

## DETERMINATION OF MICROBIAL LOAD FROM SOIL SAMPLES IN DIFFERENT AREAS OF DHAKA CITY

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### ABSTRACT

**Background:** Soil is the region on the earth's crust where geology and biology meet, the land surface that provides a home to plant animal and microbial life. Soil teems with microscopic life such as earthworms, nematodes, mites, and insects, and the root systems of plants. Soil samples from different areas of Dhaka were studied to determine microbial load and their antibiotic profile in the sample.

**Objectives:** This study was aimed to find out microbial load from soil samples, as how much gram positive & negative bacteria or other microorganisms are seen in soil samples.

**Materials & Methods:** For study purpose soil was taken as samples. Total 3 samples were collected aseptically from Hospital Based area, fish and vegetables-based area and University based area. A total of 11 single colonies were picked up and cultured in MIU medium for further analysis. All isolates were identified by standard microbiological technique and their antibiotic susceptibility was observed by Kirby-bauer method (disk diffusion method). Strict aseptic precaution was taken all through the culture systems.

**Results:** This study showed a high microbial growth in the samples. Growth in Muller Hinton agar media showed whitish, copper yellowish, some of which were sticky colony in the culture plate. MacConkey agar media showed pink colony. Eleven colonies were isolated, cultured & characterized by gram staining & biochemical test. Six isolates were found to be gram positive while five were gram negative. The antibiotic sensitivity was carried out to know the pattern of resistance. It was found that all bacterial isolates were sensitive to gentamicin, co-trimoxazole, ciprofloxacin, azithromycin and ampicillin. About 95% of the bacterial isolates were resistant to chloramphenicol, ceftazidime, cefotaxime, colistin respectively.

**Conclusion:** The outcome of this study has been able to show that diverse types of bacteria were isolated from different areas. The abundance of bacteria in this study were typical of environment with high species richness & functional diversity. This should be a subject of extension of this investigation in future.

**Keywords:** Soil Microbiology; Microbial Load; Antibiotic Resistance; Gram-Positive and Gram-Negative Bacteria; Dhaka City

## INTRODUCTION

Soil is one of the most complex and biologically active ecosystems on Earth. It serves as a natural reservoir of diverse microorganisms that play a vital role in maintaining environmental balance and sustaining life. The microbial population in soil represents a significant proportion of the Earth's total biomass and contributes substantially to global biogeochemical cycles<sup>1</sup>. These microorganisms include bacteria, archaea, fungi, actinomycetes, algae, and protozoa. Among them, bacteria are the most abundant and metabolically versatile group, capable of surviving under a wide range of environmental conditions, including extreme heat, cold, salinity, and pressure<sup>2</sup>.

Soil microorganisms are essential for nutrient cycling, decomposition of organic matter, nitrogen fixation, and maintenance of soil fertility. They contribute to plant growth promotion and ecological stability. In addition to their ecological significance, soil microbes are also of immense industrial and medical importance<sup>3</sup>. Many clinically important antibiotics such as streptomycin, tetracycline, and erythromycin were originally isolated from soil microorganisms, particularly from the genus *Streptomyces*. Soil bacteria are also known to produce various bioactive secondary metabolites, enzymes (e.g., proteases, amylases, lipases), and other compounds with pharmaceutical and biotechnological applications<sup>4</sup>.

However, the composition and abundance of soil microorganisms vary depending on environmental factors such as soil texture, moisture content, pH, temperature, organic matter, nutrient availability, and vegetation cover. Urban environments, particularly densely populated cities like Dhaka, experience continuous environmental stress due to industrialization, hospital waste disposal, market dumping sites, sewage contamination, and excessive use of antibiotics in human and veterinary medicine. These anthropogenic activities significantly influence soil microbial ecology<sup>5</sup>.

