Detection of multidrug-resistant bacteria in the eye and face makeup cosmetics collected from local markets of Dhaka

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology / Biotechnology

Department of Mathematics and Natural Sciences
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Declaration:

It is hereby declared that,

1. The thesis submitted is my/our original work while completing the degree at BRAC University.
2. The report does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The report does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Abstract

The study aimed to determine the level of contamination and evaluate the multidrug resistance of the bacteria isolated from cosmetic products commonly used by women in Dhaka city. A total of 102 samples from locally manufactured brands of available kajol, eyeshadows, eyeliners, eye serums, compact powders, foundations, primers, and loose powders were collected from different areas of Dhaka., Bacteria were found in our 98% tested cosmetic samples. The range of aerobic plate count was 45–13.7x10^{14} and 95.09% of the samples exceeded the aerobic plate count (APC) limit provided by the Food and Drug Administration (FDA). Bacterial isolates detected include *Bacillus cereus* (48%)*, Pseudomonas aeruginosa* (66.67%)*, Klebsiella pneumoniae* (77.45%)*, Escherichia coli* (14.70%)*, and Staphylococcus aureus* (83.3%). The highest antibiotic resistance was observed in Ceftazidime (70.91%), Sulfamethoxazole (83.68%), and Ampicillin (87.73%). All the gram-negative and gram-positive bacteria have shown multidrug resistance, with variations observed among samples depending on the type of bacteria. Multi-drug resistance of the gram-positive and gram-negative bacteria in cosmetic products was detected from 33% to 80%. When it comes to microbiological contamination, the presence of a large number of bacteria in cosmetic products is considered unacceptable. On the other hand, the antibacterial effect may have a major effect on the overall health of people.

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List of Acronyms

FDA: Food & Drug Administration

BSQCA: Bangladesh Standard Quality Control Authority

EU: European Union

MLB: Modified Letheen Broth

MLA: Modified Letheen Agar

TE: Tris-EDTA

EDTA: Ethylene Diamine Tetra acetic Acid

PCR: Polymerase Chain Reaction

CLSI: Clinical Laboratory Standards Institute

EUCAST: European Committee on Antimicrobial Susceptibility Testing

MHA: Mueller Hinton Agar

MIU: Motility Indole Urea

TSI: Triple Sugar Iron Agar

mm: Millimeter

ml: Milliliter

µl: Microliter

e.g: For example

et al: And others

CFU: Colony Forming Unit

spp: Species

%: Percentage

ºC: Degree Celsius

Chapter 1

Introduction

1.1 Background

The term "cosmetic" is derived from the Greek phrase "kosmetikē tekhnē". Since the 17th century, the word “cosmetic” has been used to describe ‘the art of beautifying and decorating the human body’ (Cosmetic | Etymology of Cosmetic by Etymonline, n.d.). According to the U.S. Food and Drug Administration, the concept of "cosmetic" refers to an article intended for application to the human body via rubbed, poured, dispersed, sprayed, introduced, or other means to clean, beautify, enhance attractiveness, or modify the appearance (Nutrition, 2022). In a report titled “Exploding Topics” on “The Ultimate List of Beauty Industry Stats (2024)”, it is stated that the beauty industry generates over $100 billion in revenue annually (Howarth, 2023). The worldwide cosmetics industry is expected to reach $417.24 billion by 2030, up from $313.22 billion in 2023, according to research by Fortune magazine. The market was valued at $299.77 billion in 2022 (Cosmetics Market Size, Share | Global Industry Trends [2030], n.d.). The Asia-Pacific region is widely recognized as the largest market in the cosmetics business, with a projected value of USD 16,772.63 million by 2029 (Asia-Pacific Cosmetics Market Size, Share, and Industry Analysis by 2029, n.d.). Based on market research, Bangladesh's cosmetic industry is projected to be valued at around USD 1 billion in 2023 and is anticipated to increase at a rate of 4.02% per year between 2023 and 2028 (Statista, n.d.).

Concerns about quality and safety are paramount when a product has global demand. The quality of cosmetic products depends solely on the raw materials used in them. In accordance with an article (Orth et al., 1989), the raw materials may be classified into distinct groups (Table 1). Water is the basic content of cosmetic products, which is somewhat responsible for microbial contamination. For this reason, cosmetic products cannot be expected to be non-sterile (Lundov et al., 2009). It is even supported by U.S. legislation that cosmetics do not need to be sterile, but ‘there must not be the presence of any pathogenic microorganisms’ (Nutrition, 2023). Maximum cosmetic products in local markets are imported, and the duty of ensuring the quality of cosmetics before supplying them to these markets is of the Bangladesh Standard Quality Control Authority (BSQCA). A study conducted in 2015 emphasizes the need for regular microbiological testing of all cosmetic products sold on the market to ensure their quality and safety (Noor et al., 2015).

Table 1: Raw Materials Categories

Water
Acids, Alkalis, Salts, Minerals
Oils, Waxes, Paraffin
Vitamins, Fatty acids, Alcohol, Esters
Surfactants, Emulsifier
Mica, Talc, Clay, Silica
Protein, Starches, Botanicals, Polymers, Gums and Resin
Humectants
Colour and Pigments
Preservatives, Antioxidants and Chelating agents
Fragrances, Essential oils

Additionally, the government of Bangladesh recently codified a new comprehensive law on cosmetics in the nation under the name of the Drug and Cosmetics Act 2023. The legislation has rules for the licensing, inspection, and control of cosmetics to guarantee their safety and quality (Legal, 2023). Certain levels of contamination for cosmetics should not be exceeded; for example, 1000 CFU/g should not be reached in non-eye locations, according to FDA standards for cosmetic items (Nutrition, 2023). In the eye region, the total viable count of aerobic bacteria should not be more than 10^2 CFU/ml, as per EU recommendation. The acceptable limit for aerobic bacteria in non-eye area samples is 10^3 CFU/ml. The presence of certain microorganisms, including *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Candida albicans*, in 1 ml of eye and face cosmetics is unacceptable. Cosmetic products should not include *Enterobacteriaceae* or *Escherichia coli* (Scientific Committee on Consumer Safety (SCCS), n.d.).

1.2 Literature Review

Bacterial contamination has the potential to be lethal which can cause significant harm to the eyes and skin. Multiple studies have demonstrated that the predominant microorganisms include *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and Bacillus spp* as well as other bacteria were commonly detected in cosmetics like serums, creams, and eye cosmetics (Kim et al., 2020). To enhance the microbiological quality of cosmetics, a study conducted in 1989 introduced the findings of microbial contamination in locally commercial cosmetic products such as eyeliner, and face powder. Here among the isolated bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, *Staphylococcus aureus* established a link with skin disease called impetigo and conjunctivitis (Abdelaziz et al., 1989).

Regularly using cosmetic products like powder, cream, and eyeliner contained the highest contamination rate of *Pseudomonas aeruginosa*, according to a study by "The University of Medical Science of Iran" conducted in 2016. Other organisms such *as Staphylococcus aureus, Escherichia coli, Klebsiella spp., and Bacillus spp.* were also isolated from both skin cosmetics and eye cosmetics (Dadashi & Dehghanzadeh, 2016). Usually, *Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli* are the most commonly found bacteria in both high and low-grade cosmetics. In a total of 50 cosmetic samples, like powders, foundation, eyeliner, and eye shadows from Mecca local shops, *Staphylococcus aureus* was found in both high-grade and low-grade cosmetics at 41% and 27%, respectively, which was the highest among other organisms (Alshehri, 2023). Furthermore, in the previous extension of our study, among 27 brands of local cosmetics in the Dhaka market, both Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella spp., and Shigella spp.)* and Gram-positive organisms (species of *Streptococcus, Staphylococcus, Bacillus, Lactobacillus, and Listeria monocytogenes*) were identified (Nusrat et al., 2022). In our extended study, we tested locally manufactured eye and face cosmetics of 102 samples, including kajol, eyeshadows, liners, eye serums, compact powders, foundations, primers, and loose powders, from different areas of the Dhaka market, from which five pathogenic bacterial species were isolated. Like other studies, the highest rate of bacteria found in these local cosmetics was *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Klebsiella pneumonia,* along with bacteria like *Escherichia coli and Bacillus cereus,* but we did not get any positive results for *Listeria spp., Salmonella, or Shigella*.

The endotoxins and metabolites produced by bacteria present in a cosmetic product may cause skin infections. Some allergic reactions on the skin after using cosmetic products can turn into dermatitis (Akhand et al., 2023). *Pseudomonas aeruginosa* is the cause of an eye infection, according to numerous studies. Using contaminated eye cosmetics has shown a prognosis worse than bacterial keratitis (Spencer, 1953). An article from 1979 described how using eye cosmetics caused a woman to develop a corneal ulcer. *Pseudomonas aeruginosa* was found in her eyelids and eye cosmetics through microbiological culture (Francis R. Reid, 1979). The predominant causative agent of infections affecting skin and soft tissues is *Staphylococcus aureus*. *Staphylococcus aureus*, which colonizes the skin in 20–30% of the population, is responsible for 80–90% of all skin and soft tissue infections in humans (Al Kindi et al., 2019). According to an article in CBC News, Keith Warriner, a microbiologist at the University of Guelph, tested 15 cosmetic samples of some famous brands, including MAC, Sephora, Shoppers Drug Mart, and The Body Shop, which showed 40% of *Staphylococcus aureus* presence in all brand cosmetics. Furthermore, scientists said that this percentage is enough to cause pink eyes, styes, pimples, and boils (CBC News, 2018). In a questionnaire study conducted in 2021 by Koreans, 539 patients were affected by acne vulgaris due to the use of cosmetics (Suh et al., 2020). As seen above, contaminated cosmetic products have the potential to induce skin diseases. Therefore, antibiotic susceptibility testing is required to identify the most effective antibiotics for treating the disease.

Before treating any disease, it is necessary to get confirmation of the specific species of those pathogenic bacteria, as the specific antibiotic works only on the target species of bacteria. Even though there are different strains of one species of bacteria. Different studies choose different methods for confirmation. In a study in the USA conducted in 2023, they chose the polymerase chain reaction (PCR) and gel electrophoresis methods to confirm *Bacillus cereus* among 213 bacterial strains (Yossa, 2023). The previous extension of our study conducted in 2022 had chosen a biochemical method for confirming the species of pathogenic bacteria (Nusrat et al., 2022). We found and confirmed the DNA bands of five different types of bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*e. We did this using biochemical methods and a biomolecular technique called gel electrophoresis.

The most concerning fact nowadays is the increase of mainstay antibiotics. In a study in Nigeria conducted in 2022, by the University of Yola, microbiological analysis of local cosmetics such as powder, foundation, and cream showed *Staphylococcus aureus* (42.3 %), *Escherichia coli* (23 %),

Pseudomonas aeruginosa (15.3 %), and *Klebsiella spp.* (7.7%), whose antibiotic susceptibility test depicted the highest resistance to chloramphenicol and tetracycline. Furthermore, the isolates of *Pseudomonas aeruginosa* were mostly resistant to all antibiotics used in this study (Kachalla et al., 2022). Conversely, the previous extension of our study, which was also conducted in 2022, showed the highest antibiotic resistance in ampicillin, azithromycin, cefepime, ciprofloxacin, meropenem, aztreonam, and colistin. Moreover, the isolates of *Bacillus cereus* and *Staphylococcus aureus* showed mostly resistance (Nusrat et al., 2022). Our extension study showed ceftazidime, sulfamethoxazole, and chloramphenicol as having the highest antibacterial resistance.

Though the pharmaceutical and cosmetic industries in Bangladesh have been contributing much to the economy, public health is at high risk. According to ISO 17516:2014 guidelines, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* cannot be present in 1 ml of any type of cosmetic, but the real scenario is saying a different thing. The presence of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* exceeds the limit of CFU/ml given by the FDA and EU (Scientific Committee on Consumer Safety (SCCS), n.d.). Although numerous pathogenic bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Actinomycetes* and fungi have been implicated in cosmetic contamination reports across the globe but such information is limited in Bangladesh, as stated before (Kim et al., 2020). The climate and weather conditions of Bangladesh are quite favorable for the growth of microorganisms (Noor et al., 2015). The presence of pathogenic microorganisms in these local cosmetics are capable of causing severe infections. The use of these cosmetic products has been linked to a variety of health issues, including eye infections, allergic reactions, skin rashes, swollen lips, and chemical burns (New Age, 2019). The associate professor of the Department of Pharmaceutical Technology at Dhaka University, Dr. AK Lutful Kabir, claims that contaminated cosmetics have the potential to cause skin cancer by entering the bloodstream through the skin (Daily Sun, 2020). However, there is little understanding of the bacterial pathogens found in contaminated cosmetic products due to the few studies conducted on this issue. To determine the antibiotic resistance potential of the particular bacterial pathogens that contaminated the cosmetics, the current study aimed to isolate and identify those bacteria.

