Detection of *Klebsiella pneumoniae* & *Listeria monocytogenes* and Analysis of Their Antibiotic Resistance in Bangladeshi Homemade Cosmetics

By

Sadia Zaman Mukti

ID:19326038

A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the degree of Bachelors of Science in Microbiology

Mathematics and Natural Science

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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing our degree at BRAC University.
2. The thesis 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 thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

Student's Full Name & Signature:

_____

Sadia Zaman Mukti

19326038

Approval

The project titled "***Analyzing pathogenic bacterial load and heavy metal contaminants in Bangladeshi homemade cosmetics***." submitted by Sadia Zaman Mukti (ID - 19326038) of Summer 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the Bachelor's degree in Microbiology 6th October 2025.

Examining Committee:

Supervisor:

_____

Fahim Kabir Monjurul Haque, PhD
Associate Professor, Microbiology Program
Department of Mathematics and Natural Sciences BRAC University, Dhaka, Bangladesh

Program Coordinator:

_____

Nadia Sultana Deen, PhD Associate Professor, Department of Mathematics and Natural Sciences BRAC University.

Departmental Head:

_____

Md. Firoze H. Haque, PhD Associate Professor and Chairperson, Department of Mathematics and Natural Sciences Brac University

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Abstract:

In Bangladesh, homemade organic cosmetics such as whitening night creams, whitening serums, soaps, face packs and scrubs are in high demand, particularly among women. Many of these products are marketed as pure organic and chemical-free. However, the potential risk of bacterial contamination remains largely unexplored. This study aimed to analyze the pathogenic bacterial load in Bangladeshi homemade cosmetics targeting 10 clinically significant bacteria, including *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp., Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Acinetobacter baumannii*. As part of this group project, my individual focus was on *Klebsiella pneumoniae* and *Listeria monocytogenes*, to evaluate their prevalence and potential public health implications.

A total of 22 samples were collected from online sellers who claim to sell organic homemade cosmetics. For bacterial load determination, samples were enriched in Modified Letheen Broth and plated on Modified Letheen Agar for CFU counts. Isolation and detection of *Klebsiella pneumoniae* were performed by using KPC agar followed by PCR confirmation. Moreover, *Listeria monocytogenes* was detected using Listeria Selective Oxford Agar followed by PCR confirmation as well. Antibiotic susceptibility testing (AST) was performed using the Kirby Bauer disk diffusion method against a panel of 13 antibiotics with four additional antibiotics included- Vancomycin and Linezolid for Gram-positive bacteria and Colistin and Aztreonam for Gram-negative bacteria.

Out of the 22 cosmetic products tested, 20 (90.09%) were positive for *Klebsiella pneumonia* and 2 (9.1%) were positive for *Listeria monocytogenes*. For *Klebsiella pneumoniae*, AST performed on 22 isolates that revealed high resistance to Ampicillin (92.3%), Azithromycin (84.6%), and Ciprofloxacin (76.9%) while resistance to other antibiotics ranged between 7.7% and 23.1%. However, *Listeria monocytogenes* isolates displayed mixed susceptibility patterns: one isolate was largely sensitive, while the other showed resistance to several key antibiotics including Linezolid, Vancomycin, Ampicillin and Daptomycin.

This study highlights the significant presence of pathogenic bacteria in Bangladeshi homemade cosmetic products. The high contamination rates and concerning resistance profiles are alarming for public health, emphasizing the urgent need for microbiological safety monitoring and consumer awareness.

Keywords: Homemade cosmetics*; Klebsiella pneumoniae; Listeria monocytogenes*; Antibiotic resistance; Bangladesh; Pathogenic contaminatin.

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List of Acronyms

FDA: Food & Drug Administration

BSQCA: Bangladesh Standard Quality Control Authority

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

MHA: Mueller Hinton Agar

mm: Millimeter

ml: Milliliter

µl: Microliter

CFU: Colony Forming Unit

spp: Species

Chapter 1

Introduction

1.1 Background:

Cosmetic products have been used for centuries all over the world to improve personal appearance, maintain hygiene, and provide protection. Today, a wide variety of cosmetics are available in markets, ranging from products for skin and hair care to items designed for overall body care(Das K K,2013). However, these products may be made from chemicals or natural herbal ingredients and are generally considered safe for regular use. Apart from their chemical side effects another important issue is their microbiological quality. Contamination by microorganisms in cosmetics can cause serious health risks to users, making it a growing concern for public health(Fatema K,2013).

Moreover, cosmetic products can contain various ingredients that support the growth of harmful bacteria and fungi, which may lead to skin and mucous membrane infections and sometimes skin cancer. Interestingly, this problem is more common in developing countries like Bangladesh due to poor sanitation and manufacturing, contaminated water and lack of awareness. While international guidelines such as GMP, FDA, USP, BP, and EP set strict standards for microbiological safety, cosmetic testing facilities in Bangladesh are very limited(Noor R,Zerin N,2015). As a consequence the situation is even more alarming for homemade or organic cosmetics, which are widely sold without any laboratory testing or regulatory approval by Food and Drug Administration (FDA). Since these products are often promoted as organic, natural and chemical-free, users remain unaware of the serious health risks caused by microbial contamination. Therefore, it is essential to investigate the microbiological quality of both commercial and homemade cosmetics to ensure public safety.

Furthermore, Contamination of cosmetic products by pathogenic bacteria like *Staphylococcus aureus„Streptococcus* spp., *Micrococcus* spp., *Clostridium tetani*, *Bacillus cereus*, actinomycetes and fungi has been reported worldwide(Noor R,Zerin N,2015).Until recently, Bangladeshi scientific community remains quite reluctant about cosmetic microbiology but interestingly microbiological contamination were resolved almost 30 years ago. For example:In 1946, the first notification of microbial contamination was made in talcum powder by *Clostridium tetani*, and then in 1967, *Klebsiella pneumoniae* was reported to contaminate hand creams, and finally in 1983, aqueous soaps were observed to harbor *Pseudomonas stutzeri* . (Noor R,Zerin N,2015).

However, the Drug and Cosmetics Act 2023, a new comprehensive law on cosmetics in Bangladesh, was recently codified by the government. To ensure the safety and quality of cosmetics, the law establishes guidelines for their licensing, inspection, and supervision (Legal, 2023). According to FDA guidelines for cosmetic products, certain contamination levels

shouldn't be surpassed.For instance, 1000 CFU/g shouldn't be achieved in non-eye areas (Nutrition, 2023). According to EU guidelines, the total viable count of aerobic bacteria in the eye region shouldn't exceed 10^2 CFU/ml. For samples taken from non-eye areas, the permissible maximum for aerobic bacteria 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).

1.2: Literature Review

1.2.1- Microbial Contamination of Cosmetics: A Global Concern

Studies conducted all over the world have regularly documented the widespread presence of pathogenic microbes in cosmetic products, particularly those that are not properly manufactured, handled, kept, or maintained. In a research conducted in Bangladesh that tested cosmetics manufactured locally, bacteria were found in 85.2% of the samples, and more than three-quarters (77.8%) of them exceeded the microbial safety standards established by regulatory agencies like the FDA, ISO, and BSTI. *Escherichia coli, Salmonella spp., Staphylococcus spp., Streptococcus spp., Bacillus spp., Listeria monocytogenes, Pseudomonas aeruginosa, and Klebsiella pneumoniae* were among the isolates. (Nusrat et al., 2023). Similarly*, Klebsiella spp*. and other possible infections were also found in research on baby cosmetics available in Dhaka, revealing contamination during manufacturing or storage(Feroz & Das, 2019).

Klebsiella pneumoniae was one of the pathogens recovered from creams and mascaras in an Egyptian research, highlighting how cosmetics might operate as habitats for opportunistic Gram-negative bacteria (Zeitoun et al., 2015). A study conducted in Western Saudi Arabia investigated the prevalence, species diversity, antimicrobial resistance, and virulence factors of Staphylococcus spp. in 250 cosmetic products purchased from local outlets. The results revealed a low overall contamination rate of 10.4%, with higher contamination observed in lipstick, face powder, and blusher samples. Identified species included *Staphylococcus xylosus*, *S. epidermidis*, and *S. aureus*.(A Fawziah & A Manahel,2025)

Another current study aimed to assess the prevalence of cosmetics utilization and perceived adverse effects among female students at the University of Gondar, northwest Ethiopia. A total of 403 study participants were included, with a response rate of (96%)(C Gashaw,2025).In this study, 81% of the students were using cosmetics, and 55.6% were exposed to cosmetics-related adverse reactions such as skin rash (41%) and itching (38.3%). In addition, most frequently used cosmetic products were toothpaste, lipstick, deodorant, and perfume, which accounted for 83.7%, 56.8%, and 24.7% respectively(S Wudneh,2025).

1.2.2 *Klebsiella pneumoniae* – Pathogenicity and Public Health Implications

K. pneumoniae has been increasingly recognized as a problematic opportunistic pathogen due to its high capacity to cause serious infections and its tendency to acquire multidrug resistance. The bacterium's polysaccharide capsule, siderophores, fimbrial adhesins, and biofilm formation ability enhance its virulence and survival in hostile environments, including dried surfaces and on human skin. In a study in Turkey, used cosmetic products were collected from 500 cosmetic users and a total of 101 (20.2%) bacteria were isolated from used cosmetic products. Research shows the presence of S. epidermidis (47, 46.5%), S. hominis (17, 16.8%), S. aureus (6, 5.9%), E. coli (16, 15.8%), K. pneumoniae (11, 10.9%) and P. aeruginosa (4, 4.1%) (Akgül & Bakan, 2019). Result of phenotypic antibiotic resistance evaluation revealed that 1 isolate was methicillin-resistant S. aureus (MRSA) and it was mecA gene positive. It was determined that the isolated 10 gram negative bacteria showed a profile of carbapenem and extendedspectrum beta-lactamase resistance. Only three K. pneumoniae strains were found to carry the blaOXA-48 gene in these isolates(Akgül & Bakan, 2019). However, Skin colonization, wound infections, or other dermatological conditions may result from cosmetics that were contaminated by K. pneumoniae, particularly if the user has minor scratches or damaged skin tissue. Sadly, if infections arise, antibiotic-resistant bacteria can trigger complications and make treatment more challenging.

