Assessment of multidrug resistant bacterial load in cosmetics bought from local markets of Bangladesh

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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 B.Sc. in Microbiology

Department of Mathematics and Natural Sciences

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing 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 which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help.

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Abstract

A total of 27 brands of commercially available lipsticks, powders and creams were collected from different parts of Dhaka, in order to test their level of contamination. Organisms were detected from 85.2% of the cosmetic products. The aerobic plate counts ranged from 6- 4.4×10^{15},40-6.7×10^{12} and 0-3.4×10^{13} in case of lipsticks, powders and creams respectively. The limit given by the FDA was exceeded by 77.78% of the samples. From the samples 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 monocytogens* were identified. Hemolysis was observed in 50% of Gram-positive bacteria and 14% of Gram-negative bacteria. The identified bacteria were tested for multidrug resistance. All of the Gram-positive and Gram-negative samples showed multidrug resistance. However, the percentage of multidrug resistance varied from sample to sample and depended on the type of bacteria. The Gram-positive bacteria of lipstick samples showed 77-100% multidrug resistance. In case of powder and cream samples of Gram-positive bacteria the multidrug resistance observed was 66-100% and 71-100% respectively. In Gram-negative bacteria, lipsticks powders and creams multidrug resistance observed was 28-100%, 0-68%and 0-60% respectively. High levels of microbial contamination occur during manufacturing of cosmetic products and the presence of pathogenic organisms poses a likely hazard to public health. Manufactures should ensure microbiological quality control testing and hygienic environments in order to lower the level of bacterial contamination.

Contents

List of Tables

List of Figures

Chapter 1

1.1 Introduction

Demand for makeup and skincare products has surged dramatically in recent times. In a report titled Cosmetics Market, 2021-2028," Fortune Business Insights states that the cosmetic market was valued at USD 277.67 billion in 2020("Makeup Market Size, Share & COVID-19 Impact Analysis",2021).According to the US Food, Drug and Cosmetic Act cosmetics are defined as articles except soap which is to be rubbed sprinkled or sprayed on or otherwise applied to any part of the human body for the purpose of cleansing, beautifying, promoting attractiveness, or altering the appearance ("FDA. Cosmetic Handbook", 2004). Cosmetic products are used to enhance hygiene and beauty and are used by everyone worldwide. The bases of most cosmetic products are water/oil emulsion or oil/water emulsion. According to an article (Orth et al., 1989) the raw materials used can be grouped into categories (Table 1).

Table 1: Raw Material Categories

Water
Acids, alkalis, salts
Oils, waxes, paraffin
Fatty acids, alcohol, esters
Surfactants, emulsifier
Talc, clay
Protein, starches, botanicals, gums and resin
Humectants
Colour and pigments
Preservatives, antioxidants and chelating agents
Fragrances, essential oils

As cosmetic products are considered to be non-sterile, they are prone to microbial contamination. Microbial growth is supported in the cosmetic products as they contain variable amounts of nutrients (Onurdag et al., 2010, Özalp et al.,1998, Ravita et al., 2009). According to US legislation 'cosmetics are not expected to be totally free of microorganisms when first used or to remain free during consumer use' and that 'cosmetics are not required to be sterile, but microbial contamination can pose a health hazard' ("Small Businesses & Homemade Cosmetics", 2018). As microbial contamination is capable of causing health problems it is vital to guarantee that cosmetic products as well as their raw materials are manufactured according to the guidelines of Good Manufacturing Practices and Food and Drug Administration, so they do not cause harm to the skin of consumers (Akon et al.,2015). In accordance with FDA regulations in cosmetic products level

of contamination should not exceed (non-eye area <1000cfu/g). If the limit is exceeded serious skin problem may occur in the consumer (Microbiological methods and Bacteriological manual, 2015). For cosmetics applied around the area of the eye, EU guidance states that, the total viable count for aerobic microorganisms should not be higher than 10^2 CFU per ml. For other cosmetics the total viable count for aerobic micro-organisms should not be higher than 10^3 CFU per ml. *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* i.e. organisms that potentially pathogenic must not be detectable in 1 ml of a cosmetic that is applied around the eye and in case of other products microorganisms must not be detectable in 0.1 ml. *Escherichia coli* and other *Enterobacteriaceae* is not acceptable in cosmetic products according to EU guidance ("Scientific Committee on Consumer Safety (SCCP)", 2016).

1.2 Literature Review

Even though efforts are being made to improve the microbiological quality of cosmetics, reports of microbial contamination of commercially available products are still appearing in scientific literature. *Salmonella, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa* have been isolated from mascaras, eyeliner and face powder according to studies. These studies have also determined a connection between conjunctivitis and impetigo and *Staphylococcus aureus* (Abdelaziz et al.,2016). Another study has shown that *Escherichia hermannii, S. aureus, Bacillus cereus* and *Enterobacter* species were isolated from lip glosses and lipsticks. This study also showed the presence of *Buttiauxella agrestis,* which had never been isolated before in cosmetic products. It was found in a sample of hair relaxer (Babalola & Eze, 2015). (Akin et al.,1989) investigated microbial quality and control of lipsticks, and found that of 81 samples, 42% yielded aerobic plate count and 23% consisted of mold and yeast. For creams and lotions, the bacteria and fungus isolated were *Pseudomonas aeruginosa, E. coli, Staphylococcus aureus* and *Aspergillus niger* (Aslam et al., 2017).

Bacterial contamination of products can cause human illness. Some are mild like conjunctivitis and allergy however others are more severe like systemic keratitis blood infection and whole-body inflammation (Campana et al., 2006). There have been numerous cases of eye infection as well as loss of vision due to contamination of eye cosmetics with *P. aeruginosa* (Reid et al., 1977). Even in some cases cosmetics infected with bacteria have caused death (Neza et al., 2016). According to several studies *Staphylococcus* was the most common bacterial skin pathogen (Myers et al., 1973, Aly et al., 1966, Sugeng et al., 1999). Staphylococcus is capable of causing various types of diseases such as impetigo, folliculitis, and boils which are the most common. They can also cause severe diseases such as Staphylococcal scalded skin syndrome (SSSS) which occurs mainly in infants and children as well as festering, pus-discharging skin diseases (Aly et al., 1966, Cano et al., 1998). According to a survey conducted (Wilson et al., 1975) 22 women had symptoms of bacterial blepharitis and heavy densities of *Staphylococcus epidermidis.* This microbe was found in their eyelid margins and eye cosmetics. In Singapore a study was conducted at the National Skin Center (NSK) which showed that Gram-negative organisms were responsible for 28.8% of the cases and 71.2% of skin infections was caused by Gram-positive organisms (Sugeng et al., 1999).

From the above it is seen that contaminated cosmetic products are capable of causing skin diseases, so it is necessary to determine the antibiotic susceptibly in order to determine which antibiotics are most effective in treating the disease.

In 2016 a study was conducted to observe the antibiotic susceptibility of the isolated bacteria from contaminated eye cosmetics using 10 antibiotics (Nandi & Mandal, 2016). *Bacillus* spp. was sensitive to all the test antibiotics. The isolated *Pseudomonas aeruginosa* showed resistance to vancomycin, ampicillin, cefpodoxime, trimethoprim and nalidixic acid. The highest resistance was seen by *Pseudomonas aeruginosa*, *Chromobacterium violaecium* was seen to be resistant to vancomycin, ampicillin, cefpodoxime and trimethoprim. *Listeria monocytogens* strains had resistance to cefpodoxime, trimethoprim and nalidixic acid. (Guleria A, 2014) isolated bacterial strains from different cosmetics including 'kajal' and were identified as *Escherichia coli*, *Staphylococcus sp.* and *Bacillus sp*. These bacteria were found to be resistant to one or more antibiotic such as chloramphenicol, tetracycline and streptomycin.

In a study conducted (Aslam et al., 2017) the bacteria isolated from creams and lotions were *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Aspergillus niger*. Among these *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* were sensitive to Ciprofloxacin, Amikacin and Tobramycin and *Aspergillus niger* was sensitive to Streptomycin, Ketoconazole and Clotrimazole.

According to a study, from baby lotion *Enterobacter gergoviae, Serratia marcescens, Pseudomonas aeruginosa*, and *Enterobacter cloacae* bacteria was isolated (Babalola & Eze, 2015) and they were all susceptible to Nitrofurantoin, Nalidixic acid, Gentamicin, Cotrimoxazole, and Streptomycin, but resistant to Tetracycline, Colistin and Ampicillin. Klebsiella pneumoniae was only susceptible to Nalidixic acid, Gentamicin, Tetracycline, Cotrimoxazole and Streptomycin. Among the Gram-positive isolates, 52.9% comprising *Staphylococcus aureus*, *Streptococcus lactis*, and *Micrococcus luteus*, were susceptible to Erythromycin, Amoxicillin, Gentamicin, Cotrimoxazole, and Tetracycline but resistant to Chloramphenicol and Augmentin.

In the last two decades pharmaceutical and cosmetic industries in Bangladesh have been expanding. There is a great scope to maintain public health safety and associated business as well (Shaown SA, 2011). However, unlike pharmaceutical products in Bangladesh there is a lack of cosmetic testing aptitudes due to inadequate facilities (Das et al., 2013). Even though cosmetic contamination has been reported worldwide by a variety of pathogenic bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus spp., Micrococcus spp., Clostridium tetani, Bacillus cereus*, actinomycetes and fungi such information in Bangladesh is scarce as mentioned before (Jimenez et al., 1999, Elaine B, 1989). As the weather of Bangladesh is warm and humid it is favorable for the growth of microorganisms (Akon et al., 2015, Noor et al., 2015). Since Bangladesh is a developing country which is overpopulated with a lack of knowledge of hygiene, skin diseases are more likely to occur (Akon et al., 2015, Sugeng et al., 1999, Khanom et al., 2013). As mentioned previously the knowledge of bacterial pathogens isolated in contaminated cosmetic products is inadequate. For these reasons, the present study attempted to isolate and detect the cosmetic contaminating microorganisms including specific bacterial pathogens and to determine antibiotic susceptibility of the isolated bacteria.

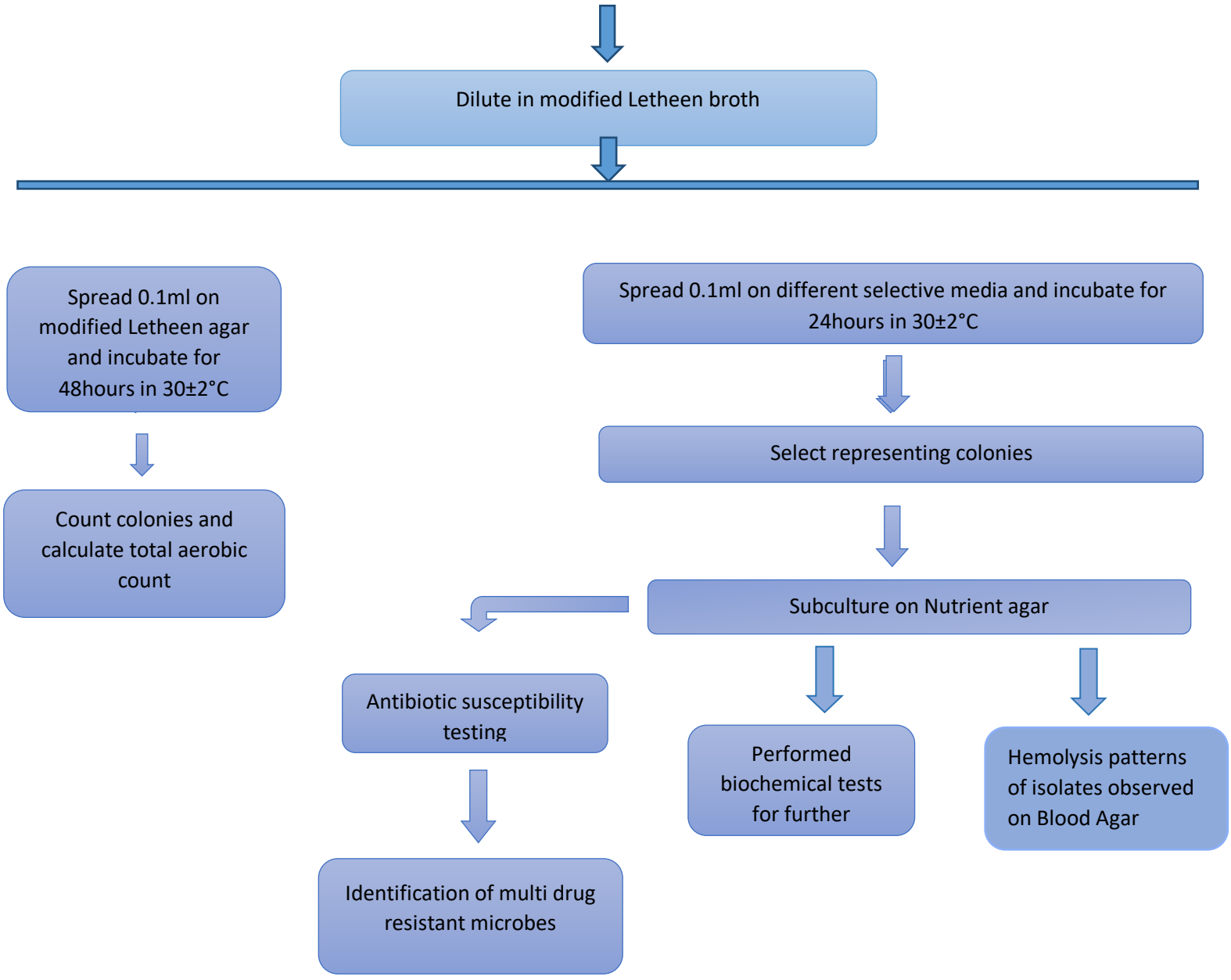
1.3 Objectives

- To determine the total aerobic count of bacteria, present in cosmetics
- Isolation and identification of bacteria isolated from cosmetics
- To do biochemical tests to identify and differentiate the microorganisms.
- Determining the hemolytic ability of the pathogens
- Determination of multidrug resistance of the isolate

Chapter 2: Materials and Methods

2.1 Flowchart of the method of the experiment:

Collecting samples and going through preliminary preparation



2.2 Sample collection:

A total of 27 cosmetic samples which includes 9 powders, 10 creams and 8 lipsticks were bought from different stores in the New Market area and Tejgaon area of Dhaka, Bangladesh. The samples were analyzed immediately after their arrival in the lab and stored at room temperature.

2.3 Sampling handling and preliminary preparation:

The FDA's Bacteriological Analytical Manual was followed in case of handling the samples, as well as preliminary preparation.

The containers of the samples were inspected properly for any irregularities and the surface was disinfected with 70% ethanol beforehand removing the contents. The surface was then dried with tissue and 1g (ml) of the sample was weighed aseptically.

The samples included powders, lipsticks, and creams so they had to go through different process for the initial preparation.

For powders, 1g of sample was aseptically removed from the container and inserted screw cap test tube containing 1ml sterile Tween 80 followed by addition of 8ml sterile MLB. The mixture was vortexed for homogenization, and it was counted as the 10^{-1} dilution.

