Thesis Report
Study on Antibiotic Resistance Profiles in Water Sample Focusing Dhaka City (Aftabnagar)

By
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A thesis submitted to the Department of Biotechnology in partial fulfillment of the requirements for the degree of B.Sc. Biotechnology .

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Declaration

It is hereby declared that

1. The thesis report submitted is my original work while completing my 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 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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Approval

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Ethics Statement

This research on the antibacterial resistance from the water sources of Bangladesh was conducted in an ethical manner so that it would be sound of a study and not harm the environment. It was an environmental study, but complete caution was taken to avoid nearby community and ecosystem impacts. Collected data will be handled in the virtue of confidentiality and intactness, so that authentic reports on results can be made without falsification and alteration. Consequently, meeting ethics on top of delivering a few ethical observations about antibiotics without violating the rights of either humans or nature.

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Abstract

Antibiotic resistant bacteria is a significant public health concern in urban surface water specially city areas like Dhaka. Aftabnagar, Dhaka, the minimum inhibitory concentration study of enteric bacteria isolated from water was conducted at a recoverable extent with antibiotic resistance profiles. This work specifically addressed three ecologically and clinically significant groups of bacteria: Escherichia coli, Salmonella spp., and Vibrio spp. Isolation was performed on selective media, such as Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar. Antibiotic susceptibility pattern of isolates was determined by Kirby-Bauer disk diffusion method. 110 bacterial isolates were recovered for this study. Of these organisms, *E. coli* was the most isolated organism at 42.73%, followed by *Salmonella spp.* at 34.55% and *Vibrio cholerae* at 22.73%. Antibiotic susceptibility results indicated recurrent resistance in each of the three bacterial groups are detected to be resistant to multiple drugs. *E. coli* showed 32.41%, *Vibrio cholerae* showed 32.31% and *Salmonella spp.* showed 40.28% resistance.These results suggest that among the studied water sources, the Aftabnagar source of water serves as probably an environmental reservoir for resistant bacteria. The findings provide first baseline data on the resistance patterns in an urban water body from Dhaka and highlight the need for continuous long-term environmental monitoring, better waste management systems, and education to raise public awareness about antibiotic resistances in aquatic environments.

Keywords: Antibacterial Resistance, Antibiotic Susceptibility Testing (AST), Escherichia coli, Gel electrophoresis, Polymerase Chain Reaction (PCR), Salmonella, Vibrio

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Acronyms

AMR - Antimicrobial Resistance

AST - Antibiotic Susceptibility Testing

DNA - Deoxyribonucleic Acid

DNTP - Deoxyribonucleotide Triphosphate

EMB - Eosin Methylene Blue

EtBr - Ethidium Bromide

MHA - Mueller Hinton Agar

NFW - water Nuclease free water

PCR - Polymerase Chain Reaction

SS agar - Salmonella-Shigella agar

TAE Buffer - Tris-Acetate-EDTA buffer

TCBS agar - Thiosulfate-Citrate-Bile Salts-Sucrose agar

TE Buffer - Tris-EDTA buffer

UV - Ultraviolet

Chapter 1

Introduction

1.1 Background of Antimicrobial Resistance

The development of resistance of microorganisms to antibiotics, called antimicrobial resistance (AMR), has emerged as one of the most significant global public health threats to date. The resistance of commonly used antibiotics, which prevents the effective inhibition or killing of pathogenic bacteria, makes many infections more difficult to treat, prolonged illness duration and greater treatment costs and thus increases mortality as well as the risk of complications. A recent large global study estimated that bacterial AMR directly caused 1.27 million deaths in 2019 and was implicated as a contributory factor in 4.95 million deaths globally, indicating that antibiotic resistance is now more than an issue of hospital hygiene but rather a global health crisis affecting the whole planet level. While resistance has long been a clinical topic of discussion, contemporary research focus is shifting to environmental reservoirs (and routes), particularly water, where resistant bacteria are enabled to persist and travel outside hospital and household walls.