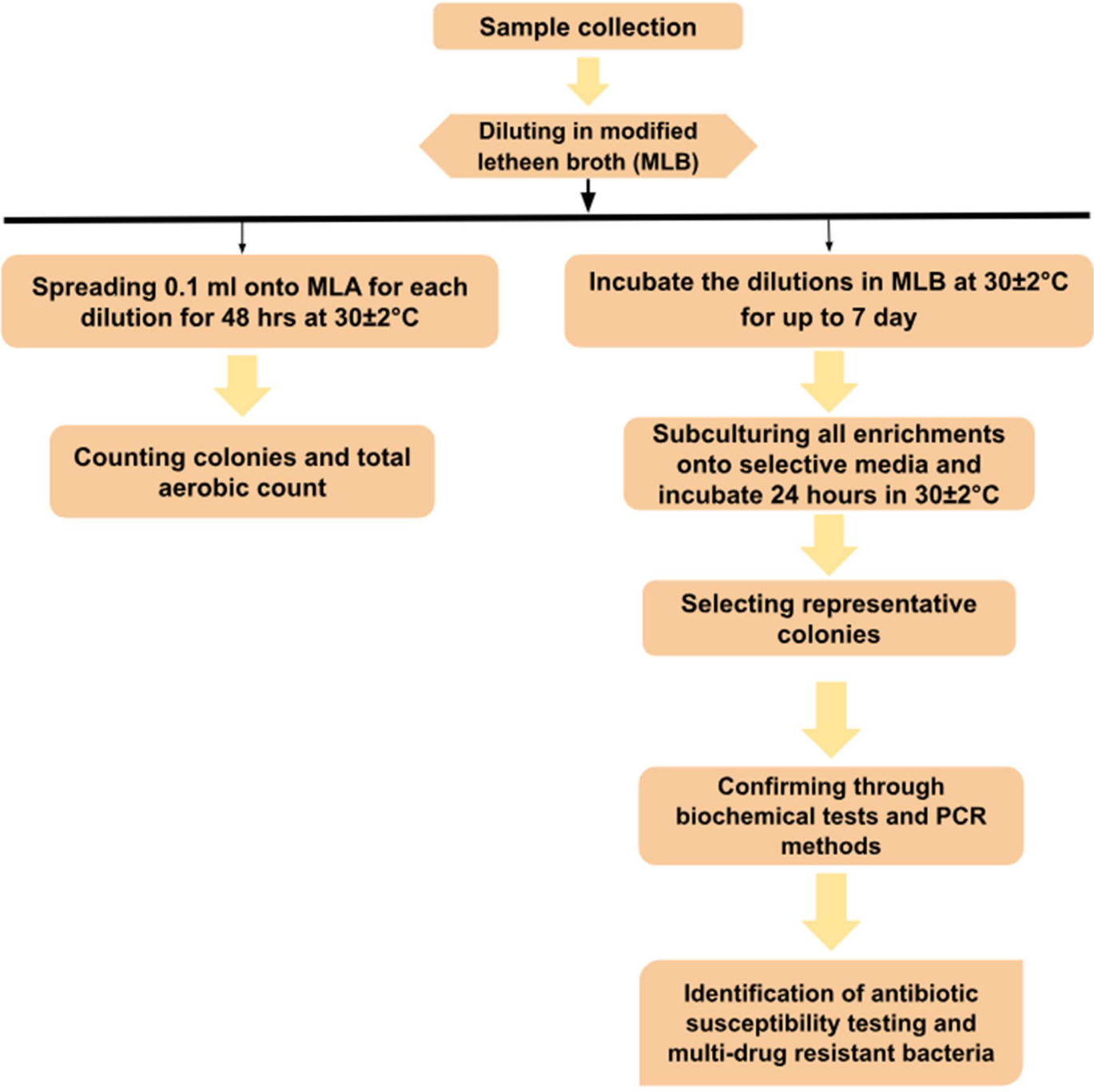
1.3 Objectives

- To isolate and identify potential pathogenic bacteria in cosmetic (eye & face) products locally sold in Dhaka city.
- To assess the antibiotic resistance pattern of the isolates found in the cosmetics.

Chapter 2

Material & Methods

2.1 Flowchart of the Method of the Experiment



2.2 Sample Collection

A total of 46 brands were tested in this study, with 102 samples of each of the following categories: kajol (5), eyeshadows (15), eyeliners (15), eye serums (15), compact powders (10), foundations (12), primers (15), and loose powders (15). The following areas of Dhaka, Bangladesh, were surveyed for the collection of these samples: Agargaon, Khilgaon, Mohakhali, Mirpur, and New Market. Each sample had a batch number, that was within its expiration date and the date of manufacture. As soon as the samples entered the laboratory, they were examined and kept at room temperature.

2.3 Sample Processing & Culture Preparation

Following the Bacteriological Analytical Manual of the FDA (Nutrition, 2023), the samples were processed, and initial preparation was carried out. Using 70% ethanol, the surface was cleaned, and both the contents and sample containers were carefully inspected for any anomalies.

Before removing the samples, a thorough examination of the containers was conducted to identify any oddities. Additionally, before opening and removing the contents, the surface of the sample containers was disinfected using an aqueous solution composed of 70% ethanol (v/v) and 1% HCl (v/v). After the surface was tissue-dried, 1 gram (ml) of the material was aseptically weighed. Due to the diverse conditions in which the collected samples were found, they required distinct initial preparation procedures. In the case of liquid-textured samples, including eyeliners, eye serums, and primers, 1g of the sample was removed aseptically from the container and placed into a screw-cap test vial containing 9 ml of Modified Letheen Broth (MLB). As powder-textured samples, eyeshadow, compact powder, and loose powder are incorporated aseptically, one gram at a time, into a test container containing eight milliliters of sterilized MLB and one milliliter of sterile Tween 80. To analyze kajols and foundations that were previously classified as oil-based and cream-textured, a 1-gram sample was carefully placed into a test tube. The test tube included 1 milliliter of sterile Tween 80 and 8 milliliters of sterile MLB, along with five to seven glass beads. Following the initial preparation of each sample, the entire 10 ml of material was tallied as a 10^{-1} dilution and vortexed for homogenization with the use of a vortex mixture.

2.4 Total Aerobic Bacterial Plate Count of Cosmetic Products

Spreading the 102 samples on Modified Letheen Agar (MLA) after processing them allowed for the calculation of the aerobic plate count according to the FDA's Bacteriological Analytical Manual (Nutrition, 2021). In MLA, 0.1 ml of sample was spread following the spread plate method to perform the aerobic plate count. The preparation was diluted decimally in MLB to get discrete, countable colonies for the count. Aseptic application of the inoculums to MLA was done using a sterile spreader. After that, the plates were inverted and incubated for 24 to 48 hours at 30±2°C to let the MLA medium absorb the inoculum. The MLA plates with colonies shown in Figure 1 were inspected for aerobic count and the findings were recorded.

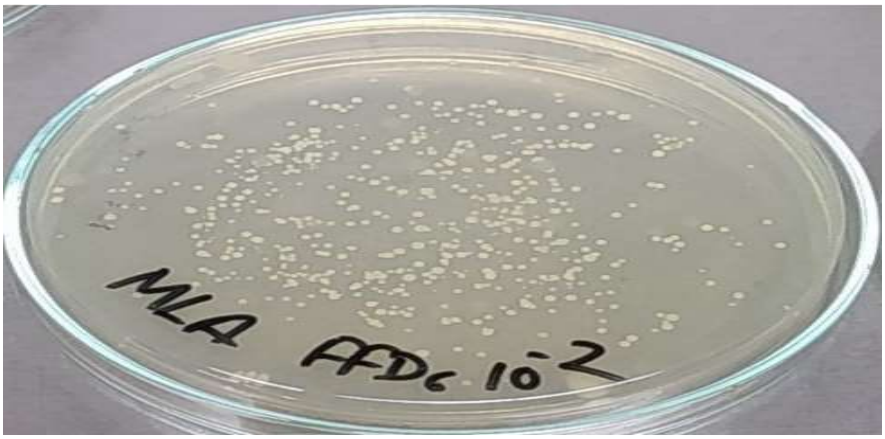


Figure 1: Using MLA to Calculate the Total Aerobic Plate Count

- For plates with 25-250 CFU:
- The following formula is used to calculate the aerobic plate count (Nutrition, 2021).

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2)] \times (d)}$$

Where, N = Number of colonies per ml or g of product

Σ c = Sum of all colonies on all plates counted

n1 = Number of plates in the first dilution counted

n2 = Number of plates in the second dilution counted

d = Dilution from which the first counts were obtained

- For plates with fewer than 25 CFU:
  When the number of colony-forming units (CFU) on plates from both dilutions was less than 25, the actual plate count was recorded. As the representing count was less than 25, we multiplied it by the dilution, where d is the dilution factor for the initial dilution.
- For plates with more than 250 CFU:
  When the plates from the two dilutions produced more than 250 colony-forming units (CFU) apiece but less than 100 CFU per square centimeter, we calculated the estimated aerobic plate count (EAPC) closest to 250 and multiplied them by the dilution.

2.5 Bacterial Culture

The identification of microorganisms was conducted following the Bacteriological Analytical Manual of the FDA (Nutrition, 2023). To culture the bacteria 0.1ml of each processed sample was spread on selective media and incubated for 24 to 48 hours at 30±2°C. The colonies were selected based on the colony morphology. The selective media that were used and the expected colony morphology are shown in Table 2.

Table 2: Colony Morphology of Specific Bacteria on Selective Media

Bacteria	Gram-positive/ Gram-negative	Media	Expected Colony Morphology
Bacillus cereus	Gram-positive	Bacillus Cereus Agar	Light blue, large, flat colonies with blue center
Staphylococcus aureus	Gram-positive	Mannitol Salt Agar (MSA)	Yellow/white colonies surrounded by yellow zone
Escherichia coli	Gram-negative	UTI Agar	Purple Colonies
Pseudomonas aeruginosa	Gram-negative	Cetrimide Agar	Yellow-green, glows under UV ray
Klebsiella pneumoniae	Gram-negative	MacConkey Agar	Pink mucoid colonies

2.6 Biochemical Tests for Further Identification

The selected isolates were subjected to biochemical tests: Motility Indole Urease (MIU), Catalase, Oxidase, Triple Sucrose Iron, and Citrate Utilization tests. The biochemical tests done for different bacteria to characterize the criteria are shown below in Table 3 and Figure 2.

Table 3 : Biochemical Test Interpretation of Different Bacteria

Organism	Motility Indole Urease (MIU)			Catalase	Oxidase	Triple Sugar Iron(TSI)		Citrate Utilization
	Motility	Indole	Urease			Gas Productution	H_2S Production	
Bacillus cereus	+ve	–ve	+ve	+ve	–ve	….	+ve	+ve
Staphylococcus aureus	–ve	–ve	+ve	+ve	–ve	-ve	–ve	+ve
Escherichia coli	+ve	+ve	–ve	+ve	–ve	+ve	–ve	–ve
Pseudomonas aeruginosa	+ve	–ve	–ve	+ve	+ve	+ve	–ve	+ve
Klebsiella pneumoniae	-ve	–ve	+ve	+ve	-ve	+ve	–ve	+ve

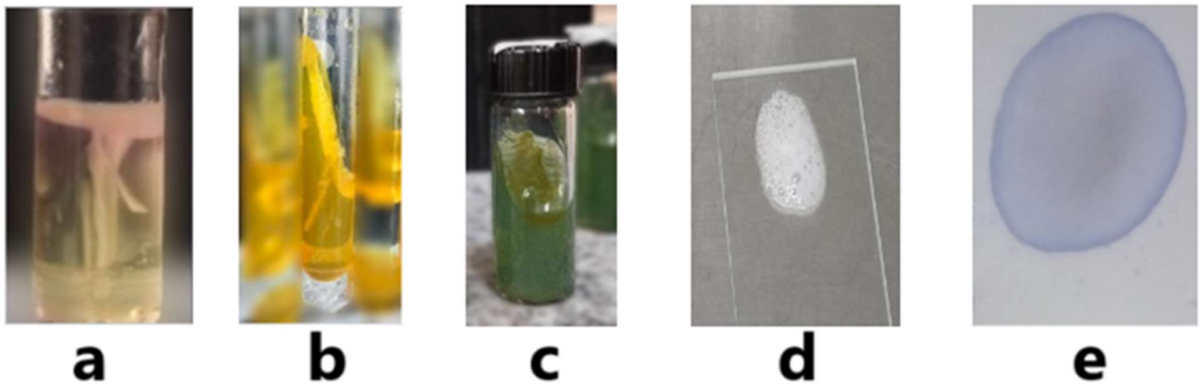


Figure 2: Biochemical tests of Different Bacteria Found in Cosmetic Samples

(a) MIU test of *Klebsiella pneumoniae*, (b) TSI test of *Pseudomonas aeruginosa*,

(c) Citrate Utilization test of *Escherichia coli*, (d) Catalase Test of *Staphylococcus aureus*,

(e) Oxidase Test of *Bacillus Cereus*

2.7 Molecular Detection of Selected Isolates

2.7.1 DNA Extraction

Genomic DNA extraction from the chosen isolates was performed using the boiling procedure. Following the boiling process, the isolates were streaked in the nutrient agar medium and then incubated at 37°C for 24h. We took one loopful of the selected isolate in Eppendorf tubes containing 150 microliter TE buffer and vortexed it. A thermocycler was used as a heat block to heat the isolate at 100°C for 15 minutes. As a result of centrifuging the cell solution at 10,000 rpm for 10 minutes at 4°C, the cell debris precipitated. The supernatant containing DNA was stored at -20°C.