1.2.3 *Listeria monocytogenes* – Beyond Foodborne Transmission

Listeria monocytogenes is a Gram-positive, facultatively anaerobic bacterium known primarily for causing listeriosis, a serious foodborne illness but its ecological versatility allows it to persist in a wide range of environments. Its resilience to low temperatures, high salt concentrations, acidic conditions, and desiccation enhances its survival potential in non-food products, including cosmetics (Nusrat et al., 2023) The bacteria may be a contaminant in items that aren't supposed to have live bacteria because of its ability to adapt to low temperatures, tolerance to settings that are acidic and high in salt, and capacity to survive drying. (Nusrat et al., 2023). According to studies, there is a higher chance of cross-contamination when Listeria species are present on surfaces and apparatus utilized in product manufacture. Contamination may occur via raw materials, production surfaces, or cross-contamination during manufacturing. While direct evidence of listeriosis linked to cosmetics use remains limited, dermal exposure particularly in immunocompromised individuals could pose risks and potentially leading to conditions such as cellulitis or cutaneous listeriosis (Noor et al., 2015). However,it causes severe conditions such as sepsis, meningitis, and encephalitis, especially in vulnerable populations like pregnant women, neonates, the elderly, and immunocompromised individuals. Thus, the presence of *L. monocytogenes* in cosmetic products, though often overlooked, warrants greater public health attention.

A case study in Japan revealed that elderly patient who had erythematous, painful, and warm skin sores on their legs, which are a sign of cellulitis, was found suffering from *Listeria*

monocytogenes bacterial infection. The lesions most likely developed as a result of bacteremia, which was made possible by persistent leg edema that interfered with lymphatic drainage. With the right antibiotic treatment, the skin lesions improved, even though the patient eventually passed away from liver malfunction. This example demonstrates that when blood cultures show Gram-positive bacilli in elderly cellulitis patients, *L. monocytogenes* should be taken into consideration because routinely used cephalosporins are ineffective against this bacterium (T Shohei,N Rika et al.,2025). In contrast, these commonly used antibiotics like cephalosporins are frequently unsuccessful in treating *Listeria monocytogenes* infections, such as bacteremia and cellulitis. Cephalosporins like cefotaxime and cefazedone have demonstrated low therapeutic efficacy for listeriosis in vivo, despite their in vitro susceptibility. While ampicillin was successful both in vitro and in vivo(B Kawaler,1984).

Collectively, microbial contamination of cosmetic products is a significant global concern, with studies from Bangladesh, Egypt, and other countries consistently reporting the presence of pathogenic bacteria including *Klebsiella pneumoniae*, *Listeria monocytogenes*, and *Staphylococcus aureus* . Such contamination not only causes dermatological risks but may also contribute to infections that are difficult to treat due to multidrug resistance. Considering these results, little study has been done expressly to examine the frequency and antibiotic resistance of microorganisms in locally produced or handmade cosmetics in Bangladesh. This emphasizes the necessity of doing comprehensive studies to assess the quality of microorganisms, detect any health hazards, and supply information for regulatory guidelines—all of which serve as the foundation for the current investigation.

1.3 Objectives:

1) To collect a set of **homemade cosmetics** specially for face and body prepared in local households or small-scale producers in Bangladesh.

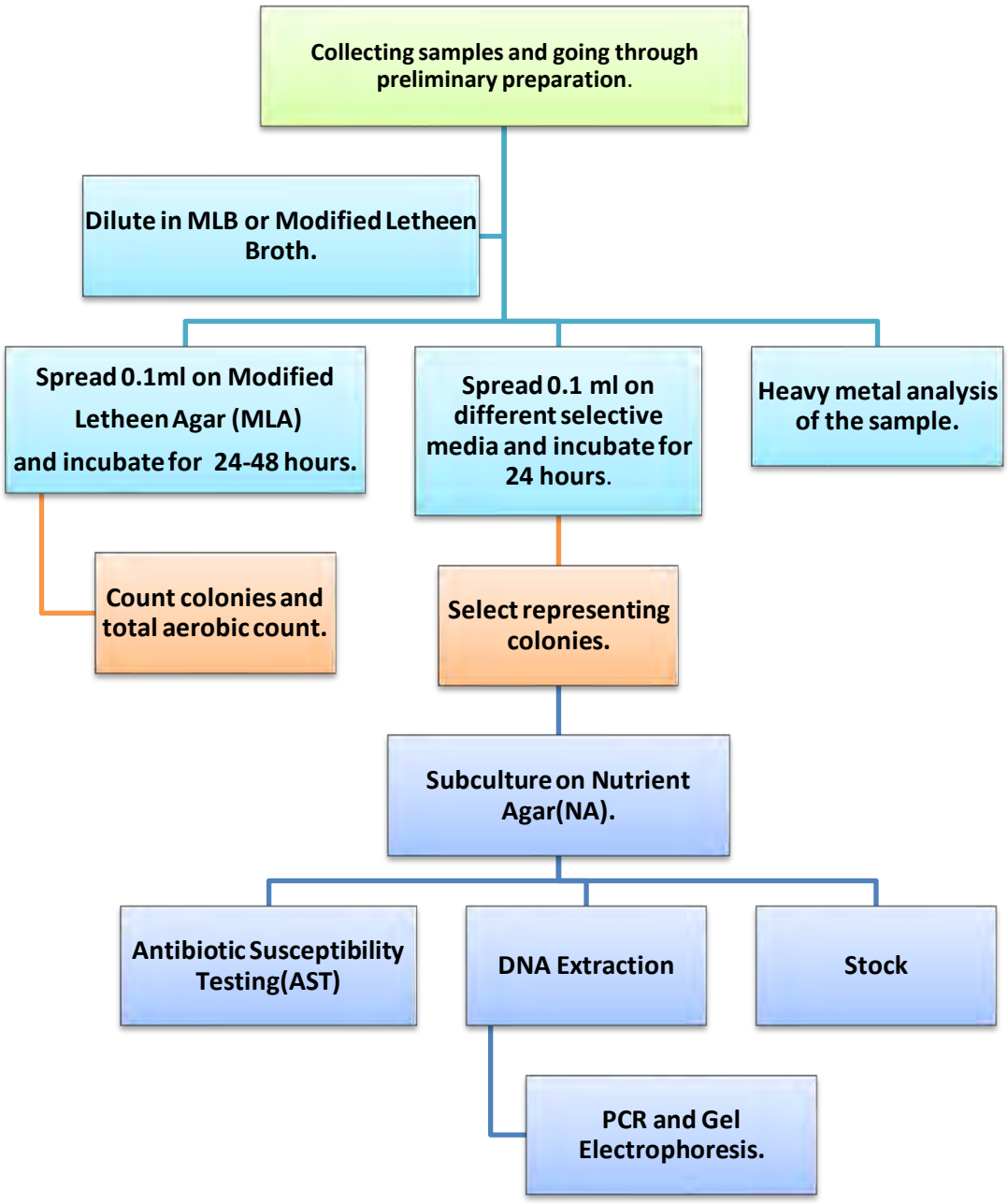
2) To isolate and identify bacterial species contaminating these homemade cosmetics using standard microbiological and molecular methods.

3) To assess the antibiotic susceptibility patterns of the isolated bacterial pathogens to determine their resistance profiles.

Chapter 2

Material & Methods

2.1 Flowchart of the Method of the Experiment:



2.2 Sample Collection:

A total of 22 cosmetic products from 17 different brands were tested in this study. The samples were collected from both online and offline renowned pages that claim to sell 100% organic, chemical-free, and homemade cosmetics. The products included Face creams (8), Body creams (4), Face serums (3), Lip balms (2), Soaps(Goat Milk) (2), Face packs (2), and Face scrubs (1). Each product was labeled with its brand name, and all were within the date of manufacture and expiration. Upon arrival at the laboratory, the samples were carefully examined and stored at room temperature until further analysis.

Figure 1.1: Samples collected from different online pages.

2.3 Sample processing and culture preparation:

The collected homemade cosmetic samples were processed by following the FDA Bacteriological Analytical Manual (Nutrition, 2023). First of all, the outer surface of each container was disinfected with 70% ethanol. After the surface was tissue dried, 1 gram (or 1 ml for liquid samples) of the sample was aseptically weighed for further analysis.

Different product textures required slightly different preparation steps. For example-for liquid or semi-liquid products such as face creams, body creams, face serums, and lip balms, 1 g of the sample was transferred into a screw-cap test tube containing 9 ml of Modified Letheen Broth (MLB). For solid samples, like soaps, face packs, and face scrubs were mixed with 8 ml of MLB ,1 g of sample and 1 ml of sterile Tween 80 to facilitate dispersion.In addition, 6-7 sterile glass beads were added and vortexed to ensure proper homogenization. Each prepared suspension was treated as a 10^{-1} dilution. From this, a series of ten-fold serial dilutions were prepared up to 10^{-4}. From each dilution (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}), 0.1 ml was spread aseptically on Modified Letheen Agar (MLA) plates. Then the inoculated plates were incubated for bacterial growth and further analysis.