1.2 Surface Water As A Reservoir Of Resistant Bacteria In The Environment

Surface water bodies e.g., rivers, lakes, ponds, canals or urban drainage-linked water systems, now widely recognised as important environmental reservoirs of antibiotic-resistant bacteria. Untreated or inadequately treated sewage, hospital waste, industrial effluents, runoff from settlements and residues of pharmaceuticals repeatedly enter these aquatic systems. These inputs not only chemically pollute water, but also make the ecological environment stay in the conditions where bacteria can endure through repeated exposure to contamination pressure. The environmental scenarios reviewed in AMR have demonstrated that wastewater associated environments are key centers for antibiotic resistant bacteria, and urban wastewater discharge plays an important environmental role as vehicles of these microorganisms delivered to wider aquatic systems. As a result surface water microbiology is being increasingly recognised as an integral component of AMR surveillance rather than merely part of the environmental discourse.

Bangladesh: 1.3 Surface Water Pollution

Surface water is an integral part of domestic use, agriculture, fisheries and urban life in Bangladesh. But several factors such as rapid urbanization, industrialisation, municipal sewage discharge and dam construction coupled with poor solid-waste management strategies and occurrence of emerging contaminants have adversely impacted water quality in a large number of regions across the country. Significant literature on Bangladesh's water contamination indicates microbial evidence attributable to both urban and non-urban water sources such as heavy metals, pesticides and pharmaceutical residues contribute therein. Antibiotic residues have also been described as emerging contaminants in Bangladesh water bodies in more recent assessments highlighting its importance particularly with respect to resistance development. Consequently, the microbiological quality of surface water in Bangladesh is an important public-health concern as well as a potential environmental issue.

1.4 Dhaka City as a Risky Urban Environment

Dhaka is one of the significant locations for environmental AMR studies conducted in cities in Bangladesh. Dhaka has a combination of very high population density, rapid growth of urban perimeter areas, and the complexity relating to water system connections draining into the environment through pipes, disposal takes place locally without treatment and sanitation management remains inadequate. These conditions allow fecal contamination and microbial pollution to transit multiple environmental transmission routes. Our results reflect the sanitation burden produced by urbanization in rapidly growing cities, where a major environmental study conducted in urban Dhaka reported fecal contamination across multiple environmental compartments. Furthermore, the metagenomic work from Bangladesh demonstrated a high abundance and diversity of resistance-related markers in urban surface waters and that human waste is an important driver of marine antibiotic resistance. These results validate Dhaka surface waters as a pertinent target for assessing non-clinical sources of bacterial resistance.

1.5 Reason for Selecting Aftabnagar, Dhaka

This study is related to a water sample of Aftabnagar, Dhaka. Study area Aftabnagar is a good choice of study for this topic as it is the part of Dhaka which has been urbanized and all

residential development around, runoff, waste deposition and human activities may impact water quality and microbial contamination. However, resistance patterns in environmental microbiology do not have a homogenous and vertical structure. They can be influenced by local contamination pressure, extent of sewage influence, hydrological conditions and surrounding human activity. That is why a specific chain survey from Aftabnagar is scientifically important in that it can develop localized baseline information as opposed to generalized city level estimations. That is critical when we are looking at resistance profiles based on a single water source and want to do this in an assessment that is structured and methodologically clear. Investigations from Bangladesh have also demonstrated heterogeneity of antimicrobial-resistant organisms in different water environments , furthering the argument for localised surveillance.

1.6 Importance of the Target Bacteria to Public Health

This study focuses on three bacterial groups : Escherichia coli, Salmonella spp. and Vibrio cholerae. Selection of these organisms was based on microbiological relevance in contaminated water, close association with enteric disease and common use in environmental water quality research. Their occurrence in surface waters suggests fecal contamination, public health risk, or both. Furthermore, the antibiotic susceptibility patterns of these bacteria are often used in environmental AMR studies to offer a solid practical assessment of the resistance burden in a contaminated aquatic source. So far, data on antimicrobial resistant enteric bacteria is inadequate in environmental water sources in Dhaka and recent work also demonstrates widespread distribution of MRD enteric bacteria across diverse environmental water sources further emphasizing the importance for local continued study.

1.6.1 Escherichia coli

Escherichia coli (E. coli) has become one of the most commonly used microbiology indicators of potable water quality. While most strains are harmless commensals, pathogenic strains can cause diarrheal disease and urinary tract infections as well as septicemia and other extraintestinal infections. Its detection in environmental water is a good indicator that it comes from human or animal waste. And in the case of Dhaka, it is even more significant because multidrug-resistant coli have already been reported from household water supplies, so not only is E. coli a fecal indicator organism but maybe an environmental vehicle to resistant bacteria as well. Due to this

dual role, E. coli is well-suited for evaluating antibiotic resistance profiles within urban water samples.