2.7.2 PCR Amplification Followed by Agarose Gel Electrophoresis

PCR amplification was performed so that species could be identified and confirmed, which include the following isolates: *Staphylococcus aureus*, *Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae*. The pair of primers that were used for PCR amplification was followed according to the conditions mentioned in Table 4.

Table 4: The Oligonucleotide Primers set for Bacterial Identification

Name of the bacteria	Targeted Gene	Sequence		Product size	PCR conditions	Agarose gel concentration
Staphylococcus aureus	Nuc	Nuc -F	5' - GCGATTGATGGTGATACGGTT - 3'	279 bp	The cycling conditions were 5 mins at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 72°C and final holding of 10 mins at 72°C.	1.5%
		Nuc-R	5' - AGCCAAGCCTTGACGAACTAAAGC -3'			
Pseudomonas aeruginosa	16S rDNA	PA- SS-F	5' - GGGGGATCTTCGGACCTCA -3'	956bp	The cycling conditions were 2 mins at 95°C followed by 25 cycles of 20 sec at 94°C, 20 sec at 58°C, 40 sec at 72°C and final holding of 1 min at 72°C.	1.4%
		PA- SS-R	5' - TCCTTAGAGTGCCCG -3'			
Klebsiella pneumoniae	16S-23S Internal Transcribed Spacer	KP Pf-F	5' - ATTTGAAGAGGTTGCAAACGAT -3'	133bp	The cycling conditions were 10 mins at 94°C followed by 30 cycles of 30 sec at 94°C, 45 sec at 60°C, 45 sec at 72°C and final holding of 10 sec at 72°C.	1.6%
		KP Prl-R	5' - TTCACTCTGAGTTTTCTTGTGTTC -3'			
Escherichia coli	16S rRNA	ECO-F	5' - GACCTCGGTTTAGTTCACAGA -3'	585bp	The cycling conditions were 10 mins at 95°C followed by 35 cycles of 30 sec at 95°C, 30 sec at 58°C, 1 min at 72°C and final holding of 7 mins at 72°C.	1.4%
		ECO-R	5' - CACCACGCTGACGCTGACCA -3'			
Bacillus cereus	16S rDNA	BC 16S-F	5' - TCGAAATTGAAAGGCGGC -3'	288bp	The cycling conditions were 10 mins at 95°C followed by 30 cycles of 15 sec at 94°C, 45 sec at 63°C, 2 mins at 72°C and final holding of 2 mins at 72°C.	1.5%
		BC 16S-R	5' - GGTGCCAGCTTATTCAAC -3'			

To begin with, PCR component mixtures of around 15 µL were prepared, where 7.5 µL was master mix, 4.5 µL was nuclease-free water, 0.5 µL was forward primer, 0.5 µL was reverse primer, and finally 2 µL of extracted DNA of the desired organisms were added. The PCR master mix contained dNTP, $MgCl_2$, and Taq polymerase. The PCR was carried out on the samples based on the conditions in Table 5 for each of the desired microorganisms. The PCR products were analyzed by electrophoresis with a 1.5–2% agarose gel concentration. The gel was stained with ethidium bromide and was visualized under a UV transilluminator.

2.8 Antibiotic Susceptibility Test

The purpose of this test was to determine whether the samples included multidrug-resistant microorganisms. The Kirby-Bauer disc diffusion method was used in this experiment, and the Clinical Laboratory Standards Institute (CLSI) standard was used to measure the disc zone sizes (Clinical and Laboratory Standards Institute, 2023). By adding bacterial colonies to 9 ml of saline solution, a bacterial suspension was generated. To rectify the turbidity, the suspension was compared to the 0.5 McFarland Standard. At 37°C, the petri dishes were incubated for an entire day. The diameter was determined following incubation by referring to the standard inhibitory zone diameter chart. According to CLSI, the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and the US Food and Drug Administration (FDA), multidrug resistance (MDR) is defined as non-susceptibility to at least one antibiotic in three antimicrobial groups. The antibiotics that were used in the Antibiotic Susceptibility Test for this study are shown in. Table 5

Table 5: List of Antibiotics Used in the Experiment

Serial No.	Antibiotics	Disc code	Disc potency (µg)	Group	Effective against	Inhibition Zone Measurements		
						Resistant	Intermediate	Sensitive
1	Amoxyclav	AMC	30	Beta-lactamase	Gram Positive Gram Negative	13	14-17	20
2	Ampicillin	AMP	10	Beta-lactamase	Gram Positive Gram Negative	13	14-16	17
3	Azithromycin	AZM	15	Macrolide	Gram Positive Gram Negative	13	14-17	18
4	Sulfamethoxazole	RL	25	Sulfonamide	Gram Positive GramNegative	10	11-15	16
5	Cefepime	CPM	30	Cephalosporin	Gram Positive Gram Negative	18	19-24	25
6	Ceftazidime	CAZ	30	Cephalosporin	Gram Positive Gram Negative	17	15-17	21
7	Ciprofloxacin	CIP	5	Fluoroquinolone	Gram Positive Gram Negative	21	22-25	26
8	Clindamycin	CD	2	Lincosamide	Gram Positive Gram Negative	14	15-20	21
9	Co-Trimoxazole	COT	25	Sulfonamides	Gram Positive Gram Negative	10	11-15	16
10	Chloramphenicol	C	30	Phenicols	Gram Positive Gram Negative	12	13-17	18
11	Tetracycline	TE	30	Protein Synthesis	Gram Positive Gram Negative	11	12-14	15
12	Doxycycline	DO	30	Tetracyclines	Gram Positive Gram Negative	12	13-15	16
13	Gentamicin	GEN	10	Aminoglycoside	Gram Positive Gram Negative	12	13-14	15
14	Imipenem	IPM	10	Carbapenem	Gram Positive Gram Negative	19	20-22	23
15	Meropenem	MRP	10	Carbapenem	Gram Positive Gram Negative	19	20-22	23
16	Streptomycin	S	10	Aminoglycoside	Gram Positive Gram Negative	11	12-14	15
17	Linezolid	LZ	10	Oxazolidinones	Gram Positive	20	21-22	23
18	Vancomycin	VA	30	Glycopeptide	Gram Positive	14	15-16	17
19	Colistin	CL	10	Polymyxin E	Gram Negative	10	11-13	14
20	Aztreonam	AT	30	Monobactam	Gram Negative	15	16-21	22

Chapter 3

Result

3.1 Total Aerobic Bacterial Plate Count of Cosmetic Products

Out of 102 cosmetic samples, while 97 samples exceeded FDA limits, only 5 cosmetic samples from each product category were within the FDA and EU limits. In Table 6 and Table 7 the total aerobic count of cosmetic products with the allowed limit of contamination according to the FDA is shown (Nutrition, 2023).

Table 6: Total Aerobic Count of Cosmetic Products in MLA

Eye Cosmetic Products	No. of samples	No. of samples contaminated			Limit of contamination allowed
		Bacterial load in CFU/ml			
		$<10^2$	10^2-10^4	$>10^4$	
Kajol	5	1	4	0	$<10^2$ CFU/g
Eyeshadow	15	0	14	1	
Eyeliner	15	1	12	3	
Eye Serum	15	1	14	1	

Face Cosmetic Products	No. of samples	No. of samples contaminated			Limit of contamination allowed
		Bacterial load in CFU/ml			
		$<10^3$	10^3-10^5	$>10^5$	
Compact Powder	10	1	8	1	$<10^3$ CFU/g
Foundation	12	0	10	2	
Primer	15	0	13	2	
Loose powder	15	1	14	0	

Table 7: Total Aerobic Count of Cosmetic Products in Different Selective Media

Eye Cosmetic Products	No. of products in which isolates were detected	Bacterial isolates	No. of samples contaminated			
			Bacterial load in CFU/ml			
			$<10^2$	10^2-10^4	$>10^4$	No growth
Kajol	3	*Staphylococcus aureus*	0	2	1	2
	2	*Bacillus Cereus*	0	2	0	3
	2	*Pseudomonas aeruginosa*	0	2	0	3
	5	*Escherichia coli*	0	4	1	0
	2	*Klebsiella pneumoniae*	0	2	0	3
Eyeshadow	14	*Staphylococcus aureus*	3	10	1	1
	6	*Bacillus Cereus*	0	4	2	9
	15	*Pseudomonas aeruginosa*	1	3	1	10
	3	*Escherichia coli*	0	3	0	12
	5	*Klebsiella pneumoniae*	0	12	3	0
Eyeliner	6	*Staphylococcus aureus*	1	4	1	9
	5	*Bacillus Cereus*	0	5	0	10
	3	*Pseudomonas aeruginosa*	0	2	1	12
	5	*Escherichia coli*	1	3	1	11
	5	*Klebsiella pneumoniae*	0	4	1	9
Eye Serum	15	*Staphylococcus aureus*	1	12	2	0
	4	*Bacillus Cereus*	1	2	1	12
	15	*Pseudomonas aeruginosa*	0	14	1	0
	0	*Escherichia coli*	0	0	0	15
	9	*Klebsiella pneumoniae*	0	8	1	6

Face Cosmetic Products	No. of products in which isolates were detected	Bacterial isolates	No. of samples contaminated			
			Bacterial load in CFU/ml			
			$<10^3$	10^3-10^5	$>10^5$	No growth
Compact Powder	9	*Staphylococcus aureus*	0	7	2	1
	9	*Bacillus Cereus*	0	7	2	1
	6	*Pseudomonas aeruginosa*	1	5	0	4
	2	*Escherichia coli*	0	2	0	8
	10	*Klebsiella pneumoniae*	1	8	1	0
Foundation	12	*Staphylococcus aureus*	2	10	0	0
	7	*Bacillus Cereus*	0	5	2	5
	12	*Pseudomonas aeruginosa*	1	10	1	0
	0	*Escherichia coli*	0	0	0	12
	11	*Klebsiella pneumoniae*	2	7	2	1
Primer	15	*Staphylococcus aureus*	2	12	1	0
	10	*Bacillus Cereus*	1	8	1	5
	15	*Pseudomonas aeruginosa*	2	11	2	0
	0	*Escherichia coli*	0	0	0	15
	12	*Klebsiella pneumoniae*	1	10	1	3
Loose Powder	11	*Staphylococcus aureus*	2	8	1	4
	6	*Bacillus Cereus*	1	5	0	9
	10	*Pseudomonas aeruginosa*	1	8	1	5
	0	*Escherichia coli*	0	0	0	15
	15	*Klebsiella pneumoniae*	0	0	0	15

3.2 Isolated Bacteria from Cosmetic Products

A total of 102 cosmetic samples were collected and analyzed. These cosmetic samples were collected from different local markets in Dhaka. From 5 kajols, 10 eyeshadows, 6 eyeliners, 15 eye serums, 10 compact powders, 12 foundations, 15 primers, and 9 loose powders, 296 isolates were obtained. These isolates were selected randomly depending on their phenotypic characteristics from five selective media: Bacillus cereus agar, Mannitol salt agar (MSA), MacConkey agar, Cetrimide agar, and UTI agar. The samples were separately cultured in these five selective media and were incubated for 24 to 48 hours at 30±2°C. The agar plate in which bacteria from the sample were cultured is shown in Figure 3.