One of the most alarming global public health concerns is antibiotic resistance. The misuse and overuse of antibiotics in human healthcare, livestock farming, aquaculture, and agriculture have accelerated the emergence and spread of resistant bacteria. In many developing countries, including Bangladesh, antibiotics are often available without prescription, increasing the risk of resistance development<sup>6</sup>. When antibiotic residues and resistant bacteria enter the environment through hospital waste, domestic sewage, and market dumping areas, they contaminate soil ecosystems. Soil thus becomes a reservoir of antibiotic-resistant bacteria and resistance genes<sup>7</sup>.

These resistant microorganisms can transfer resistance genes to other pathogenic bacteria through horizontal gene transfer mechanisms such as conjugation, transformation, and transduction. Consequently, resistant bacteria may enter the human food chain through contaminated vegetables, water, or direct contact, posing a serious threat to public health. The presence of resistant Gram-positive and Gram-negative bacteria in urban soil environments can reduce the effectiveness of commonly used antibiotics, limiting treatment options for infectious diseases<sup>8</sup>.

Dhaka city, being one of the most densely populated metropolitan areas in the world, contains diverse environmental settings including hospital surroundings, fish markets, vegetable markets, residential areas, and waste dumping sites. Each of these locations may harbor distinct microbial communities with varying microbial loads and antibiotic resistance patterns<sup>9</sup>. Therefore, systematic investigation of soil microbial load and resistance patterns in different areas of Dhaka city is essential to understand environmental contamination and potential public health risks. The present study aims to determine and compare the microbial load in soil samples collected from different areas of Dhaka city and to assess the antibiotic resistance patterns of isolated Gram-positive and Gram-negative bacteria.

## **METHODOLOGY**

The present study was a laboratory-based cross-sectional study conducted to determine the microbial load and antibiotic resistance pattern of bacteria isolated from soil samples collected from different areas of Dhaka city. All laboratory analyses were performed in the Department of Microbiology, Bangladesh University of Health Sciences (BUHS), under standard microbiological procedures and aseptic conditions. The study was carried out over a period of three months, from September 2021 to November 2021.

Soil samples were collected from three selected locations of Dhaka city, namely the BIHS General Hospital area, Boro Bazar raw vegetables market area, and Jahangirnagar University area. These sites were selected to represent hospital surroundings, commercial market environments, and comparatively less contaminated academic campus areas. A total of six soil samples were collected. Approximately 100 grams of soil was collected from each site from the upper 4–6 cm depth of the soil surface, as this layer contains the highest concentration of microbial activity. Sterile spatulas were used during collection, and the samples were transferred into clean, dry, sterile zip-lock polythene bags. Each sample was properly labeled with sampling site, date, and identification number. The samples were transported immediately to the Microbiology laboratory of BUHS for further processing.



**A. Sample Collection in Jahangirnagar area**



**B. Raw Vegetables Market Area**



**C. Sample Collection in BIHS Hospital area**  
**Figure 1: Sample Collection Area**

In the laboratory, serial dilution technique was applied for quantitative estimation of microbial load. Initially, 0.1 gram of soil sample was added into a test tube containing 10 ml of sterile Tryptone Soya Broth (TSB) and mixed thoroughly to prepare the primary suspension. From this suspension, 1 ml was transferred into another tube containing 9 ml sterile broth to obtain a  $10^{-1}$  dilution. Subsequent serial dilutions were prepared up to  $10^{-5}$  by transferring 1 ml from the previous dilution into the next tube containing 9 ml sterile broth. From each selected dilution, 0.1 ml of suspension was inoculated onto sterile Mueller-Hinton agar plates using the spread plate technique. The inoculum was evenly distributed over the surface of the agar using a sterile glass spreader. The inoculated plates were incubated aerobically at  $37^{\circ}\text{C}$  for 24 hours.

After incubation, visible colonies were observed and counted. Only plates containing 30–300 colonies were considered for calculation, as this range is regarded as statistically reliable. Plates with fewer than 30 colonies were considered too low to count, and those with more than 300 colonies were considered too numerous to count. The microbial load was calculated and expressed as Colony Forming Units per gram (CFU/g) of soil using the standard formula based on colony count, dilution factor, and inoculum volume.

