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Determinación de control microbiológico de pseudomona en máscaras de pestañas en la ciudad de Cuenca

Determination of microbiological control of pseudomonas in eyelash mascaras in the city of Cuenca

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Palabras claves:
Pseudomona sp, Máscaras de pestañas, Cuenca, NSO, importación

Resumen

Introducción: La microbiología cosmética es aquella que garantiza la inocuidad de los productos cosméticos, la presencia de patógenos nosocomiales como Pseudomona debería ser nulo, sin embargo, el mal control de las Buenas Prácticas de Manufactura (BPM) durante la manufactura del maquillaje puede generar contaminación por este microorganismo y poner en riesgo la salud del consumidor. Por lo tanto, la FDA resalta la importancia de realizar pruebas microbiológicas a todo producto destinado para el uso humano. **Objetivo:** Determinar la calidad microbiológica de máscaras de pestañas que se expenden en Cuenca, para observar si cumple con los límites máximos permitidos según la Notificación Sanitaria Obligatoria (NSO) colombiana para Pseudomona. **Metodología:** Se realizó un estudio experimental puro, en 5 marcas de máscaras de pestañas diferentes aplicando las pautas dadas por la FDA para un análisis microbiológico. La identificación de Pseudomona sp se dio con base en las pruebas de identificación realizadas en el laboratorio. **Resultados:** Se analizó un total de 5 marcas de máscaras de pestañas de las cuales, 2 son marcas ecuatorianas y 3 marcas importadas. Teniendo en cuenta que solo una de las marcas importadas presentó crecimiento positivo para Pseudomona sp esto se confirmó mediante el análisis microbiológico, con la aplicación de medios de cultivo y pruebas específicas, además de un análisis estadístico de las variables mediante la aplicación IBM SPSS 26. **Conclusión:** La identificación de Pseudomona en la marca de máscaras de pestañas señala un incumplimiento en la NSO, indicando la falta de calidad y seguridad en su aplicación. **Tipo de artículo**: Original. **Área de estudio o rama de la ciencia:** Bioquímica y Farmacia

Keywords:
Pseudomonas, eyelash mascaras, Cuenca, NSO, import

Abstract

Introduction: Cosmetic microbiology oversees guaranteeing the safety of cosmetic products; the presence of nosocomial pathogens such as Pseudomonas should be null; However, poor control of Good Manufacturing Practices (GMP) during the manufacturing of makeup can generate contamination by this microorganism and put the consumer's health at risk.

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Therefore, the FDA emphasizes the importance of performing microbiological tests on all products intended for human use. Objective: To determine the microbiological quality of masks sold in Cuenca, observing if they comply with the maximum limits allowed according to the Colombian Mandatory Health Notification (NSO, by its Spanish acronym) for Pseudomonas. Methodology: A pure experimental study was conducted on five different mascara brands, applying the guidelines given by the FDA for a microbiological analysis. The identification of Pseudomonas sp was based on identification tests performed in the laboratory. Results: Five mascara brands were analyzed, including two Ecuadorian and three imported brands. Considering that only one of the imported brands showed growth for Pseudomonas sp, this was confirmed through microbiological analysis, using culture media, specific tests, and statistical analysis of variables using IBM SPSS 26. Conclusion: Identifying Pseudomonas in the mascara brand indicates non-compliance with the NSO, indicating the application's lack of quality and safety.

Introduction

*Pseudomona aeruginosa*It is an opportunistic bacteria, a gram-negative bacillus, which is present in various ecological niches and has evolved to produce pigments thanks to its ability to take advantage of the iron present in its environment (1). This pathogen is involved in nosocomial infections which can be fatal in immunocompromised people (1). The guidelines indicated by the FDA explain that there should be no growth of Pseudomona aeruginosa in cosmetics, since this is a factor for the products to be withdrawn from the market (2), this is due to the ability to form biofilms that protect the bacteria, granting it resistance to preservatives (3). Products contaminated with Pseudomona are related to the lack of hygiene by the personnel manufacturing mascaras, this is because this microorganism is a skin saprophyte (4).

According to the author Jalón-Ortiz (4) in his study on ocular reactions produced by cosmetics in 2017, he establishes that mascara and eyeliner are the most used products by the general population, however, they generate the growth of various types of bacterial colonies (5). The study showed that the time of use of the product reduces the preservative used, which can lead to an ocular infection due to the growth of pathogens

(3). Cases of corneal infections and ocular keratitis caused by lacerations when using the contaminated mascara brush have been reported, this can generate a risk to the vision of consumers (6). In the European Union, approximately 173 cosmetic products have been withdrawn, of these 24 due to contamination with P. aeruginosa (7). The FDA highlights the importance of carrying out microbiological studies to detect the presence of Pseudomonas in cosmetics (8, 9).

