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ISOLATION AND IDENTIFICATION OF *Pseudomonas aeruginosa* IN COSMETICS AND PERSONAL CARE PRODUCTS IN IRAQ

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ABSTRACT

Cosmetics contain varying amounts of nutrients that promote microbial growth. Bacteria such as *Pseudomonas aeruginosa*, is the most common contaminants in cosmetic products. Water contamination is a likely source of organisms found in cosmetic products. Shampoo, dish washing liquid, facial cleanser, and wet wipes were all tested.

Employing rigorous isolation and identification techniques, we aim to elucidate the extent of contamination and assess the potential risks associated with the presence of this microorganism in products intended for routine use. Our research involves the systematic collection of samples from various regions of Iraq, followed by thorough microbiological analyses to accurately isolate and identify *Pseudomonas aeruginosa* strains. Our study has shown the highest prevalence of *Pseudomonas aeruginosa* in Shampoo with 3 samples out of 50 samples (6%), whereas the other tested products recorded quite the same results; dishwashing liquid scored 4%, while wet wipes and face cleaner showed 4%. By shedding light on the microbial safety of cosmetics and personal care items, this study contributes valuable insights for regulatory authorities, manufacturers, and consumers, fostering a safer and healthier consumer environment. Culture-based methods demonstrated that reliability in terms of speed, cost-effectiveness, and sensitivity.

The current finding was undertaken in response to the extensive cosmetics market and the paramount concern for consumer safety.

KEYWORDS: *Pseudomonas aeruginosa*, microbial contamination, cosmetic.

1. INTRODUCTION

Pseudomonas aeruginosa is a serious opportunistic pathogen that affects humans, animals, and plants (Al-abedi and Al-Mayahi, 2019). It is an aerobic Gram-negative rod with a respiratory metabolism. The organisms are typically (1.5-5) µm long and (0.5-1.0) µm wide, and they are motile due to the presence of flagella (Al-abedi and Al-Mayahi, 2019). This bacterium can be found in soil and water. It is a significant pathogen in immunocompromised patients, such as those with AIDS, cancer, burn wounds, and cystic fibrosis (CF) (Al-abedi and Al-Mayahi, 2019).

Cosmetics are characterized as substances applied to the external surface of the human body to enhance appearance, improve skin texture, maintain cleanliness, provide fragrance, and offer skin protection. A cosmetic product is defined as any substance or preparation designed for application to the diverse external areas of the human body, or to the teeth and mucous membranes of the oral cavity (Elmorsy and Hafez, 2016; Borgna, 2018). This classification encompasses seven primary categories: oral care products, skin care products, body care products, hair care products, sun care products, fragrance products (such as perfumes), and decorative cosmetic products (Kim *et al*., 2020).

Cosmetics consist of a blend of chemical compounds sourced from both natural and synthetic origins. Essential components of cosmetics encompass water, emulsifiers, preservatives, thickeners, pigments, glitter, and fragrances, among others. Nevertheless, these constituents serve as a conducive environment for the dissemination of pathogens in people's daily routines as these elements often promote microbial growth. Microorganisms can persist under favorable conditions of temperature, pH, moisture, and metabolites (Mohiuddin, 2019).

The microbial contamination of personal care products can originate during production, involving raw materials, ingredients, and handling processes, as well as from repeated

consumer use. Researchers have formulated a diverse array of preservatives to address contamination arising from consumer use (Sivri *et al*., 2006). Presently, the challenge lies in striking a balance between safeguarding against microbial contamination and mitigating potential health risks associated with preservative usage, a delicate equilibrium often referred to as the science of preservation (Rutala and Weber, 2015).