Distinct colonies showing different morphological characteristics such as size, shape, color, margin, and elevation were selected from countable plates for isolation. A single well-isolated colony was picked using a sterile inoculating loop and subcultured onto fresh Mueller-Hinton agar plates to obtain pure cultures. The plates were incubated at  $37^{\circ}\text{C}$  for 24 hours. Pure isolates were then subjected to Gram staining and biochemical tests for identification.

Gram staining was performed to differentiate Gram-positive and Gram-negative bacteria based on cell wall characteristics. Smears were prepared on clean glass slides, air dried, heat fixed, and sequentially stained with crystal violet, Gram's iodine, alcohol for decolorization, and safranin as counterstain. The stained slides were examined under oil immersion microscopy. Further identification was carried out by performing standard biochemical tests including catalase test, coagulase test, oxidase test, Simmons citrate test, Triple Sugar Iron (TSI) test, Motility Indole Urea (MIU) test, and urease test. Results were interpreted according to standard microbiological guidelines.

Antibiotic susceptibility testing of the isolated bacteria was performed by the Kirby–Bauer disc diffusion method on Mueller-Hinton agar. A fresh bacterial suspension was prepared in sterile normal saline and its turbidity was adjusted to match the 0.5 McFarland standard to ensure uniform bacterial density. The standardized suspension was uniformly spread over the surface of Mueller-Hinton agar plates using a sterile swab. Antibiotic discs were placed on the inoculated agar surface using sterile forceps, and the plates were incubated at  $37^{\circ}\text{C}$  for 18–24 hours. After incubation, the diameter of the zone of inhibition around each disc was measured in millimeters. The isolates were categorized as sensitive, intermediate, or resistant according to standard interpretative criteria.

Quality control procedures were strictly maintained throughout the study. Standard reference strains obtained from ATCC were used for validation of media performance and antibiotic susceptibility testing. All culture media were prepared according to manufacturer's instructions, sterilized by autoclaving, and checked for sterility before use. Laboratory procedures were conducted under aseptic conditions to prevent contamination.

All collected data, including colony counts and antibiotic susceptibility results, were recorded systematically. Microbial load values were calculated as CFU per gram of soil, and resistance patterns

of Gram-positive and Gram-negative bacteria were analyzed and compared among the different sampling sites to evaluate variation in microbial burden and antibiotic resistance across the selected areas of Dhaka city.

## RESULTS

Soil samples were collected between September and November from three selected locations in Dhaka city: the BIHS General Hospital area, Boro Bazar raw vegetables market, and Jahangirnagar University area. Following laboratory processing, the isolates obtained from these samples were subjected to Gram staining, biochemical characterization, and antibiotic susceptibility testing. The findings of these analyses are presented below.



**Figure 2: Collected three samples from different area**

After inoculation and incubation of soil samples on Mueller-Hinton agar plates, diverse bacterial colonies were observed from all three sampling locations in Dhaka city. The colonies exhibited variations in size, color, texture, and morphology. The highest colony count was recorded from the BIHS Hospital area, followed by Jahangirnagar University, while the lowest count was observed in the Mirpur Boro Bazar area. A number of representative colonies from each site were selected for subculture and further characterization.

**Table 1: Colony Characteristics and Total Bacterial Count from Soil Samples on Mueller-Hinton Agar**

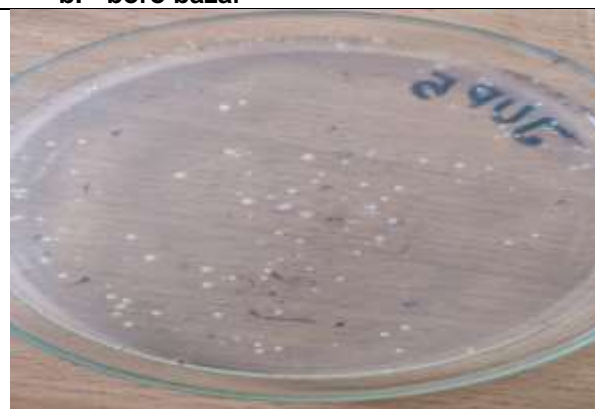
Sample Collected Area	Depending of different Colony Characteristics	Total count of colony in 5 no plate	Total subculture colony
BIHS Hospital Area	Small size, Big whitish, Sticky mucoid, copper yellowish	400	4
Mirpur Boro Bazar Area	Sticky mucoid, Small & Big size Whitish, copper yellowish, pale	93	4
Jahangirnagar University Area	Pale, small Mucoid Whitish, copper yellowish	160	3



