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Analysis of the microbial contamination in available cosmetic products from Samaru Market, Zaria, Kaduna State, Nigeria

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ABSTRACT

The purpose of this investigation was to assess the level of microbial contamination of cosmetic goods found in Samaru Market, Zaria, Kaduna State, Nigeria. Ten samples of two distinct cosmetics: face powder (labeled as FP) and kohl (labeled as K); were obtained from merchants at Samaru Market. The samples were cultivated on nutrient agar and mannitol salt for the purposes of viable cell count, isolation, and microscopic identification. To further characterize the isolates from the agar, gram staining technique and biochemical tests (catalase, urease, indole, Voges-Proskauer, citrate utilization, and methyl red test) were employed. With the exception of samples K1 and FP2, every sample had total viable bacteria. The United States Pharmacopeia (USP) and Food and Drug Administration (FDA) standards of less than 10^3 cfu/ml of bacteria load for non-eye areas were exceeded by all samples except K2 and FP1. Sample FP5 had the highest count of 3.6 x 10^4cfu/ml, while sample K2 had the lowest count of 1.2 x10^2 cfu/ml. Microscopy, revealed the isolates on mannitol salt agar as rounded, elevated, flat, small, yellowish and pinkish with short rods. The nutrient agar colonies appeared to be short rod-like, mucoid, uneven, rough, and whitish in color. The biochemical characterization of the isolates was positive for catalase, citrate, Voges Proskauer, and motility test. In conclusion, some of the face powder and kohl samples in the study are dangerous to use since *Bacillus* spp. were isolated with a bacterial count that was greater than the acceptable limit.

Keywords: *Bacillus* spp., cosmetic, face powder, kohl, Samaru Market

Citation

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INTRODUCTION

Cosmetics are skin and hair care products designed to cleanse, beautify, and accentuate the qualities that are attractive (Mansuri *et al.*, 2018). Artificial or natural materials are used as cosmetics on many parts of the human body, such as the lips and eyelids (de Oliveira *et al.*, 2020). While there have been numerous modifications to cosmetic products in the contemporary era, the fundamental idea of utilizing cosmetics to improve health-related features has remained constant (Mansuri *et al.*, 2018).

Most cosmetics are made from non-sterile basic components (e.g., natural oils, waxes etc.) (Choubey and Godbole, 2017). Water used in non-hygienic production and ingredients for cosmetics may be polluted and this could offer the perfect environment for the growth of microorganisms, which could result to a variety of diseases, from minor to serious (Alshehrei, 2023; Dadashi and Dehghanzadeh, 2016; Siegert, 2013). Since some cosmetic products (e.g., face powder, kohl etc.) are not made in a medium that has been sterilized against microbial development, they typically contain a number of ingredients that are conducive to microbial growth and this could make the products to be tainted (Siegert *et al.*, 2005). It has long been known that bacteria in cosmetic products can proliferate and develop. Microorganisms have the potential to harm consumers and induce cosmetic product deterioration or chemical alterations (Dashen *et al.*, 2011). Moisture and nutrients encourage the growth of microorganisms in cosmetics. Organoleptic alterations, including colour, viscosity, and unpleasant smells, are often caused by microorganisms (Behravan *et al.*, 2005).

There are two main reasons for the occurrence of microbes in some used cosmetics: The first is caused by applying makeup incorrectly or by engaging in non-hygienic practice such as sharing makeup applicators. Despite the fact that the products include preservatives, this allows skin-derived debris to accumulate, which promotes the growth and dissemination of bacteria (Alshehrei, 2023). The second factor contributing to microbial propagation in cosmetics is particles that are dispersed in indoor air, such as bacteria, spores, and dust (de Oliveira *et al.*, 2020). Furthermore, inadequate air quality significantly affects the development of microorganisms in cosmetics, especially in humid environments (Mohammed *et al.*, 2021). According to Halla *et al.* (2018) there are four main ways to avert microbiological contamination of cosmetics without affecting the product's properties: (1) ingredient assessment and control; (2) manufacturing process; (3) end-product delivery; and (4) customer use. It is necessary to locate and keep an eye on every potential source of contamination (Choubey and Godbole, 2017).