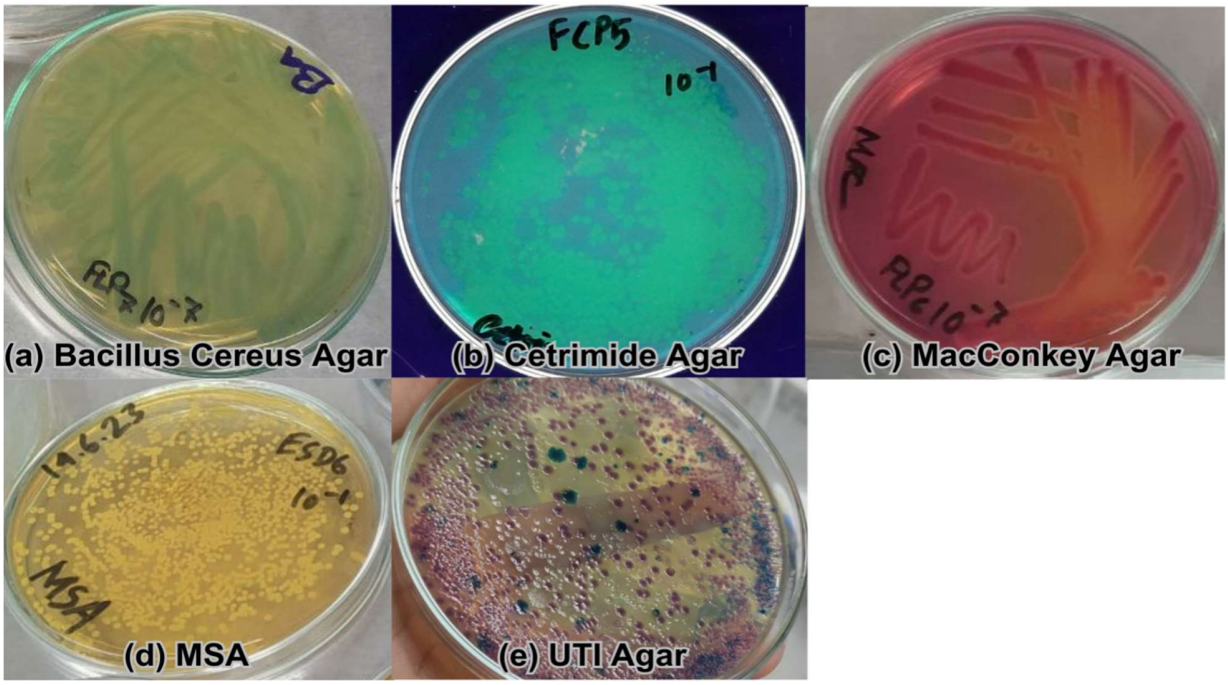


Figure 3: Growth of particular bacterial colonies on Various Selective media
(a) *Bacillus cereus* on Bacillus Cereus agar, (b) *Pseudomonas aeruginosa* in Cetrimide agar (c) *Klebsiella pneumoniae* on MacConkey agar, (d) *Staphylococcus aureus* on MSA, (e) *Escherichia coli* on UTI agar

3.3 Polymerase Chain Reaction (PCR) Confirmation Followed by Agarose Gel Electrophoresis

Specific primers and PCR conditions were applied to perform amplification on each species. Following this, the effectiveness of the PCR was determined by examining the amplified DNA on an agarose gel. The size of the DNA band was calculated using the DNA Ladder. Laddering an unknown PCR result next to the nearest band in the ladder lane on an agarose gel allows one to determine the size of the unknown fragment.

The presence of 133 bp in Figure 4 indicates the gel electrophoresis result of *Klebsiella pneumoniae*. Out of the 82 likely *Klebsiella pneumoniae* isolates, 79 had positive bands. These bands were found in eye serums, eyeshadows, eyeliners, compact powders, foundations, primers, and loose powders.

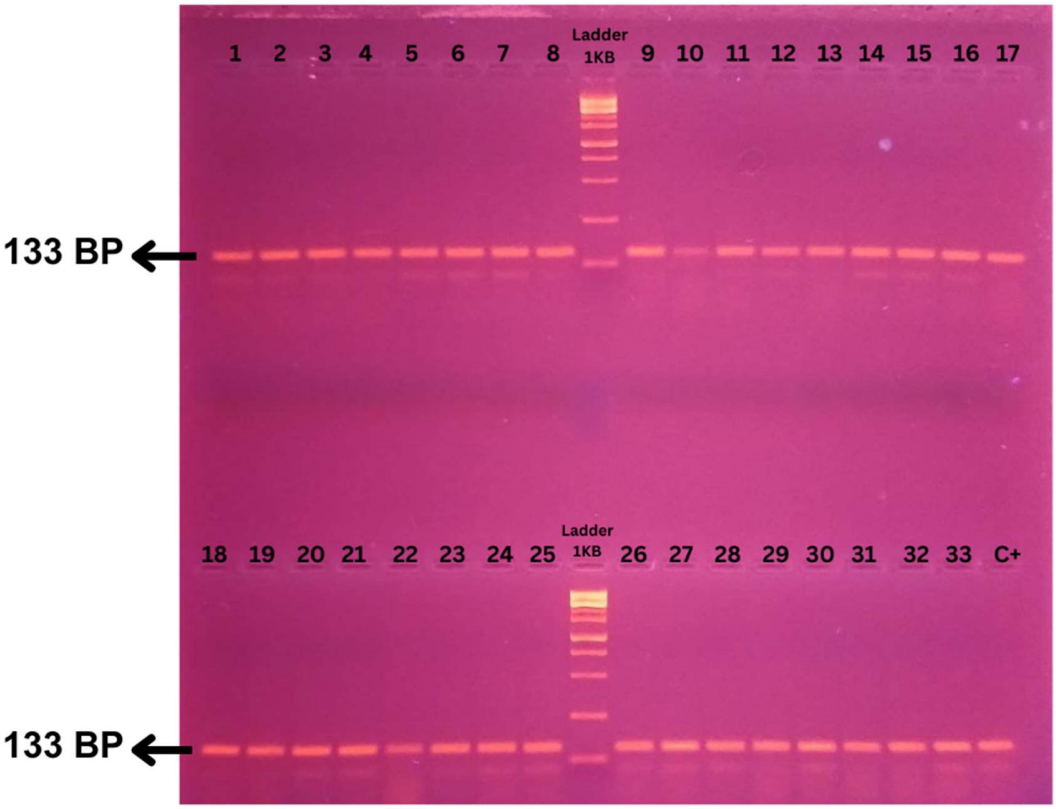


Figure 4: Gel electrophoresis of 1KB ladder and PCR product of *Klebsiella pneumoniae*

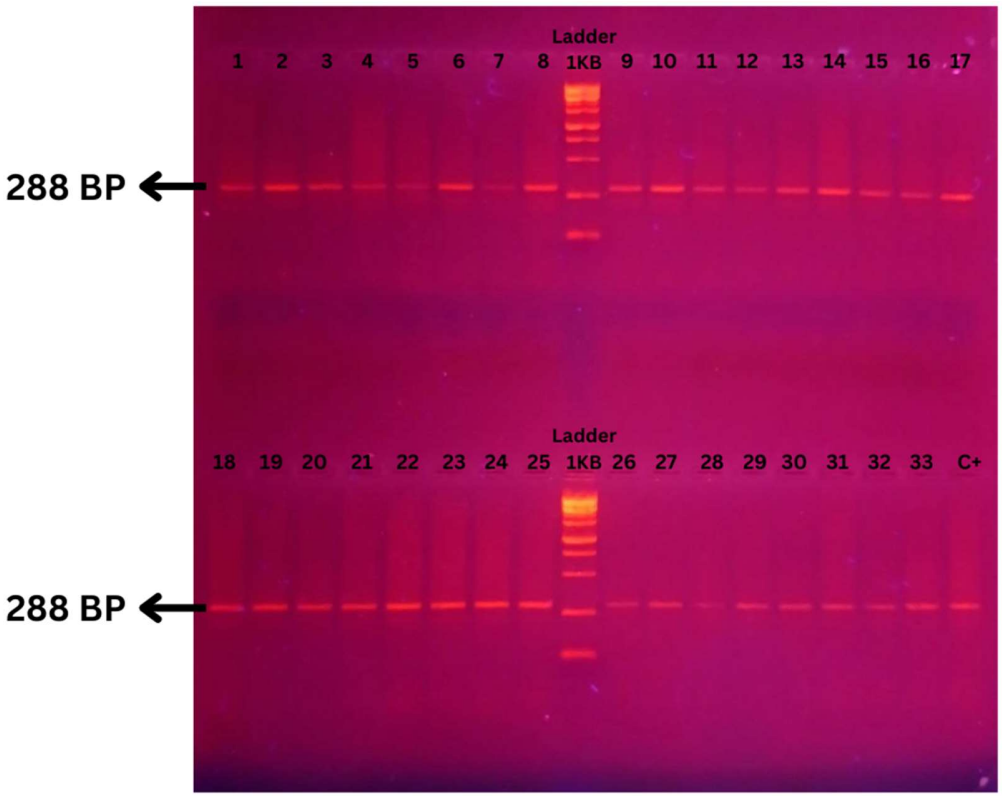


Figure 5: Gel electrophoresis of 1KB ladder and PCR product of *Bacillus cereus*

Figure 5 shows the gel electrophoresis results for Bacillus cereus with 1 kb ladder, indicating that all 49 suspected isolates tested positive. The isolates exhibited a band at 288 base pairs, confirming the presence of *Bacillus cereus*.

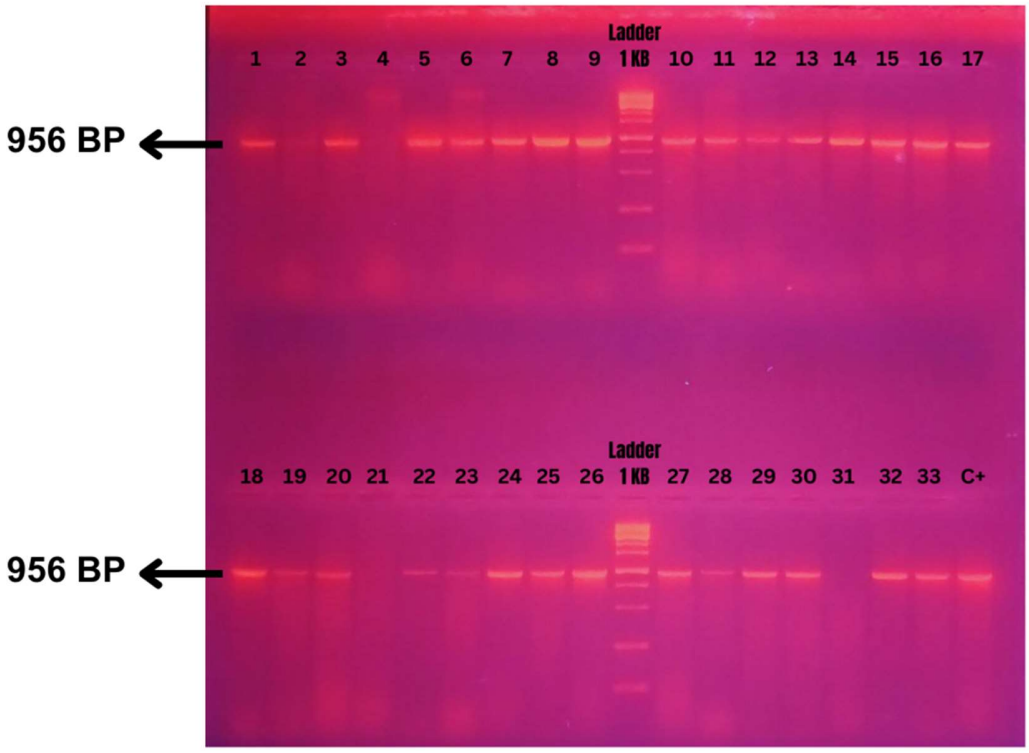


Figure 6: Gel electrophoresis of 1KB ladder and PCR product of *Pseudomonas aeruginosa*

Figure 6 shows the gel electrophoresis result of *Pseudomonas aeruginosa* with 1 kb ladder. Following the identification of the suspected *Pseudomonas spp.* through biochemical testing, all of these isolates were subjected to PCR using a species-specific primer for *Pseudomonas aeruginosa*. Out of a total of 73 potential samples of Pseudomonas aeruginosa, 68 indicated positive bands that were obtained from kajols, eyeshadows, eyeliners, eye serums, compact powders, foundations, primers, and loose powders.