**a. BIHS Area**



**b. boro bazar**



**c. Jahangirnagar area**

**Figure 1.1: Bacterial colonies Growth**

A total of 11 distinct single colonies were selected from the primary culture plates and subcultured in MIU medium for further identification and characterization. To determine the morphological and biochemical properties of the isolates, Gram staining and a series of standard biochemical tests were performed. Among the isolates, approximately 60% were identified as Gram-positive bacteria and 40% as Gram-negative bacteria. Microscopic examination revealed different cellular morphologies, including cocci (round-shaped), bacilli (rod-shaped), and cluster-forming organisms. Biochemical analysis demonstrated the presence of important enzymes such as catalase, coagulase, and oxidase in several isolates, indicating metabolic diversity among the soil bacteria. The isolated organisms were further evaluated for their susceptibility to commonly used clinical antibiotics in order to assess their resistance patterns. Antibiotic susceptibility testing was performed separately for Gram-positive and Gram-negative isolates using selected antibiotic discs. The detailed antibiotic profile and resistance pattern of the isolates are presented in the following tables.

**Table 2: Biochemical Characterization and Gram Staining Results of Isolates from BIHS Hospital Area**

ID no.	Oxidase	Catalase	Coagulase	M	I	U	TSI	Citrate	Gram Stain
S1A	-	+	+	-	-----	+	Butt- yellow Slunt- Red	-	Gram (+) rod shape
S1	-	+	-	-	-----	+	Butt- red Slunt- red H2s weakly(+)	+	Gram (-) rod shape
S2P	-	-	-	-	-----	-	Butt- yellow Slunt- red	+	Gram (-) round shape
S2	-	+	+	-	-----	-	Butt- yellow Slunt- red H2s weakly(+)	-	Gram (+) rod shape



a. Growth of isolates on Muller Hinton Agar



**Figure 2.1: Culture Characteristics of Isolates on Selective and Non-Selective Media**

**Table-2.1: Antibigram chart for BIHS HOSPITAL AREA Sample for Gram Positive Samples**

ID no.	Antibiotics Disk	Test Result
S1A	1.Cotrimoxazole	S
	2.Azithromycine	I
	3.Doxycycline	S
	4.Ampicilin	S
	5.Gentamicine	S
	6.Ceftazidime	R
	7.Cefotaxime	R



**Figure 2.2: Antibiogram test for Sample S1A**

**Table-2.2: Antibiogram chart for BIHS HOSPITAL AREA Sample for Gram Positive Samples**

ID no.	Antibiotics Disk	Test Result
S2	1.Cotrimoxazole	S
	2.Azithromycine	I
	3.Doxycycline	R
	4.Ampicilin	I
	5.Gentamicine	S
	6.Ceftazidime	R
	7.Cefotaxime	R



Figure 2.3: Antibiogram test for Sample S2

Table-2.3: Antibiogram chart for BIHS HOSPITAL AREA Sample for Gram Negative Samples

ID no.	Antibiotics Disk	Test Result
S1	1.Chloramphenical	R
	2.Ciprofloxacin	I
	3.Colistin	I
	4.Aztreonam	R
	5.Gentamicine	S
	6.Ceftazidime	R
	7.Cefoxitin	R



Figure 2.4: Antibiogram test for Sample S1

**Table-2.4: Antibiogram chart for BIHS HOSPITAL AREA Sample for Gram Negative Samples**

ID no.	Antibiotics Disk	Test Result
S2P	1.Chloramphenical	R
	2.Ciprofloxacin	R
	3.Colistin	S
	4.Aztreonam	R
	5.Gentamicine	S
	6.Ceftazidime	R
	7.Cefoxitin	R