In order to verify the microbiological safety and quality, samples will be analyzed to determine the number of bacteria present under a process known as bacteriological quality (Yusha'u *et al.*, 2017). The most researched microorganisms influencing composition are bacteria, particularly Enterobacteria and Staphylococci (Bashir and Lambert, 2020; Dashen *et al.*, 2011). *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two pathogenic bacteria that have been identified in cosmetic products (Detmer *et al.*, 2010; Sreeparna *et al.*, 2017). *Staphylococcus epidermidis* and *S. aureus* are the most frequent bacteria that cause illnesses in humans, including skin infections alongside deadly filamentous fungi that are connected to both mycotoxin toxicity and sporadic infections (Hayleeyesus and Manaye, 2014).

Conservatives are necessary for two main reasons: (1) to stop microbes from spoiling things, and (2) to stop diseases that are brought about by microbes (Nasir and Qasim, 2020). Additionally, a variety of chemicals found in cosmetics, including pigments, perfumes, preservatives, and additional compounds referred to as skin sensitizers, may be detrimental to one's overall health (Panico *et al.*, 2019). Preservatives must be added to cosmetics to keep them hygienic and safe, as well as to guard against contamination and loss of money (Siya *et al.*, 2019).

Microbiological contamination of cosmetic products is a significant risk to the industry and might be a primary factor in both financial and goods loss (Alshehrei, 2023). This study investigates the level of microbial contamination in cosmetic products sold at Samaru

Market, Zaria, Kaduna State, Nigeria. By analyzing the presence and type of microbes in these products, this research aims to contribute to ensuring consumer safety and product quality.

MATERIALS AND METHODS

Study Area

The cosmetic product samples used in this study were obtained from Samaru Market, Zaria in Kaduna state. Samaru is situated within latitude 11.0819°N and longitude 7.7160°E, at an altitude of 550-700 meters. It is about 13 km from Zaria-city on the Sokoto road, 8 km to Shika, and 7 km from Bassawa.

Collection of Samples

Ten (10) samples overall, representing two (2) distinct cosmetics—face powder (labeled as FP) and kohl (labeled as K)—were collected from merchants at Samaru Market within 2 months period (June-July, 2023). The samples were placed in sterile aseptic labelled containers and immediately transferred to the laboratory for microbiological analysis and prior to the analysis the samples were stored in refrigerator under 4°C.

Sterilization of Materials

Conical flasks, beakers, test tubes, and bottles were among the glassware that was carefully washed with detergents and then cleaned with sterile distilled water. They were then dried at 160°C in an analytical oven. Additionally, the media were autoclaved for 15 minutes at 121°C to sterilize them (Cheesbrough, 2006).

Preparation of Culture Media

The culture media for the microorganisms' growth and isolation were prepared according to the instructions provided by the manufacturer of the several types of agars utilized in this investigation.

Nutrient Agar (Microgen, Central Drug House Pvt. Ltd., New Delhi, India)

In a clean flask, 28g of nutrient agar (NA) powder was dissolved in 1000ml of distilled water. After plugging the flask's mouth with non-absorbent cotton wool, the flask's neck was covered with aluminum foil. The mixture in the flask was shaken to allow the NA power to dissolved properly. It was autoclaved for 15 minutes at 121°C, then cooled to 45°C to achieve sterilization. It was poured aseptically into a petri dish.

Peptone Water **(Titan Biotech Limited. India)**

One hundred milliliters of distilled water were used to dissolve 0.1g of peptone powder to create peptone water. After thoroughly mixing, it was autoclaved for 15 minutes at 121°C to sanitize it. Peptone water was used as a growth medium.

Plate Count Agar (Microgen, Central Drug House Pvt. Ltd., New Delhi, India)

To prepare, 23.5g of the agar was dissolved in 1000 ml of distilled water. It was sterilized by autoclaving at 121°C for 15 minutes after being gently heated to dissolve it. It was poured aseptically into a petri dish and allowed to cool. This medium was used for the total aerobic bacteria plate count.