For creams and lipsticks, 1g of sample was aseptically removed from the container and inserted screw cap test tube containing 1ml sterile Tween 80 and five to seven glass beads. The total contents were homogenized with the help of a vortex mixture. 8ml of sterile MLB was added to adjust total volume to 10ml and mixed properly for the 10^{-1} dilution.

2.4 Aerobic plate count (APC):

In case of aerobic plate count the FDA's, Bacteriological Analytical Manual was also followed. Aerobic plate count was done using the spread plate method on MLA. The preparation was diluted decimally in MLB to get discreet countable colonies for the count. The inoculums were spread on MLA with a sterile spreader in an aseptic way. The plates were then let to absorb the inoculum before inverting and incubating for 48h at 30±2°C

The plates are then observed, and the colonies are counted from each aerobic plate and the numbers were then recorded.

2.5 Identification of Microbes:

For identification of microbes the FDA's Bacteriological Analytical Manual was followed. To identify the presence of target microorganisms, 0.1ml of each dilution was spread on different selective media and incubated for 48h at 30±2°C. After incubation, the morphology of the colonies was inspected for the primary identification of the microorganism and gram stained. The tables below were followed for primary inspection for the assumed microorganisms:

Table 2: Colony Morphology of Specific Bacteria on Selective Media

Organism	Gram Positive/Negative	Media	Expected colony morphology

Bacillus cereus	Gram positive	HiCrome Bacillus agar	light blue, large, flat colonies with blue center
Lactobacillus spp.	Gram positive	MRS media	Round, creamy, white colonies
Listeria monocytogenes	Gram positive	Listeria Selective Oxford agar base media	positive reaction for esculin hydrolysis, blackening of medium around the colony
Staphylococcus aureus	Gram positive	MSA agar	yellow/white colonies surrounded by yellow zone
Staphylococcus epidermidis	Gram positive	MSA agar	red
Enterococcus faecalis	Gram positive	KF streptococcal media	Red maroon with yellow zone
Escherichia coli	Gram negative	EMB agar	purple with black center and green metallic sheen
Salmonella spp.	Gram negative	XLD Agar	pink, red with black centers
Shigella spp.	Gram negative	XLD Agar	colorless
Pseudomonas aeruginosa	Gram negative	Cetrimide Agar	yellow green, glows under UV ray
Klebsiella pneumoniae	Gram negative	HiCrome ESBL Agar (without added antibiotic)	Bluish green
Klebsiella pneumoniae	Gram negative	HiCrome KPC Agar (without added antibiotic)	Bluish green

2.6 Biochemical tests for further identification:

After the observation of colony morphology on selective media and gram staining, a plethora of biochemical tests were performed which includes Motility-indole-urease test (MIU), Catalase test, Oxidase test, Triple sugar iron test, Citrate utilization test, Hemolysis test.

Table 3: Biochemical Test Interpretation for Different Organisms

Organism	Motility	Indole	Urease	Catalase	Oxidase	MR	VP	Citrate utilization	Glucose Ferm.	Sucrose ferm	Lactose Ferm.	Gas Prod.	H_2S Prod.	Hemolysis
Bacillus cereus	+ve	-ve	+ve	+ve	-ve	- ve	+ ve	+ve	+ve	-ve	-ve	.	+ve	Beta
Bacillus spp.	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	Alpha/ Beta/Gamma
Lactobacillus spp.	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	Gamma
Listeria monocytogenes	+ve	-ve	-ve	+ve	-ve	+ve	+ ve	-ve	+ve	+ve	+ve	-ve	-ve	Beta
Staphylococcus aureus	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	Beta
Staphylococcus epidermidis	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	Gamma
Enterococcus faecalis	+ve	-ve	-ve	-ve	-ve	.	+ve	-ve	+ve	+ve	+ve	.	-ve	Alpha/Beta
Streptococcus spp.	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	.	-ve	Beta
Escherichia coli	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	variable	+ve	+ve	-ve	Alpha/ Beta/ Gamma
Salmonella spp.	+ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	Gamma
Shigella spp.	-ve	variable	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	Gamma
Pseudomonas aeruginosa	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	Beta
Klebsiella pneumoniae	-ve	-ve	+ve	+ve	-ve	-ve	+ ve	+ve	+ve	+ve	+ve	+ve	-ve	Gamma

2.7 Antibiotic susceptibility Test:

This test was done to find out multidrug resistant organisms present in the samples. This experiment was done following the Kirby-Bauer disc diffusion protocol and the disc zone sizes were interpreted according to the CLSI standard.

To test the multi-drug resistance of the organism, representatives of different antibiotic groups was taken. From the Clinical Laboratory Standards Institute (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the United States Food and Drug Administration (FDA). MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Table 4: List of Antibiotics used in the Experiment

Serial no	Antibiotic	Group	Effective against	Disc code	Disc potency (µg)
1	Gentamicin	Aminoglycoside	Gram positive and gram negative	GEN	10
2	Ampicillin	Beta lactamase	Gram positive and gram negative	AMP	10
3	Meropenem	Carbapenem	Gram positive and gram negative	MEM	10
4	Cefepime	Cephalosporin	Gram positive and gram negative	CPM	30
5	Piperacillin tazobactam	Penicillin and beta- lactamase inhibitor	Gram positive and gram negative	PIT	100/10
6	Imipenem	Carbapenem	Gram positive and gram negative	IMI	10
7	Azithromycin	Macrolide	Gram positive and gram negative	AZM	15
8	Amikacin	Aminoglycoside	Gram positive and gram negative	AK	30
9	Ciprofloxacin	Fluoroquinolone	Gram positive and gram negative	CIP	5
10	Tigecycline	Glycylcyline	Gram positive and gram negative	TGC	15
11	Vancomycin	Glycopeptide	Gram positive	VA	30
12	Linezolid	Oxazolidinones	Gram positive	LZ	30
13	Aztreonam	Monobactam	Gram negative	AT	30
14	Colistin	Polymyxin E	Gram negative	CT	10

Chapter 3: Results

3.1 Total aerobic bacterial plate count of Lipsticks, Powders and Creams

After processing the 27 samples they were spread on modified Letheen agar in order to obtain the aerobic plate count. The amount spread on modified Letheen agar was 0.1ml.

Formula used for calculating aerobic plate count ("Center for Food Safety and Applied Nutrition", 2021):

- For plates with 25-250 CFU:

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

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 first dilution counted

n2 = Number of plates in second dilution counted

d = Dilution from which the first counts were obtained

- For plates with fewer than 25 CFU:

When plates from both dilutions yield fewer than 25 CFU each, record actual plate count but record the count as less than 25 × 1/d when d is the dilution factor for the dilution from which the first counts were obtained.

- For plates with more than 250 CFU.
  When plates from both 2 dilutions yield more than 250 CFU each (but fewer than 100/cm^2), estimate the aerobic counts from the plates (EAPC) nearest 250 and multiply by the dilution.

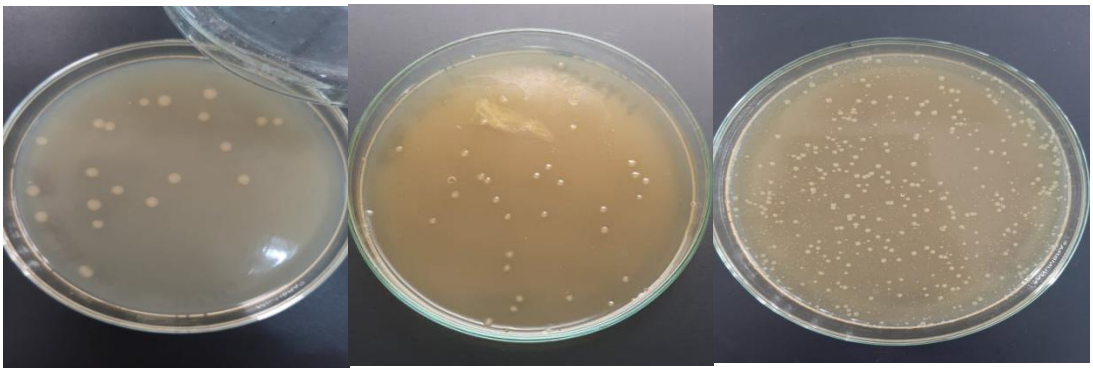


Figure 1: Modified Letheen Agar used in order to obtain the Total Aerobic Plate Count

Table 5: Total Aerobic Plate Count for Lipsticks, Powders and Creams

Lipsticks								Powders									Creams						
Lipstick 1	Lipstick 2	Lipstick 3	Lipstick 4	Lipstick 5	Lipstick 6	Lipstick 7	Lipstick 8	Powder 1	Powder 2	Powder 3	Powder 4	Powder 5	Powder 6	Powder 7	Powder 8	Powder 9	Cream 1	Cream 2	Cream 3	Cream 4	Cream 5	Cream 6	
4.4×10^{15}	3.9×10^{14}	1×10^{13}	54000	58000	5.5×10^{7}	50000	6	6.7×10^{12}	5.4×10^{13}	40	6.4×10^{8}	<2500	4.7×10^{8}	<2500	2×10^{8}	3×10^{9}	3.4×10^{13}	3×10^{12}	39700	4400	<2500	0	

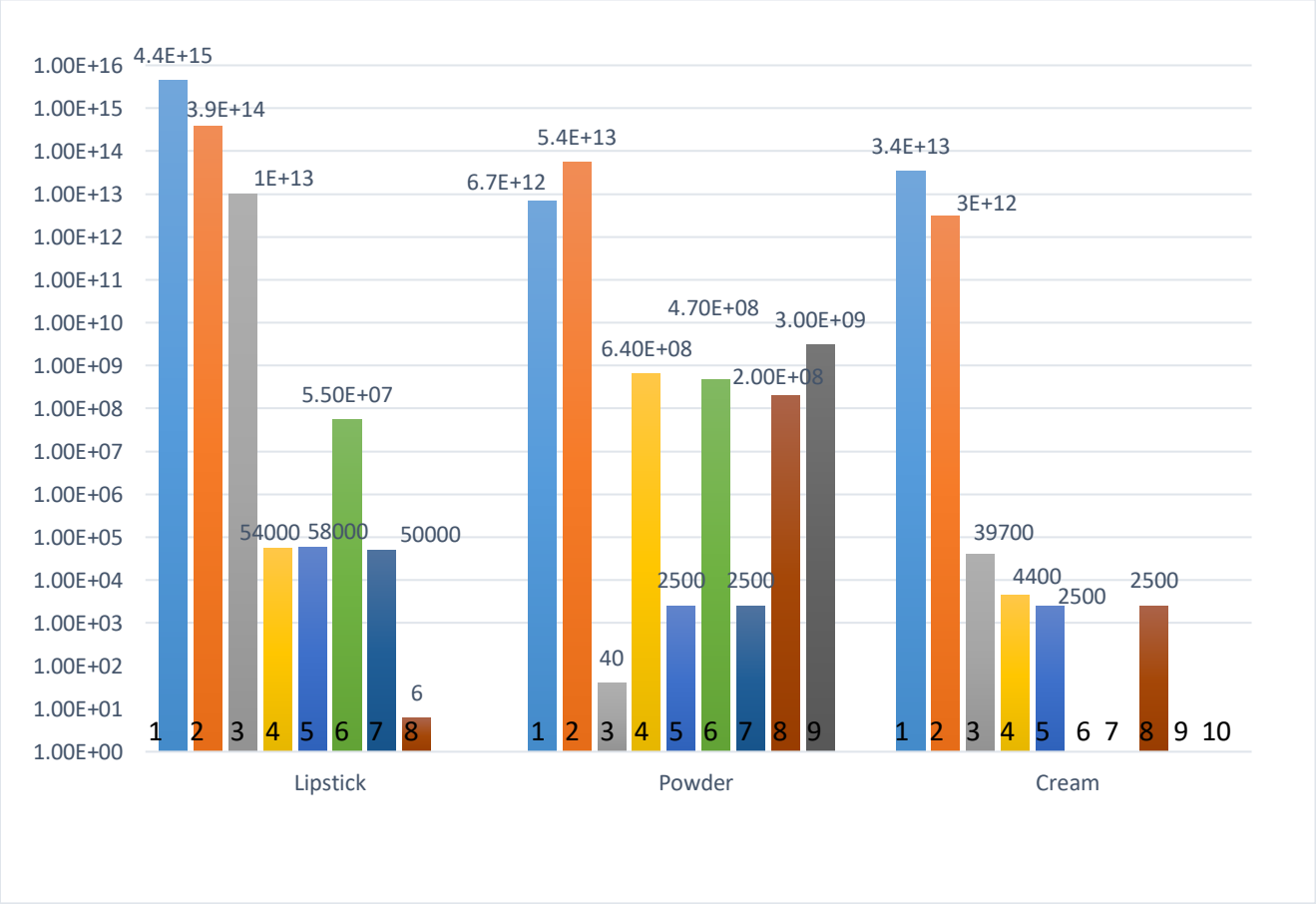
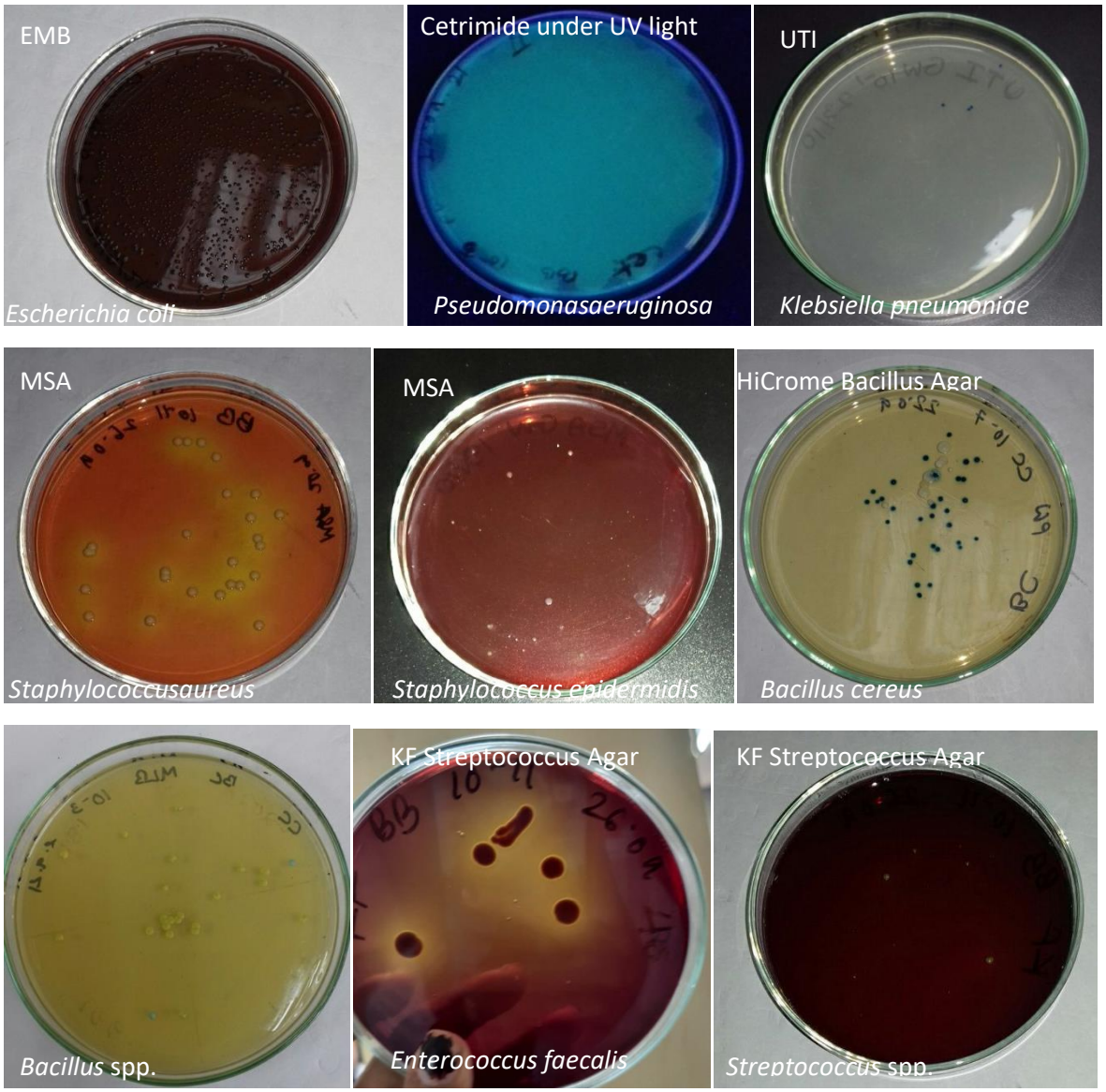


Figure 2: Total Aerobic Plate Count for Lipsticks, Powders and Creams

3.2 Based on morphology on selective media

Then the samples were spread on selective media for identification of bacteria. They were further identified using gram staining, biochemical tests and testing the hemolytic ability of the isolates.