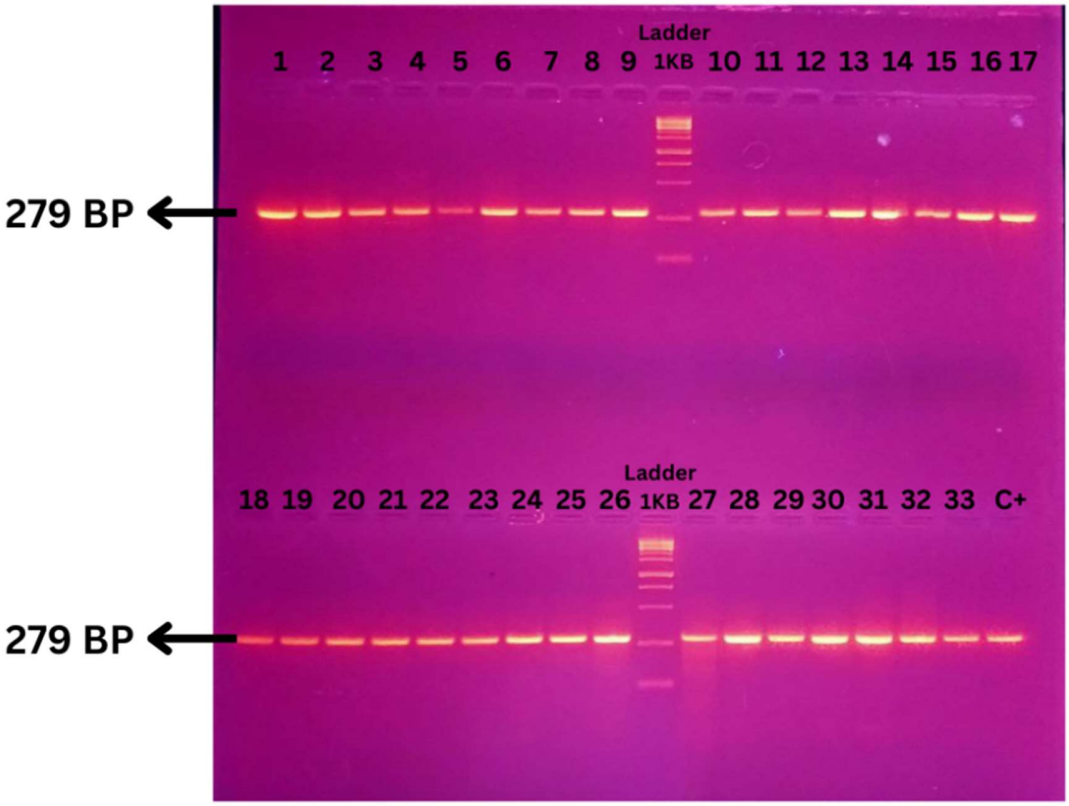


Figure 7: Gel electrophoresis of 1KB ladder and PCR product of *Staphylococcus aureus*

Figure 7 represents the gel electrophoresis of the PCR product of *Staphylococcus aureus*, as evidenced by the presence of a 279 base pair band with a 1 kb ladder. Initially, all potential *Staphylococcus spp.* organisms were identified and confirmed using species-specific primers. Following the confirmation of *Staphylococcus aureus*, a PCR analysis was conducted using a primer sequence unique to the species to confirm if the isolate was *Staphylococcus aureus* or not. Out of the 89 *Staphylococcus spp.*, 85 of them were identified as *Staphylococcus aureus*.

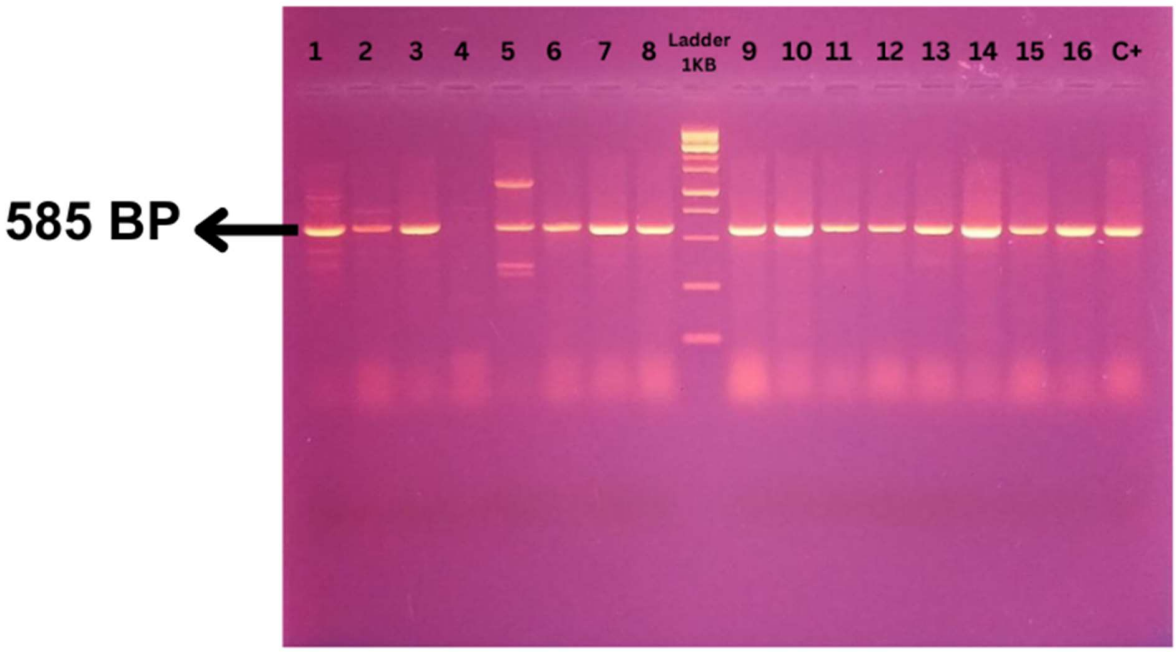


Figure 8 : Gel electrophoresis of 1KB ladder and PCR product of *Escherichia coli*

Figure 8 presents the gel electrophoresis results of the 1KB ladder and PCR product obtained from *Escherichia coli*. An arrow indicates the presence of a band (585 bp) that is specific to *Escherichia coli*. All potential Escherichia coli isolates exhibited bands confirming their predicted identity, except one after gel electrophoresis.

After the confirmation of 5 bacteria isolated from 102 cosmetics, we got the highest presence of *Staphylococcus aureus* in the locally manufactured cosmetics. The number of bacteria we isolated from various cosmetic samples is shown in Table 8. The overall percentage of isolated bacteria from cosmetics samples is outlined in Figure 9.

Table 8: Number of Isolated Bacteria Found in Various Cosmetic Samples

Cosmetic product category (N)	**Name of bacteria**	**Number of cosmetic with bacterial growth**
Kajol(5)	*Staphylococcus aureus*	3
	Bacillus Cereus	2
	Pseudomonas aeruginosa	2
	Escherichia coli	5
	Klebsiella pneumoniae	2
Eyeshadow(15)	*Staphylococcus Aureus*	14
	Bacillus Cereus	6
	Pseudomonas aeruginosa	5
	Escherichia coli	3
	Klebsiella pneumoniae	15
Eyeliner(15)	*Staphylococcus Aureus*	6
	Bacillus Cereus	5
	Pseudomonas aeruginosa	3
	Escherichia coli	5
	Klebsiella pneumoniae	5
Eye Serum(15)	*Staphylococcus Aureus*	15
	Bacillus Cereus	4
	Pseudomonas aeruginosa	15
	Escherichia coli	Not Found
	Klebsiella pneumoniae	9

Cosmetic product category (N)	Name of Bacteria	Number of cosmetic with bacterial growth
Compact Powder (10)	*Staphylococcus Aureus*	9
	Bacillus Cereus	9
	Pseudomonas aeruginosa	6
	Escherichia coli	2
	Klebsiella pneumoniae	10
Foundation (12)	*Staphylococcus Aureus*	12
	Bacillus Cereus	7
	Pseudomonas aeruginosa	12
	Escherichia coli	Not Found
	Klebsiella pneumoniae	11
Primer (15)	*Staphylococcus Aureus*	15
	Bacillus Cereus	10
	Pseudomonas aeruginosa	15
	Escherichia coli	Not Found
	Klebsiella pneumoniae	12
Loose powder (15)	*Staphylococcus Aureus*	11
	Bacillus Cereus	6
	Pseudomonas aeruginosa	10
	Escherichia coli	Not Found
	Klebsiella pneumoniae	15

N= Number of cosmetic samples

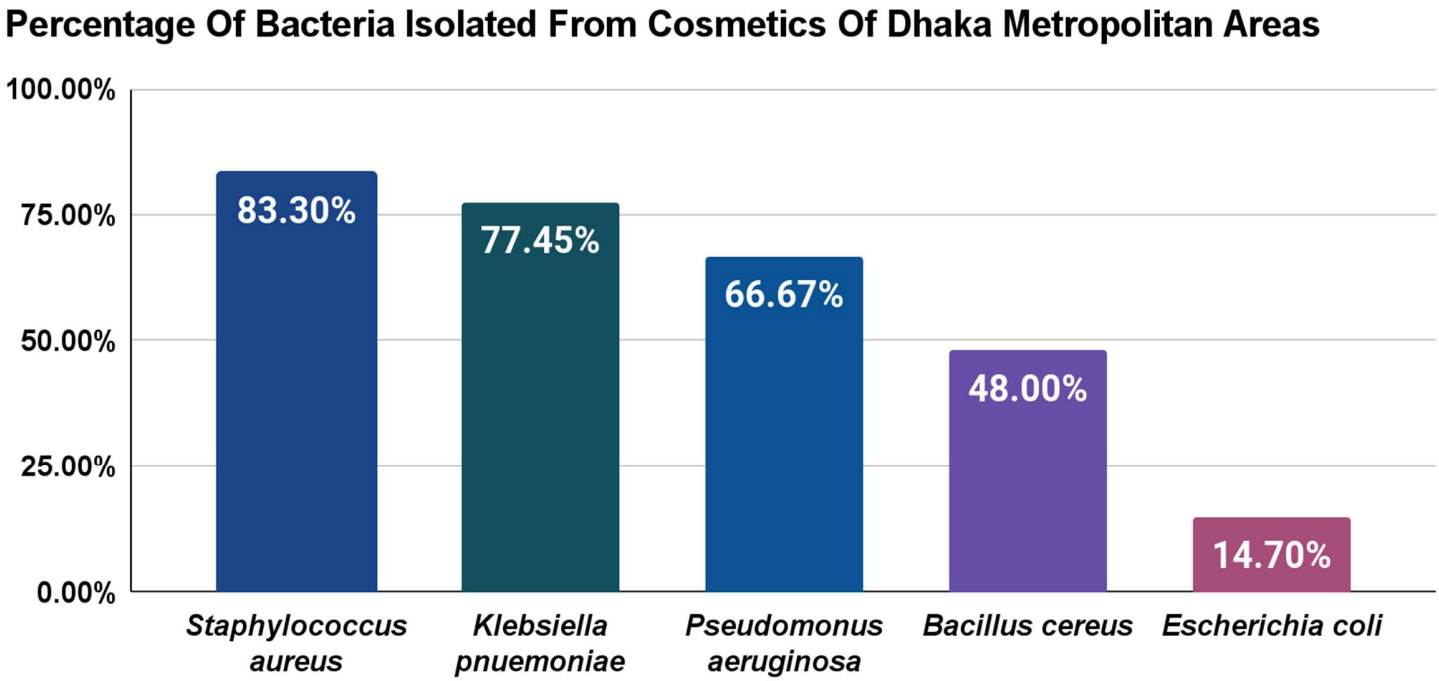


Figure 9: The overall percentage of the isolated bacteria from cosmetics sample

3.4 Antibiotic Susceptibility Test

Using the antibiotic disc diffusion technique, the antibiotic susceptibility test of 296 isolates of 102 cosmetic samples was done on the Mueller-Hinton Agar plate that is shown in Figure 10 following the Clinical Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2023). Tables 9 and 10 below illustrate the percentage of antibiotic resistance and multi-drug resistance in the bacteria. Figures 11, 12, 13, and 19 show the graphical percentage view of bacterial antibiotic resistance and multi-drug resistance. Figures 14, 15, 16, 17, and 18 depict the graphical representation of antibiotic resistance found among five bacteria.