2.4 Total Aerobic Bacterial plate count of cosmetic products:

The aerobic bacterial plate count of the cosmetic products was determined by following the FDA's Bacteriological Analytical Manual (Nutrition, 2021). After serial dilution of each sample up to 10^{-4} in Modified Letheen Broth or MLB, 0.1 ml from each dilution from 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} was spread onto Modified Letheen Agar (MLA) plates using the spread plate method. Here a sterile glass spreader was used for uniform distribution of the inoculum. After that all the plates were incubated in an inverted position at 30 ± 2 °C for 24–48 hours to allow bacterial growth. After incubation period, plates containing discrete and countable colonies were selected for enumeration. The total aerobic bacterial count was recorded in colony-forming units per gram (CFU/g) or per milliliter (CFU/ml) of sample, depending on the product type. Representative plates showing colony growth are presented in Figure 1.2

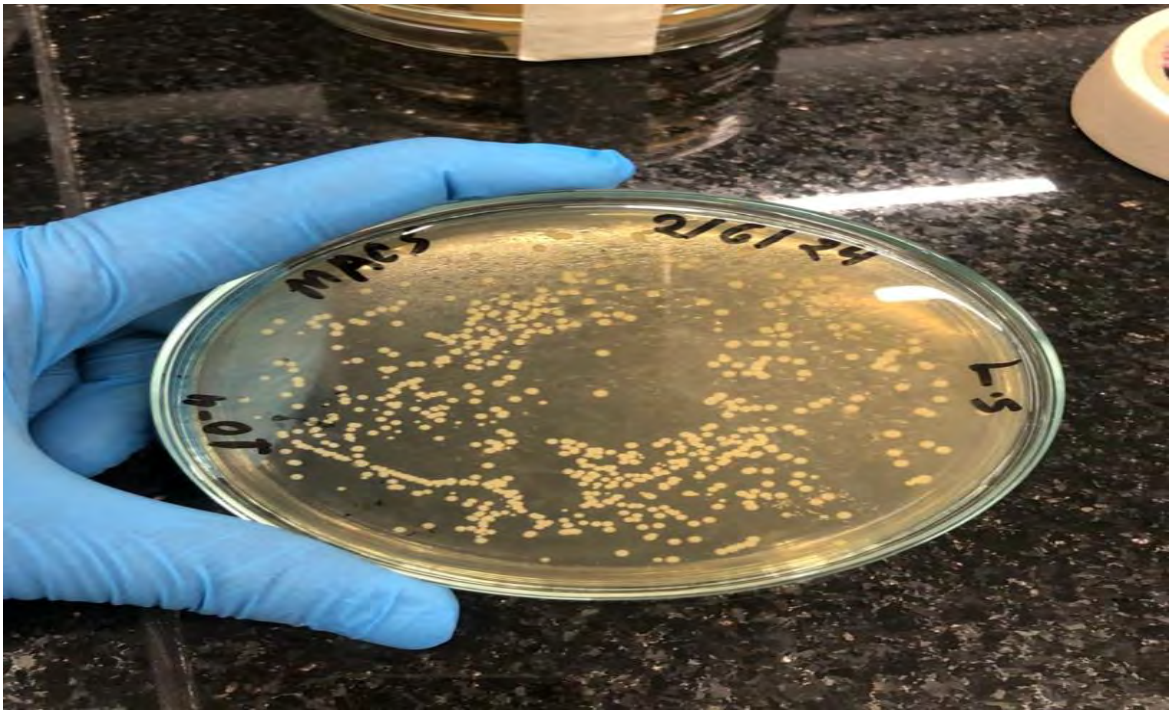


Figure 1.2: Using MLA to Calculate the Total Aerobic Plate Count

For plates with 25-250 CFU following formula is used to calculate the aerobic plate count:

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

Here,

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 and isolation of microorganisms from the collected cosmetic products were performed according to the FDA's Bacteriological Analytical Manual (Nutrition, 2023). For the primary culture, 0.1 ml of each processed sample was spread onto selective agar media(KPC and Listeria oxford agar) and incubated in an inverted position at 37 ± 2 °C for 24–48 hours. The incubation conditions were carefully monitored to allow the growth of both Gram-negative and Gram-positive organisms. The colonies were selected based on the colony morphology. The selective media that were used and the expected colony morphology are shown in **Table 1.**

Table 1: Colony Morphology of Specific Bacteria on Selective Media

Bacteria	Gram-Positive/ Gram Negative	Media	Expected Colony Morphology
Klebsiella pneumoniae	Gram-Negative	HiCrome KPC Agar (without added antibiotic)	Bluish green
Listeria monocytogenes	Gram-Positive	Listeria Selective Oxford Agar	Positive reaction for esculin hydrolysis, blackening of the medium around the colony.

However, the main focus of this study was on *Klebsiella pneumoniae* and *Listeria monocytogenes*. For the detection of *Klebsiella pneumoniae*, KPC agar was employed. Colonies suspected as *Klebsiella pneumoniae* were typically large, round, glistening, and mucoid due to the presence of a polysaccharide capsule. On KPC agar, the colonies appeared as distinctive blue-colored**,** which made them easier to differentiate from other Gram-negative bacilli. On the other hand, for the detection of *Listeria monocytogenes*, Listeria Selective Agar (Oxford Agar) was used. Colonies suspected as *Listeria monocytogenes* were observed as small, round, and greyish colonies, often with a black halo surrounding them due to esculin hydrolysis(Blackening of the media). Their typical pinpoint size and unique appearance made them distinguishable from other microorganisms. Colonies showing the expected morphology for either *Klebsiella pneumoniae* or *Listeria monocytogenes* were carefully picked and subjected to further confirmatory tests which ensured accurate identification and differentiation from other closely related organisms.

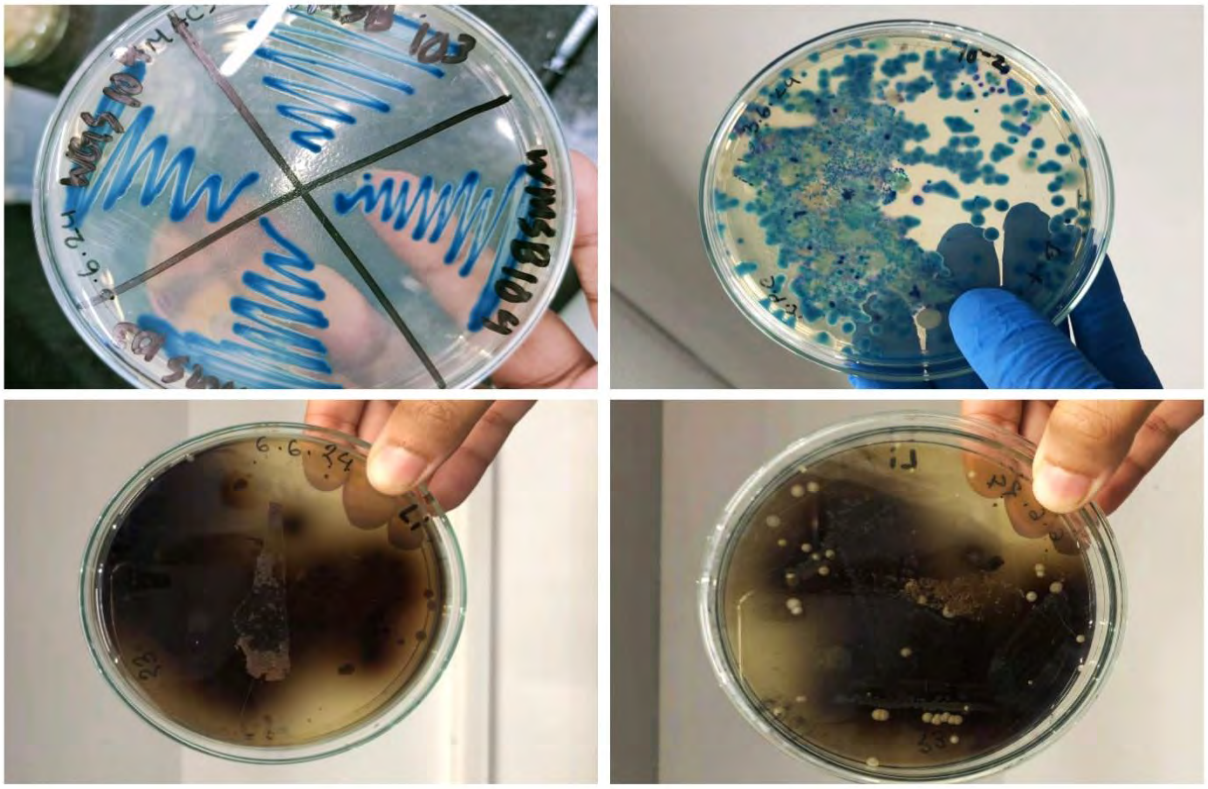


Figure 1.3: Growth of *Klebsiella pneumoniae* showing blue colonies on KPC agar & growth of *Listeria monocytogenes* showing greyish colonies with blackeningof the media on Oxford Listeria Agar.

2.6 Molecular Detection of Selected Isolates.

2.6.1 Bacterial DNA extraction :

The bacteria were inoculated into Nutrient Agar and were incubated at 37 °C for 24 h. A single colony of the bacteria was selected. Then, a single colony of bacteria was added to 400 µl of TE buffer. Then it was vortexed for 15 seconds. After that, the samples were centrifuged (13000RPM,10 min) at 25°C . Discarded the supernatant and again mixed the pellet with 400 µl of TE buffer. Again it was vortexed for 15 seconds. A thermocycler was used as a heat block to heat the isolate at 100°C for 10 minutes. Kept the mixture in room temparute for 5 to 10 min. After that again centrifuged the mixture(13000 RPM for 10 min) at 25°C ,the cell debris precipitated. The supernatant containing DNA was transferred in 1.5 µl microcentrifuge tube or MCT and stored at -20°C.