**Figure 2.5: Antibiogram test for Sample S2P**

**Table-3: Result interpretation chart for Mirpur Boro Bazar Sample**

ID no.	Oxidase	Catalase	Coagulase	M	I	U	TSI	Citrate	Gram Stain
BBS5 P1	-	-	-	-	-----	-	Butt-red Slunt-yellow	+	Gram (+) cocci
BBS5 P2	-	-	-	-	-----	-	Butt-yellow Slunt-yellow	+	Gram (-) bacilli

BBS5 P3	-	Slightly (+)	+	-	-----	-	Butt- red Slunt- red	-	Gram (-) bacilli
BBS5 P4	-	+	+	-	-----	+	Butt- yellow Slunt- yellow	-	Gram (+) Cocci



Figure 3.1: Growth of isolates subculture on Muller Hinton Agar

Table-3.1: Antibigram chart for Mirpur Boro Bazar Sample for Gram Positive Samples

ID no.	Antibiotics Disk	Test Result
BBS5 P1	1.Cotrimoxazole	S
	2.Azithromycine	R
	3.Doxycycline	R
	4.Ampicilin	R
	5.Gentamicine	S
	6.Ceftazidime	I
	7.Cefotaxime	I



Figure 3.2: Antibiogram test for Sample BBS5P1

Table-3.2: Antibiogram chart for Mirpur Boro Bazar Sample for Gram Positive Samples

ID no.	Antibiotics Disk	Test Result
BBS5 P4	1.Cotrimoxazole	S
	2.Azithromycine	S
	3.Doxycycline	S
	4.Ampicilin	R
	5.Gentamicine	S
	6.Ceftazidime	R
	7.Cefotaxime	R



Figure 3.3: Antibiogram test for Sample BBS5P4

Table-3.3: Antibiogram chart for Mirpur Boro Bazar Sample for Gram Negative Samples

ID no.	Antibiotics Disk	Test Result
BBS5 P2	1.Chloramphenical	R
	2.Ciprofloxacin	I
	3.Colistin	S
	4.Aztreonam	R
	5.Gentamicine	I
	6.Ceftazidime	R
	7.Cefoxitin	R



Figure 3.4; Antibiogram test for Sample BBS5P2

Table-3.4: Antibiogram chart for Mirpur Boro Bazar Sample for Gram Negative Samples

ID no.	Antibiotics Disk	Test Result
BBS5 P3	1.Chloramphenical	R
	2.Ciprofloxacin	S
	3.Colistin	S
	4.Aztreonam	R
	5.Gentamicine	S
	6.Ceftazidime	R
	7.Cefoxitin	R



Figure 3.5: Antibiogram test for Sample BBS5P3

Table-4: Result interpretation chart for Jahangirnagar University

ID no.	Oxidase	Catalase	Coagulase	M	I	U	TSI	Citrate	Gram Stain
JUS1	-	+	+	-	-----	-	Butt- red Slant- red	+	Gram(+) cocci
JUS2	-	+	+	-	-----	-	Butt- yellow Slant- yellow	Slightly (+)	Gram (+) cluster shape cocci
JUS3	+	+	-	+	-----	+	Butt- yellow Slant- yellow	-	Gram (-) Bacilli



Figure 4.1: Growth of isolates subculture on Muller Hinton Agar

Table-4.1: Antibigram chart for Jahangirnagar University for Gram Positive Samples

ID no.	Antibiotics Disk	Test Result
JUS1	1.Cotrimoxazole	S
	2.Azithromycine	I
	3.Doxycycline	S
	4.Ampicilin	R
	5.Gentamicine	S
	6.Ceftazidime	R
	7.Cefotaxime	R