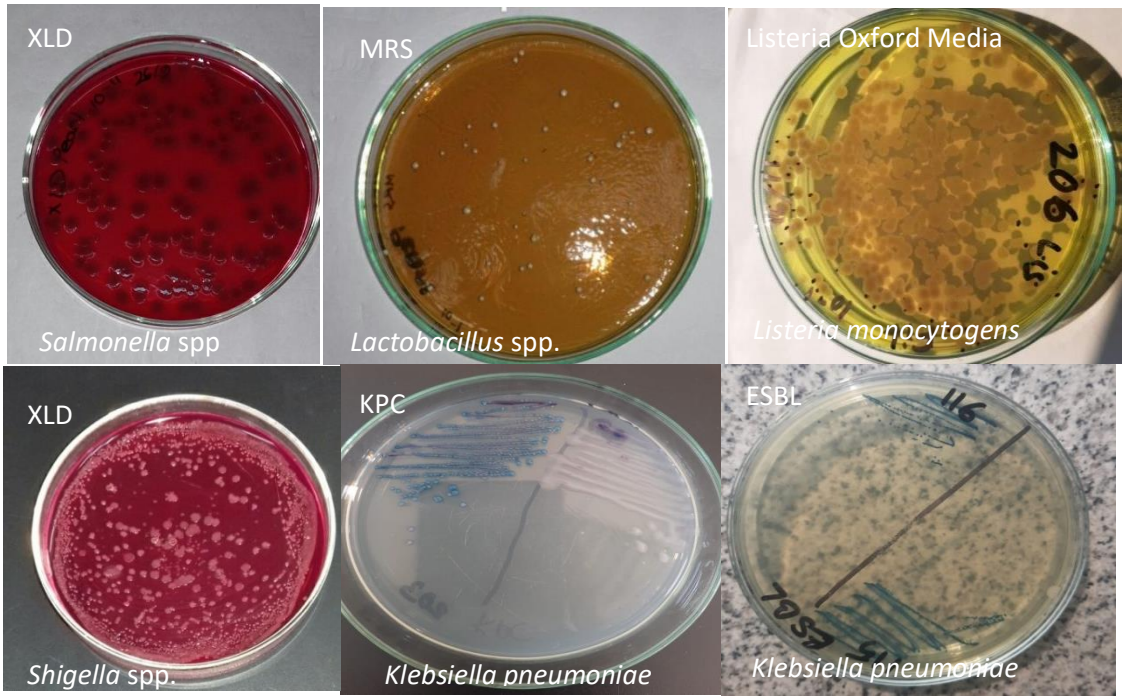


Figure 3: Different Selective Media used to isolate Different Organisms. The Top Left indicates the Media used and the Bottom left indicates the Organism isolated

3.3 Based on gram staining

Smears were prepared with the colony taken from selective media and gram staining was done. Followed by inspection under microscope:

3.4 Isolation of organisms

In order to isolate *Escherichia coli* Eosin Methylene Blue, (EMB) Agar was used. From Xylose Lysine Deoxycholate (XLD) Agar *Shigella spp.* and *Salmonella spp.* was isolated. HiCrome UTI Agar was used to isolate *Klebsiella pneumoniae*. From cetrimide agar *Pseudomonas aeruginosa* was isolated. For isolating *Staphylococcus aureus* and *Staphylococcus epidermidis.* Mannitol Salt Agar was used. From HiCrome Bacillus Agar *Bacillus cereus* and other *Bacillus* spp was isolated. From KF Streptococcal Agar *Enterococcus faecalis* and *Streptococcus spp.* were isolated. MRS Agar (de MAN, Rogosa and Sharpe) was used for isolating *Lactobacillus spp.*

Table 6: CFU/ml of Different Bacteria Found in Various Samples

CFU/ml in original sample				
Name of Bacteria	*Escherichia coli*	*Shigella spp*	*Salmonella spp*	*Klebsiellapneumoniae*
Media used	EMB Agar	XLD Agar	XLD Agar	Hicrome UTI Agar
Lipsticks	Lipstick 1	5120000	370	No growth	No growth
	Lipstick 2	1.5×10^{11}	No growth	2×10^{10}	No growth
	Lipstick 3	No growth	No growth	No growth	No growth
	Lipstick 4	4500000	No growth	No growth	No growth
	Lipstick 5	4.2×10^{7}	No growth	No growth	6.92×10^{7}
	Lipstick 6	100	No growth	No growth	20
	Lipstick 7	4870	No growth	No growth	No growth
	Lipstick 8	4930	No growth	350	No growth
Powders	Powder 1	No growth	No growth	1.92×10^{14}	No growth
	Powder 2	5×10^{12}	No growth	No growth	No growth
	Powder 3	630	No growth	No growth	2.5×10^{8}
	Powder 4	8.9×10^{6}	No growth	No growth	1.1×10^{9}
	Powder 5	1.6×10^{5}	No growth	No growth	3.04×10^{5}
	Powder 6	No growth	No growth	6.27×10^{7}	No growth
	Powder 7	1.18×10^{6}	No growth	No growth	50000
	Powder 8	55000	No growth	No growth	No growth
	Powder 9	No growth	No growth	No growth	No growth
Creams	Cream 1	7.7×10^{13}	No growth	1.17×10^{14}	No growth
	Cream 2	4.12×10^{6}	No growth	No growth	No growth
	Cream 3	23100	No growth	No growth	No growth
	Cream 4	2×10^{5}	No growth	No growth	400
	Cream 5	No growth	No growth	No growth	8.9×10^{6}
	Cream 6	No growth	No growth	No growth	No growth
	Cream 7	No growth	No growth	No growth	No growth
	Cream 8	7×10^{13}	No growth	No growth	No growth
	Cream 9	4.07×10^{6}	No growth	No growth	No growth
	Cream 10	No growth	No growth	No growth	No growth

CFU/ml in original sample	Creams	Cream 10	No growth	No growth	No growth
		Cream 9	No growth	No growth	No growth
		Cream 8	No growth	No growth	No growth
		Cream 7	No growth	No growth	No growth
		Cream 6	No growth	No growth	No growth
		Cream 5	No growth	No growth	No growth
		Cream 4	No growth	210	No growth
		Cream 3	No growth	30	No growth
		Cream 2	No growth	No growth	No growth
		Cream 1	5×10^{12}	No growth	250
	Powders	Powder 9	No growth	3.48×10^{10}	No growth
		Powder 8	No growth	3.92×10^{8}	No growth
		Powder 7	No growth	1.44×10^{6}	1.2×10^{4}
		Powder 6	No growth	No growth	No growth
		Powder 5	No growth	340000	300
		Powder 4	No growth	5.29×10^{9}	3×10^{7}
		Powder 3	1.2×10^{6}	3×10^{7}	5680
		Powder 2	20	2.56×10^{14}	4.3×10^{5}
		Powder 1	8.8×10^{13}	7000	2.7×10^{13}
	Lipsticks	Lipstick 8	4070	10	550
		Lipstick 7	No growth	80	20
		Lipstick 6	No growth	860	20
		Lipstick 5	5.96×10^{7}	5.36×10^{7}	5.36×10^{7}
		Lipstick 4	No growth	280	1×10^{5}
		Lipstick 3	No growth	2.76×10^{14}	No growth
		Lipstick 2	1420	6490	6.12×10^{14}
		Lipstick 1	50	2×10^{12}	No growth
	Media used		Cetrimide	MSA	MSA
	Name of Bacteria		*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Staphylococcus epidermidis*

CFU/ml in original sample				
Creams	Cream 10	No growth	No growth	No growth
	Cream 9	No growth	No growth	No growth
	Cream 8	No growth	No growth	No growth
	Cream 7	No growth	No growth	No growth
	Cream 6	2×10^{5}	4.5×10^{6}	No growth
	Cream 5	No growth	No growth	1×10^{5}	No growth
	Cream 4	No growth	5×10^{5}	No growth	No growth
	Cream 3	No growth	No growth	No growth	No growth
	Cream 2	No growth	5.7×10^{13}	No growth	No growth
	Cream 1	9.1×10^{10}	No growth	No growth	1600
Powders	Powder 9	No growth	1.66×10^{10}	1.89×10^{4}	No growth
	Powder 8	1.9×10^{9}	4.44×10^{10}	No growth	No growth
	Powder 7	No growth	No growth	No growth	No growth
	Powder 6	2.62×10^{7}	No growth	2.4×10^{5}	No growth
	Powder 5	1.7×10^{5}	No growth	8000	No growth
	Powder 4	1×10^{6}	1.71×10^{8}	8.4×10^{9}	No growth
	Powder 3	No growth	No growth	2800	1.04×10^{4}
	Powder 2	No growth	2.58×10^{11}	No growth	6.09×10^{11}
	Powder 1	No growth	5×10^{9}	No growth	5×10^{12}
Lipsticks	Lipstick 8	No growth	No growth	No growth	No growth
	Lipstick 7	No growth	No growth	No growth	No growth
	Lipstick 6	No growth	No growth	No growth	No growth
	Lipstick 5	No growth	No growth	3.07×10^{7}	No growth
	Lipstick 4	No growth	No growth	No growth	No growth
	Lipstick 3	No growth	1.5×10^{10}	No growth	1.4×10^{4}
	Lipstick 2	6×10^{14}	No growth	No growth	4.88×10^{14}
	Lipstick 1	6×10^{6}	1.64×10^{6}	No growth	5×10^{12}
Media used		HiCrome Bacillus	HiCrome Bacillus	KF Streptococcal	KF Streptococcal
Name of Bacteria		*Bacillus cereus*	*Bacillus* spp	*Enterococcus faecalis*	*Streptococcus* spp

Lactobacillus spp	MRS Agar	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	8×10^{9}	2.6×10^{9}	No growth	No growth	No growth	3440	No growth	2.11×10^{3}	700	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Listeria monocytogens	Listeria Oxford Medium	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth

3.5 Biochemical test results:

Colonies were selected from the selective media based on their morphology and subcultured on nutrient agar. This step was then followed by different biochemical tests to further identify the organisms. The table aforementioned in the materials and methods shows the result of the performed biochemical tests

3.6 Hemolysis

Figure 4: Hemolysis on Blood Agar

Table 7: Percentage of Observed Hemolytic Organisms

Hemolytic Pattern	Samples	Percentage of specific hemolytic pattern	Percentage of hemolytic organisms found in total samples
Alpha hemolysis	Lipsticks	4.76	1.56
	Powders	0	
	Creams	0	
Beta hemolysis	Lipsticks	61.90	61.72
	Powders	56.36	
	Creams	59.09	
Gamma hemolysis	Lipsticks	33.33	36.72
	Powders	43.64	
	Creams	40.90	

The results from different experiments were then complied and a comprehensive chart was made which is given below:

Table 8: Different Bacteria Found in Various Samples

Sample	Organism found
Lipstick 1	*E. coli, Shigella spp, Salmonella spp, P. aeruginosa, S. aureus, B. cereus, Streptococcus spp., Bacillus spp*
Lipstick 2	*E. coli, Salmonella spp, P. aeruginosa, S. aureus, S. epidermidis, B. cereus, Streptococcus spp, Bacillus. Spp*
Lipstick 3	*E. coli, S. aureus, Bacillus spp, streptococcus spp*
Lipstick 4	*E. coli, S. aureus, S. epidermidis*
Lipstick 5	*E. coli, K. pneumoniae, P. aeruginosa, S. aureus, S. epidermidis, E. faecalis*
Lipstick 6	*E. coli, K. pneumoniae, P. aeruginosa, S. aureus, S. epidermidis*
Lipstick 7	*E. coli, S. aureus, S. epidermidis*
Lipstick 8	*E. coli, Salmonella spp, P. aeruginosa, S. aureus S. epidermidis*
Powder 1	*E. coli, Salmonella spp, P. aeruginosa, S. aureus, S. epidermidis, B. spp, streptococcus spp, Lactobacillus spp*
Powder 2	*E. coli, P. aeruginosa, S. aureus, S. epidermidis, Bacillus spp Streptococcus spp., Lactobacillus spp*
Powder 3	*E. coli, Shigella spp, K. pneumoniae, P. aeruginosa S. aureus, S. epidermis, E. faecalis, Streptococcus spp*
Powder 4	*E. coli, K. pneumoniae, S. aureus*

Powder 5	*E. coli, K. pneumoniae, S. aureus, S. epidermdis, B. cereus, E. faecalis*
Powder 6	*Salmonella spp, S. aureus, B. cereus, E. faecalis, L. monocytogenes*
Powder 7	*E. coli, K. pneumoniae, S. aureus, S. epidermidis*
Powder 8	*E. coli, S. aureus, B. cereus, Bacillus spp, E. faecalis* *L. monocytogenes*
Powder 9	*E. coli, S. aureus, Bacillus spp, E. faecalis* *L. monocytogenes*
Cream 1	*E. coli, Salmonella spp, P. aeruginosa, S. epidermidis, B. cereus,* *Streptococcus spp*
Cream 2	*E. coli, Salmonella spp, Bacillus spp.*
Cream 3	*E. coli, S. aureus*
Cream 4	*E. coli, S. aureus, K. pneumoniae, Bacillus spp*
Cream 5	*E. coli, K. pneumoniae, S. aureus, E. faecalis*
Cream 6	*B. cereus, Bacillus spp*
Cream 7	-
Cream 8	*E. coli*
Cream 9	-
Cream 10	-

3.7Antibiotic resistance observed in different organisms

In this study 209 agar plates have been randomly selected to identify the antibiotic resistance of different organisms. Gentamicin, Ampicillin, Meropenem, Cefepime, Piperacillin, Imipenem, Azithromycin, Amikacin, Tigecycline and Ciprofloxacin was used to determine the antibiotic susceptibility of Gram-negative bacteria as well as Gram-positive bacteria. For testing specifically Gram-negative bacteria Colistin and Aztreonam antibiotics were used. For Gram-positive bacteria Vancomycin and Linezolid antibiotics were used.