2.6.2 PCR Amplification:

For each bacterial sample, 2 µl template DNA, 7.5 µl Master Mix, 2.5 µl of nuclease-free water, 0.5 µl forward primer (10µM) and 0.5 µl reverse primer were adjusted to be a 13 µL of final solution for PCR. The primers, PCR thermocycler conditions and amplicon size is mentioned in Supplementary Table 2. Then the PCR products were examined by Gel Electrophoresis in a 2% agarose gel using 1X TBE buffer, stained with Ethidium Bromide. The products were observed under a UV transilluminator.

Table 2: The Oligonucleotide Primers set for Bacterial Identification

Name of Bacteria	Primer Designation	Target gene	Primer Sequence	Product Size	PCR Conditions
Klebsiella pneumoniae	KP Pf-F: KP Pr1-R:	*phoE* gene	5′-ATTTGAAGAGGTTGCAAACGAT-3′ 5′-TTCACTCTGAAGTTTTCTTGTGTTC-3′	130bp	The cycling conditions were 10 min at 94 °C followed by 35 cycles of 30s at 94 °C, 20s at 57 °C and 20s at 72 °C, followed by a 10 min hold at 72 °C.
Listeria monocytogenes	hlyA-F hlyA-R	hlyA gene	LL5: 5'-AAC CTA TCC AGG TGC TC-3' LL4: 5'-CGC CAC ACT TGA GAT AT-3'	520bp	95 °C for 3 min and then 35 cycles consisting of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min with a final extension step at 72 °C for 7 min

2.7 Antibiotic Susceptibility Test:

The purpose of this antibiotic susceptibility test was to evaluate whether the bacterial isolates obtained from cosmetic samples exhibited multidrug resistance. For this, the Kirby-Bauer disc diffusion method was performed following the Clinical and Laboratory Standards Institute-CLSI guidelines (CLSI, 2023). A bacterial suspension was prepared by transferring colonies into 9 ml of sterile saline, and the turbidity was adjusted to match the 0.5 McFarland Standard. The inoculated plates were incubated at 37 °C for 24 hours, after which the diameters of inhibition zones were measured and interpreted using the CLSI 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 agent in three or more antimicrobial categories. In this study, the antibiotics were selected based on their clinical relevance and frequent use against bacterial pathogens associated with face and body skin and cosmetic related infections. These included broad-spectrum antibiotics such as β-lactams,Cephalosporin,Carbapenem, Sulfonamide, fluoroquinolones etc. as they are commonly prescribed for the treatment of skin and soft tissue infections, which may arise from contaminated cosmetic products. The specific antibiotics used, are presented in **Table 3**.

Table 3: List of Antibiotics Used in the Experiment

Serial No.	Name of the Antibiotics	Disc Code	Disc Potency (µg)	Group	Effective against	Inhibition Zone Measurement.
1.	Ampicillin	AMP	10	Beta-lactamase	Gram Positive, Gram Negative	R ≤ 13, I = 14–16, S ≥ 17
2.	Azithromycin	AZM	15	Macrolide	Gram Positive	R ≤ 13, I = 14–17, S ≥ 18
3.	Cefepime	FEP	30	Cephalosporin	Gram Positive, Gram Negative	R ≤ 18, I = 19–24, S ≥ 25
4.	Ciprofloxacin	CIP	5	Fluoroquinolone	Gram Positive, Gram Negative	R ≤ 21, I = 22–25, S ≥ 26
5.	Co-trimoxazole	COT	25	Sulfonamide	Gram Positive, Gram Negative	R ≤ 10, I = 11–15, S ≥ 16
6.	Chloramphenicol	C	30	Phenicol	Gram Positive, Gram Negative	R ≤ 12, I = 13–17, S ≥ 18
7.	Imipenem	IMP	10	Carbapenem	Gram Positive, Gram Negative	R ≤ 19, I = 20–22, S ≥ 23
8.	Meropenem	MRP	10	Carbapenem	Gram Positive, Gram Negative	R ≤ 19, I = 20–22, S ≥ 23
9.	Piperacillin	PRL	100	Beta-lactam	Gram Positive, Gram Negative	R ≤ 17, I = 18–20, S ≥ 21
10.	Amikacin	AK	30	Aminoglycoside	Gram Negative	R ≤ 14, I = 15–16, S ≥ 17
11.	Dancomycin	DAN	30	Lipopeptide	Gram Positive	R ≤ 19, I = 20–22, S ≥ 23
12.	Vancomycin	VA	30	Glycopeptide	Gram Positive	R ≤ 14, I = 15–16, S ≥ 17
13.	Linezolid	LZ	10	Oxazolidinone	Gram Positive	R ≤ 20, I = 21–22, S ≥ 23
14.	Colistin	CL	10	Polymyxin E	Gram Negative	R ≤ 10, I = 11–

						13, S ≥ 14
15.	Aztreonam	AT	30	Monobactam	Gram Negative	R ≤ 15, I = 16–21, S ≥ 22
16.	Clindamycin	CD	2	Lincosamide	Gram Positive	R ≤ 14, I = 15–20, S ≥ 21

Chapter 3

Result:

3.1 Total Aerobic Bacterial Plate Count of Homemade Cosmetic Products.

Out of the 22 homemade cosmetic samples analyzed in this study, the majority showed aerobic bacterial counts that exceeded the acceptable microbial limits set by international guidelines such as the FDA and EU standards. The ISO standard requires non-sensitive cosmetics (non-eye, non-mucous) to include not more than 1×10^3 CFU/g or mL of aerobic bacteria, yeasts, or molds. Our microbiological study revealed that the majority of the tested skincare products surpassed this limit. Face creams, serums, and lip balms were particularly contaminated, with extremely high microbe counts, indicating poor microbiological quality and posing a danger to consumer safety. However, only a small proportion of the samples fell within the permissible range for total viable counts.

3.2 Isolated Bacteria from Homemade Cosmetic Products

A total of 22 homemade cosmetic samples were collected and analyzed in this study. These samples were obtained from renowned facebook pages and small scale producers in Dhaka. The products included Face creams (8), Body creams (4), Face serums (3), Lip balms (2), Soaps (Goat Milk) (2), Face packs (2), and Face scrubs (1). From these 22 samples, a total of 34 bacterial isolates*(K. pneumonia & Listeria monocytogenes*) were obtained. The isolates were selected randomly based on their phenotypic characteristics and cultured on two selective media: HiCrome KPC Agar(without added antibiotic) and Listeria Selective Oxford Agar. Each sample was separately cultured on this two selective media and incubated at 30 ± 2°C for 24–48 hours. Representative agar plates showing bacterial growth from the cosmetic samples are presented in Figure 3.1.

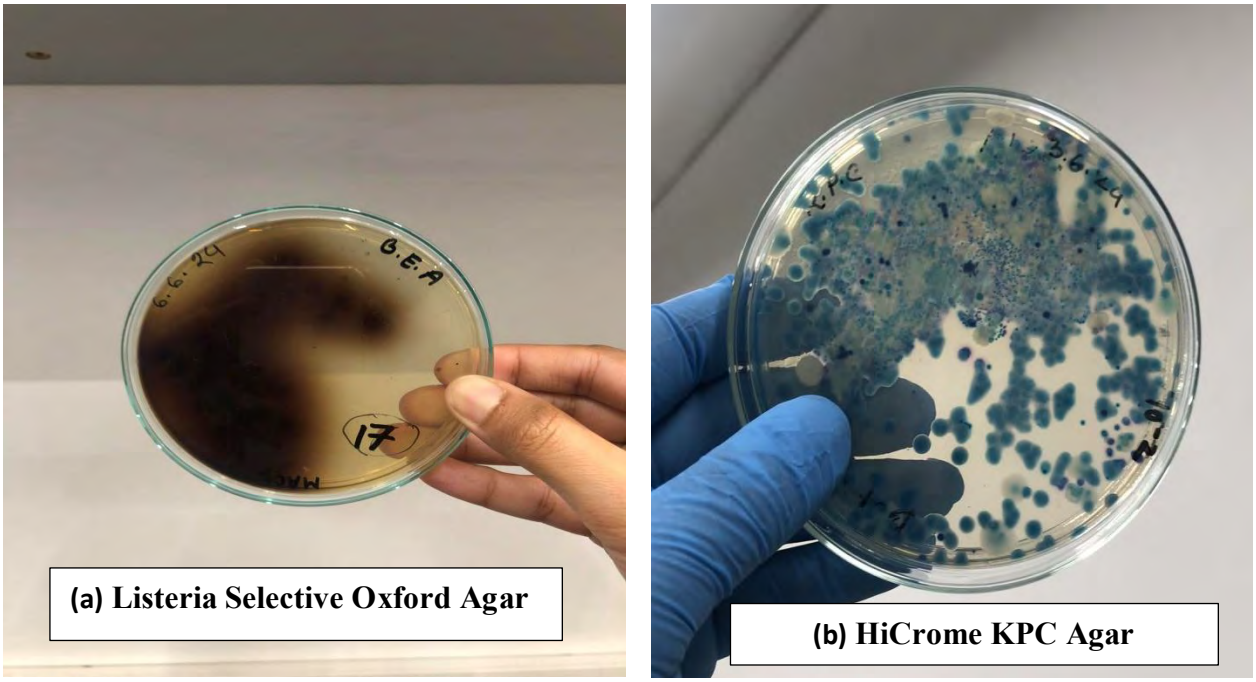


Figure 3.1: Growth of particular bacterial colonies on two selective media- HiCrome KPC Agar(without added antibiotic) and Listeria Selective Oxford Agar.

(a) *Listeria monocytogenes* on and Listeria Selective Oxford Agar
(b) *Klebsiella pneumoniae* on HiCrome KPC Agar.

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

PCR was performed by using specific primers for ***Klebsiella pneumoniae*** and ***Listeria monocytogenes*** to confirm the existence of the suspected bacteria. In order to confirm the results, the size of each fragment of DNA was compared with a standard DNA ladder after the PCR products were examined on Agarose Gel Electrophoresis.
However, a distinct band was seen at **133 bp** for ***Klebsiella pneumoniae*** (Figure 3.2). 20 samples from the suspected isolates were positive and showed the distinctive band. Several cosmetic items, such as face creams, serums, soaps, face packs, and scrubs, have been identified that contained ***Klebsiella pneumonia***.