Mannitol Salt Agar (MSA) (MicroMedica Laboratories Private Limited)

To prepare, 50.03g of MSA powder was measured and mixed in 1000 ml of distilled water. It was left for ten minutes to settled. Following that, it was agitated, blended, and autoclaved for 15 minutes at 121°C to achieve sterilization. It was then given time to cool before being poured into petri dishes.

Serial Dilution

One gramme of every face powder and kohl sample was weighed and added to 9 ml of peptone water to create a stock solution for the serial dilution process. Nine (9) milliliters of distilled water were introduced into sterile test tubes that were stacked in a plastic rack. Next, 1 ml of the stock solution was transferred into the first test tube, which was labeled 10-1, in order to perform a 10-fold serial dilution. After it was well combined, 1 milliliter was removed from the 10-1 dilution tube and placed into the subsequent test tubes (designated 10-2). Before every transfer, a thorough shake was given to each test tube as illustrated by Olu *et al.* (2021)**.** The samples were plated using the spread plate technique. For each of the face powder and kohl samples, 0.1 ml of the dilution 10-2 was obtained with a sterile pipette, added to the surface of a solidified plate count agar, and then evenly spread with a bent glass rod and was incubated at 37°C. for 48 hours

Viable Cell Count

A colony counting machine was utilized to determine the overall plate count of aerobic bacteria as illustrated by Petersen and McLaughlin (2016).

Gram Staining Technique

Almost all gram-positive and gram-negative bacteria may be distinguished using the widely used Gram staining procedure. Each isolate was smeared and heat-fixed on a sanitized, grease-free slide. After applying crystal violet primary stain for 45 seconds, the area was gently cleaned with running water. Afterwards, Lugol's iodine was applied and then rinsed. After using acetone to decolorize it, they were cleaned with clean water. After 30 seconds of 30% safranin counterstaining, the slides were cleaned and dried. After being allowed to air dry, they were inspected using a microscope's oil immersion lens (x100) (Paray *et al*., 2023).

Biochemical Test

Catalase Test

The catalase test is performed to find out if a microbe produces the enzyme, catalase. A spotlessly clean, grease-free slide held a loop containing the culture. The culture (e.g., isolated colony from both Nutrient and Mannitol salt agar plate) was emulsified on the slide using a loop full of 3% hydrogen peroxide (H_2O_2), and the reaction was checked right away to see if the organism was catalase positive or negative (Khatoon *et al*., 2022).

Urease Test

Using phenol red indicator, this test was employed to detect bacteria, especially those that generate the urease enzyme, which breaks down urea into NH_3 and CO_2 and causes the pH to increase. The test organism was injected into urea agar and incubated for 24 hours at 37°C after that.

Indole Test

Weighing 0.1g into 100ml of distilled water produced peptone water. After that, 5 ml was placed into sterile test tubes, which were then loosely capped and autoclaved at 121°C. After allowing it to cool to 30°C, it was inoculated and incubated for 48 hours at 35°C. Kovac's reagent (0.5 ml) was then added, and the mixture was gently mixed. The appearance of a red ring after 10 mins will indicate a positive response (MacWilliams, 2016).

Voges-Proskauer Test

As the growing medium, phosphate-buffered glucose was produced, sanitized, and allowed to cool for 24 hours at room temperature. Next, three drops of alpha-naphthol and one milliliter of 40% KOH were added. Before being observed, the tube was vigorously shaken and let to stand for five minutes (Shanmugaraj *et al*. 2021).

Citrate Utilization Test

The Simon's citrate agar method was used to conduct this test. Test tubes were cleaned and the citrate agar was poured into them. After 15 minutes of sterilization at 121°C, slopes of roughly 2.54cm were created. The test tubes were inverted to allow them to set, then a loop filled with the culture (e.g., isolated colony from both Nutrient and Mannitol salt agar plate) was streaked across the slopes to inoculate them. For 48 hours, they were incubated at 37°C, and their use of citrate was monitored (Sapkota, 2022).

Methyl Red Test

The 9 ml of the glucose phosphate broth medium was poured into sterile test tubes, which were then sterilized for 15 minutes at 121°C with a loose closure. After allowing it to cool to 30°C, duplicates of the isolated colony from both Nutrient and Mannitol salt agar plate were injected. A few drops of methyl red reagent were added to the broth culture and it was observed after incubating at 37°C for 24-hour incubation period (Shanmugaraj *et al*., 2021).