1.6.2 Salmonella spp.

Salmonella spp. are Gram-negative enteric pathogens that are usually responsible for food and water-borne diseases. They can lead to gastroenteritis, enteric fever and in a few cases invasive systemic infection. Due to their relevance, presence in environmental water generally indicates a poor sanitary quality and has a direct association with public health risk where untreated or contaminated water might be used in household activities or for food handling. Salmonella is a globally significant diarrheal disease pathogen and was thus included in the current study, at least partly because of its capacity for environmental persistence. (Popa & Papa, 2021).

1.6.3 Vibrio cholerae

Vibrio cholerae is particularly relevant in Bangladesh as it is a water borne disease-causing aerobic bacterium responsible for large outbreaks of cholera. In contrast to many enteric pathogens that merely reflect fecal contamination conditions, V. cholerae are an autochthonous inhabitant of aquatic environments and they may survive in water under favorable ecological niche conditions. Bangladesh finally needed national-level cholera surveillance, and V. cholerae remains a significant cause of diarrheal disease burden in the country where choleralike transmission has been observed for long. In Bangladesh, similar antibiotic-resistant Vibrio isolates have been identified in water sources as reported by environmental studies , lending further support for the inclusion of V. cholerae in water microbiology studies focused on resistance.

1.7 Importance of Antibiotic Susceptibility Testing

Antibiotic susceptibility testing is a laboratory method to assess whether a bacterial isolate is susceptible, intermediate or resistant to defined antimicrobial agents. Through environmental studies, at hospitals and beyond clinical settings in the U.S., AST helps to unveil just how extensive resistance has spread. It also gives a characteristic of the phenotypic behavior of bacterial performance toward pharmaceutical agents used frequently in practice. That

information is useful for grasping the local landscape around resistance, tracking multidrug-resistant isolates and creating baseline evidence to monitor future change.

1.8 Most Used Antibiotics for Treatment in Bangladesh

Antibiotics are compounds made by microorganisms, or synthetically manufactured for medical purposes that kill or slow the growth of bacteria. Antibiotics are often used as treatment for a bacterial infection, available as narrow-spectrum or broad-spectrum antibiotics based on their range of activity (Antibiotic, 2023).

Common groups of antibiotics used in Bangladesh include:

Aminoglycosides: Directly inhibit protein synthesis, used to treat serious infections such as urinary, respiratory and bloodstream infections.

Cephalosporins: It targets the bacterial cell wall, common uses include respiratory, urinary tract, skin, bone and bloodstream infections.

Chloramphenicol: Inhibits protein synthesis. It is used for special eye, skin and certain other bacterial infections.

Fluoroquinolones: Directly interfere with bacterial DNA replication and are used for diarrhea, pneumonia, gonorrhea, and urinary tract infections

Macrolide - It also inhibits protein synthesis , used for respiratory tract infections, skin infections and some sexually transmitted disease.

Penicillins: work as bacterial cell wall synthesis inhibitor, used for respiratory tract, urinary tract and other systemic infections.

Tetracyclines: Inhibit protein synthesis, used in rickettsial infections, pneumonia and some skin infections (such as acne).

1.9 Study Objectives

- To obtain the water sample from the designated source at Aftabnagar, Dhaka, employing appropriate aseptic techniques to facilitate the examination of the microbiological quality of the water body in a laboratory setting.
- To isolate and purify bacterial colonies from the obtained water sample utilizing selective and differential culture conditions to recover microorganisms typically linked to contaminated urban water.
- To identify the target bacterial isolates, specifically Escherichia coli, Salmonella spp., and Vibrio cholerae, based on their culture features, colony shape, and confirmatory laboratory techniques.
- To conduct antibiotic susceptibility testing on the verified isolates against selected antibiotics frequently utilized in Bangladesh using the standard Kirby–Bauer disk diffusion method.

