




## Determinación de *Staphylococcus aureus* en delineadores líquidos, en la ciudad de Cuenca, Ecuador

### *Determination of Staphylococcus aureus in liquid eyeliners*

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**Palabras claves:**

Contaminación  
Cuenca  
Delineadores  
Microbiológico  
S. aureus

**Resumen**

**Introducción:** En la industria cosmética, la calidad y la seguridad de los productos es fundamental para proteger la salud del consumidor. La presencia de microorganismos patógenos, como *Staphylococcus aureus*, en delineadores líquidos representa un riesgo para la salud ocular y cutánea de los usuarios. **Objetivo:** Identificar la presencia de *Staphylococcus aureus* en delineadores de ojos comercializados en el centro comercial "El Arenal" de Cuenca. **Metodología:** Se realizó una investigación exploratoria-descriptiva para cuantificar Unidades Formadoras de Colonias de *Staphylococcus aureus* en delineadores líquidos. Se ejecutó un muestreo por conveniencia, se escogieron 30 puestos de venta de cosméticos en el centro comercial "El Arenal", seleccionando 5 puestos a conveniencia. Se eligieron 15 muestras en total. Las muestras se prepararon, utilizando caldo de enriquecimiento Letheen con Tween. Se sembraron en tres medios de cultivo selectivos: Modified letheen agar, manitol salado y Macconkey, se incubaron a 37°C para su lectura a 24 y 48 horas. Su identificación se realizó por observación e identificación de morfología de las colonias y pruebas bioquímicas confirmatorias: catalasa y coagulasa. **Resultados:** Los delineadores líquidos seleccionados mostraron presencia de *S. aureus* en 4 de los 5 locales de expendio evaluados. Las pruebas bioquímicas confirmaron la presencia de *S. aureus* dando resultado positivo el 40% total de las muestras. **Conclusión:** La presencia de *S. aureus*, resalta la importancia de la vigilancia y control de la calidad cosmética que deben llevar los organismos de control en el país para proteger la salud pública y garantizar la seguridad del consumidor. **Área de estudio general:** Bioquímica farmacéutica. **Área de estudio específica:** Microbiología. **Tipo de estudio:** Artículos originales

**Keywords:**

Eyeliners  
S. aureus  
Identification  
Pollution  
Microbiological

**Abstract**

**Introduction:** Product quality and safety in the makeup industry are essential to protect the consumer health. The presence of pathogenic microorganisms, such as *Staphylococcus aureus*, in liquid eyeliners represents a risk to users' eye and skin health. **Objective:** To identify the presence

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of *Staphylococcus aureus* in eyeliners sold in the "El Arenal" shopping center in Cuenca. Methodology: An exploratory-descriptive research was conducted to quantify *Staphylococcus Aureus* Colony Forming Units in liquid eyeliners. A convenience sampling was carried out, 30 cosmetics sales stands were chosen in the "El Arenal" shopping mall, selecting five stands for convenience. A total of 15 samples were chosen. The samples were prepared using Lethen enrichment broth with Tween. They were seeded in three selective culture mediums: Modified Lethen agar, salted mannitol, and Macconkey, and incubated at 37 °C for reading after 24 and 48 hours. They were identified by observing and recognizing colony morphology and confirmatory biochemical tests: catalase and coagulase. Results: The selected liquid eyeliner samples showed the presence of *S. aureus* in four out of the five stands tested. Biochemical tests confirmed the presence of *S. aureus*, with positive results in 40% of the total samples. Conclusion: The presence of *S. aureus* highlights the importance of cosmetic quality surveillance and control that the country's regulatory agencies must carry out to protect public health and guarantee consumer safety.

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## Introduction

The presence of certain bacterial species belonging to the genera *Staphylococcus*, *Streptococcus* and *Pseudomonas* are a cause for great concern due to their association with numerous diseases related to respiratory problems and chronic diseases due to their ability to produce toxins (1).

In 2022, in a study conducted by Gurav in India, at the Shriram Institute within the area of Microbiology, he investigated the microbial contamination present in mascaras that he purchased in various cosmetic stores located in the suburbs of Delhi, for which he collected 15 samples to analyze them. The results revealed that 27% of the samples studied corresponded to the presence of *Staphylococcus aureus*, on the other hand, he found that 40% of the samples were contaminated with *Pseudomonas aeruginosa*, a bacteria that can cause opportunistic infections in susceptible individuals. In addition to this, he detected the presence of the yeast, *Candida albicans*, in 47% of the samples analyzed (2). The presence of pathogenic bacteria and fungi in these cosmetic products poses potential risks to the eye health of consumers. These results underline the

importance of carrying out thorough quality controls in the cosmetics industry and of promoting awareness about hygiene and the safe use of beauty products (3).