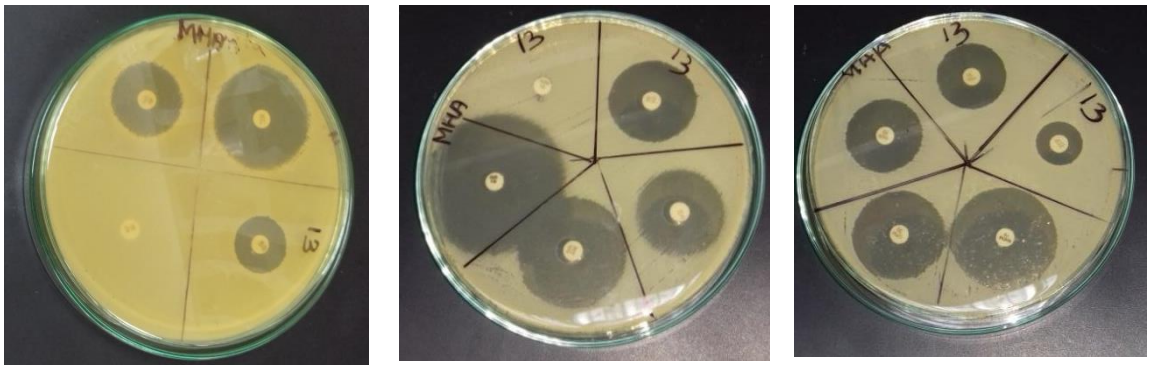


Figure 5: Antibiogram done on MHA agar

Table 9: Percentage of Resistance Observed

Name of Bacteria	Lipsticks	Powders	Creams
	Percentage of Resistance Observed		
Escherichia coli	68.4%	68.1%	60%
Shigella spp.	100%	0%	No isolates observed
Salmonella spp.	66.67%	50%	50%
Klebsiella pneumoniae	100%	62.5%	66.67%
Pseudomonas aeruginosa	57.1%	60%	0%
Staphylococcus aureus	77.77%	75%	85.71%
Staphylococcus epidermidis	100%	72.73%	100%
Bacillus cereus	100%	100%	100%
Bacillus spp.	100%	88.89%	100%
Enterococcus faecalis	100%	88.89%	100%
Streptococcus spp.	100%	100%	100%
Lactobacillus spp.	No isolates observed	100%	No isolates observed
Listeria monocytogens	No isolates observed	100%	No isolates observed

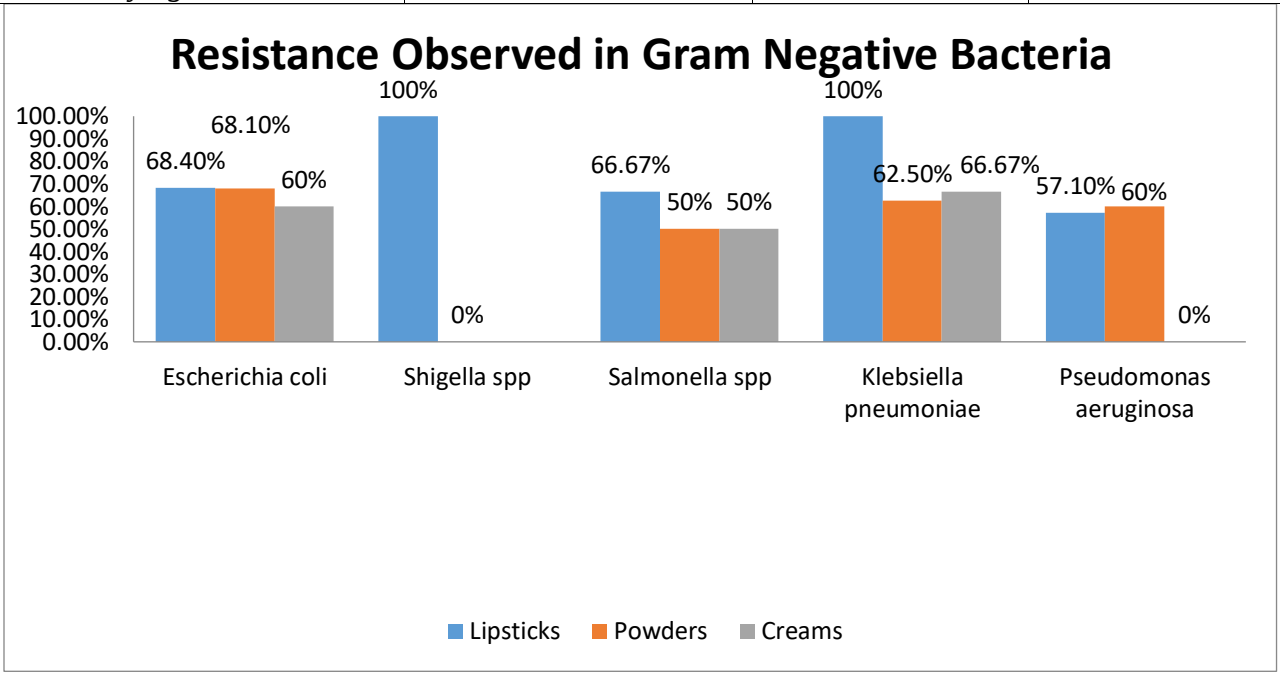


Figure 6: Resistance Observed in Gram Negative Bacteria

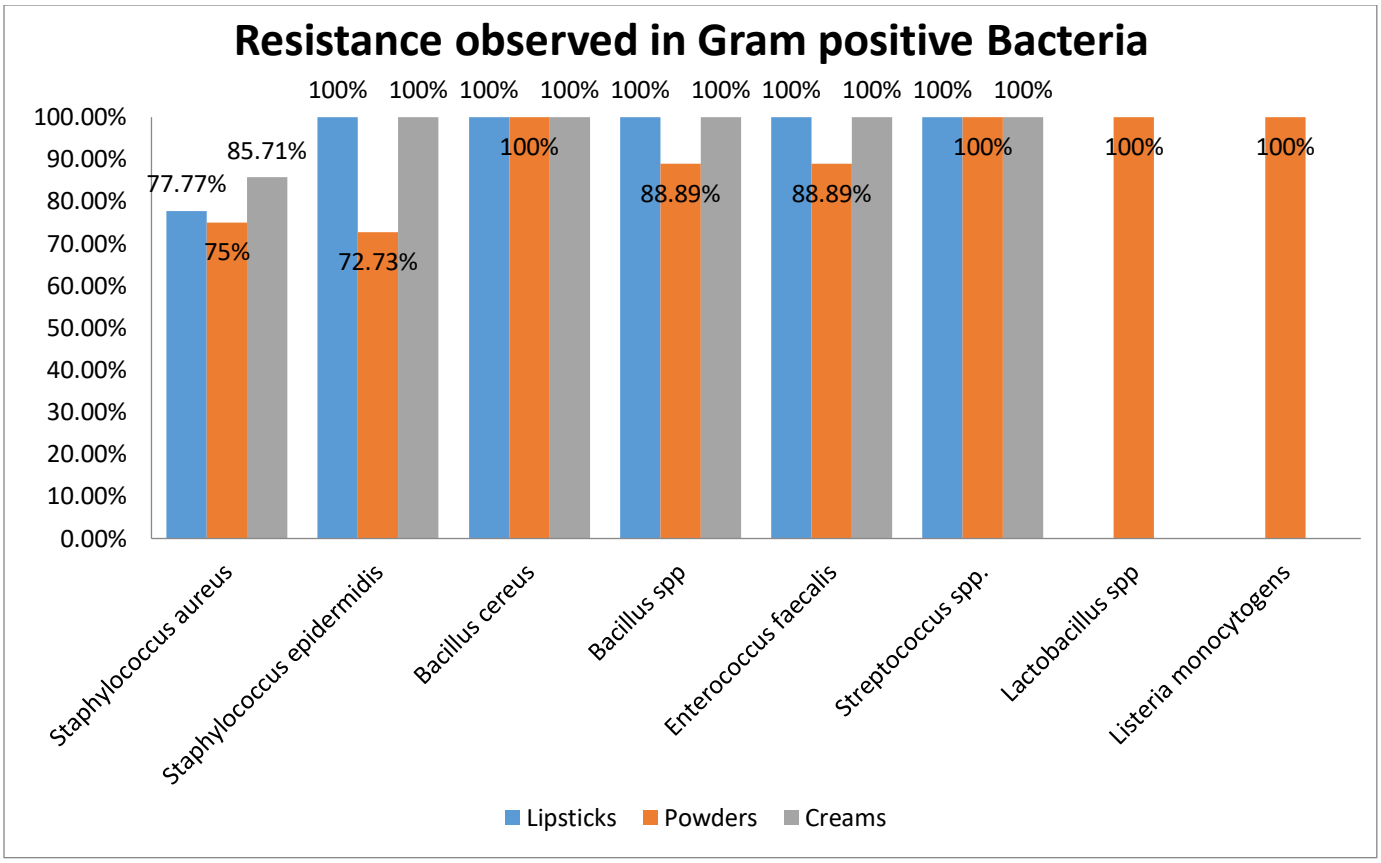


Figure 7: Resistance Observed in Gram Positive Bacteria

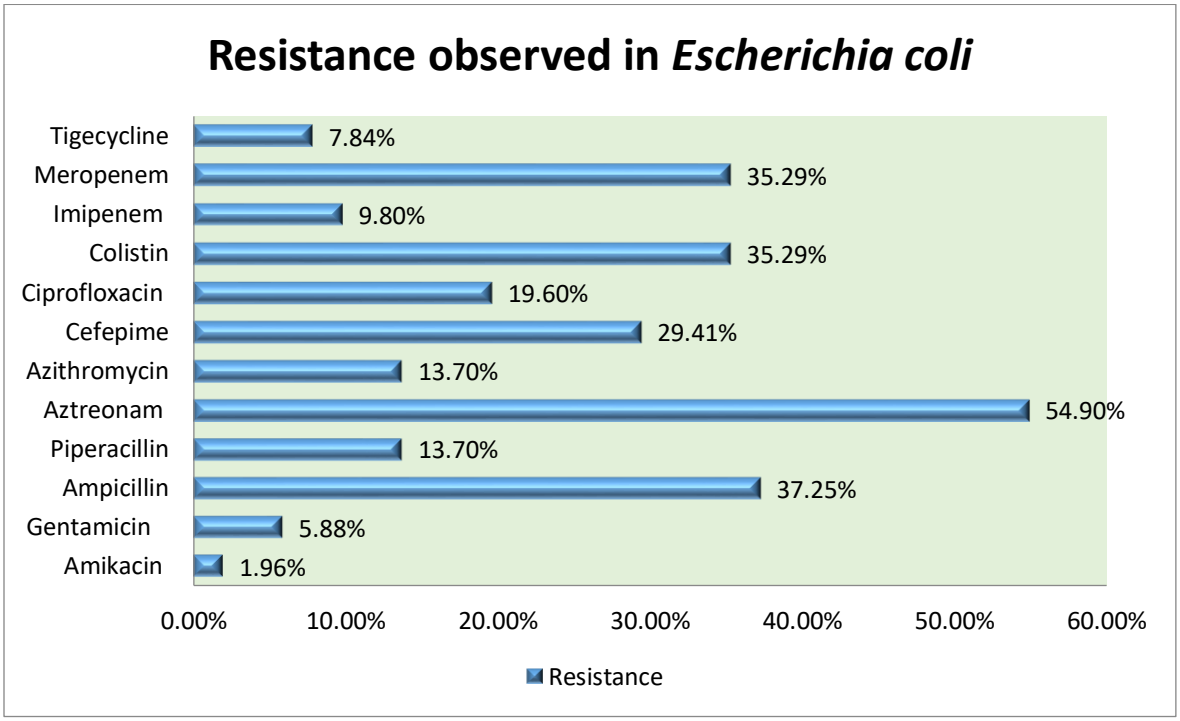


Figure 8: Resistance Observed in *Escherichia coli*

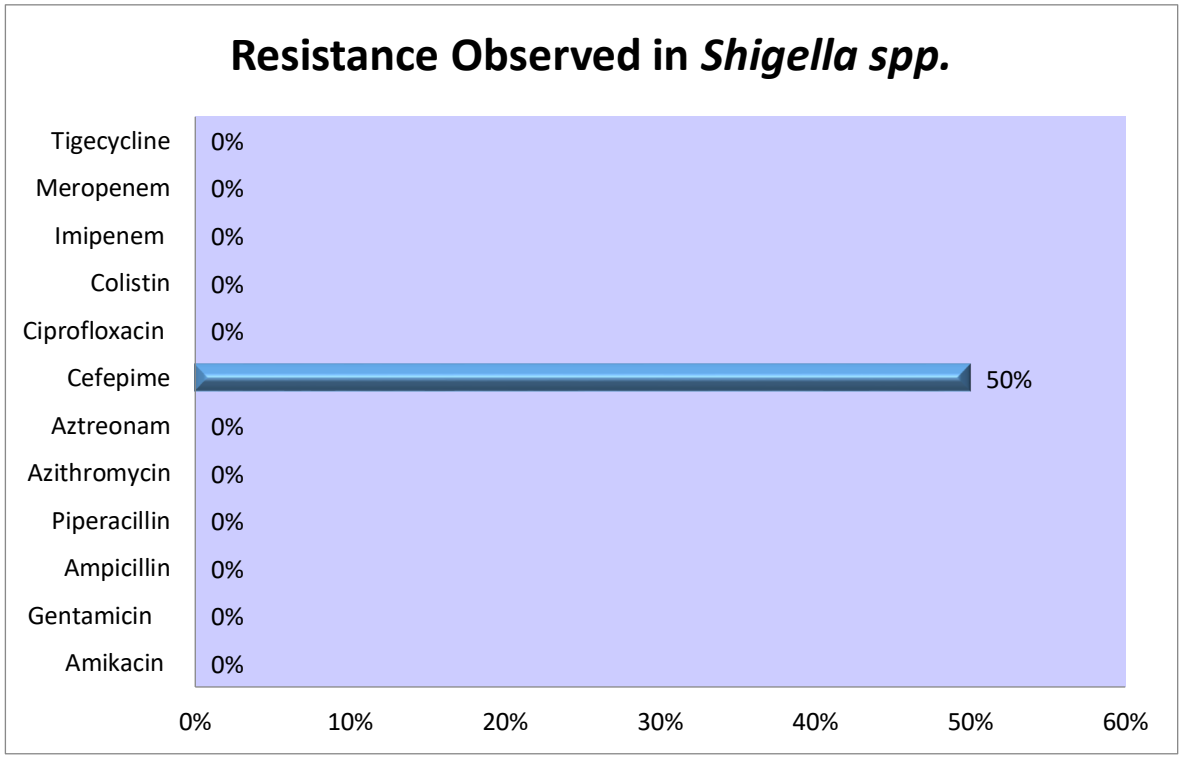

Resistance Observed in *Shigella spp.*
Tigecycline
Meropenem
Imipenem
Colistin
Ciprofloxacin
Cefepime
Aztreonam
Azithromycin
Piperacillin
Ampicillin
Gentamicin
Amikacin
0%
0%
0%
0%
0%
50%
0%
0%
0%
0%
0%
0%
0%
10%
20%
30%
40%
50%
60%