Figure 4.2: Antibigram test for Sample JUS1

**Table-4.2: Antibiogram chart for Jahangirnagar University for Gram Positive Samples**

ID no.	Antibiotics Disk	Test Result
JUS2	1.Cotrimoxazole	S
	2.Azithromycine	R
	3.Doxycycline	I
	4.Ampicilin	R
	5.Gentamicine	S
	6.Ceftazidime	R
	7.Cefotaxime	R

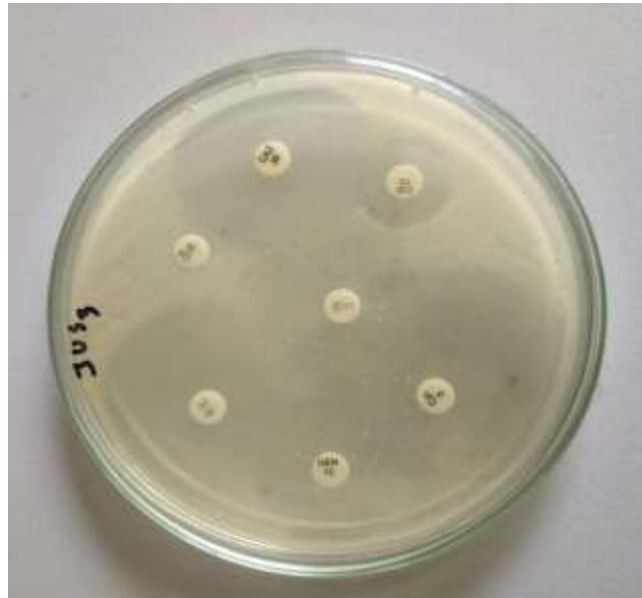


**Figure 4.3: Antibiogram test for Sample JUS2**

**Table-4.3: Antibiogram chart for Jahangirnagar University for Gram Negative Samples**

ID no.	Antibiotics Disk	Test Result
JUS3	1.Chloramphenical	R
	2.Ciprofloxacin	S
	3.Colistin	R
	4.Aztreonam	R

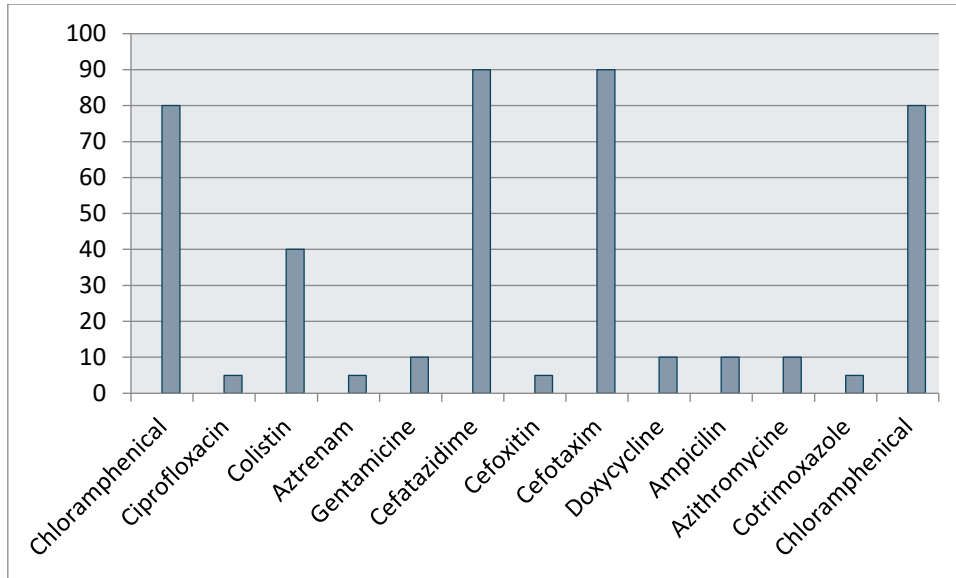
	5.Gentamicine	S
	6.Ceftazidime	R
	7.Cefoxitin	R