In Ecuador, the National Health Plan focuses on addressing microbial resistance present mainly in hospitals and health centers, emphasizing the poor control of nosocomial infections (10). Despite using beta-lactams to treat P. aeruginosa infections, resistance to this antibiotic has been observed since 2017, with a progressive increase in subsequent years (10), demonstrating that initiatives to curb antimicrobial resistance are ineffective so far (10).

In our country, it is not mandatory to perform microbiological tests to check if cosmetics are meeting the established manufacturing parameters; they are only guided by the requirements requested by the Colombian NSO. This regulation establishes minimum and maximum growth limits, however, it does not standardize the processes to carry out correct control. In the city of Cuenca-Ecuador, certain makeup distributors are experiencing an increase in sales due to the low prices at which makeup is offered, so the microbiological quality of mascaras that are issued in the city of Cuenca is being determined and whether they comply with the provisions of the regulations.

Methodology

This study is purely experimental in nature, applying a non-probabilistic sampling approach. It was developed in the period of July 2023. The procedure was performed on 5 different brands of mascaras, of which 2 are Ecuadorian brands and 3 are imported brands. The experimental procedure was carried out according to the parameters specified by the FDA in the Bacteriological Analytical Manual (BAM) (Chapter 23), where the sampling methodology for oil- and cream-based cosmetic products is defined.

Experimentation and data selection techniques

Five mascaras were obtained from various stores and distributors in the city of Cuenca, which were of different brands and the style of the packaging was different, and which were taken to the microbiology laboratories of the Catholic University of Cuenca to be analyzed.

Sample collected

Following the instructions in Chapter 23 of the FDA, the 5 mascaras were analyzed. A 10 -1 solution was prepared under completely aseptic conditions, from which 1 mL of

mascara was extracted and placed in screw-top tubes previously prepared with 1 mL of Tween 80. The samples must be homogenized in a vortex before adding 8 mL of modified Letheen Broth Base (MLB). They are incubated at 37°C for 24 hours (11).

Reading the results

Of the 5 brands obtained, only one showed growth of gram-negative, oxidase-positive bacilli. Therefore, 4 mascaras of the same brand were purchased, but in different batches, from different distributors in the city and the procedure indicated by the FDA, BAM 23, was repeated.

Identification of Pseudomona aeruginosa

The modified Letheen broth has the necessary properties to neutralize preservatives in cosmetic products and generate successful bacterial growth (12). The tubes were cultured for 24 hours at 35°C. The direct microbiological culture was performed on Cetrimide agar, which is specific and differential for Pseudomona. The Letheen broth culture was inoculated with a sterile loop and was then cultured by exhaustion on the cetrimide agar. The media was cultured for 24 hours at 35°C, giving positive results of bacterial growth. When subjected to ultraviolet light, the presence of bright green fluorescence was observed, as shown in Figure 1.

To corroborate the Pseudomona result, the oxidase test was performed, and it was grown on TSI (Triple Sugar Iron), Macconkey, Citrate and Muller Hilton agar.

Statistical analysis

Statistical analysis was performed using the IBM SPSS 26 application on all samples analyzed with the variables: Pseudomona sp, product content, health notification, packaging status; it is performed once the microbiological analysis was completed; this with the objective of determining the influence of each variable mentioned above on the presence or absence of Pseudomona. For this, the 9 samples that showed or did not show growth in the microbiological analysis were statistically analyzed, without exclusion parameters.

Results

To validate the growth obtained on cetrimide agar, confirmatory tests for Pseudomona were performed, emphasizing the positivity of the oxidase test. TSI agar is used to isolate gram-negative bacilli, which are differentiated by the color change in the medium. In the case of Pseudomona, the medium changes its pH due to the way in which this bacteria assimilates nutritional components, generating a turn of the medium to red. Likewise, it is not a gas producer, nor a sulfuric acid producer, as seen in Figure 3b.

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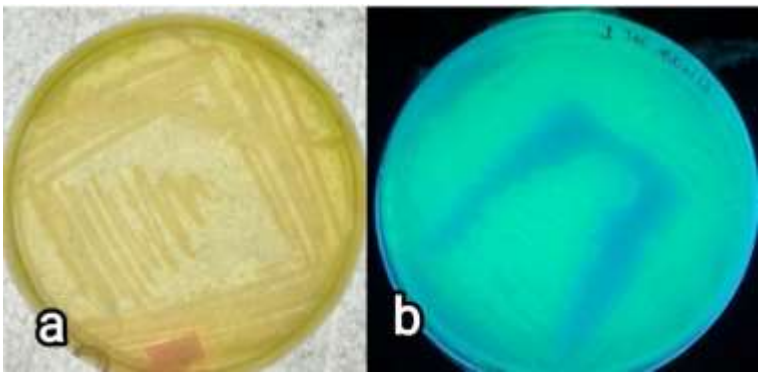

Figure 1.a. Cetrimide Agar: Positive growth, green coloration of the medium. b. Exposure to UV light with positive fluorescence

Macconkey agar helps to distinguish lactose-fermenting bacteria, which have significant growth of pink colonies (13,14,15). Pseudomona species do not ferment lactose, resulting in growth of clear or brown colonies, as shown in Figure 2.