Figure 10: Antibiogram done on Mueller-Hinton Agar

Table 9: Antibiotic resistance observed in different bacteria based on cosmetics

Name of Bacteria	Percentage of Resistance Observed							
	Kajol	**Eyeshadow**	**Eyeliner**	**Eye Serum**	**Compact Powder**	**Foundation**	**Primer**	**Loose Powder**
Klebsiella pneumoniae	80.56%	100%	77.77%	88.89%	80.55%	73.73%	81.01%	100%
Pseudomonas aeruginosa	63.89%	100%	70.37%	65.18%	76.98%	100%	53.32%	100%
Staphylococcus aureus	43.66%	88.89%	72.22%	38.89%	55.65%	30.95%	34.4%	77.78%
Bacillus Cereus	30.55%	37.03%	61.1%	31.55%	33.32%	100%	39.89%	37.06%
Escherichia coli	24.44%	27.78%	23.6%	No growth	23.6%	No growth	No growth	No growth

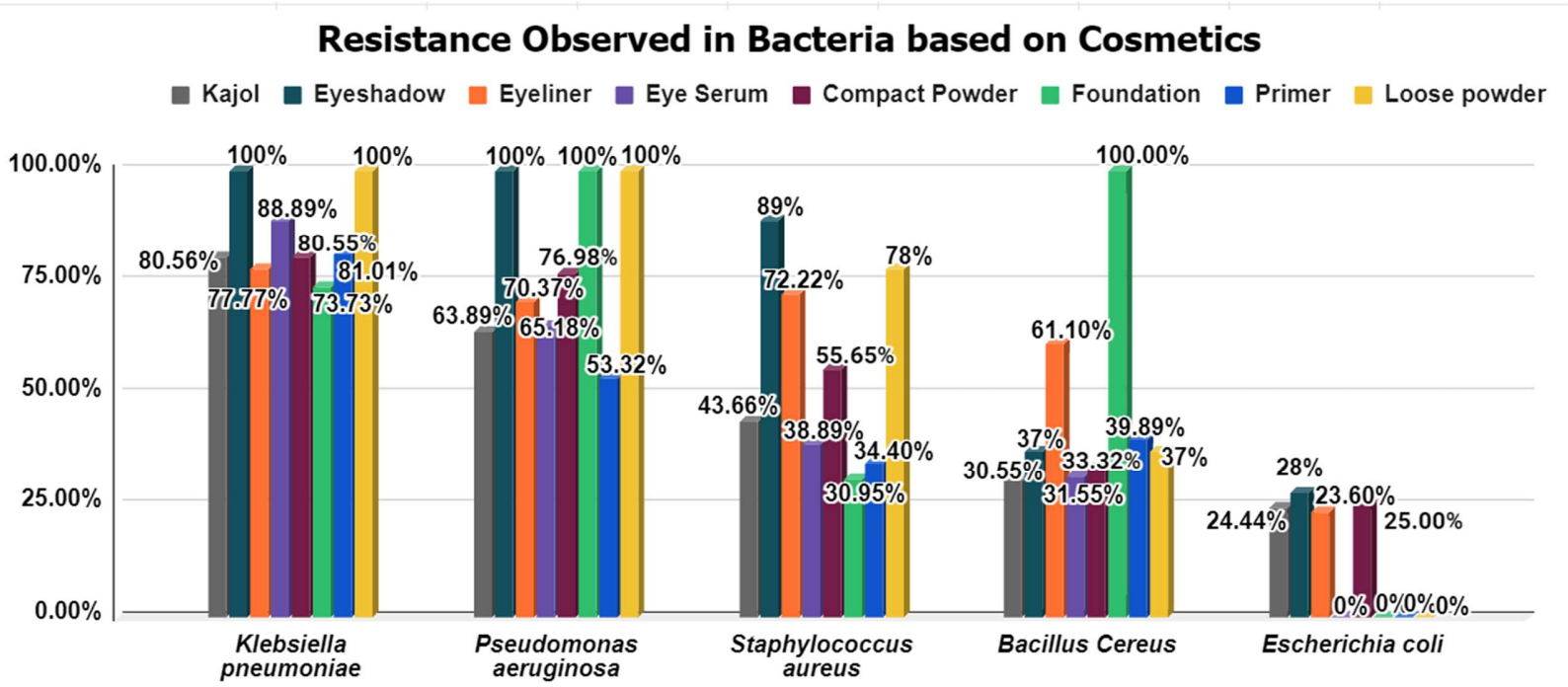


Figure 11: Resistance Observed in Different Bacteria Based on Cosmetics.

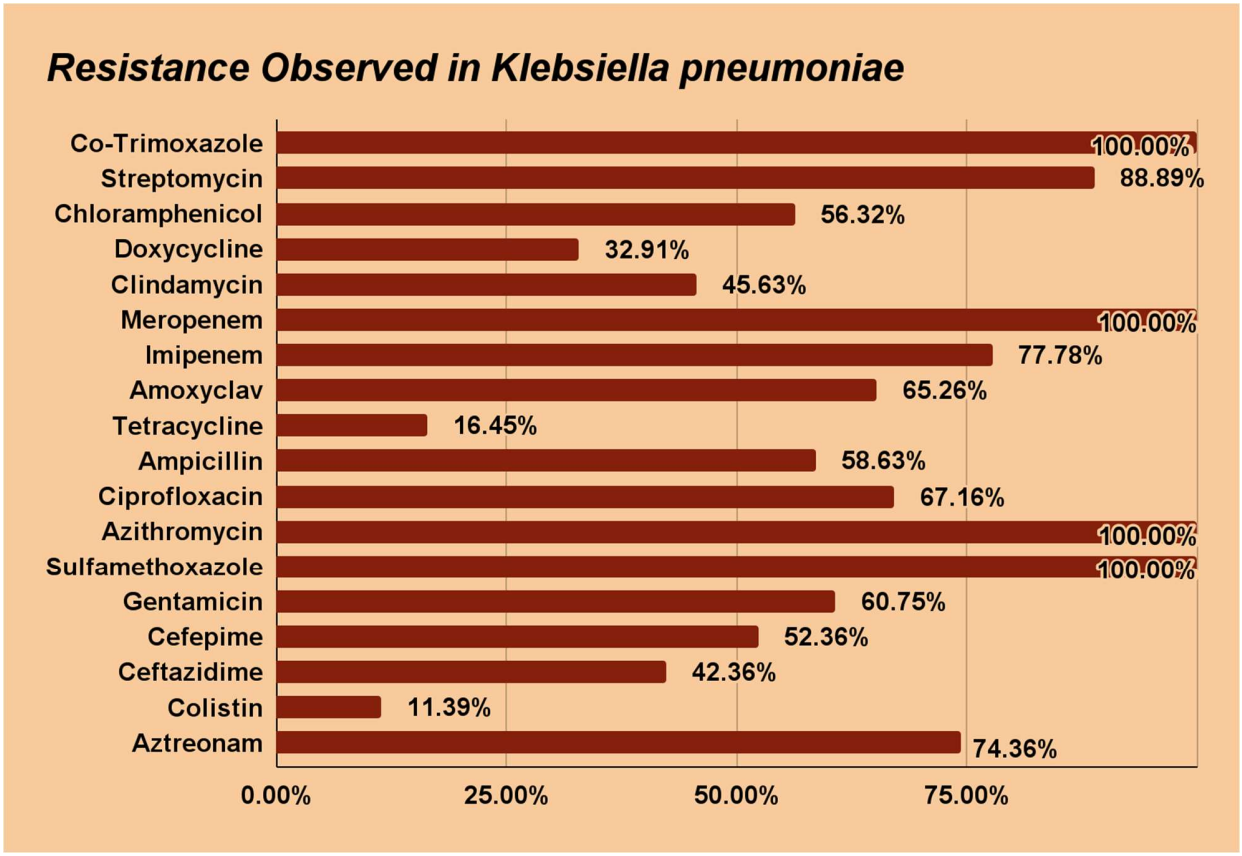


Figure 12: Resistance Observed in *Klebsiella pneumoniae*.