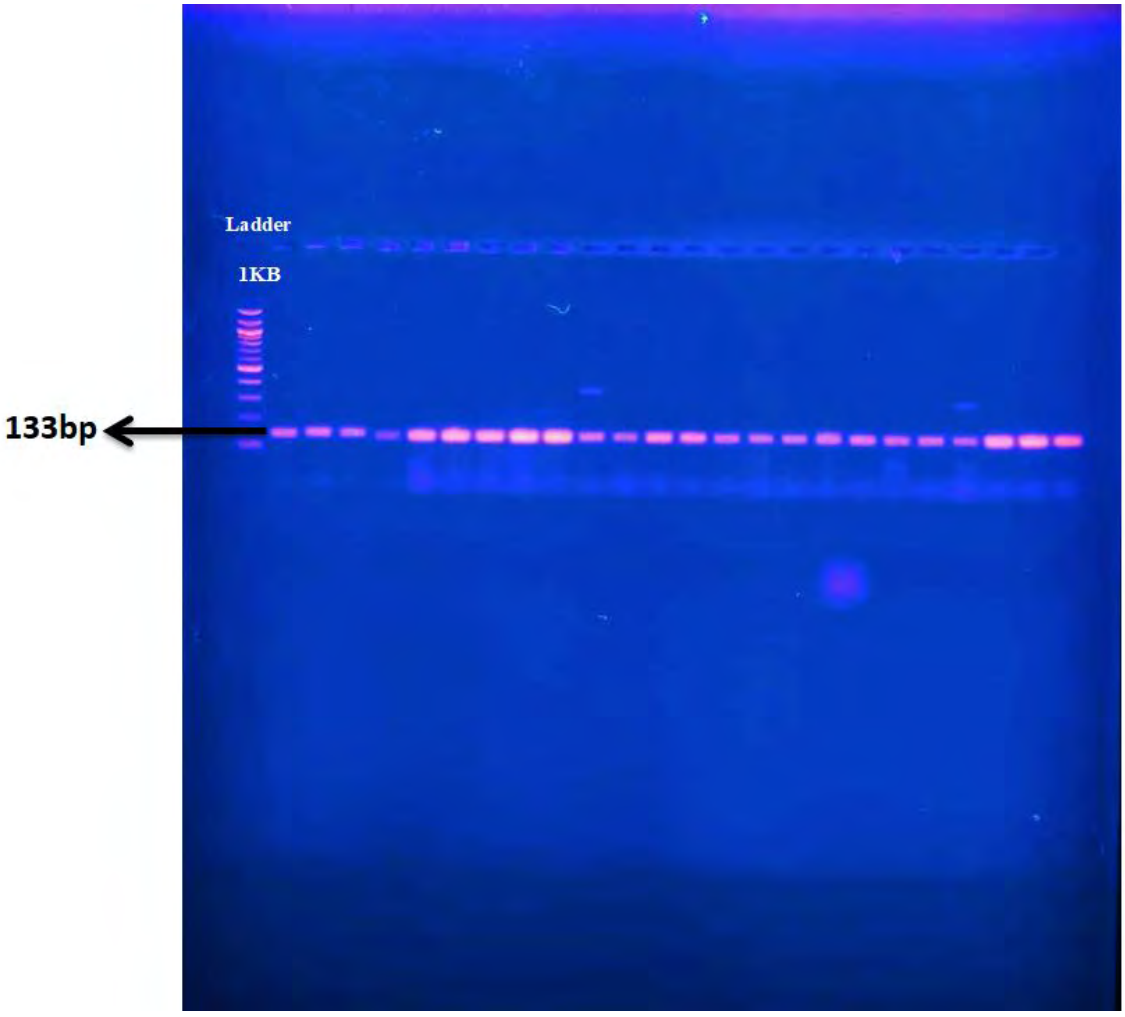


Figure 3.2: Gel electrophoresis of 1KB ladder and PCR product of ***Klebsiella pneumonia.*** An arrow indicates the presence of a band (133bp) that is specific to ***Klebsiella pneumonia.***

On the other hand,a clear band at **520 bp** verified the presence of ***Listeria monocytogenes*** (Figure 3.3). ***Listeria monocytogenes*** has been identified in face serum and lip balm, and two of the examined samples tested positive. These results demonstrate that common homemade cosmetic items may contain the opportunistic pathogens like ***Klebsiella pneumoniae*** and ***Listeria monocytogenes*** which might threaten the health of consumers.

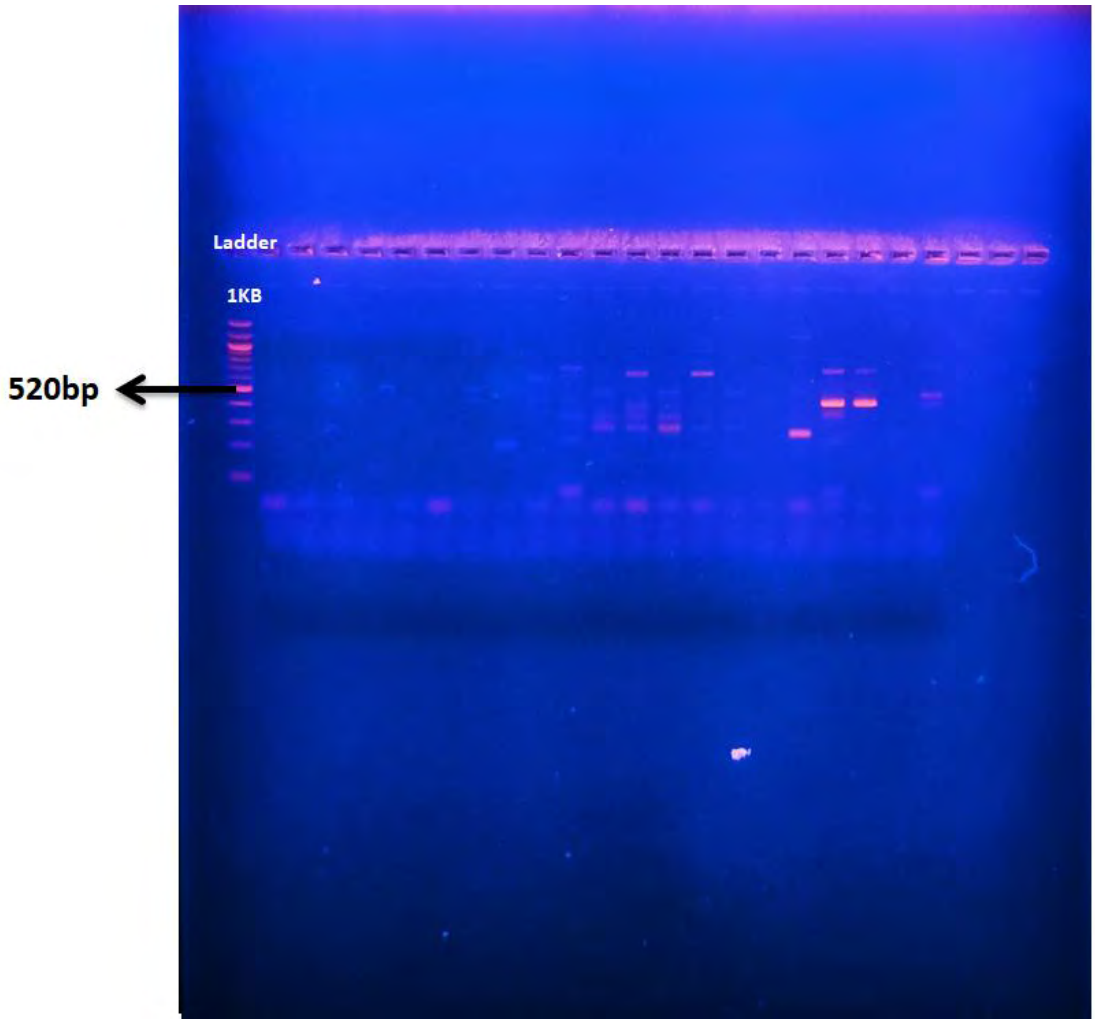


Figure 3.3: Gel electrophoresis of 1KB ladder and PCR product of ***Listeria monocytogenes***. An arrow indicates the presence of a band (520 bp) that is specific to ***Listeria monocytogenes.***

3.4 Bacterial Isolation and Distribution in Cosmetic Samples

Out of the 22 homemade cosmetic products that were tested, two bacterial species have been identified after microbiological assessment and molecular confirmation. *Klebsiella pneumoniae* was the most frequently detected bacteria, with *Listeria monocytogenes* presenting in only a few percent of samples.

Klebsiella pneumoniae was detected in 20 samples, making it the most common bacterial contamination in these products. It has been identified in a variety of cosmetics, including face creams, serums, soaps, face packs, and scrubs, showing that it is commonly found in handmade formulations. In contrast, *Listeria monocytogenes* was found only in two samples: lip balm and face serum. Although less common than *Klebsiella pneumoniae*, the presence of *Listeria monocytogenes* is especially troubling because it is a pathogenic bacterium associated with severe infections and is not generally seen in cosmetic items.
Table 4 summarizes the numerical distribution of bacteria extracted from cosmetic samples, and **Figure 3.4** shows the total percentage of the discovered bacteria. These findings give solid evidence of considerable microbiological contamination in locally manufactured homemade cosmetics, highlighting the urgent need for rigorous safety assessment and regulatory monitoring.