Figure 2. Mackonkey Agar: Round, shiny brown colonies. Releases pigment into the medium.

Bacteria using citrate as the sole carbon source cause a blue color change on Simmons citrate agar, as can be seen in Figure 3a.

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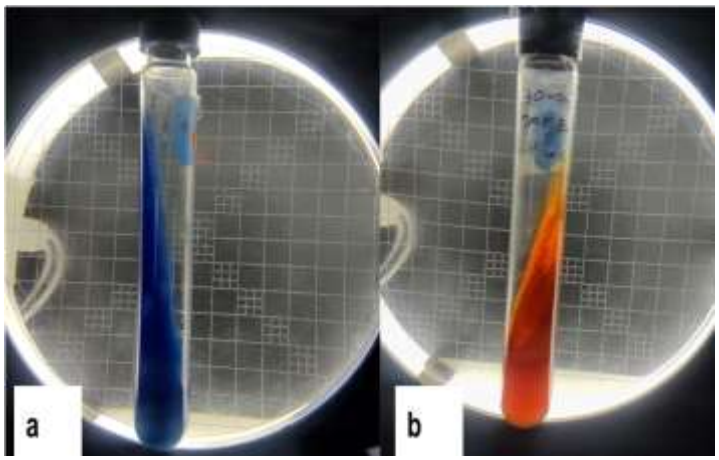

Figure 3. a. Simmons Citrate Agar, medium staining blue. b. TSI Agar, medium staining red.

Muller Hilton agar is used to observe the bacterial growth and coloration characteristic of Pseudomona, which produces a greenish pigmentation that diffuses into the medium, as can be seen in Figure 4.

Figure 4. Muller Hilton Agar

After performing the microbiological analysis of the different samples, the positive result for Pseudomona sp in a foreign brand is confirmed.

Statistical Analysis

The percentage of each independent variable (table 2, 3, 4) is analyzed with respect to the presence or absence of Pseudomona dependent variable (table 1) in the 9 mascara samples.

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Table 1. *Result of statistical analysis of the variable Pseudomona sp.*

Pseudomona sp.					
		Frequency	*Percentage*	*Valid percentage*	*Cumulative percentage*
lido	*Presence*	5	55.6%	55.6 %	55.6%
	Absence	4	44.4%	44.4%	44.4%
	Total	9	100.00%	100.00%	

Of the total of 9 samples analyzed, 55.6% of the sample (5 masks) showed the presence of Pseudomona, while 44.40% (4 masks) did not present said bacteria, which indicates that the majority of the sample showed growth of Pseudomona.

Table 2. *Statistical analysis result of health notification*

Health Notification					
		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	*Without health notification*	5	55.6%	55.6%	55.6%
	With health notification	4	44.4%	44.4%	44.4%
	Total	9	100.00%	100.00%	100.00%

Table 2 describes the analysis regarding the health notification variable. Of the total analyzed, 55.6% (5 masks) do not have a health notification, however, 44.40% of the total samples (4 masks) have a health notification, so the health notification variable significantly affects the presence or not of Pseudomona sp (Table 1).

Table 3. Statistical analysis result of the packaging condition variable

Packaging status					
		Frequency	*Percentage*	*Valid percentage*	*Cumulative percentage*
Valid	*Closed*	9	100.00%	100.00%	100.00%

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Regarding the packaging status variable (Table 3), it shows that 100% of samples (9 masks) have closed packaging, therefore, this variable is not influential in the presence or not of Pseudomona sp (Table 1).

Table 4. Statistical analysis result of the net product content variable

Net content of the product					
		Frequency	*Percentage*	*Valid percentage*	*Cumulative percentage*
Valid	*7ml*	6	66.7	66.7	66.7
	10ml	3	33.3	33.3	33.3
	Total	9	100.00%	100.00%	100.00%

In the variable of net product content (table 4), it indicates that 66.7% (6 masks) of the total samples (9 masks) contain 7 ml, and that 33.3% (3 masks) contain 10 ml, therefore, this variable does not influence the presence or absence of Pseudomona sp (table 1).