**Figure: 4.4 Antibiogram test for Sample JUS3**

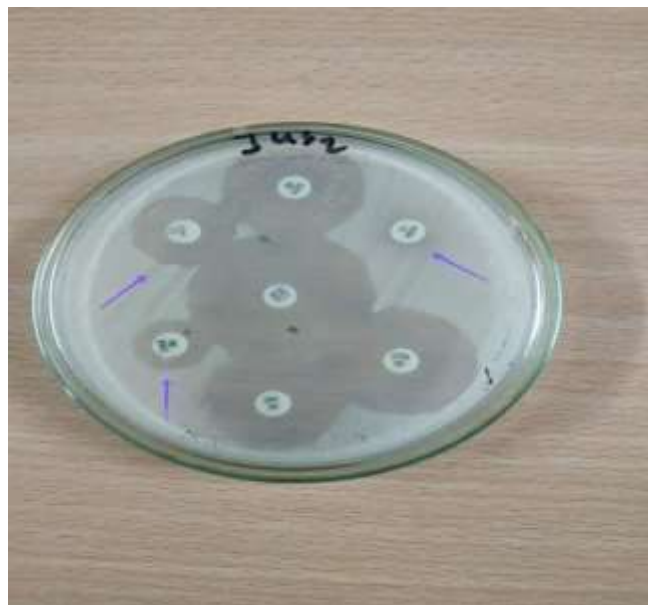
Among the Gram-positive bacterial isolates obtained from soil samples, a high level of resistance was observed against several commonly used antibiotics. Approximately 95% of the isolates demonstrated resistance to ceftazidime, cefotaxime, and ampicillin. Considerable resistance was also noted against doxycycline and azithromycin. In contrast, a high level of susceptibility (approximately 95%) was observed for cotrimoxazole and gentamicin, indicating their comparatively better effectiveness against the Gram-positive isolates.

For Gram-negative bacterial isolates, an overall high resistance pattern was also observed. Out of the seven antibiotic discs tested, four antibiotics demonstrated consistently high resistance among the isolates. These included chloramphenicol, aztreonam, ceftazidime, and cefoxitin. Colistin showed resistance in only one isolate, while the remaining isolates exhibited susceptibility. Other tested antibiotics showed relatively better sensitivity profiles compared to the highly resistant drugs. These findings indicate the presence of multidrug-resistant bacterial strains in the soil samples studied.



**Figure 5: Percent of antibiotic resistance against isolated soil bacteria**

In this study, soil-derived bacterial isolates were evaluated to determine their resistance patterns against antibiotics commonly used in clinical practice. It is noteworthy that more than 80% of currently used antibiotics have originally been derived from soil microorganisms, highlighting the ecological and medical importance of soil bacteria. The susceptibility testing results demonstrated that all isolates were highly sensitive to gentamicin, cotrimoxazole, ciprofloxacin, and azithromycin. High resistance was observed against chloramphenicol, ceftazidime, and cefotaxime, with approximately 95% of isolates showing resistance to these antibiotics. Moderate levels of resistance were recorded for ampicillin (40%) and ceftaxitin (30%), while lower resistance rates were observed for aztreonam (10%) and doxycycline (5%).