1.10 Literature Review

Recent studies indicate that surface water significantly contributes to the dissemination of antibiotic resistance. Raw sewage, wastewater, and unclean pharmaceutical waste sometimes end up in lakes, rivers, canals, and other bodies of water in cities. This makes it easier for germs that are resistant to antibiotics to live and spread. This is why aquatic ecosystems are now seen to be important areas where antimicrobial resistance can grow, not merely regions where pollution happens (Berendonk et al., 2015; Rizzo et al., 2013). This situation is even more concerning in Bangladesh because dirty water is still a major public health issue. Dhaka is especially stressed out since it is growing quickly, has a lot of people living there, bad sanitation, and rubbish that isn't thrown away correctly. Research in Bangladesh has shown that urban areas are heavily polluted with waste, and more recent studies from Dhaka have shown that environmental water sources are full of enteric bacteria that are resistant to antibiotics. These results suggest that urban water bodies in Dhaka may operate as reservoirs for resistant bacteria, potentially increasing human exposure via direct or indirect contact .Escherichia coli, Salmonella spp., and Vibrio cholerae are three species of bacteria that are often studied because they are significant for public health and are intimately associated with diseases that spread through water and fecal

contamination. Finding out what kinds of drugs these organisms are resistant to can help us figure out how safe urban water sources are from bacteria. Antibiotic susceptibility testing is still a common approach to find out how bacteria are resistant and how much they damage the environment .

Chapter 2

Materials and Methods

2.1 Study Place

The research laboratory work for this project took place in the Biotechnology, Microbiology, and Molecular Biology Laboratory, which is part of the school of Life Science,
of BRAC University.

2.2 Study period

The study project spanned from August 2024 to January 2025.

2.3 Experimental Procedure

- ➔ The water sample was collected from the location in sterilized bottles.
- ➔ Sample filtration was done by Whatman Filter Paper to eliminate impurity.
- ➔ 1mL of filtered sample was inoculated in 4mL of Luria-Bertani (LB) broth and kept for 4 hours in a shaker incubator.
- ➔ Different media (TCBS for *Vibrio cholerae,* EMB for *Escherichia coli* and SS for *Salmonella spp*) were selected in order to culture the sample.
- ➔ Serial dilution of the enriched sample was done to perform the spread plate method in the selected media and incubated for 18-24 hours at 37°C .
- ➔ Colony Forming Units (CFU) were determined.
- ➔ Isolated colonies were streaked in different plates of selective media via streak plate method and incubated at 37°C for 18-24 hours.
- ➔ Pure colonies were selected, picked and stabbed with sterilized needles into soft agar future work (stock).
- ➔ DNA extraction from selected bacterial colonies from streak plates though boiling method was performed and extracted DNA was stored at -20°C.
- ➔ PCR was performed to get genomic DNA.
- ➔ Agarose gel electrophoresis was performed for visualisation of the PCR products.

- ➔ Re-streaking from the stock was done for Antibiotic Susceptibility Test (AST).
- ➔ Bacterial suspension in saline was prepared, lawned in Mueller-Hinton Agar (MHA) plates, antibiotic disks were placed and plates were incubated for 18-24 hours at 37°C
- ➔ After incubation, diameter zones were measured.

2.4 Types of Equipment

- Autoclave machine
- Electric balance
- Glassware and Instruments : Glass pipettes, micropipettes, test tubes, Glassware, pH meter, Beaker, Glassrod
- Incubator
- Laminar air flow cabinet
- Petri dishes, spreader, metallic loop, Bunsen burner
- Shaker incubator
- Vortex machine

2.5 Collection and Processing of Water Sample

In this study, the water sample was aseptically collected with sterile, pre-labeled sampling bottles from the selected water source, Aftabnagar. The site chosen is anthropogenically affected, thus more likely to demonstrate contamination which may impact not only microbial diversity but antibiotic resistance profiles. Right after collection, the sample was immersed in a cooled insulated ice box to achieve and maintain a low temperature and uniform conditions for transportation of sample, which preserves the original microbial composition of the sample. After collection, the sample was immediately processed on-site within the same day at BRAC University's Biotechnology Laboratory in order to maximize the precision and representativeness of microbiological quality as well as to minimize degradation or external contamination.

2.6 Bacteria Collection

The water sample was first passed through 0.4 mm Whatman filter paper to remove suspended particles and debris prior to bacterial isolation. A certain amount of filtered sample was then aseptically inoculated into a sterile autoclaved falcon tube with LB for enrichment. The mixture

was cultured on a shaking incubator at 37°C for 4 hours to meet the best growth requirement of bacteria. To lower bacterial concentrations serial dilution was performed after enrichment using sterile 0.9% sodium chloride (NaCl) solution in autoclaved test tubes to determine viable counts. Simultaneously, to separately target the isolation of specific bacterial species from the original water sample a small volume of the raw unprocessed sample was inoculated directly onto selective culture media.