Microbial contamination in cosmetic products is a widespread global health concern, causing inconvenience for consumers, manufacturing industries, and clinicians alike. The susceptibility of cosmetics to contamination arises from impurities in raw materials, exposure to a contaminated environment during production, or inadequate personal hygiene practices (Jung *et al*., 2019). This issue poses challenges and concerns at both the consumer and industrial levels. Cosmetic products are susceptible to contamination due to impurities in raw materials, exposure to contaminated environments, or inadequate personal hygiene practices (Janetos *et al*., 2019). Microbes not only alter the physical attributes such as color, viscosity, flavor, and scent of these products but also degrade essential components, leading to significant consequences. Microbial interference can generate toxic compounds and metabolites, triggering severe allergic reactions on the skin (Bashir and Lambert, 2020). This underscores the importance of maintaining microbial quality in cosmetic formulations.

Contaminated cosmetic products pose potential health risks, lead to a spectrum of illnesses ranging from mild to severe (Zirwas, 2019). Researchers have identified pathogenic microbes, such as Staphylococcus aureus, *Pseudomonas aeruginosa*, Enterobacter, and Klebsiella pneumonia, in cosmetics (Khan and Alam, 2019). These microorganisms can result in various conditions, from severe skin allergies to respiratory infections, as well as bacteremia and urinary tract infections (Khan and Alam, 2019). These opportunistic pathogens, lead to severe infections in mechanically ventilated patients, individuals with compromised immune systems, and those diagnosed with malignancies or HIV infection (Migiyama *et al*., 2016; Moradali *et al*., 2017). It highlights the importance of ensuring the microbiological safety of cosmetic formulations to safeguard consumer health.

A precise and timely diagnosis of a disease is imperative to mitigate prolonged effects and complications. Accurate diagnosis enhances the efficacy of the necessary treatment for infection, avoiding unnecessary procedures and medications. Additionally, a precise disease diagnosis plays a pivotal role in preventing disease outbreaks and minimizing the emergence of antibiotic resistance (Fernández *et al*., 2020). This emphasizes the significance of precise diagnostic methods in healthcare for improved patient outcomes and public health. The primary objective of this study is to conduct a comprehensive isolation and identification process for *Pseudomonas aeruginosa* in imported cosmetics and personal care products within the context of the Iraqi market.

MATERIAL AND METHOD

2.1. Sample collection

A comprehensive total of 150 cosmetic samples, encompassing Shampoo (n=50), dishwashing liquid (n=50), wet paper (wet towel n=25), and face cleaner (n=25), were systematically acquired from various sources. We meticulously examined the containers for any signs of leakage or swelling and checked their expiration dates to ensure their physical integrity. We deliberately omitted cosmetic items approaching expiration within three months from the study, as per established protocols (reference). Concurrently, we procured supplementary samples from diverse local establishments specifically to isolate *P. aeruginosa*.

2.2 Standard strain

A reference strain of *pseudomonas aeruginosa* was obtained from the American Type Culture Collection (ATCC) under the number (ATCC 27853) as seen in figure 1. The specific strain that was used in this study was available at New-Standard Company for testing and quality control, Microbiology Department, Duhok province, Iraq.