RESULTS AND DISCUSSION

In order to prevent skin infections, cosmetic goods should be free of pathogens and have a total aerobic bacterial load below certain threshold (Aleem *et al*., 2020). This is because cosmetics include suitable nutrients that stimulate the growth of microorganisms (Siegert, 2012). We investigated the total aerobic bacterial count in various cosmetic samples (face powder and kohl). As shown in Table 1, all samples except K1 and FP2 exhibited some bacterial growth. The bacterial counts ranged from 1.2 x 10^2 cfu/ml (K2) to 3.6 x 10^4 cfu/ml (FP5). Only sample F2 and FP1 exceeded the United States Pharmacopeia (USP) and Food and Drug Administration (FDA) standard of less than 10^3 cfu/g for non-eye area cosmetic. Two samples (K4 and FP3) had colonies too numerous to count (TNTC). These findings are consistent with previous studies by

Aleem *et al*. (2020) and Das *et al*. (2013) who also reported significant bacterial contamination in Cosmetic products.

Table 1: Total aerobic mesophilic bacterial count.

Sample code	Dilution	Aerobic count (cfu/ml)
K1	10^{-2}	NG
K2	10^{-2}	1.2×10^{2}
K3	10^{-2}	3.1×10^{4}
K4	10^{-2}	TNTC
K5	10^{-2}	1.79×10^{4}
FP1	10^{-2}	2×10^{2}
FP2	10^{-2}	NG
FP3	10^{-2}	TNTC
FP4	10^{-2}	2.3×10^{4}
FP5	10^{-2}	3.6×10^{4}

KEY: K- kohl, FP- face powder, TNTC- Too numerous to Count, NG- No growth

Samples exceeding the USP/FDA limit were further analyzed for colony morphology on mannitol salt agar (Table 2). Observed colony characteristics included round, raised, flat, small, large, moist, yellowish and pinkish colonies. Microscopic examination revealed, short rod-like bacteria. In contrast to these findings. Mansuri *et al*., (2018) reported no bacterial growth on mannitol salt agar from their cosmetic samples. This discrepancy maybe due to different sample types, storage conditions or inherent microbial flora of the cosmetic product.

Table 2: Cultural and Microscopic Characteristics of Isolates on Mannitol Salt Agar and Nutrient Agar

Isolate Code	Cultural Characteristics on Agar Plate		Microscopic Characteristics
	MSA	NA	
K3	Round, raised, large, moist, yellowish colonies.	Flat, irregular, mucoid whitish colonies.	Short rods
K4	Round, flat, large, moist pinkish colonies.	Flat, smooth, mucoid whitish colonies.	Short rods
K5	Round, flat, small, moist pinkish colonies.	Flat, Smooth, Mucoid, whitish colonies.	Short rods
FP3	Flat, small, moist pinkish colonies.	Flat, irregular, mucoid whitish colonies.	Short rods
FP4	Round, raised, large, moist yellowish colonies.	Flat, rough, mucoid, whitish colonies.	Short rods
FP5	Round, raised, large, moist yellowish colonies.	Flat, rough, mucoid, whitish colonies.	Short rods

Key: Mannitol Salt Agar – MSA; Nutrient Agar – NA**,** K- kohl, FP- face powder

Table 3 shows that under a microscope, the colonies on nutrient agar were flat, rough, uneven, mucoid, whitish in color, and resembled short rods.

As shown in Tables 2 for mannitol salt and nutrient agar, respectively, there were no fungi found in any of the samples used in this investigation. Skowron *et al.* (2017) similarly reported that fungi were absent from cosmetics used by many individuals. Conversely, it was reported by Shitu *et al.* (2022) and Gamal *et al.* (2015) that certain types of fungi were identified from certain cosmetic items.