Table 4: Number of Isolated Bacteria Found in Various Cosmetic Samples

Product Category	Name of Bacteria	Number of cosmetic with bacterial growth
Face creams (8)	***Klebsiella pneumoniae***	**7**
	Listeria monocytogenes	**Not found**
Body creams (4)	***Klebsiella pneumoniae***	**4**
	Listeria monocytogenes	**Not found**
Face serums (3)	***Klebsiella pneumoniae***	**2**
	Listeria monocytogenes	**1**
Lip balms (2)	***Klebsiella pneumonia***	**2**
	Listeria monocytogenes	**Not found**
	Klebsiella pneumonia	**2**

Soaps (Goat Milk) (2)	***Listeria monocytogenes***	**Not found**
Face packs (2)	***Klebsiella pneumoniae***	**2**
	Listeria monocytogenes	**Not found**
Face scrubs (1)	***Klebsiella pneumoniae***	**1**
	Listeria monocytogenes	**1**

The pie chart represents the distribution of verified positive bacterial samples isolated from Bangladeshi homemade cosmetics. Initially, a presumptive screening was performed using selective culture and preliminary laboratory tests.

- *Klebsiella pneumoniae* showed 22 presumptive positive samples, out of which 20 were confirmed through further molecular tests.
- *Listeria monocytogenes* had 12 presumptive positive samples, but only 2 were confirmed after confirmation tests.

The following chart highlights that *Klebsiella pneumoniae* was the predominant bacteria, constituting about 91% of the confirmed positive samples, whereas *Listeria monocytogenes* represented only 9%**.** This demonstrates that while several samples might appear positive in preliminary testing, confirmation is essential to accurately identify the bacterial load in cosmetic products

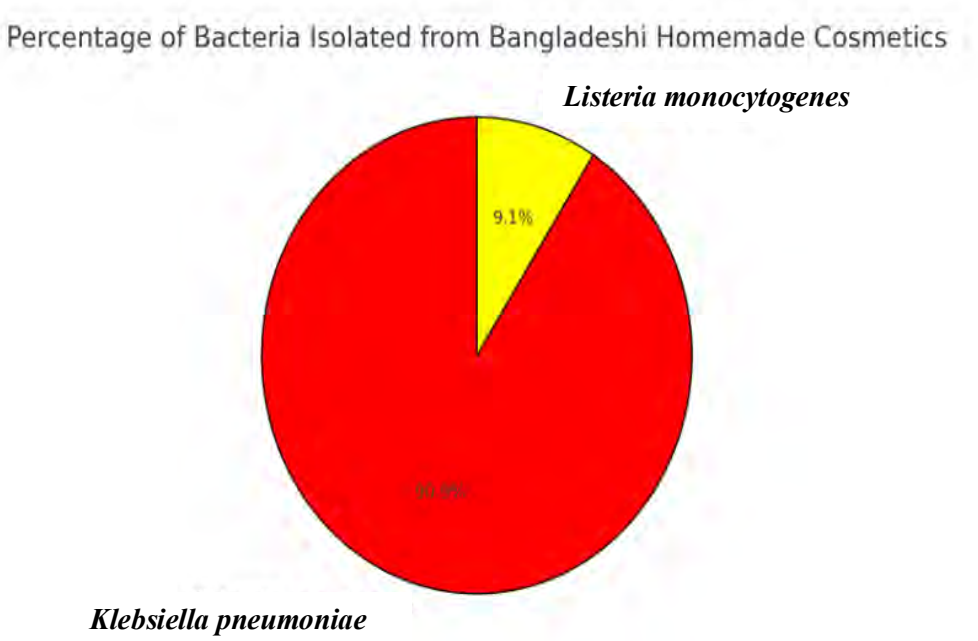


Figure 3.4: The overall percentage of the isolated bacteria from cosmetics sample.

Chapter 4

Antibiotic Susceptibility Test

The antibiotic susceptibility test for bacterial isolates from homemade cosmetics in Bangladesh was examined by the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) plates, as suggested by the Clinical and Laboratory Standards Institute (CLSI, 2023). This test was performed on a total of 86 bacterial isolates obtained from 22 cosmetic samples to assess their resistance to a panel of widely used antibiotics. The process was carried out under aseptic circumstances, and the diameter of the inhibitory zones was measured in millimeters and interpreted by CLSI standards to classify the isolates as resistant, intermediate, or sensitive.

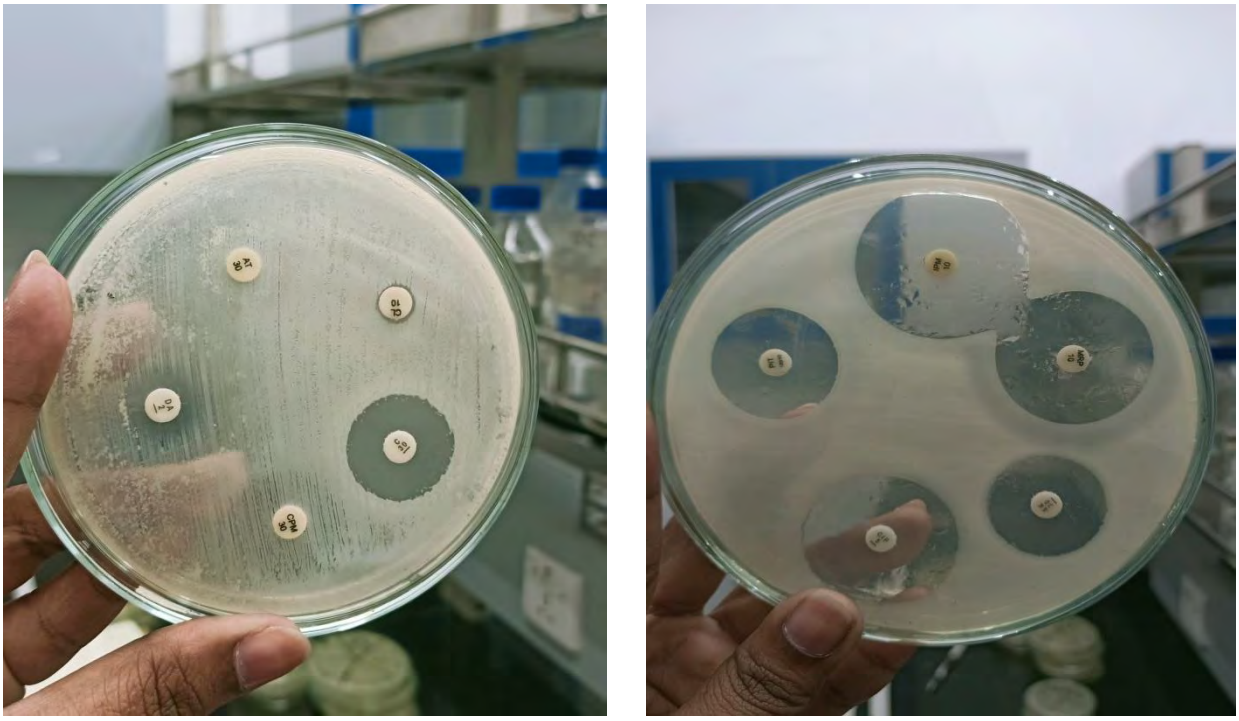


Figure 4.1: Antibiogram done on Mueller-Hinton Agar

The results of the antibiotic susceptibility testing are summarized in table 4 and Figure 4.2 which shows the percentage distribution of antibiotic resistance among the isolates. Additionally, to gain a more specific understanding, the resistance profiles of individual bacterial species were analyzed. **Figures 4.3 and 4.4** display the graphical representation of the antibiotic resistance

patterns observed in the two major bacterial pathogens isolated during this study. This includes resistance trends for *Klebsiella pneumoniae* and *Listeria monocytogenes*, the two most significant organisms identified from the cosmetic samples.

The analysis highlights the alarming prevalence of antimicrobial resistance in bacteria isolated from cosmetic products, suggesting that these products could serve as potential reservoirs for resistant strains. Such findings emphasize the need for stricter monitoring, regulation, and public awareness regarding the microbial quality of homemade cosmetic products available in the Bangladeshi market.

Table 5: Antibiotic resistance observed in different bacteria based on cosmetics.

Name of Bacteria	**Percentage of resistance observed**						
	Face cream	**Body Cream**	**Face Serum**	**Lip balm**	**Soap**	**Face pack**	**Face scrubs**
Klebsialla pneumoniae	**32.14%**	**31.79%**	**19.05%**	**28.57%**	**39.29%**	**28.57%**	**28.7%**
Listeria monocytogenes	**No growth**	**20%**	**6.7%**	**No growth**	**No growth**	**No growth**	**No growth**