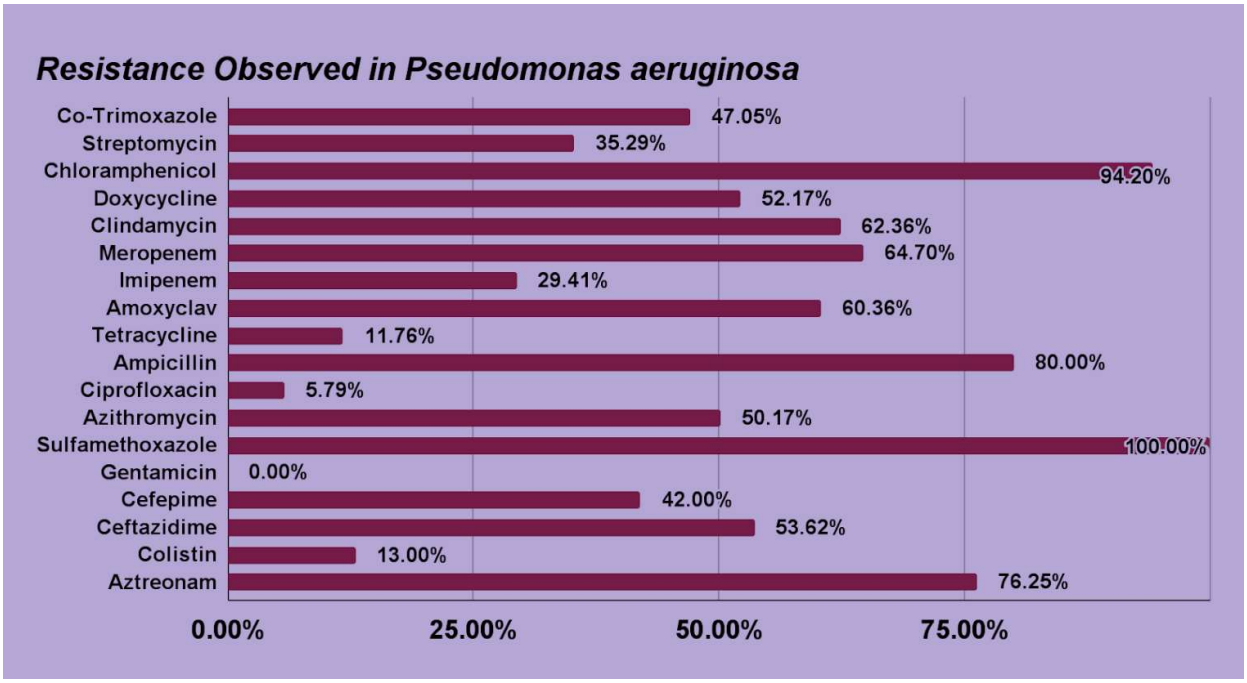


Figure 13: Resistance Observed in *Pseudomonas aeruginosa*

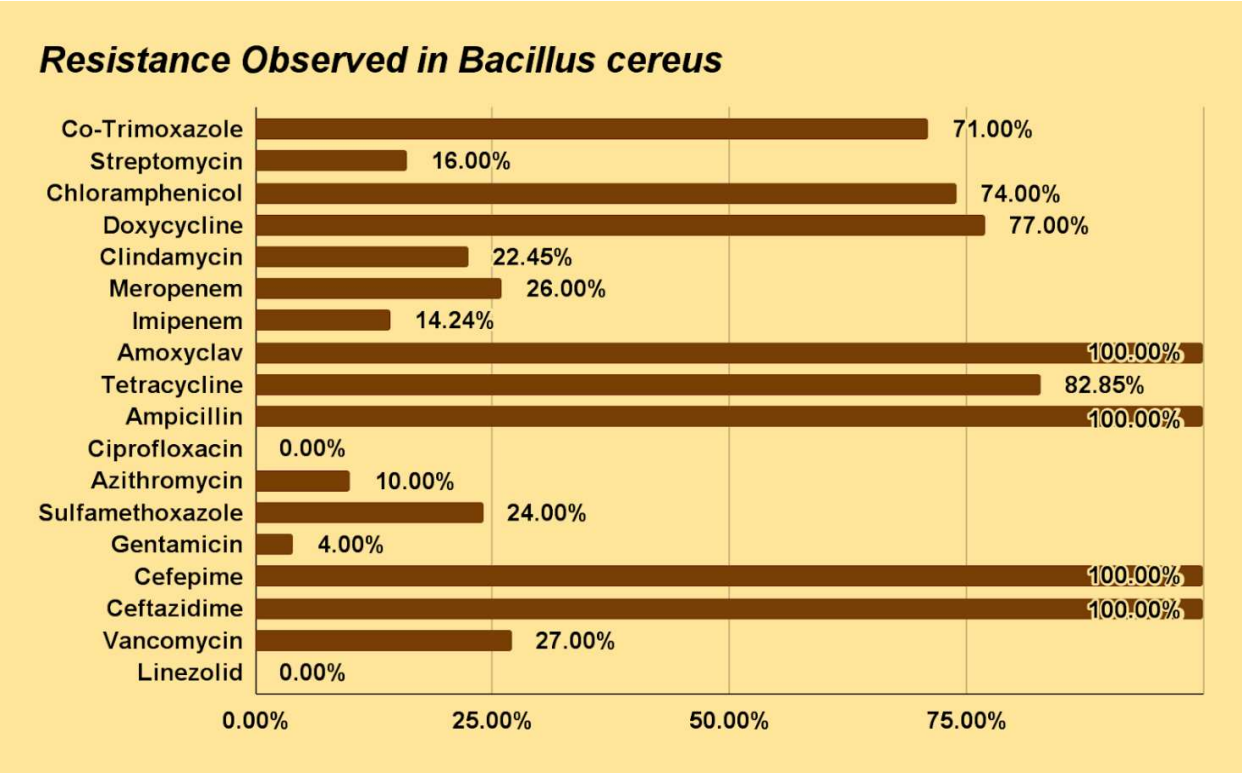


Figure 14: Resistance Observed in *Bacillus Cereus*

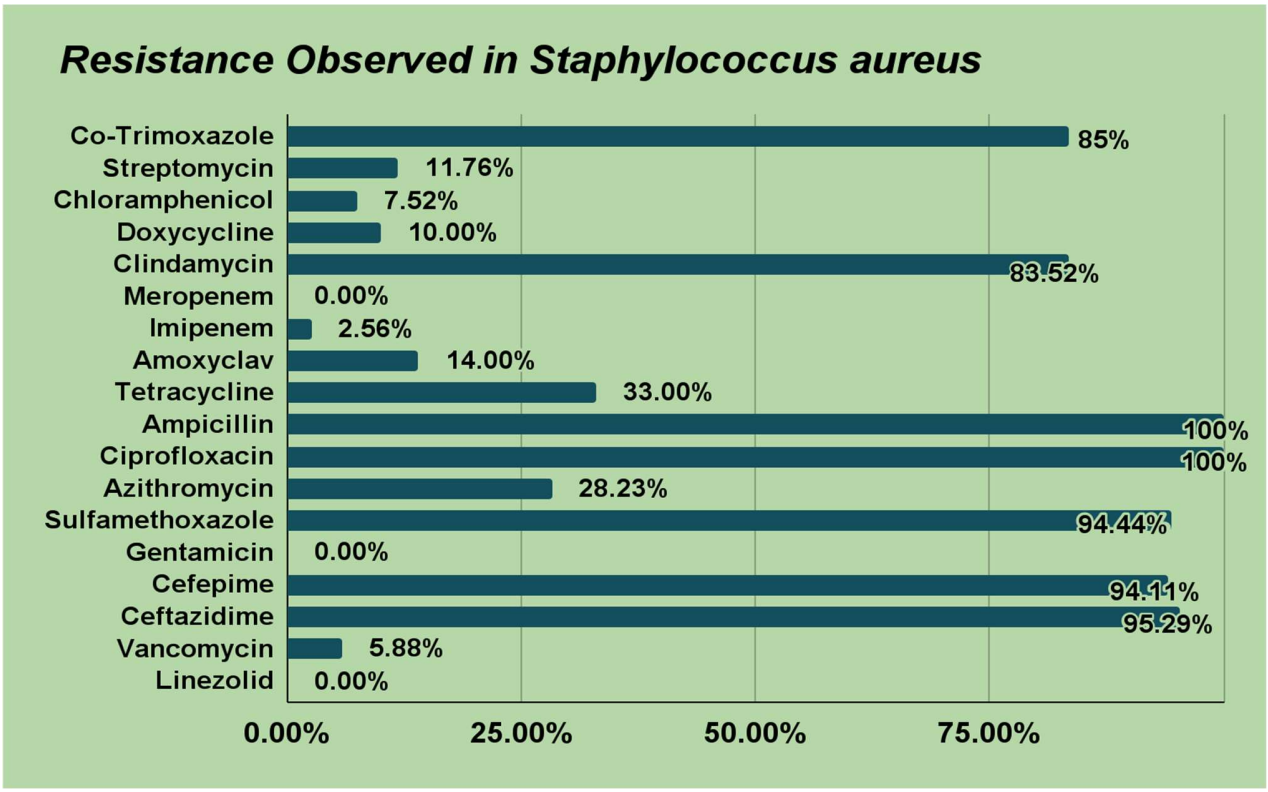


Figure 15: Resistance Observed in *Staphylococcus aureus*

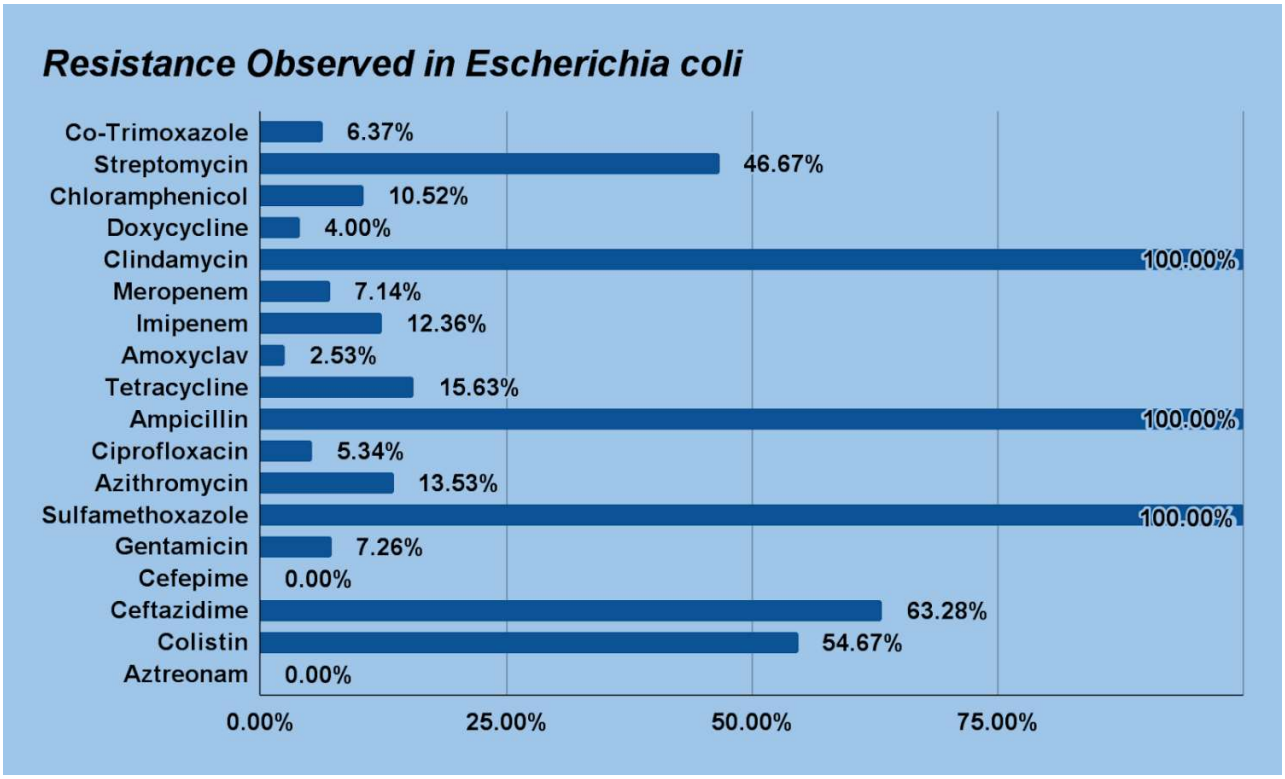


Figure 16: Resistance Observed in *Escherichia coli*

Table 10: Multi-drug resistance observed in different organisms based on cosmetic samples

Name of Organism	Percentage of Multi-drug Resistance							
	Kajol	**Eyeshadow**	**Eyeliner**	**Eye Serum**	**Compact Powder**	**Foundation**	**Primer**	**Loose Powder**
Klebsiella pneumoniae	100%	66.67%	60%	66.67%	70%	36.36%	75%	53.33%
Pseudomonas aeruginosa	50%	40%	33.33%	46.67%	66.67%	41.67%	46.67%	60%
Staphylococcus aureus	33.33%	42.85%	50%	40%	55.56%	25%	46.67%	54.54%
Bacillus Cereus	100%	50%	60%	75%	44.44%	57.14%	30%	66.67%
Escherichia coli	40%	33.33%	20%	No isolates observed	50%	No isolates observed	No isolates observed	No isolates observed

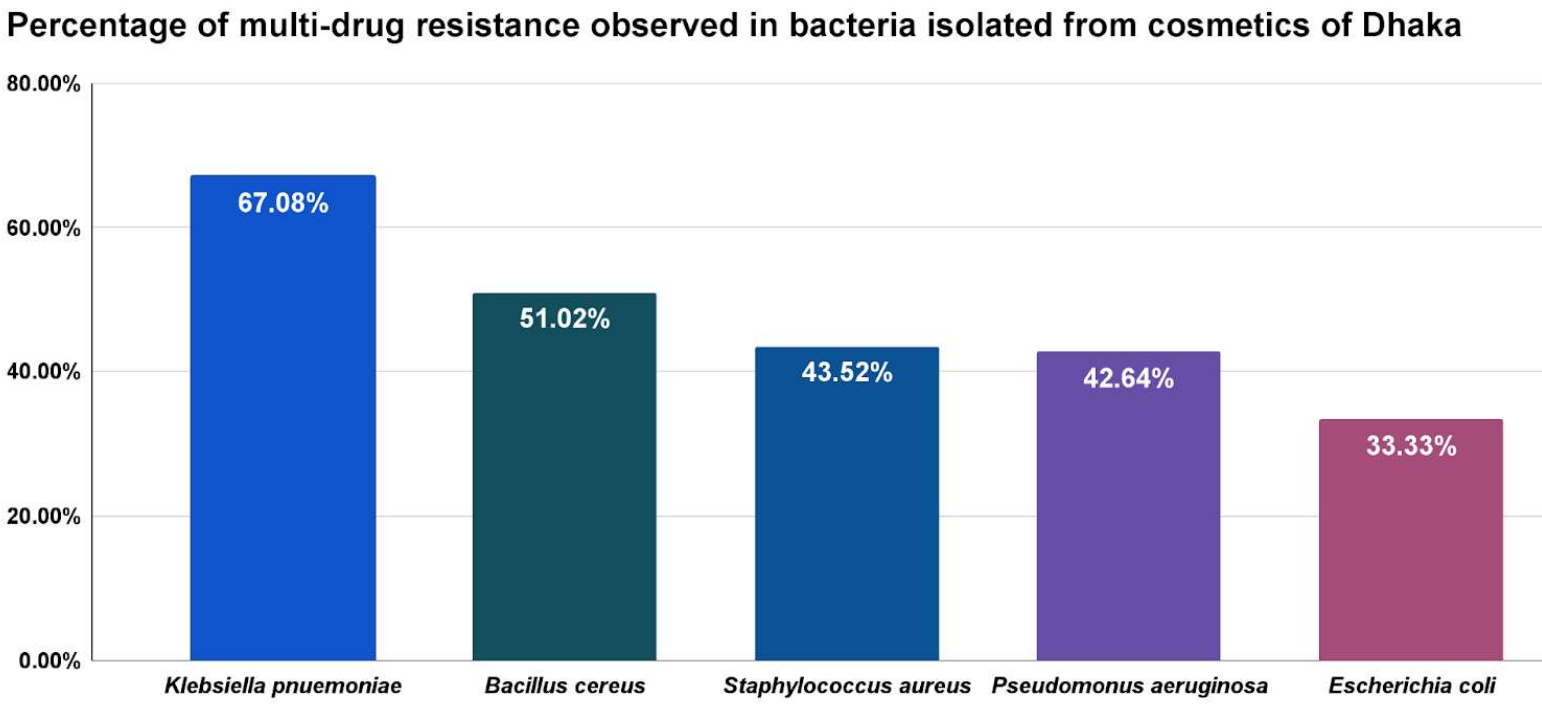


Figure 17: Percentage of multi-drug resistance observed in bacteria isolated from cosmetics

Chapter 4: Discussion

As cosmetic items are regarded as essential components of our daily schedule, they should be secure and contamination-free. As like many other countries of the world, microbial contamination is the greatest concern regarding the quality and safety of cosmetic items even in Bangladesh. (Almukainzi et al., 2022). But in Bangladesh, there is not enough study about microbial contamination in cosmetic products even though some of the studies that had been done about the microbiological quality of cosmetic products till now have pathogenic bacteria like *Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus aureus* (Akon et al., 2015). The purpose of this study was to determine the total aerobic plate count and CFU/ml of bacteria, as well as to identify bacteria isolated from cosmetics using selective media and multidrug resistance.

A selection of kajol, eyeshadow, eyeliner, eye serum, compact powder, foundation, primer, and loose powder were tested for microbial contamination. In the case of kajol, 5 samples were tested. In the case of kajol 1, the aerobic plate count was 5.5×10^{10}. The aerobic Plate count was 9.3×10^{5} for kajol 2. For kajol 3, the aerobic plate count was 14.3×10^{7}. For kajol 4, the aerobic plate count was 45. In the case of kajol 5, the aerobic plate count was 3.6×10^{7}.

In the case of eyeshadow, 15 samples were tested. For the 1st sample, the aerobic plate count was 2.1×10^{14}. For the 2nd sample, the aerobic plate count was 11.4×10^{14}. In the 3rd sample, the aerobic plate count was 6.3×10^{12}. For the 4th sample, the aerobic plate count was 8.7×10^{12}. For the 5th sample, the estimated aerobic plate count was 1.2×10^{8}. In the 6th sample, the aerobic plate count was 2.6×10^{7}. In the 7th sample, the estimated aerobic plate count was 71.3×10^{12}. In the 8th sample, the aerobic plate count was 4×10^{5}. For the 9th sample, the aerobic plate count was 13.7×10^{14}. For the 10th sample, the aerobic plate count was 5.2×10^{12}. In the 11th sample, the aerobic plate count was 12.3×10^{6}. For the 12th sample, the estimated aerobic plate count was 1×10^{9}. In the 13th sample aerobic plate count was 57000. In the 14th sample, the estimated aerobic plate count was 4.8×10^{12}. In the last sample, the aerobic plate count was 2.7×10^{5}.