Table 3 displays the biochemical characteristics of the isolates. All of the isolates tested negative for urease, methyl red, indole, and catalase, but positive for citrate, Voges Proskauer, and motility test. Furthermore, every isolate had gram positive characteristics. The findings of this investigation aligned with the Bacillus species isolated in the Mansuri *et al.* (2018) study, where the microorganisms tested positive for citrate and the Voges Proskauer test but negative for the catalase test. Conversely, none of the microorganisms recovered in the Kachalla *et al.* (2022) investigation was *Bacillus spp*. The presence of *Bacillus spp.* could be the cause of the bad odor and spoiling of cosmetic items (Das *et al.*, 2013; Mansuri *et al.*, 2018; Perry, 2011).

Table 3: Biochemical characterization of isolates.

Isolate code	CAT	I	MR	CIT	U	VP	MOT	Gram Staining	Inference
K3	+	-	-	+	-	+	+	+	*Bacillus spp.*
K4	+	-	_	+	_	+	+	+	*Bacillus spp.*
K5	+	_	_	+	_	+	+	+	*Bacillus spp.*
FP3	+	_	_	+	_	+	+	+	*Bacillus spp.*
FP4	+	_	_	+	_	+	+	+	*Bacillus spp.*
FP5	+	_	_	+	_	+	+	+	*Bacillus spp.*

KEY: - = negative, + positive, CIT = citrate, MR= Methyl red, VP= Voges Proskauer, MOT= Motility, CAT =Catalase, I = Indole, U= urease

CONCLUSIONS

According to the study's findings, bacterial contamination was found in the cosmetic samples that were examined. All of the face powder and kohl samples that were purchased from various vendors in the Samaru Market are potential health risks associated with exceeding bacterial limits and *Bacillus spp*. presence. It is likely that vendors' inadequate implementation of good handling standards has an impact on the microbiological load of cosmetics and their products, which is concerning for consumer safety. Maintaining cosmetics correctly requires taking the necessary precautions to avoid contamination both during and after manufacture.

This work adds a great deal to our understanding of the microbial spread in cosmetic goods. The results also show how important it is that the highest standards of hygiene be followed by manufacturing facilities, tools, equipment, storage containers, etc. Additionally, adherence to the ISO 22716 Good Manufacturing Practice (GMP) procedures is required.

AUTHOR(S) CONTRIBUTIONS

YUSUF, H.O.: Designed the study, wrote the protocol, managed the literature searches, wrote the first draft of the manuscript.
MALLAM-DAUDA, M.: Gave technical guidance and support in the research and input on the manuscript.
AGAKWU, M.U..: Edited the first draft of the manuscript.
ASUQUO, I.E.: Edited the manuscript and read through the regulatory protocols.
ELEBE, V.C.: Was involved in the sample collection

IBRAHIM, H.S.: Was involved in the sample collection

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CONFLICT OF INTEREST

There is no conflict of interest in this project.

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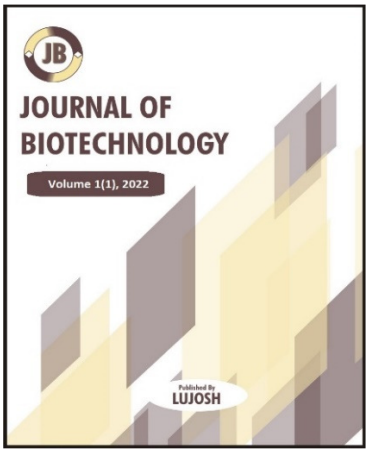

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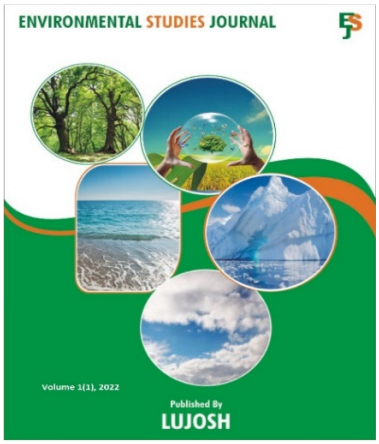

Environmental Studies Journal
https://www.esj.com.ng

International Journal of Biosafety, Biosecurity & Bioscience
https://www.ijbbb.com

Journal of Agricultural Sciences & Technology
https://www.jast.com.ng

Journal of Biochemistry & Molecular Biology
https://www.jbmb.com.ng

Plant Science Journal
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