Figure 9: Resistance Observed in *Shigella spp*

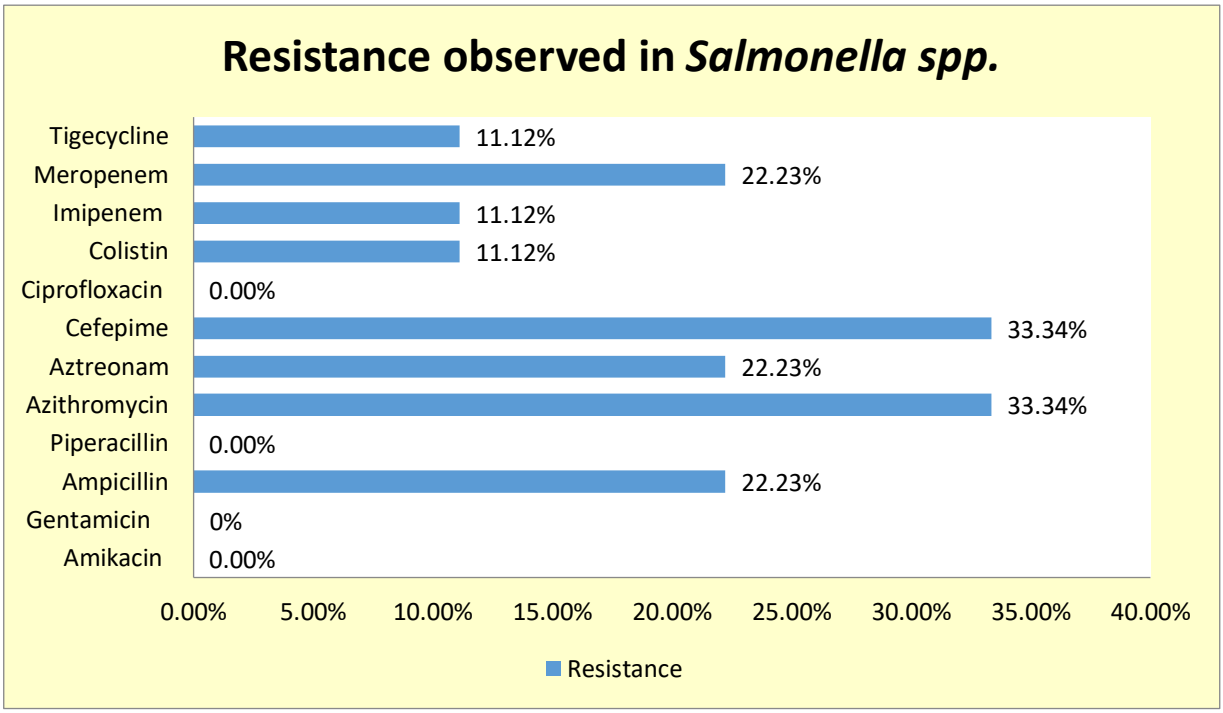


Figure 10: Resistance Observed in *Salmonella spp.*

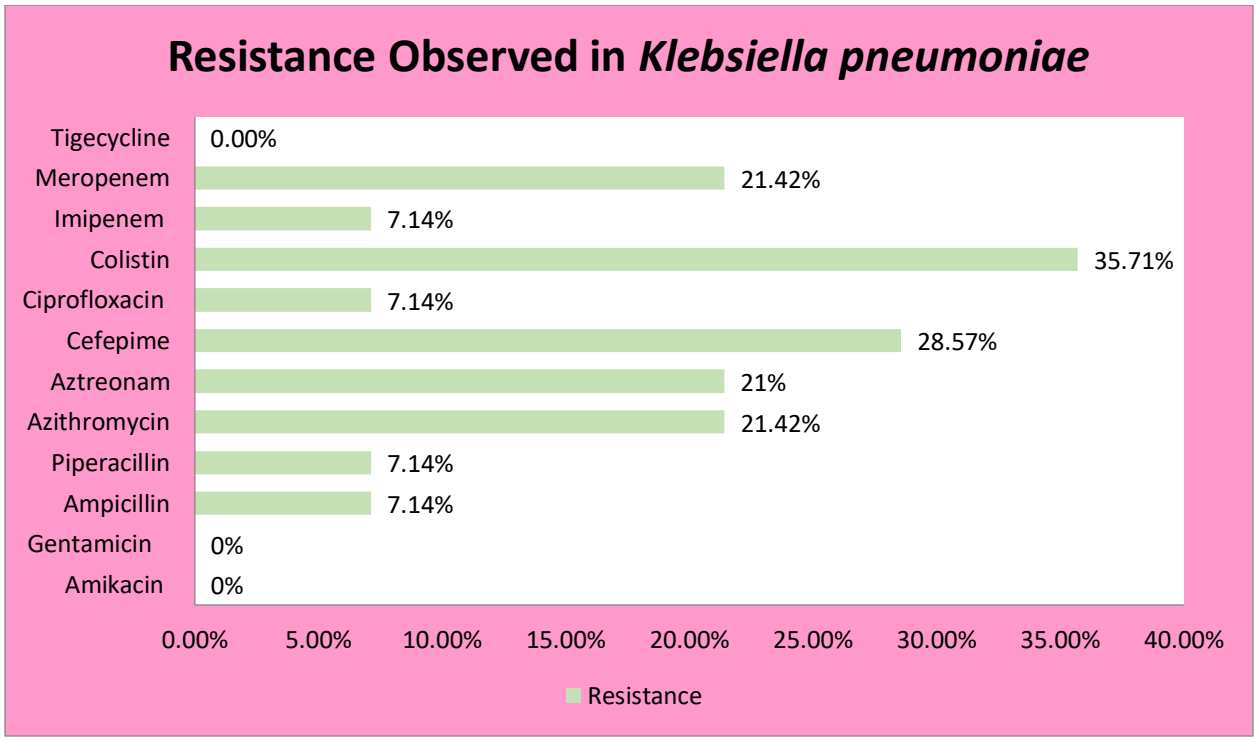


Figure 11: Resistance Observed in *Klebsiella pneumoniae*

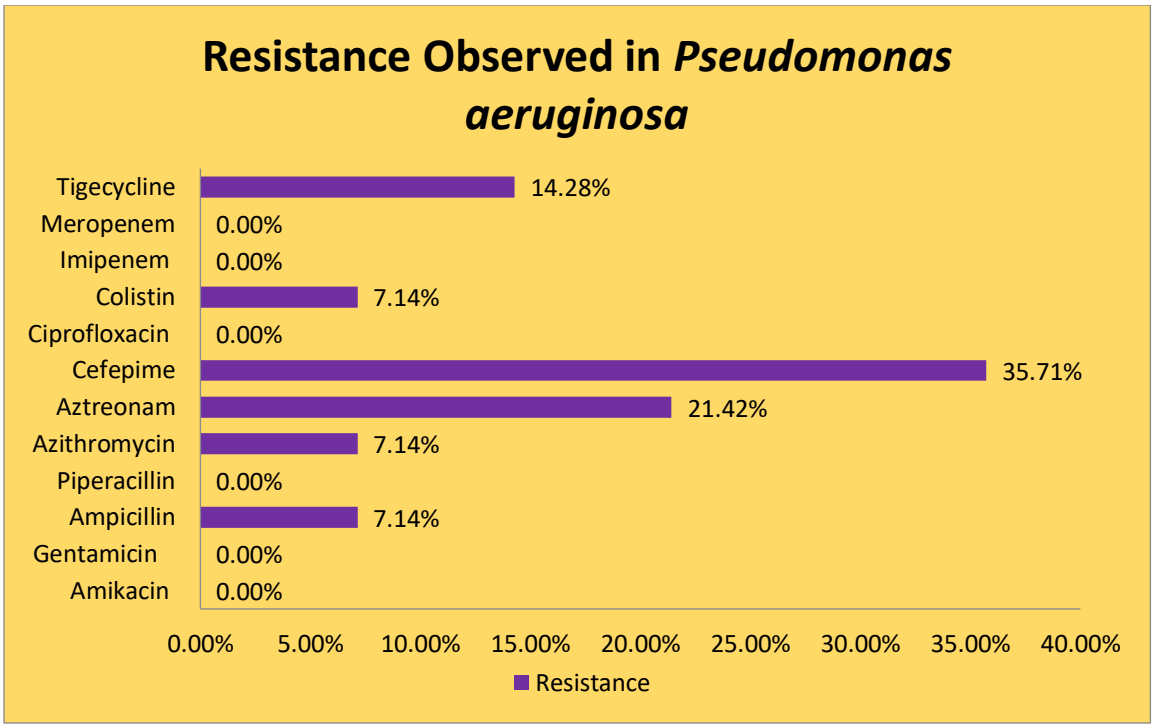


Figure 12: Resistance Observed in *Pseudomonas aeruginosa*

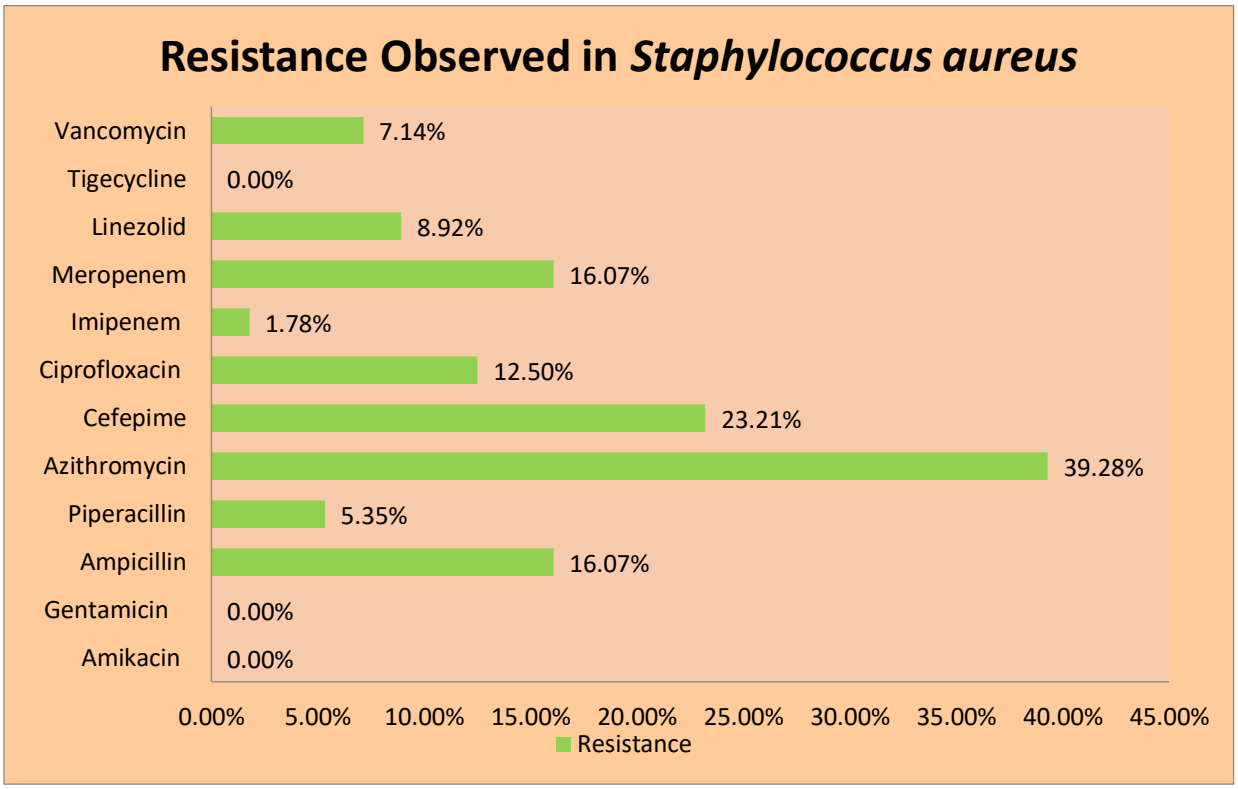


Figure 13: Resistance Observed in *Staphylococcus aureus*

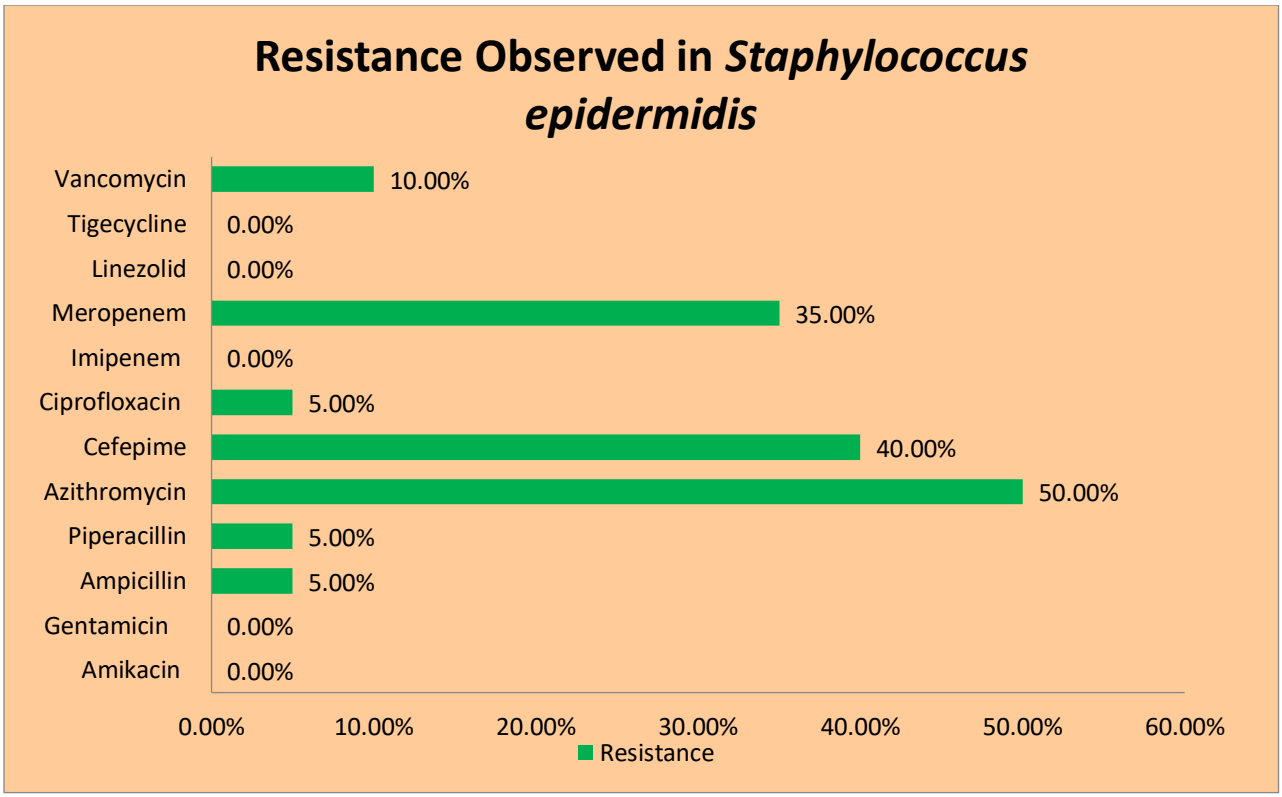


Figure 14: Resistance Observed in *Staphylococcus epidermidis*

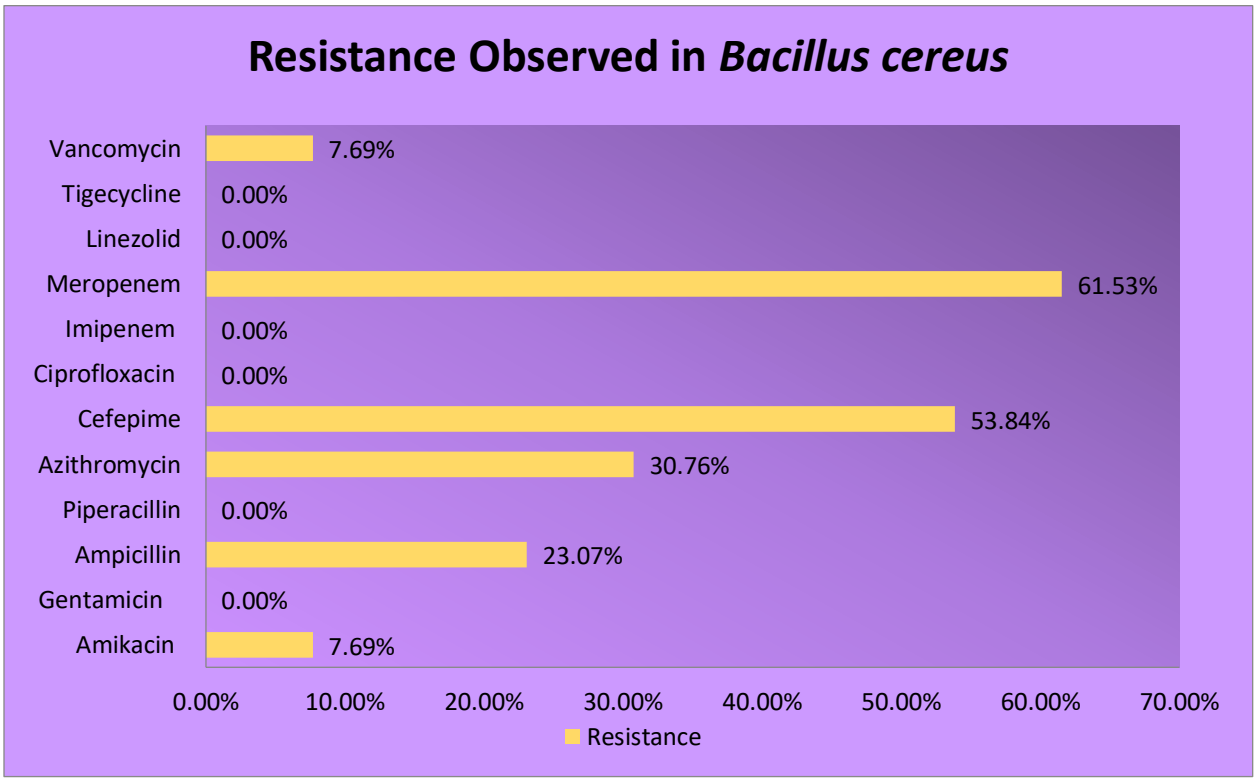


Figure 15: Resistance Observed in *Bacillus cereus*

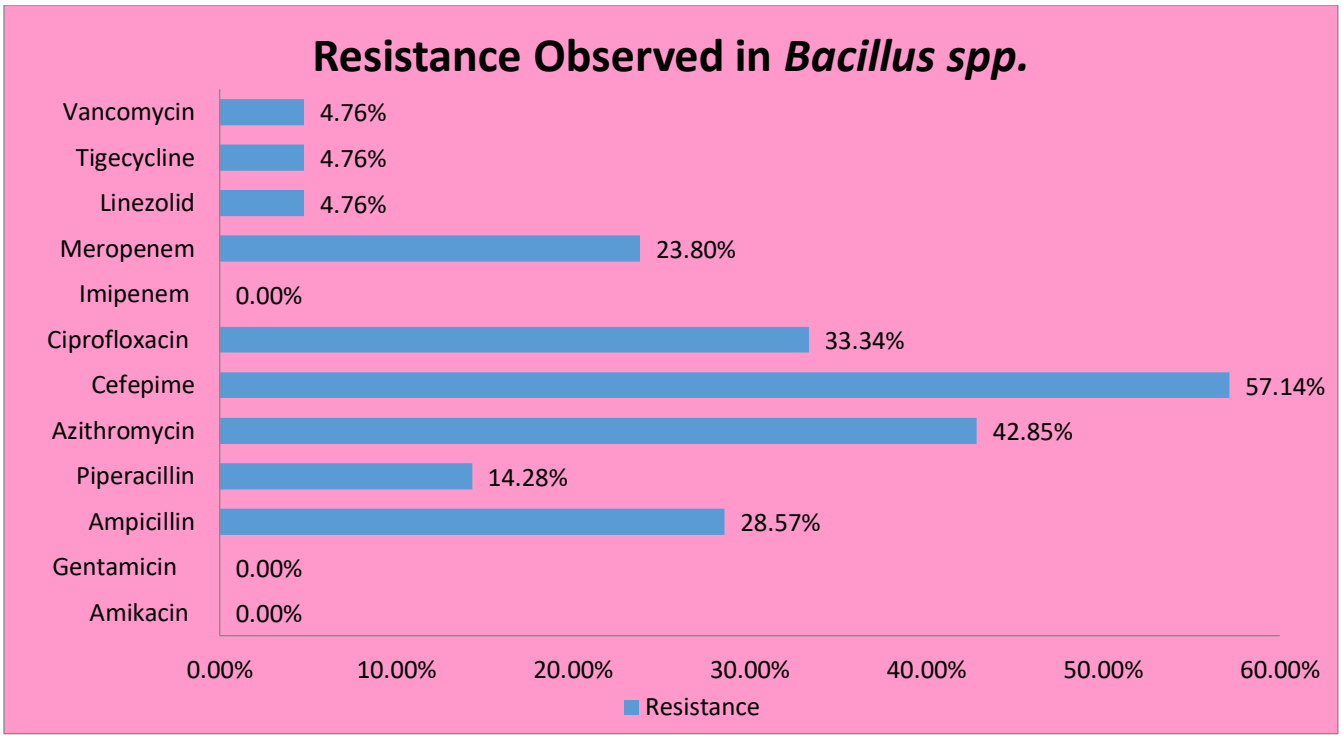


Figure 16: Resistance Observed in *Bacillus spp.*

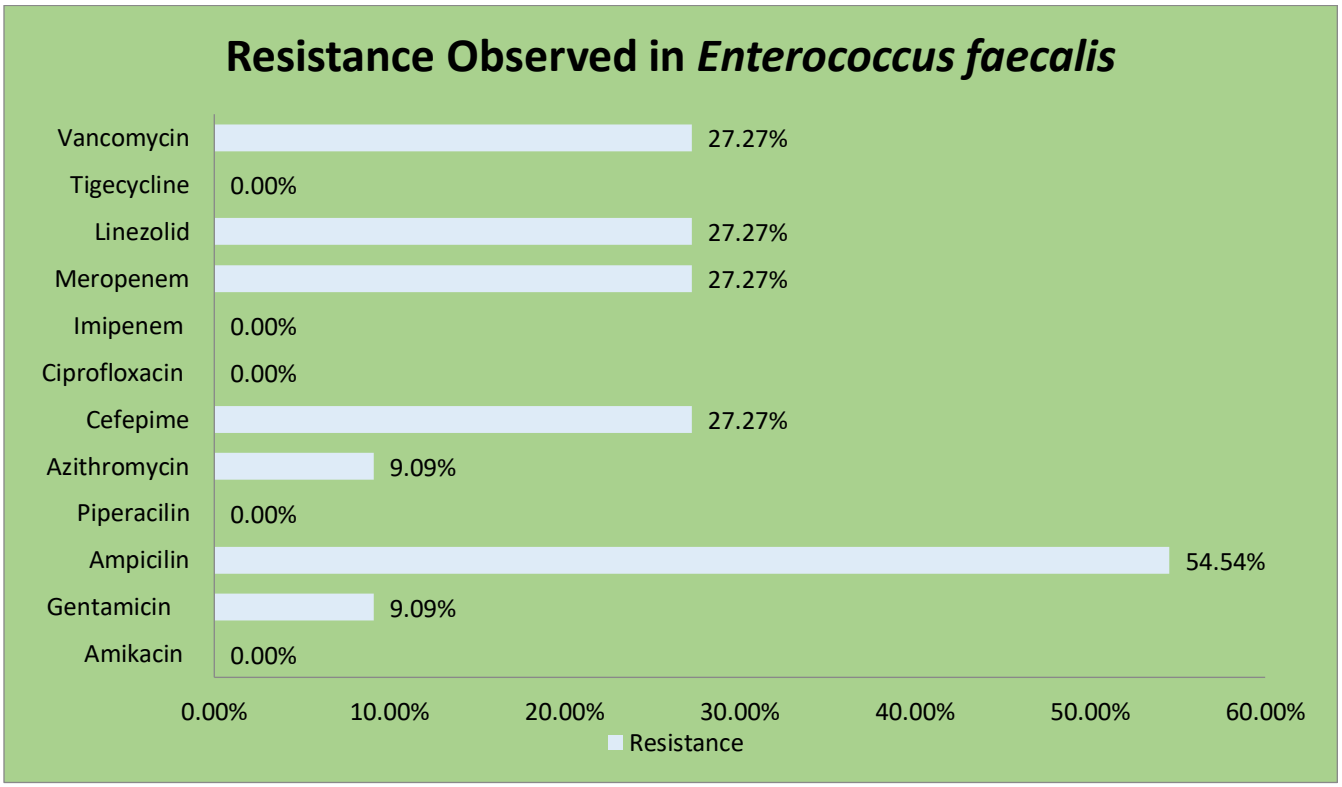


Figure 17: Resistance Observed in *Enterococcus faecalis*

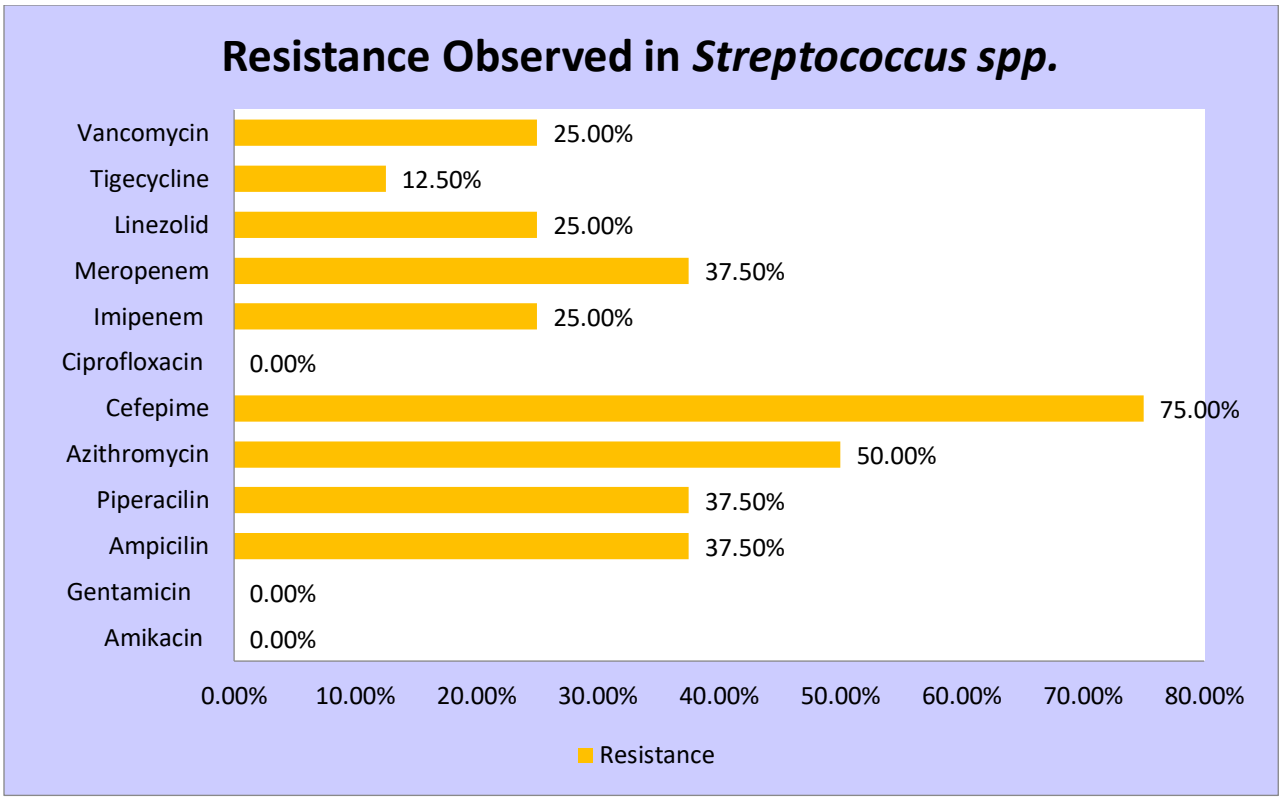


Figure 18: Resistance Observed in *Streptococcus spp.*

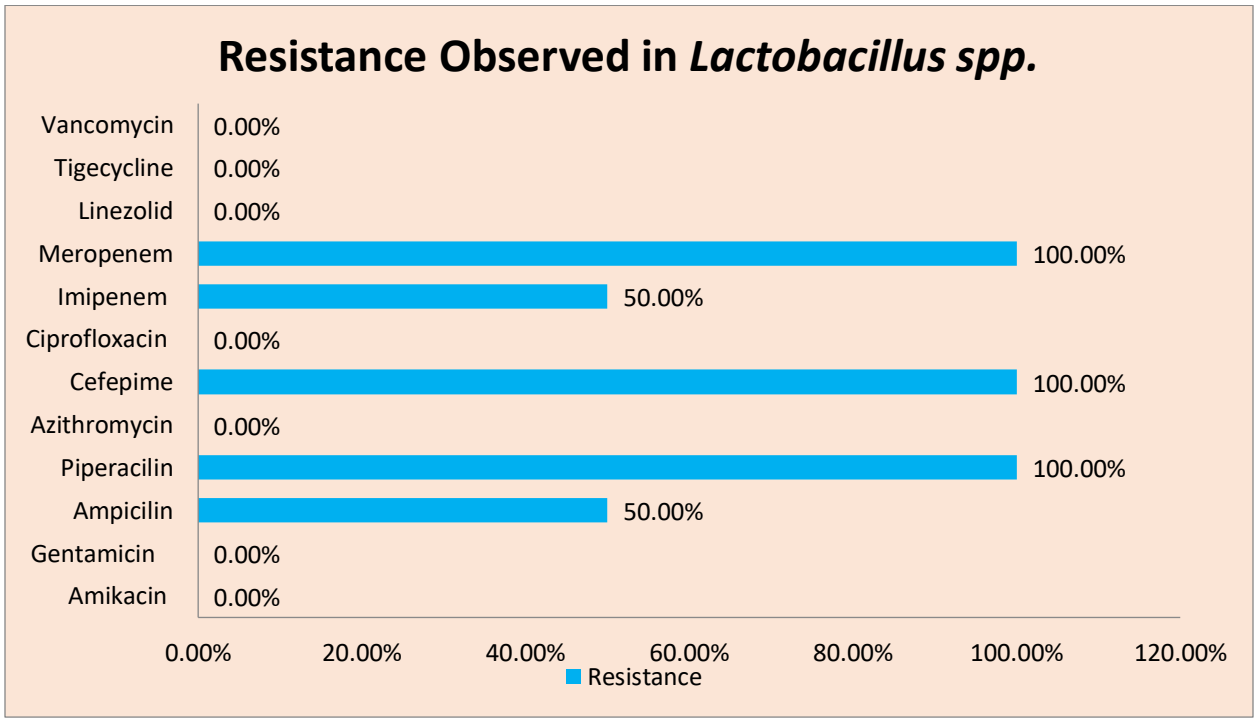


Figure 19: Resistance Observed in *Lactobacillus spp.*

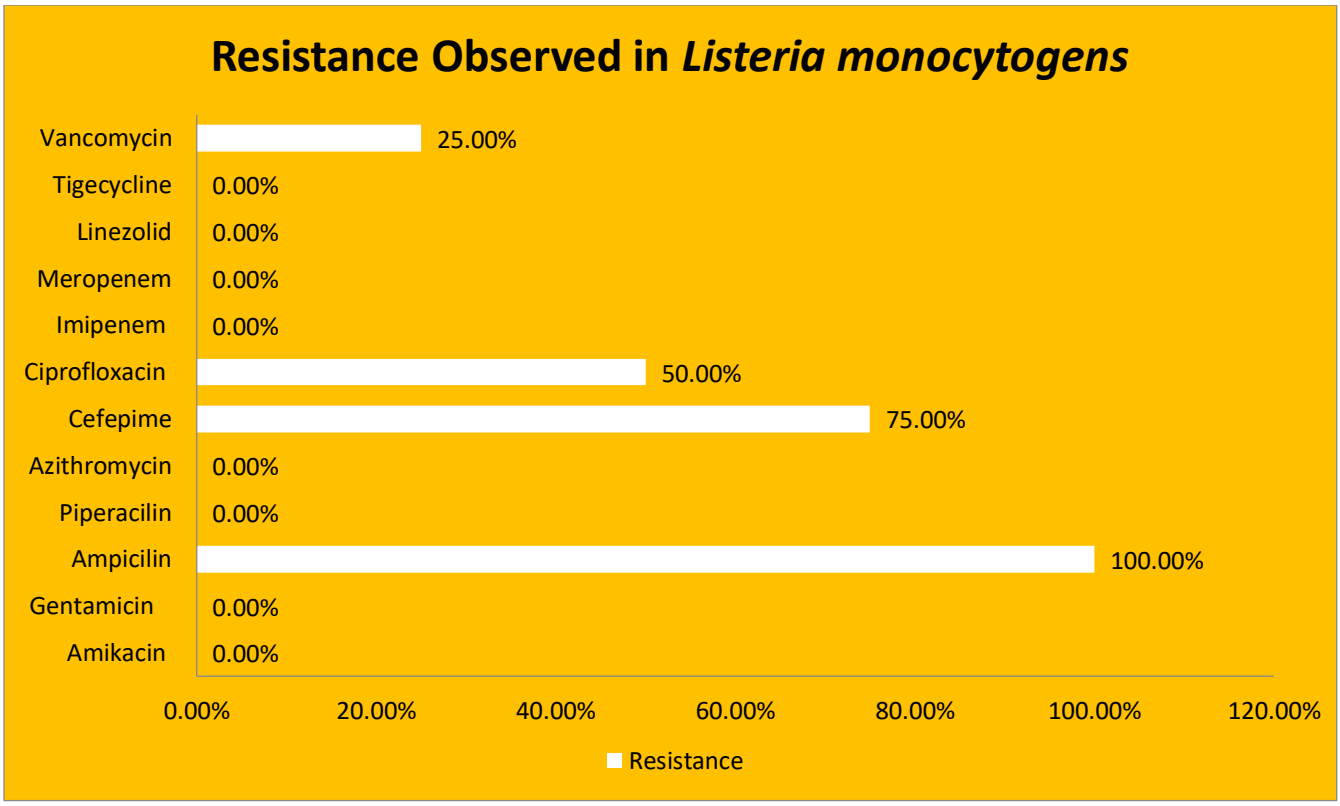


Figure 20: Resistance Observed in *Listeria monocytogens*

Table 10: Percentage of Multidrug Resistance Observed

	Name of Bacteria	Lipsticks	Powders	Creams
		Percentage of Multidrug Resistance Observed		
Gram- negative Bacteria	*Escherichia coli*	63.15%	33.4%	50%
	Shigella spp.	100%	0%	No isolates observed
	Salmonella spp.	50%	33.4%	0%
	Klebsiella pneumoniae	66.7%	25%	50%
	Pseudomonas aeruginosa	40%	0%	0%
Gram-positive Bacteria	*Staphylococcus aureus*	15.3%	11.7%	11.1%
	Staphylococcus epidermidis	28.5%	9%	0%
	Bacillus cereus	25%	71.4%	50%
	Bacillus spp.	62.5%	66.7%	75%
	Enterococcus faecalis	100%	62.5%	100%
	Streptococcus spp.	50%	50%	100%
	Lactobacillus spp.	No isolates observed	100%	No isolates observed

	Listeria monocytogens	No isolates observed	100%	No isolates observed

Chapter 4: Discussion

Cosmetic contamination by microbes is a massive health problem that affects the public (Hugbo et al.,2013). Pharmaceutical products in Bangladesh undergo sterility testing however there is a lack of cosmetic testing aptitudes due to inadequate facilities (Akon et al., 2015). In Bangladesh there is a scarcity of data about microbial contamination in cosmetics, even though cosmetic contamination has been reported worldwide by a variety of pathogenic bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus spp., Micrococcus spp., Clostridium tetani, Bacillus cereus,* actinomycetes and fungi (Jimenez et al., 1999, Elaine B, 1989). This study aimed to find the total aerobic plate count and cfu/ml of bacteria, identify the bacteria isolated from cosmetics using selective media and biochemical tests, determining the hemolytic ability of the pathogens as well as multidrug resistance.

A selection of lipsticks, powders and creams were tested for microbial contamination. In case of lipsticks 8 samples were tested. In case of Lipstick 1 the aerobic plate count was 4.4×10^{15}. The aerobic plate count was 3.9×10^{14} for Lipstick 2. For Lipstick 3 aerobic plate count was 1×10^{13}. For Lipstick 4, the aerobic plate count was 54000. In case of Lipstick 5, the aerobic plate count was 58000. For Lipstick 6, the aerobic plate count was 5.5×10^{7}. In case of Lipstick 7 the aerobic plate count was 50000. Lastly, for Lipstick 8 the aerobic plate count was 6.