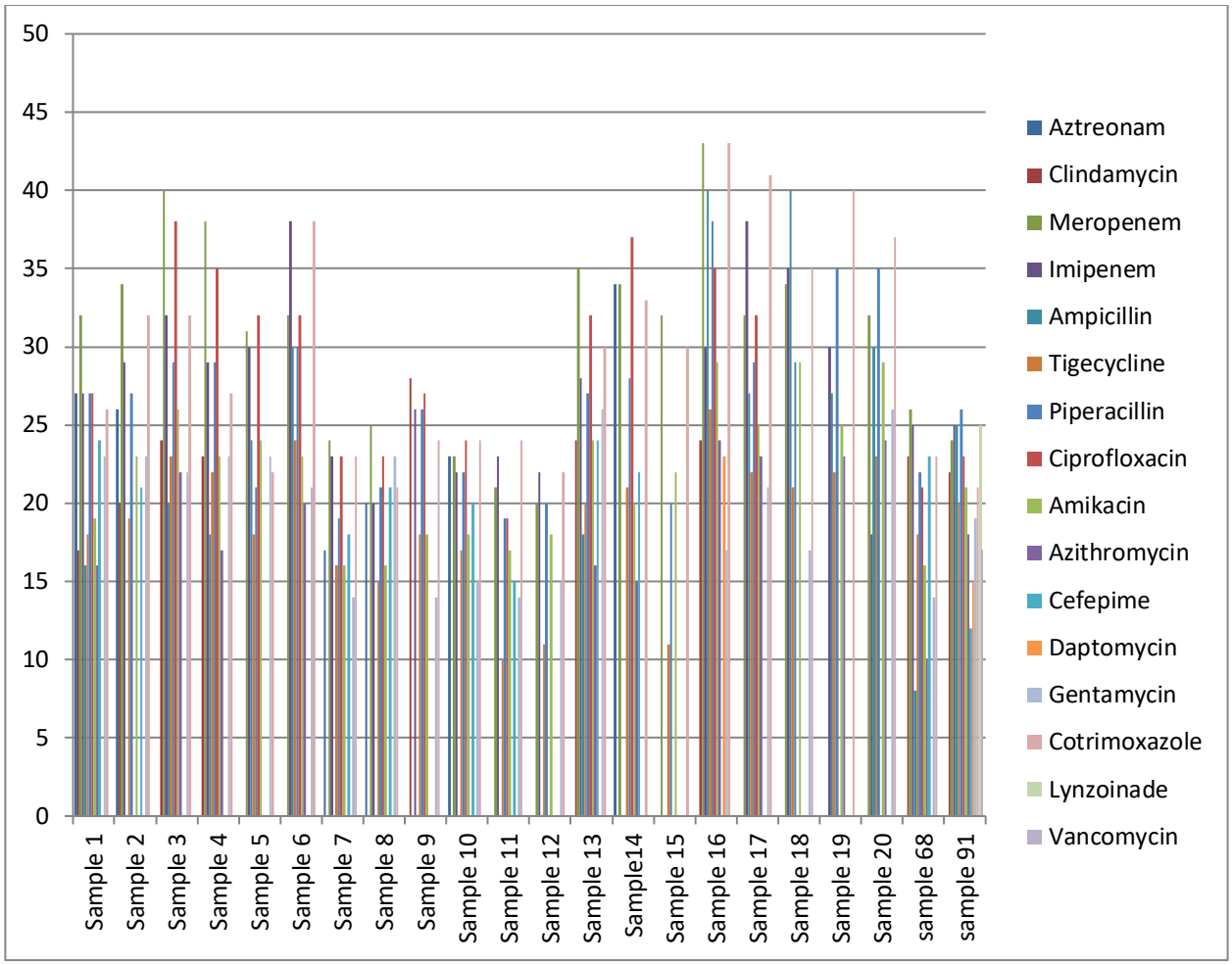


Figure 4.2: Antibiotic susceptibility profile of *Klebsiella pneumoniae* (n = 20) and *Listeria monocytogenes* (n = 2) isolates against 16 different antibiotics.

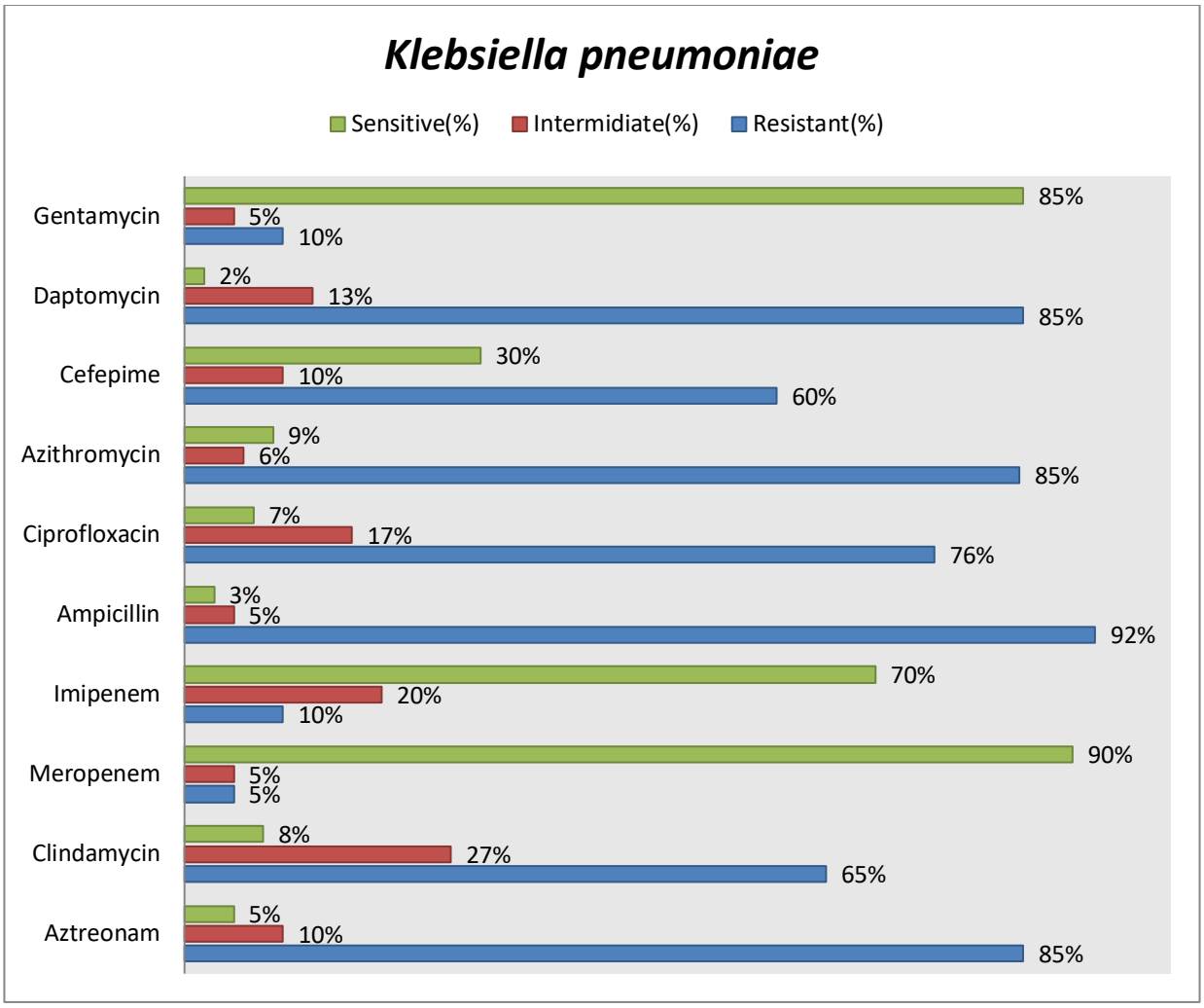


Figure 4.3: Resistance Observed in ***Klebsiella pneumoniae***.

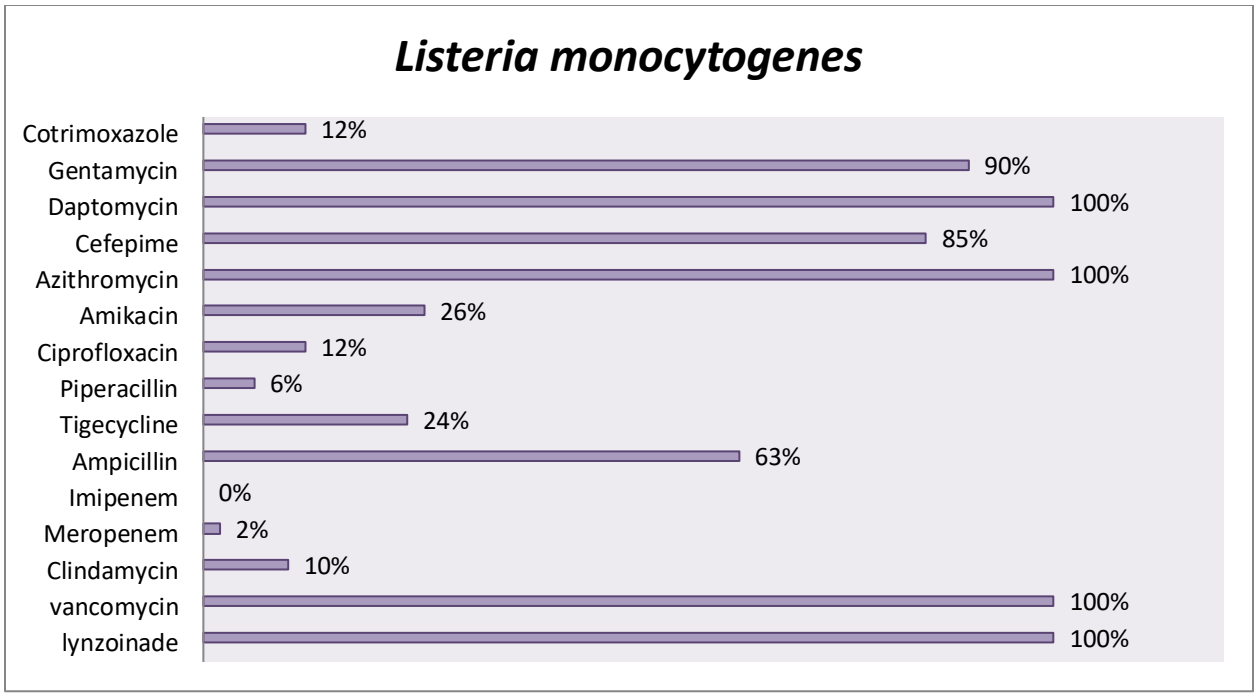


Figure 4.4: Resistance Observed in ***Listeria monocytogenes***.

4.1 Percentage of multi-drug resistance observed in bacteria isolated from homemade cosmetics.

Figure 4.5 shows the overall MDR percentage for ***Klebsiella pneumoniae*** and ***Listeria monocytogenes*****,** highlighting the levels of resistance among these organisms in the tested homemade cosmetics. In contrast, Figure 4.6 shows the multidrug resistance (MDR) patterns seen among bacterial isolates from multiple cosmetic product categories.