The presence of *Staphylococcus aureus* in eyeliners, a beauty cosmetic used in the eye area, is an important problem that requires attention from the control authorities of our country (4). This microorganism is commonly found on the skin and mucous membranes of humans and although it may be harmless under normal circumstances, it can cause serious infections such as bacterial conjunctivitis, blepharitis and styes in consumers with a weakened immune system (5). Therefore, it is important to verify the final quality of the products, that is, that they comply with the Good Manufacturing Practices (GMP) standards and with the requirements established by the Ecuadorian standard NTE INEN-ISO 22718 – 2014. The control and identification of microorganisms dictated in this standard must be carried out through a reliable and precise identification method to detect the presence of *Staphylococcus aureus* in eyeliners. This implies that cosmetic products must comply with the quality standards established by the National Agency for Regulation, Control and Sanitary Surveillance (ARCSA) prior to obtaining the Mandatory Sanitary Notification (NSO), a necessary requirement for products imported into Ecuador, which specify the total absence of *Staphylococcus aureus* in a sample of 1 gram or 1 milliliter of product (13).

The identification of *Staphylococcus aureus* in eyeliners can be essential to prevent potential health risks and improve the quality and safety of cosmetic products, resulting in greater consumer protection (6). Routine monitoring at the outlets selling this type of cosmetic products can prevent contamination and the spread of infectious diseases (7).

The present investigation aimed to determine the presence of *Staphylococcus aureus* in samples of liquid eyeliners, sold in the "El Arenal" shopping center in Cuenca.

### Methodology

The methodology followed for the identification of *S. aureus* in liquid products is the one established by the Food and Drugs Administration (FDA) in chapter "G" Preliminary sample preparation, of its "Bacteriological Analytical Manual" (BAM), chapter 23, methods for cosmetics. In this experimental investigation, the quantification of Colony Forming Units (CFU) of *Staphylococcus aureus* was verified and performed in order to check compliance with the NTE INEN-ISO 22718 - 2014 Regulation COSMETOLOGY. MICROBIOLOGY. DETECTION OF STAPHYLOCOCCUS AUREUS.

Non-probabilistic convenience sampling was applied, 15 units of eyeliners were collected, that is, liquids of different brands and colors that are sold in the shopping center "El Arenal", located in the city of Cuenca, province of Azuay. The samples were obtained during 6 days from the cosmetics vending stands where 5 stands were sampled, selected

for presenting the same types of cosmetics. That is, the same brand, in which 3 different units were selected in each stand.

To counteract the investigation, a purity control was carried out by incubating the Lethen culture broth with Tween (MLB) as well as the media used: salted mannitol agar, Modified letheen agar (MLA) and MacConkey. In addition, an ATCC-23x923 strain of *S. aureus* was incubated in the Lethen culture broth with Tween (MLB) for positive control of the same, subsequently the sowing was carried out in salted mannitol agar, Modified letheen agar (MLA) and MacConkey, in which growth could be observed in all the media used.

For the sample identification procedure, each position was numerically labeled from 1 to 3 according to the corresponding brand, taking into account that each sample refers to a type of brand.

### **Sampling**

For the sample collection, the condition of the liquid eyeliner packaging at the time of purchase was verified. It was observed whether the product was sealed or not. If the eyeliner was sealed, it was recorded as such. In the event that the product was not sealed and did not have the Mandatory Health Notification (NSO), a collection procedure was followed in sterile bags, maintaining adequate aseptic conditions during the process. This collection was carried out at all the selected marketing posts and was subsequently transferred to the Microbiology laboratories of the Biochemistry and Pharmacy Degree of the Catholic University of Cuenca for the respective analyses.

### **Identification of *Staphylococcus aureus***

Following the procedures established by the FDA, BAM chapter 23 for liquid products, readings were taken at 24H and 48H of the seeded plates that were incubated at 37°C. The samples were seeded in three different culture media: mannitol salt agar, Modified letheen agar (MLA) and MacConkey, the growth of *S. aureus* colonies was observed; biochemical confirmation tests were performed: catalase and coagulase, established by the Clinical and Laboratory Standards Institute (CLSI). The biochemical confirmation tests are reflected in table 1.

**Table 1:** Biochemical Tests Catalase and Coagulase.

Positions	Samples	Catalase	Coagulase	Incubation hours
1	1	Positive	Positive	48h
2	1	Positive	Positive	24h
3	1	Positive	Positive	24h
5	1	Positive	Positive	24h
5	1	Positive	Positive	24h
5	1	Positive	Positive	48h
1	2	Positive	Positive	24h

### Results

The results of the microbiological analysis revealed the presence of *S. aureus* in 6 of the 15 samples selected from the stalls sampled in the "El Arenal" shopping center, giving a positive result in 4 of the 5 stalls selected, therefore, it is inferred that 40% in total of the samples present *S. aureus*.