In case of powders 9 samples were tested. For the 1st sample the aerobic plate count was 6.7×10^{12}. For the 2nd sample the aerobic plate count was 5.4×10^{13}. In the 3rd sample, the aerobic plate count was 40. For the 4th sample the aerobic plate count was 6.4×10^{8}. For the 5th sample the estimated aerobic plate count was <2500. In the 6th sample aerobic plate count was 4.7×10^{8}. In the 7th sample the estimated aerobic plate count was <2500. In the 8th sample the aerobic plate count was 2×10^{8}. In the last sample the aerobic plate count was 3×10^{9}.

In case of creams 10 samples were tested. The aerobic plate count l of the 1st sample was 3.4×10^{13}. For the 2nd sample the aerobic plate count was 3×10^{12}. For the 3rd sample the aerobic plate count was 39700. The aerobic plate count of the 4th sample was 4400. In the 5th sample the estimated aerobic plate count was <2500. In the 6th sample no growth was found. No growth was seen in the 7th sample. In the 8th sample the aerobic plate count was <2500. In the 9th and 10th sample no growth was seen in any of the samples.

As mentioned previously, according to the FDA regulations in case of cosmetic products level of contamination should not exceed <1000cfu/g for non-eye area. According to the EU the total aerobic count for microorganisms should not be higher than 10^2 CFU per ml for eye cosmetics and for non-eye area the total aerobic count for micro-organisms should not be higher than 10^3 CFU per ml ("Scientific Committee on Consumer Safety (SCCP)", 2016). However, in the lipstick samples all of them except Lipstick 8 had higher aerobic plate count than the level allowed. This does correspond with a study conducted in Dhaka (Akon et.al., 2015) where the load of bacteria was up to10^5 CFU/ml which exceeded the FDA limit. In case of powder samples all of the

samples except Powder 5 and Powder 7 had high levels of aerobic plate count than the limit. However, in a study conducted in 2020 (Jairoun et al., 2020) showed that the powder samples they tested for contamination were within the limits. In case of the cream samples Cream 6 and Cream 7 had no growth. In Cream 8 there was growth, but it was within the acceptable limit. Cream 9 and 10 had no growth as well. The rest of the samples had cfu/ml ranging from 3×10^4 - 1.03×10^{15}. This is higher than the acceptable limit. This is in accordance with a study conducted (Aslam.S et.al., 2017). In that study the cfu/ml of creams varied from 2.7×10^4 - 1.84×10^{10}. Although it is to be noted that some cfu/ml obtained is significantly higher than the results found in the study.

In case of *Escherichia coli* isolates resistance observed was amikacin (1.96%), gentamicin (5.88%), ampicillin (37.25%), piperacillin/tazobactam (13.7%), colistin (35.29%), meropenem (35.29%), imipenem (9.8%) cefepime (29.41%), azithromycin (13.7%), aztreonam (54.9%), ciprofloxacin (19.6%) and tigecycline (7.84%). This partially corresponded with findings of a study conducted in 2017 (Aslam.S et.al., 2017) where ciprofloxacin and amikacin were sensitive and colistin was resistant. In another study (Akgül.O & Bakan.K, 2021) *E. coli* isolates were found to show the highest antibiotic resistance to ampicillin (31.2%), and gentamicin (31.2%) antibiotics. The levels of resistance shown in other antibiotics were cefepime (6.3%), imipenem (6.3%), meropenem (0%), piperacillin/tazobactam (18.8%), amikacin (12.5%), ciprofloxacin (18.8%), tigecycline (6.3%) and colistin (0%). This study did not correspond with our findings. The level of resistance found in the study completely differs from our findings.

