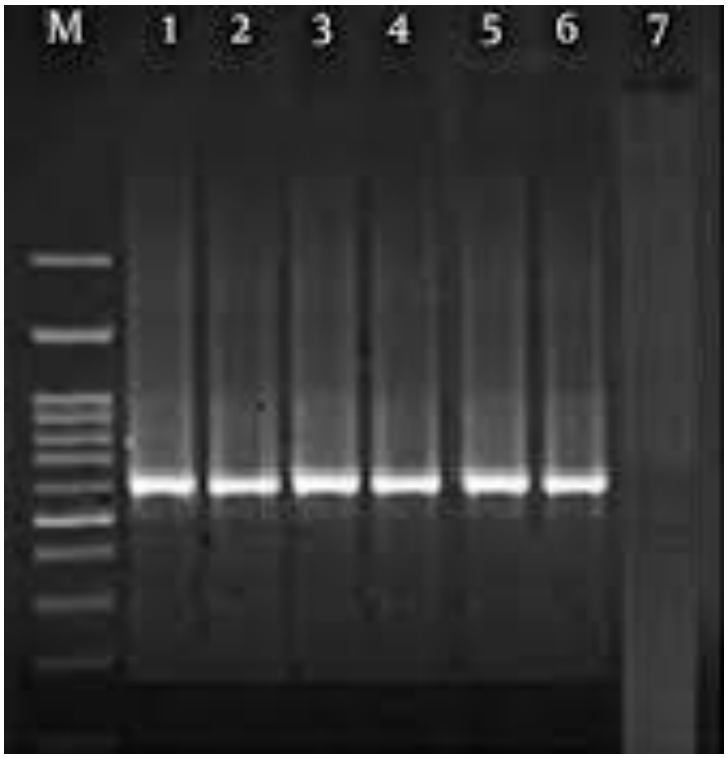

Figure 3. Electrophoresis in agarose gel reveals portion of the 563 bp for pml gene

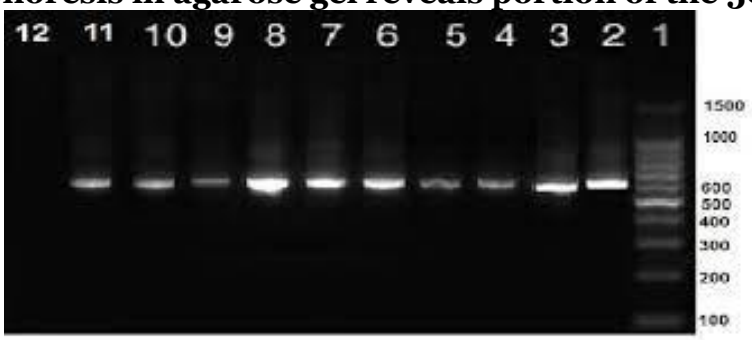

Figure 4. Electrophoresis in agarose gel reveals portion of the 623 bp for uidA gene

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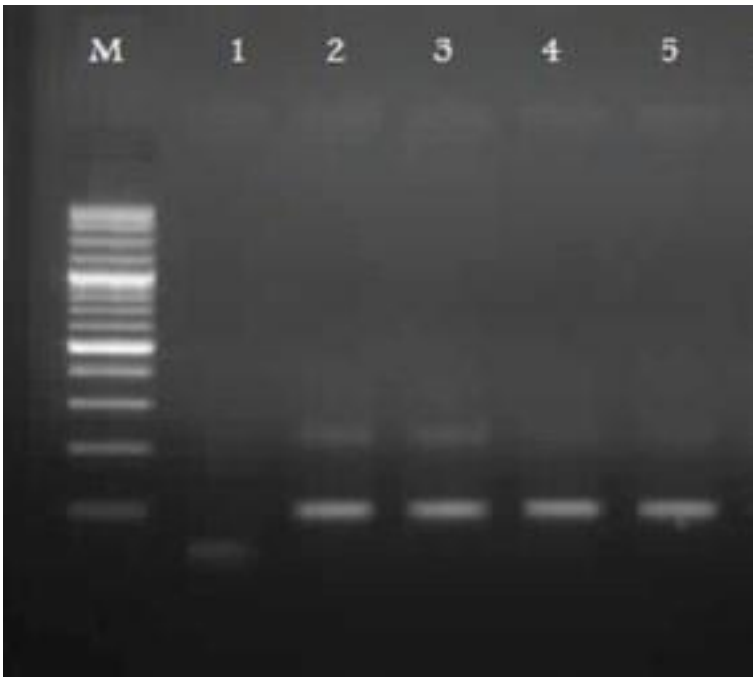

Figure 5. Electrophoresis in agarose gel reveals portion of the 101 bp for 16s rRNA gene

Figure 6. Electrophoresis in agarose gel reveals portion of the 222 bp for gyrb222 gene

The field of microbiology has benefited greatly from recent developments in molecular methods. The time and money needed to carry out these molecular procedures are gradually reducing as a consequence of technological advancements, which have led to new discoveries and rising demand. To cut down on the time and money wasted on incorrectly characterizing of isolates, however, it is necessary to verify the organism's identification first (30).

Conclusion:

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Staphylococcus aureus was shown to be most bacteria that isolated from lip sticks and face cream.

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