In the case of eyeliner, 15 samples were tested. For the 1st sample the aerobic plate count was 3×10^{5}. For the 2nd sample the aerobic plate count was 1×10^{4}. In the 3rd sample, the aerobic plate count was 8.2×10^{12}. For the 4th sample the aerobic plate count was 5×10^{6}. For the 5th sample the estimated aerobic plate count was 12×10^{6}. In the 6th sample aerobic plate count was 1.6×10^{14}. In the 7th sample the estimated aerobic plate count was 2.9×10^{12}. In the 8th sample the aerobic plate

count was 5.6×10^{14}. In the 9th sample, the aerobic plate count was 7.7×10^{14}. For the 10th sample, the aerobic plate count was 1.5×10^{5}. In the 11th sample, the aerobic plate count was 400. For the 12th sample, the estimated aerobic plate count was 3.9×10^{5}. In the 13th sample aerobic plate count was 1.6×10^{3}. In the 14th sample, the estimated aerobic plate count was 7×10^{12}. In the last sample, the aerobic plate count was 1×10^{5}.

In the case of eye serum, 15 samples were tested. For the 1st sample, the aerobic plate count was 4.7×10^{12}. For the 2nd sample, the aerobic plate count was 5.3×10^{12}. In the 3rd sample, the aerobic plate count was 6.2×10^{14}. For the 4th sample, the aerobic plate count was 5.1×10^{11}. For the 5th sample, the estimated aerobic plate count was 3.4×10^{14}. In the 6th sample, the aerobic plate count was 11.1×10^{14}. In the 7th sample, the estimated aerobic plate count was 3.9×10^{10}. In the 8th sample, the aerobic plate count was 1.3×10^{14}. For the 9th sample, the aerobic plate count was 2×10^{10}. For the 10th sample, the aerobic plate count was 7×10^{10}. In the 11th sample, the aerobic plate count was 8.5×10^{12}. For the 12th sample, the estimated aerobic plate count was 1.610^{12}. In the 13th sample aerobic plate count was 6.4×10^{14}. In the 14th sample, the estimated aerobic plate count was 150. In the last sample, the aerobic plate count was 4.5×10^{12}.

In the case of compact powder, 10 samples were tested. For the 1st sample, the aerobic plate count was 6×10^{12}. For the 2nd sample, the aerobic plate count was 3.1×10^{12}. In the 3rd sample, the aerobic plate count was 2.6×10^{10}. For the 4th sample, the aerobic plate count was 12.2×10^{14}. For the 5th sample, the estimated aerobic plate count was 9×10^{7}. In the 6th sample, the aerobic plate count was 3×10^{5}. In the 7th sample, the estimated aerobic plate count was 10.9×10^{14}. In the 8th sample, the aerobic plate count was 190. For the 9th sample, the aerobic plate count was 4.3×10^{12}. In the last sample, the aerobic plate count was 1×10^{5}.

In the case of the foundation, 12 samples were tested. For the 1st sample, the aerobic plate count was 2×10^{13}. For the 2nd sample, the aerobic plate count was 410^{5}. In the 3rd sample, the aerobic plate count was 13.5×10^{14}. For the 4th sample, the aerobic plate count was 5.2×10^{14}. For the 5th sample, the estimated aerobic plate count was 2.3×10^{12}. In the 6th sample, the aerobic plate count was 11.3×10^{14}. In the 7th sample, the estimated aerobic plate count was 6.3×10^{14}. In the 8th sample, the aerobic plate count was 7.2×10^{14}. For the 9th sample, the aerobic plate count was 3×10^{10}. For the 10th sample, the aerobic plate count was 1×10^{4}. In the 11th sample, the aerobic plate count was 5560. In the last sample, the aerobic plate count was 7.7×10^{14}.

In the case of primer, 15 samples were tested. For the 1st sample, the aerobic plate count was 11.7×10^{14}. For the 2nd sample, the aerobic plate count was 1×10^{5}. In the 3rd sample, the aerobic plate count was 8×10^{14}. For the 4th sample, the aerobic plate count was 4.710^{14}. For the 5th sample, the estimated aerobic plate count was 23.2×10^{7}. In the 6th sample, aerobic plate count was 610^{5}. In the 7th sample, the estimated aerobic plate count was 5.2×10^{14}. In the 8th sample, the aerobic plate count was 3.5×10^{14}. For the 9th sample the aerobic plate count was 2.9×10^{7}. For the 10th sample the aerobic plate count was 6.7×10^{14}. In the 11th sample, the aerobic plate count was 9.6×10^{14}. For the 12th sample the estimated aerobic plate count was 4.2×10^{14}. In the 13th sample aerobic plate count was 8.2×10^{14}. In the 14th sample, the estimated aerobic plate count was 3950. In the last sample, the aerobic plate count was 4.6×10^{14}.

In the case of loose powder, 15 samples were tested. For the 1st sample, the aerobic plate count was 11.2×10^{14}. For the 2nd sample, the aerobic plate count was 2.4×10^{9}. In the 3rd sample, the aerobic plate count was 9×10^{12}. For the 4th sample, the aerobic plate count was 6.3×10^{14}. For the 5th sample, the estimated aerobic plate count was 5.1×10^{14}. In the 6th sample, aerobic plate count was 3×10^{12}. In the 7th sample, the estimated aerobic plate count was 11.6×10^{14}. In the 8th sample, the aerobic plate count was 2×10^{8}. For the 9th sample the aerobic plate count was 4.6×10^{12}. For the 10th sample the aerobic plate count was 3.2×10^{14}. In the 11th sample, the aerobic plate count was 340. For the 12th sample the estimated aerobic plate count was 6.3×10^{7}. In the 13th sample aerobic plate count was 3×10^{6}. In the 14th sample, the estimated aerobic plate count was 1.7×10^{12}. In the last sample, the aerobic plate count was 1.3×10^{7}.

According to FDA and EU guidelines, the total aerobic count limit should not exceed more than 500 CFU/g or CFU/ml for any cosmetic products used in the eye area and for other products, the total aerobic count limit should not exceed more than 1000 CFU/g or CFU/ml ("Scientific Committee on Consumer Safety (SCCP)", 2016). However, in the Kajal and Eyeliner samples, except Kajal 4 and Eyeliner 11 all of them exceed the limit for eye area cosmetics set by the FDA and EU. In the case of other products, except Compact Powder 8, Loose Powder 11 and Eye Serum 14 samples, all of them exceed the limit for non-eye area cosmetics set by the FDA and EU. These cosmetic products may not exceed the range but there was growth. The rest of the samples had CFU/ml ranging from 1.6×10^{3}–13.7×10^{14}. This is higher than the acceptable limit.

In case of *Escherichia coli* isolates resistance observed was Co-Trimoxazole (6.37%), Streptomycin (46.67%), Chloramphenicol (10.52%), Doxycycline (4.00%), Clindamycin (100%), Meropenem (7.14%), Imipenem (12.36%), Amoxyclav (2.53%), Tetracycline (15.63%), Ampicillin (100%), Ciprofloxacin (5.34%), Azithromycin (13.53%), Sulfamethoxazole (100%), Gentamicin (7.26%), Ceftazidime (63.28%) and Colistin (54.67%). Comparing the findings with the previous study (Nusrat et al., 2023) of this extension, both studies are showing high resistance to Ampicillin. There is a drastic change in the resistance percentages of Meropenem, Aztreonam, Ciprofloxacin, and Cefepime which showed these antibiotics are sensitive towards *Escherichia coli*. In another study (Kachalla et al., 2022), E. coli isolates were found highly antibiotic-resistant to Chloramphenicol (100%), Tetracycline (100%), and Streptomycin (100%) antibiotics. The level of resistance shown in other antibiotics were Gentamicin (50%), Ciprofloxacin (67%), and Co-Trimoxazole (0%). This study did not correspond with our findings. The level of resistance in the study completely differs from our findings.

In case of *Klebsiella pneumoniae* the resistance observed in the antibiotics was Co-Trimoxazole (100%), Streptomycin (88.89%), Chloramphenicol (56.32%), Doxycycline (32.91%), Clindamycin (45.63%), Meropenem (100%), Imipenem (77.78%), Amoxyclav (65.26%), Tetracycline (16.45%), Ampicillin (58.63%), Ciprofloxacin (67.16%), Azithromycin (100%), Sulfamethoxazole (100%), Gentamicin (60.75%), Cefepime (52.36%), Ceftazidime (42.36%), Colistin (11.39%) and Aztreonam (74.36%). In the previous study (Nusrat et al., 2023) of this extension, the antibiotic resistance levels were Meropenem (21.42%), Imipenem (7.14%), Aztreonam (21%), Azithromycin (21.42%), Ampicillin (7.14%), Ciprofloxacin (7.14%), Collistin (35.71%) and Gentamicin (0%) which have shown increment in our findings but sensitive to Colistin. In another study (Kachalla et al., 2022) conducted in 2022, the isolates were found resistant to Chloramphenicol, Gentamicin, Tetracycline, and Ciprofloxacin. This study's findings partially corresponded with our levels of antibiotic resistance.

In case of *Pseudomonas aeruginosa* isolates resistance observed in the antibiotics was Co-Trimoxazole (47.05%), Streptomycin (35.29%), Chloramphenicol (94.20%), Doxycycline (52.17%), Clindamycin (62.36%), Meropenem (64.70%), Imipenem (29.41%), Amoxyclav (60.36%), Tetracycline (11.76%), Ampicillin (80.00%), Ciprofloxacin (5.79%), Azithromycin (50.17%), Sulfamethoxazole (56.23%), Gentamicin (0.00%), Cefepime (42.00%), Ceftazidime (53.62%), Colistin (13.00%) and Aztreonam (76.25%). These findings are somewhat

confirmed by the previous study of this extension (Nusrat et. Al.,2023) where Imipenem, Colistin, Ciprofloxacin, and Gentamicin were sensitive to *Pseudomonas aeruginosa*. In another study (Kachalla et al., 2022) conducted in 2022, the resistance levels of the antibiotic were Chloramphenicol (100%), Gentamicin (50%), Tetracycline (100%), Ciprofloxacin (50%), Streptomycin (50%) and Co-trimoxazole (75%). These findings partially corresponded with our findings.

In case of *Staphylococcus aureus* isolates the resistance observed in the antibiotics was Co-Trimoxazole (83.33%), Streptomycin (11.76%), Chloramphenicol (7.52%)

Doxycycline (10.00%), Clindamycin (66.67%), Meropenem (0.00%), Imipenem (2.56%), Amoxyclav (14.00%), Tetracycline (94.44%), Ampicillin (100%), Ciprofloxacin (100%), Azithromycin (28.23%), Sulfamethoxazole (77.64%), Gentamicin (0.00%), Cefepime (94.44%), Ceftazidime (88.89%), Vancomycin (5.88%) and Linezolid (0.00%). These findings partially corresponded with the previous study (Nusrat et al., 2023) of this extension where Vancomycin, Linezolid, Meropenem and Gentamicin were sensitive to *Staphylococcus aureus*. In another study (Kachalla et al., 2022) conducted in 2022, except Ciprofloxacin, the levels of antibiotic resistance did not correspond.

In case of *Bacillus cereus* isolates, resistance observed in the antibiotics was Co-Trimoxazole (71.00%), Streptomycin (16.00%), Chloramphenicol (74.00%), Doxycycline (77.00%), Clindamycin (22.45%), Meropenem (26.00%), Imipenem (14.24%), Amoxyclav (100%), Tetracycline (82.85%), Ampicillin (100%), Ciprofloxacin (0.00%), Azithromycin (10.00%), Sulfamethoxazole (24.00%), Gentamicin (4.00%), Cefepime (100%), Ceftazidime (100%), Vancomycin (27.00%) and Linezolid (0.00%). The finding of the previous study (Nusrat et al., 2023) of this extension partially corresponded with our finding. The percentage of the resistance found in Linezolid, Ciprofloxacin and Gentamicin matched with our findings and differed for the rest of the antibiotics.

Chapter 5: Conclusion

When it comes to the application of cosmetics, quality, and safety are among the most dynamic and crucial considerations. According to our study, the majority of cosmetics marketed in Dhaka's urban marketplaces include bacteria like *Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus aureus*. The detected contamination level exceeded the limit set by the FDA. Gram-positive and gram-negative bacteria, which are prevalent opportunistic skin pathogens, are the primary source of concern due to their abundant presence. The etiology of bacterial skin folliculitis and various other skin maladies has been linked to both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, according to multiple research studies (Winters, 2023, Sugeng et al., 1999, Wu et al., 2011). *Escherichia coli* has been identified as the causative agent of bacterial eye infections, including conjunctivitis and keratitis (Nunes et al., 2022). Additionally, it was disconcerting to discover that a significant proportion of the antibiotics examined on the bacterial samples exhibited multidrug resistance. Strict microbiological quality control testing along with upholding and enhancing personal hygiene might be a strategy to lower the amount of contamination in cosmetic items.

Chapter 6: References

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