In *Shigella spp.* none of the samples were multidrug resistant. Resistance observed in the antibiotics was amikacin (0%), gentamicin (0%), ampicillin (0%), piperacillin/tazobactam (0%), colistin (0%), meropenem (0%), imipenem (0%), cefepime (50%), azithromycin (0%), aztreonam (0%) ciprofloxacin (0%) and tigecycline (0%). A study conducted in 2009 (Razooki.A et. al., 2009,) had isolated *Shigella* spp. from cosmetics but the study had not tested the antibiotic susceptibility of the isolates.

In case of *Salmonella* spp. all the isolates were multidrug resistant. Resistance observed in the antibiotics was amikacin (0%), gentamicin (0%), ampicillin (22.23%), piperacillin/tazobactam (0%), colistin (11.12%), meropenem (22.23%), imipenem (11.12%), cefepime (33.34%), azithromycin (33.34%), aztreonam (22.23%), ciprofloxacin (0%) and tigecycline (11.12%). In previously conducted studies (Dadashi&Dehghanzadeh, 2016, Akon et.al.,2015) *Salmonella spp.* was isolated; however, the *Salmonella spp.* isolates were not tested for their antibiotic susceptibility.

In case of *Klebsiella pneumoniae* the resistance observed in the antibiotics was amikacin (0%), gentamicin (0%), ampicillin (7.14%), piperacillin/tazobactam (7.14%), colistin (35.71%), meropenem (21.42%), imipenem (7.14%), cefepime (28.57%), azithromycin (21.42%), aztreonam ((100%), ciprofloxacin (7.14%) and tigecycline (0%). In a study conducted in 2021 (Akgül.O & Bakan.K, 2021) the resistance levels of the antibiotics were gentamicin (0%), amikacin (0%) ampicillin (45.5%), piperacillin/tazobactam (27.3%), colistin (0%), meropenem (9.1%), imipenem (9.1%), cefepime (9.1%), ciprofloxacin (0%) and tigecycline (0%). This study corresponded with our level of resistance findings.

In case of *Pseudomonas aeruginosa* isolates resistance observed in the antibiotics was amikacin (0%), gentamicin (0%), ampicillin (7.14%), piperacillin/tazobactam (0%), colistin (7.14%), meropenem (0%), imipenem (0%), cefepime (35.71 %), azithromycin (7.14%), aztreonam (21.42%), ciprofloxacin (0%) and tigecycline (14.28%). These findings are somewhat confirmed by a previous study (Aslam.S et.al., 2017) where amikacin, colistin and ciprofloxacin were sensitive to *Pseudomonas aeruginosa.* In another study conducted in 2021 (Akgül.O & Bakan.K, 2021) the resistance levels of the antibiotics were amikacin (0%), gentamicin (0%), ampicillin (0%), piperacillin/ tazobactam (0%), colistin (0%), meropenem (25%), imipenem (25%), cefepime (25%), ciprofloxacin (25%) and tigecycline (0%). This partially corresponded with our findings. The percentage of resistance found in amikacin, gentamicin and piperacillin/tazobactam matched with our findings and differed for rest of the antibiotics.

In case of *Staphylococcus aureus* isolates the resistance observed in the antibiotics was amikacin (0%), gentamicin (0%), ampicillin (16.07%), piperacillin/tazobactam (5.35%), meropenem (16.07%), imipenem (1.78%), cefepime (23.21%), azithromycin (39.28%), ciprofloxacin (12.5%), vancomycin (7.14%), linezolid (8.92%), and tigecycline (0%). This partially corresponded with findings of a study conducted in 2017 (Aslam.S et.al., 2017) where ciprofloxacin and amikacin were sensitive and vancomycin and colistin were resistant. In another study conducted in 2021 (Akgül.O & Bakan.K, 2021) the resistance levels of the antibiotics were gentamicin (50%), ampicillin (33.4%), ciprofloxacin (33.4%), erythromycin (16.7%), vancomycin (0%), linezolid (0%) and tigecycline (0%). Except for tigecycline the levels of antibiotic resistance did not correspond.