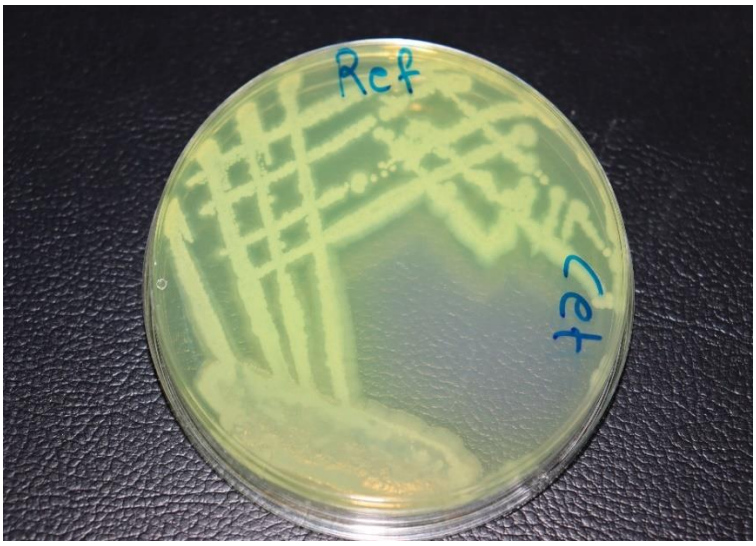

Fig. (1): Reference strain of *P. aeruginosa* on cetrimide agar

2.3 Sample preparation and enrichment

The methodology employed for the isolation and identification of *P. aeruginosa* in this study adhered to the established procedures outlined in the International Standard Organization (ISO) document ISO 22717:2015. One-gram samples should be carefully and aseptically transferred into vials that hold 9 ml of Modified Letheen Broth (MLB), which is obtained from NEOGEN Laboratories, United Kingdom. Following thorough mixing by vortex for 30 seconds, the aliquots were incubated at 32.5˚C ±2.5˚C for at least 20 h. (Maximum 72 h).

2.4 Isolation of *pseudomonas aeruginosa*

From incubated enrichment broth, a loopfull of subsequent culture was streaked on the surface of cetrimide agar medium (NEOGEN, United Kingdom) in order to obtain isolated colonies and inverted the Petri dishes then incubate at 32.5˚C ±2.5˚C for 24 h. (maximum 48 h.). It was observed yellow-green pigment (pyocyanin), which fluoresces under UV light. All the cultured media and reagents that were used in this study with originally manufactured countries are presented in Table 1.

Table (1): Media and reagent was used in this study with original manufactures

Media and reagent name	Original country
Cetrimide agar medium	NEOGEN, United Kingdom
Modified letheen broth	NEOGEN, United Kingdom
Oxidase test stick	Liofilchem, Italy
Gram stain	Scharlau, Spain

Table (2): indicates the name of the product, main category, country of origin

Product name	Category	Original country
Shampoo	Hair and scalp care	Turkey
Dishwashing liquid	Body care preparation	Turkey
Wet wipes	Face and neck care	Turkey
Face cleaner	Face and neck care	Turkey

2.5 VITEK-2 compact systems

The presumed colonies of *P. aeruginosa* were selected and introduced into a tube containing 3.0 mL of a 45% sodium chloride solution. The turbidity of the bacterial suspension was standardized to 0.5 McFarland standards using VITEK Densicheck (bioMerieux). Subsequently, the ID-Gram Negative (ID-GN) protocol was employed in conjunction with the VITEK-2 system, adhering to the procedural guidelines outlined in the manufacturer's manual (BioMerieux, France) as previously described by Tayeb *et al*., 2020.

2. RESULTS

In the current study, all samples were tested for the occurrence of *pseudomonas aeruginosa* using the enrichment method recommended by ISO 22717, which is focused on traditional and biochemical identification of suspected colony growth on cetrimide agar plates. A colony of this bacteria appeared with yellow-green pigment as shown in (figure 2), which fluoresces under UV light (figure 3) (ISO 22717:2015), a selective media recommended for the isolation of *pseudomonas aeruginosa* from cosmetics and personal care products. A total of 150 samples were examined for the presence of *pseudomonas aeruginosa*, only seven samples (4.7%) were contaminated with this pathogen. Table 3 shows that among the different types of cosmetics and personal care products tested, the highest percentage positivity of *pseudomonas aeruginosa* was recorded in shampoo items 50 (6.0%) compared to other cosmetic and personal care products, followed by dishwashing liquid 50 (4.0%). There were several types of wet wipes and face cleaners included in the current research. In a total of 25 samples for both wet wipes and face cleaners, only one for each type sample (4.0%) has been shown to be contaminated with this pathogen. The suspected items were verified as positive via conventional culturing techniques and biochemical testing.