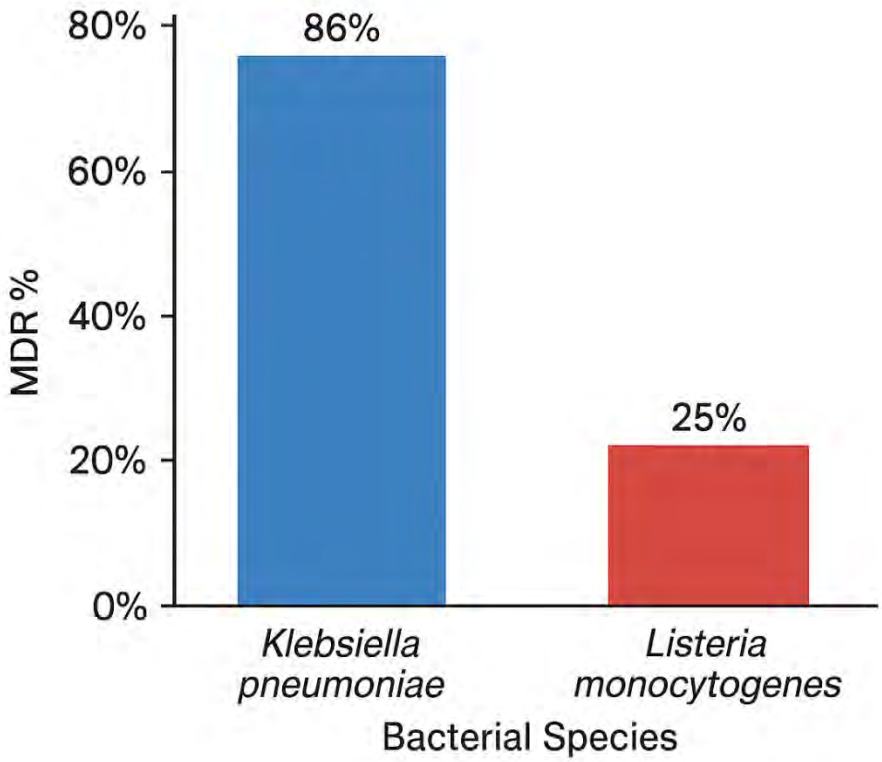


Figure 4.5: Percentage of multi-drug resistance observed in bacteria isolated from cosmetics.

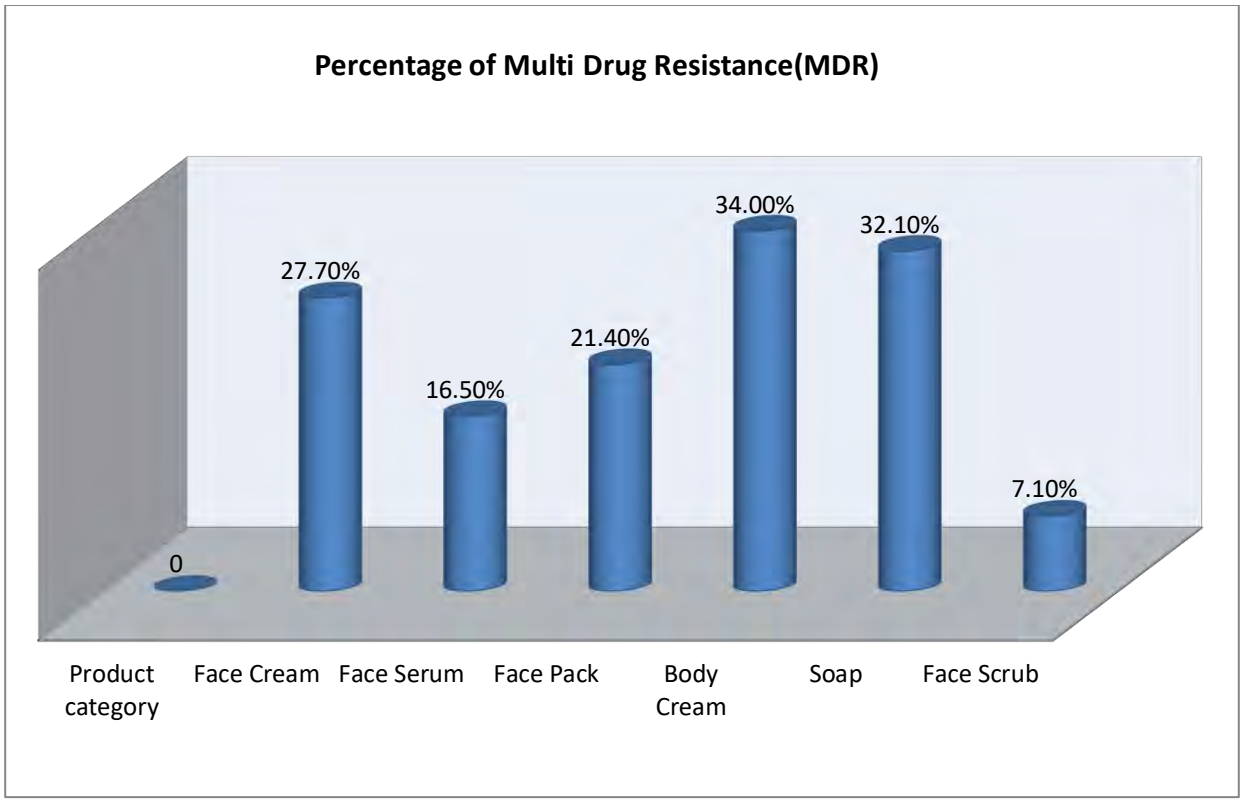


Figure 4.6: Multi-drug resistance observed based on cosmetic samples.

Chapter 5

Discussion:

This research focused on the widespread presence of pathogenic microbes, particularly ***Listeria monocytogenes*** and ***Klebsiella pneumoniae*** in homemade cosmetics that are frequently sold both online and offline in Bangladesh. 20 out of 22 tested samples confirmed positive for ***Klebsiella pneumonia*** but a small proportion tested positive for ***Listeria monocytogenes.*** These results indicate to a concerning rate of contamination in products that are marketed as chemical-free and organic. However, the high prevalence of ***Klebsiella pneumoniae*** is particularly alarming, given its known pathogenic potential and ability to cause a variety of infections, including skin infections, wound infections, and opportunistic systemic infections, especially in immunocompromised individuals. Similarly, ***Listeria monocytogenes*** is a known as a foodborne and opportunistic bacteria that can cause serious infections, especially in certain individuals, although being found less commonly.

Our results align with earlier studies that have shown microbial contamination in cosmetics, especially locally manufactured or home-made products. According to a study *Klebsiella pneumoniae* was one of the most prevalent bacteria found in 85.2% of cosmetic samples examined in Dhaka(Nusrat et al,2023). Similarly, another study discovered that *Klebsiella pneumoniae* isolates from clinical settings exhibited resistance to multiple antibiotics, including ampicillin, cefradine, and chloramphenicol(Tanni et al.2021). Furthermore, a study found that the prevalence of pathogenic microorganisms in cosmetic items and the associated public health risks. It highlights the necessity of routine microbiological testing and legislative measures to mitigate contamination problems(Noor et al.2015). All of these studies support our own findings that pathogenic and multidrug-resistant bacteria can be detected in Bangladeshi homemade cosmetic products and highlight the importance of adequate hygiene and quality control throughout the entire manufacturing procedure.

The finding of this research highlight not only the microbiological risks associated with Homemade cosmetics but also the immediate needs of identifying the human behavioral aspect of these hazards. Many people in Bangladesh still use homemade or so-called "organic" cosmetics, frequently without realizing the potential microbiological risks. Conducting **Knowledge, Attitude, and Practice (KAP) studies** among both producers and consumers would reveal important information on their awareness, attitudes, and handling habits with regard to product safety. This data might be used to create regulatory and educational initiatives that would lower contamination and enhance product quality.

Along with microbiological risks, **chemical pollutants including heavy metals (such as lead, arsenic, cadmium, and mercury)** that have been found in different cosmetic formulations in other nations may also be present in homemade cosmetics. In order to provide a more comprehensive assessment of risk, future research might include chemical testing in addition to microbiological tests. This combination strategy will generate data that is extremely pertinent to public health authorities and might help create stricter laws that protect Bangladeshi consumers.

Chapter 6:

Conclusion

This research draws attention to the potential microbiological risks connected to the widely used handmade cosmetics in Dhaka, Bangladesh. *Listeria monocytogenes* and *Klebsiella pneumoniae* were the most common pathogenic bacteria found in majority of the tested samples, however other opportunistic pathogens were occasionally found as well. A serious public health issue was raised by the contamination levels found in multiple items, which were higher than permitted safety limits.

However, these isolates' multidrug resistance (MDR) characteristics increase the possible health concerns because infections brought on by these strains may be challenging to cure. Higher contamination and MDR rates were found in face creams and serums, which are frequently used on sensitive skin areas. This suggests that the manufacturing processes and storage conditions of certain product types may make them more prone to bacterial development. Subsequently, our results highlight the necessity of more stringent hygiene standards while handling, preparing, and storing homemade cosmetics. Furthermore, there is compelling evidence to support the use of Knowledge, Attitude, and Practice (KAP) studies to evaluate consumer and producer awareness and actions regarding product safety. In order to provide a thorough assessment of the safety of DIY cosmetics, future research should also take into account complementary chemical analyses for heavy metals and other pollutants.

All things considered, this study highlights that even while homemade cosmetics are promoted as "organic" and safe, they may include harmful and multidrug-resistant microorganisms. To guarantee the safety of cosmetic items and secure the health of Bangladeshi consumers, regulatory supervision, public awareness initiatives, and regular chemical and microbiological testing are all essential tactics.

Chapter 7

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