In *Staphylococcus epidermidis* isolates resistance observed was amikacin (0%), gentamicin (0%), ampicillin (5%), piperacillin/tazobactam (5%), meropenem (35%), imipenem (0%), cefepime (40%), azithromycin (50%), ciprofloxacin (5%), vancomycin (10%), linezolid (0%), and tigecycline (0%). In a study conducted in 2021 (Akgül.O & Bakan.K, 2021) the resistance levels of the antibiotics were gentamicin (10.6%), ampicillin (36.1%), ciprofloxacin (2.1%), erythromycin (19.1%), vancomycin (0%), linezolid (4.2%) and tigecycline (6.3%). These findings do not correspond with our study.

In case of *Bacillus cereus* isolates, resistance observed in the antibiotics was amikacin (7.69%), gentamicin (0%), ampicillin (23.07%), piperacillin/tazobactam (0%), meropenem (61.53%), imipenem (0%), cefepime (53.84%), azithromycin (30.76%), ciprofloxacin (0%), vancomycin (7.69%), linezolid (0%), and tigecycline (0%). A previous study conducted (Turnbull et.al. 2004) confirmed these findings where the *B. cereus* isolates showed resistance to ampicillin, cephalosporins, penicillin and sensitivity to aminoglycosides, ciprofloxacin erythromycin, imipenem, and vancomycin.

In case of *Bacillus spp.* resistance observed was amikacin (0%), gentamicin (0%), ampicillin (28.57%), piperacillin/tazobactam (14.28%), meropenem (23.8%), imipenem (0%), cefepime (57.14%), azithromycin (42.85 %), ciprofloxacin (33.34%), vancomycin (4.76%), linezolid (4.76%), and tigecycline (4.76%). According to a study conducted in 2016 (Mandal et.al.2016) *Bacillus spp.* isolates were sensitive to amikacin, gentamicin, ampicillin, ciprofloxacin, meropenem, nalidixic acid and vancomycin antibiotics. This partially corresponds with our

findings where *Bacillus spp.* isolates are sensitive to amikacin, gentamicin, and vancomycin antibiotics.

In *Enterococcus faecalis* isolates resistance observed in the antibiotics was amikacin (0%), gentamicin (9.09%), ampicillin (54.54%), piperacillin/tazobactam (0%), meropenem (27.27%), imipenem (0%), cefepime (27.27%), azithromycin (9.09%), ciprofloxacin (0%), vancomycin (27.27%), linezolid (27.27%), and tigecycline (0%). Till now there is no peer reviewed journal that has detected the presence of *Enterococcus faecalis* in cosmetics.

In *Streptococcus* species isolates resistance observed was amikacin (0%), gentamicin (0%), ampicillin (37.5%), piperacillin/tazobactam (37.5%), meropenem (37.5%), imipenem (25%), cefepime (75%), azithromycin (50%), ciprofloxacin (0%), vancomycin (25%), linezolid (25%), and tigecycline (12.5%). Studies (Onurdağ et.al., 2010) have isolated *Streptococcus spp.* from cosmetics, however the isolates were not tested for antibiotic susceptibility.

In *Lactobacillus spp*. all the isolates were multidrug resistant. Resistance observed was amikacin (0%), gentamicin (0%), ampicillin (50%), piperacillin/tazobactam (100%), meropenem (100%), imipenem (50%), cefepime (100%), azithromycin (0%), ciprofloxacin (0%), vancomycin (0%), linezolid (0%), and tigecycline (0%). Lactobacillus spp. was isolated in a study (Bashir et. al., 2019) but the isolates were not tested for their antibiotic susceptibility.

In *Listeria monocytogens* all the isolates were multidrug resistant. Resistance observed was amikacin (0%), gentamicin (0%), ampicillin (100%), piperacillin/tazobactam (0%), meropenem (0%), imipenem (0%), cefepime (75%), azithromycin (0%), ciprofloxacin (50%), vancomycin (25%), linezolid (0%), and tigecycline (0%). This is partially in accordance with a study (Mandal et.al., 2016) where *Listeria monocytogens* is sensitive to gentamicin, amikacin, ampicillin, ciprofloxacin and meropenem. However, in this study ampicillin was completely resistant to *Listeria monocytogens*.

Chapter 5: Conclusion

In our study nearly all the cosmetics were found to contain a high level of contamination. The level of contamination found exceeded the FDA limit. Pathogenic organisms such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Listeria monocytogens* species of *Salmonella, Shigella Streptococcus, Staphylococcus, Bacillus and Lactobacillus* were identified. This is a cause for concern as Staphylococcus is the most common bacterial skin pathogen (Myers et al., 1973, Aly et al., 1966, Sugeng et al., 1999) and can cause various skin diseases. *Enterococcus faecalis* can cause meningitis. Another concerning finding was that most of the antibiotics tested on the bacterial samples were found to be multidrug resistant. A way to reduce the level of contamination in cosmetic products could be to implement rigid microbiological quality control testing as well as maintaining and improving personal hygiene.

Chapter 6: References

- Abdelaziz, A. A., Ashour, M. S. E., Hefni, H., & El-Tayebt, O. M. (1989). MICROBIAL CONTAMINATION OF COSMETICS AND PERSONAL CARE ITEMS IN EGYPT—SHAVING CREAMS AND SHAMPOOS. *Journal of Clinical Pharmacy and Therapeutics*, *14*(1), 29–34. https://doi.org/10.1111/j.1365-2710.1989.tb00218.x

- Akin, A., & Altanlar, N. (1989). Yurdumuzda uretilen ve kullanilan dudak boyalarinin mikrobiyolojik karlite kontrolleri üzerinde araştirmalar [Microbiological quality control of lipsticks which are on the market in our country]. Mikrobiyoloji bulteni, 23(4), 369–378.

- AKGÜL, M., & BAKAN, K. (2021). AEROBIC BACTERIA ISOLATED FROM USED COSMETIC PRODUCTS AND EVALUATION OF ANTIBIOTIC RESISTANCE. Ankara Universitesi Eczacilik Fakultesi Dergisi. https://doi.org/10.33483/jfpau.850561

- Aly, R. (1996). Microbial Infections of Skin and Nails. In S. Baron (Ed.), Medical Microbiology. (4th ed.). University of Texas Medical Branch at Galveston.

- Aly, R. (1999). Staphylococcal infections. Atlas of Infections of the Skin. (3rd ed) New York: Churchill Livingstone

- Aslam, S., Rahman, S. U., Sabir, Z., & Maqbool, B. (2017). EVALUATION OF COSMETICS FOR THEIR POTENTIAL CONTAMINANTS AND DRUG RESISTANT MICROORGANISMS. *Acta Scientifica Malaysia*, *1*(2), 16–19. https://doi.org/10.26480/asm.02.2017.16.19

- Babalola, M., & Eze, M. (2018). Microbiological Quality and Characterization of Potential Pathogens Associated with Selected Brands of Commercial Cosmetic Products in

Nigeria. *Microbiology Research Journal International*, *9*(5), 1-17. https://doi.org/10.9734/BMRJ/2015/14083

- Cano, R. J., & Colome, J. S. (1988). *Essentials of Microbiology*. Thomson Learning.
- Campana, R., Scesa, C., Patrone, V., Vittoria, E., & Baffone, W. (2006). Microbiological study of cosmetic products during their use by consumers: health risk and efficacy of preservative systems. Letters in applied microbiology, 43(3), 301–306. https://doi.org/10.1111/j.1472-765X.2006.01952.x
- Center for Food Safety and Applied Nutrition. (2021, June 16). BAM Chapter 3: Aerobic Plate Count. U.S. Food and Drug Administration. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count
- Center for Food Safety and Applied Nutrition. (2020, August 24). *Small Businesses & Homemade Cosmetics: Fact Sheet*. U.S. Food and Drug Administration. https://www.fda.gov/cosmetics/resources-industry-cosmetics/small-businesses-homemade-cosmetics-fact-sheet
- Center for Food Safety and Applied Nutrition. (2021b, December 23). *Bacteriological Analytical Manual (BAM)*. U.S. Food and Drug Administration. https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam
- Dadashi, L., & Dehghanzadeh, R. (2016). Investigating incidence of bacterial and fungal contamination in shared cosmetic kits available in the women beauty salons. Health Promotion Perspectives, 6(3), 159–163. https://doi.org/10.15171/hpp.2016.25
- Elaine, B. (1989). The hazards of cosmetics. New York, Hasrper and Row, 1-5.
- Eldesoukey, R. M. M., & S Alqhtani, B. (2016). Comparative Microbiological Study between Traditional and Modern Cosmetics in Saudi Arabia. *Enzyme Engineering*, *05*(02). https://doi.org/10.4172/2329-6674.1000146
- Feroz, F., & Das, K. K. (2020). Presence of microorganisms in commonly used baby cosmetics, available in Dhaka City. *Stamford Journal of Microbiology*, *9*(1), 9–11. https://doi.org/10.3329/sjm.v9i1.45650
- Guleria, A. (2012). Isolation And Identification of Bacteria from Different Cosmetic Samples and to Check Antimicrobial Activity of Antibiotics on Bacteria Isolated. *International Journal of Scientific Research*, *3*(2), 462–465. https://doi.org/10.15373/22778179/feb2014/153

- Hugbo, P. G., Onyekweli, A. O., & Igwe, I. (2005). Microbial contamination and preservative capacity of some brands of cosmetic creams. *Tropical Journal of Pharmaceutical Research, 2*(2). https://doi.org/10.4314/tjpr.v2i2.14604

- Jairoun, A. A., Al-Hemyari, S. S., Shahwan, M., & Zyoud, S. H. (2020). An Investigation into Incidences of Microbial Contamination in Cosmeceuticals in the UAE: Imbalances between Preservation and Microbial Contamination. *Cosmetics*, *7*(4), 92. https://doi.org/10.3390/cosmetics7040092

- Jimenez, L., Ignar, R., Smalls, S., Grech, P., Hamilton, J., Bosko, Y., & English, D. (1999). Molecular detection of bacterial indicators in cosmetic/pharmaceuticals and raw materials. *Journal of Industrial Microbiology and Biotechnology*, *22*(2), 93–95. https://doi.org/10.1038/sj.jim.2900611

- Khanom S, Das KK, Banik S & Noor R. (2013). Microbiological analysis of liquid oral drugs available in Bangladesh. International Journal of Pharmacy and Pharmaceutical Science, 5(4):479- 482. https://www.researchgate.net/publication/256324356_Microbiological_analysis_of_liquid_oral_drugs_available_in_Bangladesh

- Makeup Market Size, Share & COVID-19 Impact Analysis, By Product (Powder, Gels, Lotions, and Others), Application Area (Lips, Eyes, Face, and Others), Distribution Channel (Supermarkets/Hypermarkets, Exclusive Brand Stores, Online/e-commerce Channels, and Others), and Regional Forecast, 2021–2028. (21–11). Fortune Business Insights. https://www.fortunebusinessinsights.com/makeup-market-102587

- Myers, G. E. & Pasutto, F. M. (1973) Microbial contamination of cosmetics and toiletries. Can J Pharm Sci, 8, 19–23.

- Nandi, S., & Mandal, S. (2016). Bacteriological Profiling of Commercially Available Eye Cosmetics and their Antibiotic Susceptibility Pattern. *Translational Biomedicine*, *7*(3). https://doi.org/10.21767/2172-0479.100080

- Neza, E., & Centini, M. (2016). Microbiologically Contaminated and Over-Preserved Cosmetic Products According Rapex 2008–2014. *Cosmetics*, *3*(1), 3. https://doi.org/10.3390/cosmetics3010003

- Noor, R., Zerin, N., Das, K. K., & Nitu, L. N. (2015). Safe usage of cosmetics in Bangladesh: a quality perspective based on microbiological attributes. *Journal of Biological Research-Thessaloniki*, *22*(1). https://doi.org/10.1186/s40709-015-0033-4

- Office of the Commissioner. (2019, April 1). U.S. Food and Drug Administration. https://www.fda.gov/cosmetics/cosmetics-labeling-regulations/cosmetics-labeling-guide

- Onurdağ, F. K., Özgen, S. & Abbasoğlu, D. (2010). Microbiological Investigation of Used Cosmetic Samples. Hacettepe University Journal of the Faculty of Pharmacy, (1), 1-16. https://dergipark.org.tr/en/pub/hujpharm/issue/49839/639115

- Orth, D. S. (1993). *Handbook of Cosmetic Microbiology (Cosmetic Science and Technology, v. 12)* (1st ed.). Marcel Dekker, Inc.

- Özalp, M (1998). Kozmetik Ürünlerde Görülen Mikrobiyolojik Kontaminasyonlar, Türkiye Klinikleri Kozmetoloji Dergisi, 1(3), 167-176. https://www.turkiyeklinikleri.com/article/en-kozmetik-urunlerde-gorulenmikrobiyolojik-kontaminasyonlar-32341.html

- Ravita, T. D., Tanner, R. S., Ahearn, D. G., Arms, E. L., & Crockett, P. W. (2009). Post-consumer use efficacies of preservatives in personal care and topical drug products: relationship to preservative category. Journal of industrial microbiology & biotechnology, 36(1), 35–38. https://doi.org/10.1007/s10295-008-0468-9

- Razooki, R.A., Saeed, E.N., & Hamza, H. (2009). A Study on Cosmetic Products Marketed in Iraq: Microbiological Aspect. Iraqi Journal of Pharmaceutical Sciences, 18, 20-25.

- Reid, F. R., & Wood, T. O. (1979). Pseudomonas corneal ulcer. The causative role of contaminated eye cosmetics. Archives of ophthalmology (Chicago, Ill.: 1960), 97(9), 1640–1641. https://doi.org/10.1001/archopht.1979.01020020208002

- *Scientific Committee on Consumer Safety (SCCS)*. (n.d.). Public Health. https://ec.europa.eu/health/scientific-committees/scientific-committee-consumer-safety-sccs_en

- Shaown SA. Pharmaceutical Industry of Bangladesh. Dhaka: IDLC finance limited; 2011.

- Smart, R., & Spooner, D. F. (1972). Microbiological spoilage in pharmaceuticals and cosmetics. J Soc Cosmet Chem, 23, 721-737.

- Sugeng, M. W., Ang, P., Tan, H. H., & Goh, C. L. (1999). Characteristics of bacterial skin infections in children compared to adults at a tertiary dermatologic center. International

journal of dermatology, 38(8), 582–586. https://doi.org/10.1046/j.1365-4362.1999.00740.x

- Turnbull, P. C., Sirianni, N. M., LeBron, C. I., Samaan, M. N., Sutton, F. N., Reyes, A. E., & Peruski, L. F., Jr (2004). MICs of selected antibiotics for Bacillus anthracis, Bacillus cereus, Bacillus thuringiensis, and Bacillus mycoides from a range of clinical and environmental sources as determined by the Etest. Journal of clinical microbiology, 42(8), 3626–3634. https://doi.org/10.1128/JCM.42.8.3626-3634.2004

- Wilson, L. A., & Ahearn, D. G. (1977). Pseudomonas-induced corneal ulcers associated with contaminated eye mascaras. American journal of ophthalmology, 84(1), 112–119. https://doi.org/10.1016/0002-9394(77)90334-8

- Wilson, L. A., Julian, A. J., & Ahearn, D. G. (1975). The survival and growth of microorganisms in mascara during use. American journal of ophthalmology, 79(4), 596–601. https://doi.org/10.1016/0002-9394(75)90798-9