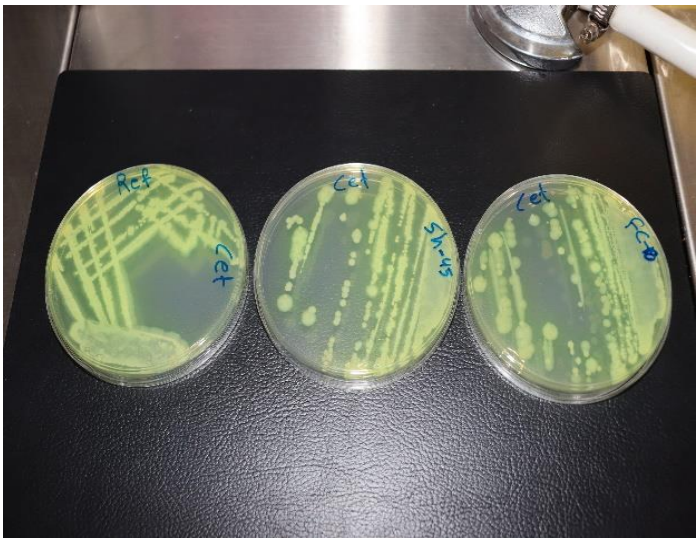

Fig. (2): Growth of *P. aeruginosa* on cetrimide agar after overnight at 32.5 ˚C

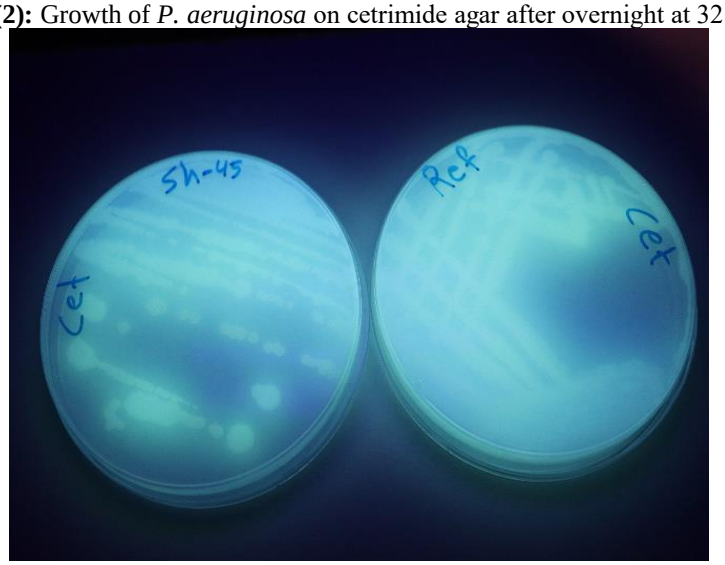

Fig. (3): Growth of *P. aeruginosa* on cetrimide agar after overnight at 32.5 ˚C under UV light

Table (3): Isolation of *P. aeruginosa* in selected cosmetics and personal care products samples

Sample type	No. of samples	positive		negative	
		n	%	n	%
Shampoo	50	3	6	47	94
Dishwashing liquid	50	2	4	48	96
Wet wipes	25	1	4	24	96
Face cleaner	25	1	4	24	96
total	150	7	4.7	143	95.3

3.1 Biochemical confirmation

For confirmation the presumptive colonies of *P. aeruginosa* from the cetrimide agar plates the following microbiological and biochemical tests were performed; gram stain, oxidase reaction and VITEK-2 compact systems as shown in table 4. The reference strain of *pseudomonas aeruginosa* (ATCC 27853) was used for control purposes in all confirmation procedures.

Table (4): Microbiological and biochemical tests of *P. aeruginosa*

Sample name	**No. of positive sample**	**Cultural and morphological characteristics**		**VITEK-2 compact system**	**oxidase**
		CAM (greenish fluorescence colonies under UV light)	MEG (gram negative long rods		
Shampoo	3	+	+	+	+
Dishwashing liquid	2	+	+	+	+
Wet wipes	1	+	+	+	+
Face cleaner	1	+	+	+	+

MEG= microscopic examination of Gram staining (Gram negative long rods), CAM=cetrimide agar medium (Greenish fluorescence colonies under UV light),

3.2 Confirmation by VITEK-2 compact System

This system demonstrated an impressive level of confidence in identifying the target organism, with a probability of 99% certainty, identifying it as *P. aeruginosa*. For the remaining isolates originating from the suspected colonies, the system consistently provided identification values ranging from (93% - 98%), reaffirming their pathogenic nature.