Discussion

Mascara is the most used product at the level of eye cosmetics, according to several studies carried out by Jalón-Ortiz (4) and Bashir & Lambert (16), in the study Microbiological study of used cosmetic products: highlighting possible impact on consumer health carried out in 2020, they concluded that it is necessary to carry out microbiological studies on cosmetics, since they increase bacterial proliferation once the packaging is opened and during the time of use (4). Therefore, the time of use, once opened, directly affects the concentration and effectiveness of preservatives used in its formulation against microbial development, making cosmetics a danger to health (3). The present study was based on determining whether mascaras (eyelash masks) marketed in Cuenca have the maximum limits allowed according to the Colombian NSO, for this, the study was divided into two stages, the first was an exploratory phase in which 5 brands of eyelashes "A, B, C, D, E" are analyzed, in this a microbiological study is carried out following the instructions of the FDA and a positive result for Pseudomona is confirmed in a foreign brand (E) which, despite the fact that the packaging arrives closed, does not have the relevant health notification.

Various tests are also carried out to confirm the result of Pseudomona aeruginosa and the second part of the study or verification phase is carried out. To do this, 4 mascaras of the same brand “E” are obtained, from different batches and collected at different points in the city “E1, E2, E3, E4”. In this way, the procedure indicated by the FDA is repeated and it is found that in the 4 samples of mascaras the growth of the bacteria is positive for Pseudomona aeruginosa.

Pseudomona is a nosocomial microorganism with genetic factors that favor a pathogenic corneal invasion, even invading the corneal stroma (3). Ulcerative keratitis is an ocular infection that can be caused by Pseudomona, this disease is high risk for the eye, being one of the main causes of vision loss worldwide.(3). However, for an infection by this microorganism to occur, there must be a previous injury to the eye. This bacteria takes 24 hours to produce an infection and less than a week to perforate the cornea (7). Incorrect handling of mascara can lead to the consumer injuring the cornea with the brush (7). Visual diseases caused by this microorganism are difficult to treat because this pathogen has the ability to form biofilms that serve as a barrier against antimicrobial agents, generating resistance to the antibiotic and making it impossible to end the infection (3).

For a cosmetic to be safe for human use, it must have been produced in companies that have good manufacturing practices (GMP), and physical-chemical and microbiological analyses must be carried out to ensure that it does not represent a danger (3). Cosmetic microbiology is the one that evaluates the microbiological quality of the products, avoiding premature deterioration of the formulation (3). A cosmetic product can become contaminated in the manufacturing or packaging stages, either due to incorrect cleaning of equipment or by the personnel themselves. To prevent this from happening, the operators in charge must be aware of the hygiene and GMP standards imposed by the company (3).

In Ecuador, cosmetics are imported which do not have the necessary microbiological studies to be considered a quality product. The fact that they do not have a health notification and can be marketed in several distributors, puts the health of consumers at risk. The NSO establishes the requirements and procedures that cosmetic products must comply with to be marketed in the Andean subregion (17). In this way, control and surveillance can be carried out in the market and human health can be assured (17). However, the certificate requested must contain information approved by the local authority declaring the product as competent in compliance with the technical, location and hygienic-sanitary conditions (17).

This is how Resolution No. 2120 of the Andean Regulation on microbiological technical specifications of cosmetic products approves cosmetic products that have complied with the physicochemical parameters and are free of microbiological contamination (17). Determining that cosmetic products for use in the eye area or that come into contact with mucous membranes must have an absence of Pseudomona aeruginosa in 1 g or ml of product. In this case, contamination is present in several products of the “E” brand by Pseudomona aeruginosa, following what is indicated by the NSO, this brand is not suitable for human use.

Conclusions

- It is important to note that Pseudomona is a genus of bacteria that can cause eye infections when it comes into contact with the eyes. The Colombian NSO, as a health standard, establishes standards and requirements to guarantee the safety and quality of mascaras; indicating that the presence or growth of Pseudomona in these products should be zero.
- The presence of Pseudomona sp in the brand of the products analyzed (mascaras) is due to a lack of compliance with the NSO regulations, which indicates a failure to comply with Good Manufacturing Practices (GMP) by the factory staff.
- The relevance of this research is to emphasize the importance of carrying out microbiological analyses on all cosmetic brands entering the country, especially if these are products that come into contact with mucous membranes. In this way, consumer well-being is preserved.

Conflict of interest

We, Dayanarha Zoilanhy Aguilera Buele, Tania Valeria Figueroa Figueroa and Maria Viviana Araujo Campoverde declare that there is no conflict of interest in the development of the project "Determination of Microbiological Control of Pseudomona in Mascaras in the city of Cuenca", we assure that our impartiality will not be compromised in any decision or action related to the project.

Authors' contribution statement

The authors Dayanarha Aguilera, Tania Figueroa and Viviana Araujo have actively participated in the organization, design and interpretation of results. They also took part in the development and experimental analysis. They critically reviewed the final version of the work and agree with its publication in the journal Anatomía digital.

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