### MLA agar

The results of the MLA plate analysis revealed that 5 of the 15 samples inoculated were positive. These correspond to products from stations #1, 2, 3 and 5 after incubation at 37 °C for 24 and 48 hours.

### AgarMacConkey

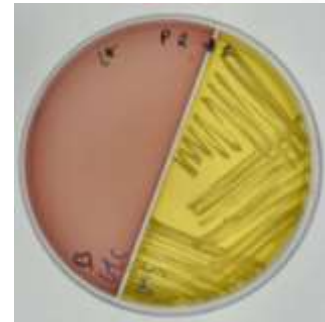
As expected, all samples cultured in MacConkey culture medium were negative upon culture and observation at 24H and 48H at 37°C of incubation.

### AgarMannitolSalty (MLA)

Regarding the sowing in the MLA culture medium, 5 of the 15 samples sown were positive. These correspond to the products from positions #1, 2, 3 and 5 after their incubation at 37 °C at 24H and 48H. The results are reflected in figure 4.



**Figure 4:** Growth results of *S. aureus* in Mannitol Salt medium



**Figure 5:** Growth of *S. aureus* in Mannitol Salt medium

To confirm the results obtained, the positive samples were inoculated in duplicate on Mannitol Salt Agar. This information can be compared in Table 2.

**Table 2.** Results confirming *S. aureus* in mannitol salt medium

Position	Sample	TO	B	Incubation hours
1	1	Positive	Positive	48 h
2	1	Positive	Positive	24 h
3	1	Positive	Positive	24 h
5	1	Positive	Positive	24 h
5	1	Positive	Positive	48 h
5	2	Positive	Positive	24 h

A: plate 1 of the duplicate.

B: plate 2 of the duplicate.

### Confirmatory biochemical tests: catalase and coagulase

#### Catalase

Of the 5 samples that showed direct growth of *S. aureus* colonies in the medium, 100% of them were positive for the catalase confirmation test, performed in duplicate.

## Coagulase

For the coagulase test, two types of serum were used, the first extracted in a tube with Ethylene Diamine Tetraacetic Acid (EDTA) and the second serum from the tube with Citrate, in order to rule out false positives, that is, some bacteria use citrate in their metabolism that after four hours of incubation or more can give false positive reactions for the coagulase activity; however, the serum with EDTA works for this type of bacteria that do not use EDTA and there will be no false positive reactions.

Of the 5 samples that showed direct growth of *S. aureus* colonies in the selective medium, 100% of them were positive for the coagulase confirmation test, which was performed in duplicate. The detection of the extracellular staphylocoagulase enzyme, which activates prothrombin present in plasma and produces its coagulation, verifies bacterial contamination in the analyzed samples of liquid eyeliners from the "El Arenal" shopping center.

## Discussion

The presence of *S. aureus* in samples of liquid eyeliners sold at the "El Arenal" shopping centre in Cuenca was confirmed by the analyses carried out, resulting in 4 of the 5 evaluated stands being positive. This contamination could represent a risk to the health of users, since *S. aureus* is a known pathogen that can cause a wide range of infections, including serious eye infections. This is crucial in the context of consumer protection and the prevention of adverse effects derived from the use of cosmetics (9).

The results found differ from the research carried out by Tirado (10) who investigated the microbiological quality of liquid eyeliners sold in the "Uyustus" zonal market in the city of La Paz, in which using the confirmatory medium salt mannitol, he found the absence of *S. aureus* in 7 of the samples analyzed. However, in 2 samples, he reported counts of 80 CFU/ml and 720 CFU/ml of total aerobic microorganisms, respectively. This discrepancy could be due to differences in manufacturing, storage or handling practices of cosmetic products in different geographic regions, as well as variations in environmental and sanitary conditions (11).

Likewise, Minacapilli (12) when carrying out a microbiological control of cosmetic products used in the ocular area, in the Sambil Shopping Center in the city of Santo Domingo, Ecuador, found that of the 7 samples collected, all were negative despite the fact that 5 of them did not have Mandatory Sanitary Notification (NSO).

## Conclusions

- It was found that 6 out of 15 total samples showed the presence of *S. aureus*, i.e. 4 out of 5 stands selling eyeliners were positive in this study. These findings



underline the importance of monitoring and controlling the microbiological quality of cosmetic products, as well as the need to implement preventive measures to protect public health and ensure consumer safety.

### Conflict of interest

The authors of this article declare that they have no conflicts of interest.

### Authors' contribution statement

The authors of this article declare an equal contribution to the research and development of this article.

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