3. DISCUSSION

A total of 150 cosmetic samples were collected from the Cosmetic lab located in Ibrahim Khalil border. Cultural and biochemical identification revealed that only 7 (4.7%) of the samples tested positive for *P. aeruginosa*, according to the finding conducted in central laboratory in Ibrahim Khalil border in Duhok region.

Table (3) shows that shampoo samples had the highest *P. aeruginosa* bacterial counts when compared to other cosmetic samples, followed by dish wash gel, wet wipes, and face cleaner samples. Shampoos were more susceptible to Contamination than other products presumably because they contain surfactants fig (3) and table (3).

P. aeruginosa bacteria were recovered from 6% of shampoos. Whereas, 4% of total samples found in wet wipes, facial cleanser, washing liquid where found to be contaminated with *P. aeruginosa.*

The present work is in agreement with Al-Derzi stated that the prevalence of 5.2% of *P. aeruginosa* was contaminated in his conducted research regarding cosmetic products. Furthermore, 63 *P. aeruginosa* isolates were recovered from 158 samples in Karbala, Iraq, using cultural and biochemical identification (Al-abedi and Al-Mayahi, 2019). This study backup our finding in term of identification of *P. aeruginosa.* Also Hasan *et al.* found 21.6% P. aeruginosa isolates in 185 swab samples from Kirkuk City, Iraq. The variation in *P. aeruginosa* isolation percentage can be attributed to geographical, climatic, and sanitary factors.

Our findings are consistent with (Razooki *et al.*, 2017) report, which found that Gram-negative bacteria were present in these investigations; however, Hugbo's report differs gram-positive cocci, such as staphylococci, were the most common; gram-negative isolates were scarce.

Cosmetics are becoming a significant part of daily life and are excessively used for sun protection, face cleaner and clearing extraneous matter (Onurdağ *et al*., 2010). The presence of *P. aeruginosa* in cosmetics and personal care products can cause various hygiene risks, including skin and eye infections. Individuals with immunocompromised systems (Spernovasilis *et al*., 2021). Also, this pathogenic bacterium carries a very high risk of infection, as they might infect the lips or eyes (Bashir and Lambert, 2020; Burleson and Martinez-Vaz, 2011). According to Alshehrei, (2023), *P. aeruginosa* and *Enterobacter gergovia* were isolated from cosmetic productions and showed higher resistance to parabens and formaldehyde-releasing preservatives.

In recent years, concerns about biological contamination of cosmetic and personal care items have grown. As the use of these products has grown, a lot of studies have been done to assess the microbial contamination in these items. The number of individuals purchasing these things has increased, and the retail sector has grown faster than the population. This study examined the levels of microbiological contamination in cosmetics and personal care items on the Iraqi market and identified the most significant risk factors that cause this kind of contamination (Alshehrei, 2023).

A noteworthy aspect of the research is the comparative examination amongst different product categories, shampoo showed a higher contamination rate 6.0%, while, dishwashing liquid, wet wipes and face cleaner, and all showed a 4.0% contamination rate. The positive findings for every sample category indicate the presence several issues or contaminants in a small percentage of the items. The nature of these contaminants and any possible effects on health or safety must be determined. Contrary to expectations, our study shows a higher percentage of positive results for the presence of *P. aeruginosa* in shampoo compared to other examined products. This unexpected finding prompts a closer examination into the factors contributing to the prevalence of this bacterium in a product normally connected with hygiene and cleanliness. Therefore, while preservatives are commonly used in personal care products to suppress microbial development, the efficiency of these preservatives varies. Shampoo formulations may pose unique challenges in maintaining a balance between preventing bacterial contamination and guaranteeing the safety of the product and the skin tolerance of the user.