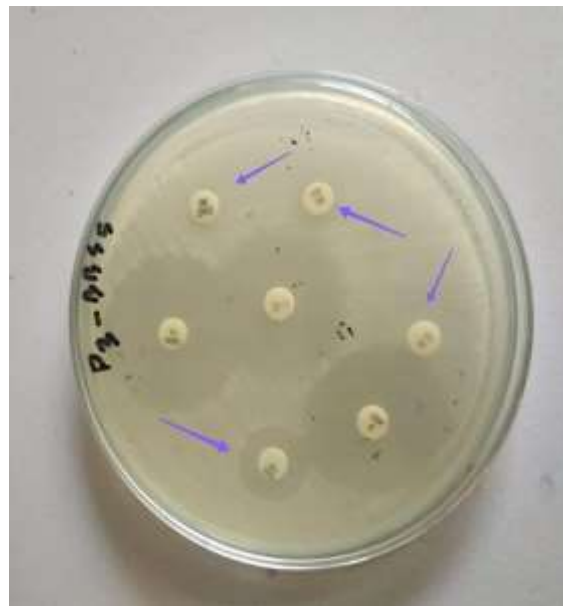


**Figure 6.1: Resistance pattern of JUS2**



**Figure 6.2: Resistance pattern BBS5P2**

The increasing prevalence of antibiotic resistance has made it essential to develop novel antimicrobial agents to address the emerging challenges in the treatment of infectious diseases. In this context, the potential of soil bacterial isolates to produce bioactive compounds capable of inhibiting the growth of other microorganisms was investigated.



**Figure 6.3: Resistance pattern BBS5P3**

All isolates were evaluated for antibacterial activity against both Gram-positive and Gram-negative test organisms. Several soil-derived bacterial isolates demonstrated bactericidal activity against pathogenic bacteria, indicating their ability to inhibit microbial growth. These findings suggest that the isolates may produce bioactive compounds with inhibitory or bactericidal properties.

## DISCUSSION

Due to the warm and humid climatic conditions of Bangladesh, waste dumping areas provide a favorable environment for bacterial growth and proliferation. In the present study, bacterial populations were investigated in different soil environments of Dhaka city, including fish and vegetable waste dumping sites, hospital-based areas, and university campus areas. Soil samples collected from these locations revealed diverse bacterial colonies, which were initially differentiated based on colony morphology and pigmentation.

A total of 11 distinct colonies were selected and subcultured on Mueller-Hinton agar for further characterization. Gram staining and standard biochemical tests were performed to identify the isolates. Among the total isolates, approximately 60% were Gram-positive and 40% were Gram-negative bacteria. Microscopic examination showed both cocci (round-shaped) and bacilli (rod-shaped) forms. Preliminary biochemical screening demonstrated the presence of important enzymes such as oxidase, catalase, and coagulase among several isolates.

The study also revealed a concerning pattern of antibiotic resistance among the isolates. Several clinically important antibiotics showed high resistance rates, which is alarming from a public health perspective. The highest bacterial load was observed in hospital-based and vegetable market soil samples, likely due to higher nutrient availability and continuous waste deposition. In contrast, the lowest bacterial count was recorded in university campus soil samples. This finding differs from some previous reports from other regions, where park and garden soils showed the highest bacterial counts<sup>10</sup>. Compared to earlier local studies, the resistance pattern observed in this study appears comparatively higher. Multiple antibiotic resistance was detected in both Gram-positive and Gram-negative isolates, highlighting the potential role of environmental soil as a reservoir of resistant bacteria<sup>11</sup>.

In recent years, the emergence of bacterial pathogens resistant to multiple antibiotics has become a major global concern. The increasing resistance trend emphasizes the urgent need to explore and develop novel antimicrobial agents. In this context, attention was given to evaluating whether the isolated soil bacteria possess the ability to produce bioactive compounds capable of inhibiting the growth of other microorganisms<sup>12</sup>.

## Conclusion

The present study revealed that soil samples from different areas of Dhaka city contain diverse Gram-positive and Gram-negative bacteria. Bacterial isolates were identified from hospital, vegetable market, and university soils, indicating widespread environmental contamination. The presence of bacteria in vegetable market soil suggests a potential risk for food-borne infections. A high level of antibiotic resistance was observed among the isolates, highlighting the public health significance of environmental monitoring and the need for rational antibiotic use. Further investigation is required to better understand resistance mechanisms and their potential impact on human health.

## Recommendation

Based on the findings of this study, continuous surveillance of environmental soil bacteria is recommended to monitor emerging antibiotic resistance patterns. Proper waste management, especially in hospital and market areas, should be strengthened to minimize environmental contamination.

Furthermore, soil microorganisms may serve as a valuable source for the discovery of novel antimicrobial compounds. Therefore, advanced research should be conducted to isolate, purify, and characterize potential bioactive substances produced by soil bacteria, which may contribute to the development of new therapeutic agents for the treatment of infectious diseases.

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