Additionally, cosmetic and personal care products may become contaminated by microbes due to factors such as the growing and harvesting circumstances, the storage and transportation of raw materials, and/or the manufacturing environment utilized to produce the finished product. Therefore, high manufacturing process standards should be adhered to, and all raw materials, particularly those that come from natural sources must be tested for contamination and verified to ensure that they fall within suitable bounds (Panico *et al*., 2019).

Such items are regularly exposed to air. Furthermore, the natural constituents in these products, such as bentonite, Fuller's earth, and talc, may raise levels of contamination (Kulkarni *et al*., 2011). These items are regularly exposed to air. Furthermore, the natural constituents in these products, such as bentonite, Fuller's earth, and talc, may raise levels of contamination (Kulkarni *et al*., 2011). Cosmetic items with a high moisture content are the most likely to have microbial contamination; these products' components should be changed to avoid endangering consumer health (Lundov *et al*., 2009). Moreover, microbiological contamination is still a major cause of product recalls globally, particularly in underdeveloped countries in the tropics (Neza and Centini, 2016).

When comparing our results to those of older studies, it must be pointed out that, based on the outcome of this research, the comprehensive examination of 150 (4.7%) samples across different product categories (shampoo, dishwashing liquid, wet wipes and face cleaner) reveals critical insights into the quality and attributes of these consumer products. The focus on positivity and negativity percentages provides a nuanced perspective on the overall performance of each product type and underscores broader considerations for both makers and customers. Also, In Saudi Arabia, Nasser (2008) conducted an investigation into the microbial contamination of 75 cosmetic

samples. The study revealed the highest fungal counts in lip cosmetic products, with 13 and 24 species of mesophilic and thermophilic fungi, respectively, belonging to 6 and 2 genera. Aspergillus was identified as the most prevalent fungal genus. Additionally, approximately 36.7% of the tested samples were contaminated with E. coli, while Pseudomonas and Bacillus were frequently detected (Neza and Centini,2016). Notably, a cosmetic production facility yielded *Pseudomonas aeruginosa* and *Enterobacter gergovia*, exhibiting increased resistance against parabens and formaldehyde-releasing preservatives (Alshehrei, 2023).

Furthermore, the investigation in Italy explored microbial contamination levels in 91 cosmetic samples before, during, and after use (Campana *et al*., 2006). The findings revealed no contamination in the samples prior to use. However, approximately (6) six samples were found to be contaminated during use, primarily with Staphylococcus spp. Furthermore, all samples exhibited contamination after use (Elmorsy and hafez, 2016).

The study have shown that the presence of contaminations in a proportion of the examined samples. In particular, *P. aeruginosa*, a bacterium that can have health implications, was detected in a small percentage of the products. These results highlight the need for stringent quality control measures in the production of these samples, as a few contaminated products can pose potential hygiene risks to consumers.

The result now provides evidence to in terms of other forms of microbial contamination, this research found positive outcomes, with no samples displaying any contamination from *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, or *Escherichia coli*. Overall these findings are not in accordance with findings reported by those of some European studies, which found that the majority of cosmetic contamination was caused by Gram-negative bacteria, chiefly *Pseudomonas aeruginosa* and *Enterobacter gergoviae*; *Staphylococcus aureus* was the most frequently identified of the Gram-positive bacteria. The same research found that *Candida albicans* was the most prevalent fungus.

4. CONCLUSION

This experiment adds to a growing corpus of research showing that the isolation and identification of *Pseudomonas aeruginosa* in cosmetics and personal care products in Iraq is a critical study area that directly impacts consumer safety and public health. The use of various microbiological and biochemical techniques is essential for accurate detection. Companies in the cosmetics industry must prioritize quality control and adhere to regulatory requirements to protect consumers from potential health risks associated with microbial contamination in these products.

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