2.7 Culture Media used for Bacterial Growth

Choice of culture media is largely dependent on the type of bacteria being studied, the goal of the experiment, and what facilities are available in a given lab. Selective and differential media is fundamental for good isolation and identification because of different nutritional requirements, and physiological conditions of each group of bacteria. Three selective media were utilized within this study: Eosin Methylene Blue (EMB) agar, and Salmonella - Shigella (SS) agar, Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar. Serial dilution was performed, and the final samples were inoculated on these media (by spread plate method) and incubated at 37°C for 18–24 hours. When colonies were visible, the mixed communities were sub-cultured on the same selective media using the streak plate technique to obtain isolated colonies for identification and downstream analysis.

2.7.1 Eosin Methylene Blue (EMB) Agar

Eosin Methylene Blue (EMB) agar was used as a selective and differential medium to isolate Gram-negative enteric bacteria, especially Escherichia coli. It contains eosin and methylene blue dyes in its medium, and thus, it is selective as it inhibits the growth of most Gram-positive bacteria. It also makes an important distinction between lactose fermenters and non-lactose fermenting bacteria. Dark colonies with a strong green metallic sheen due to acid production suggest strong lactose fermenters (E coli), while weak fermenters may appear pink or brown. On the contrary, Non-Lactose Fermenting organisms like Salmonella and Shigella usually produce colonies without color (colorless). EMB agar was prepared by standard laboratory method and used for gram negative bacterial culture and preliminary identification of lactose fermenting bacteria.

2.7.2 Salmonella-Shigella (SS) Agar

Salmonella species were isolated on a Salmonella-Shigella (SS) agar which was used as a selective and differential medium. and certain Shigella species. Bile salts, sodium citrate and brilliant green are contained in the medium to inhibit Gram-positive bacteria and most coliform organisms from growing but allowing the target enteric pathogens to proliferate. Differential components are lactose which helps to differentiate between lactose fermenters and non-lactose fermenters, neutral red acts as a pH indicator. Those that do ferment lactose produce red or pink colonies, and the non-lactose fermenters, like for example Salmonella and Shigella appear colorless. Hydrogen sulfide production can also be detected in the presence of thiosulfate and ferric salts, resulting in black centers of Salmonella colonies. SS agar was used for selective isolation and presumptive identification of Salmonella spp. in the present study. from the collected water samples.

2.7.3 Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar

Use of thiosulfate-citrate-bile salts-sucrose (TCBS) agar as a selective and differential culture medium for Vibrio species isolation, especially Vibrio cholerae. It was a highly alkaline pH medium with sodium citrate, bile salts (ox bile and sodium cholate), which would suppress other Gram-positive bacteria and many non-targeted species of Gram-negative organisms. Vibrio cholerae is a sucrose-fermenter which produces yellow colonies due to acid production altering the pH indicators, while non-sucrose fermenters will produce green or blue–green colonies. It also contains sodium chloride to promote growth of halophilic Vibrio species. Multiple colonies are isolated on the selective culture medium—TCBS agar, and preliminary identification achieved by plating these organisms from water samples.

2.8 Buffer Formulation

Running Buffer

- Added 50 ml of 20X buffer.
- 700 ml of distilled water was added.

- If the pH is higher than 8.3, add HCl (hydrochloric acid) to bring it down to 8.3. Add NaOH (Sodium Hydroxide) pellets if the pH is less than 8.3.
- Added distilled water brought the total volume up to 250 ml.
- The pH was measured again.

1X TE Buffer

- 10 mM of each Tris-HCl and EDTA was added.
- Added 10 ml of distilled water.
- If the pH is higher than 8.3, add HCl (hydrochloric acid) to get the final pH to 8.3. If the pH is lower than 8.3, add NaOH (sodium hydroxide) pellets.
- Added distilled water produced the final volume 20 ml.
- The pH was examined once again.

2.9 Plating Methods for bacterial inoculation

Inoculation methods are basic lab techniques, since they involve introducing microorganisms to culture media to keep them viable under suitable conditions and enable proper study of the organisms. This section described that serial dilutions were performed to obtain appropriate bacterium cell concentrations for culture. The diluted samples were inoculated onto the culture media by means of the spread plate method, which helps homogeneous distribution of bacteria over an agar surface and allows easy differentiation between individual colonies after incubation. Subsequently, single colonies from mixed bacterial growth were isolated and purified using the streak plate method. This stepwise method was essential for acquiring distinct strains and obtaining precise identification and analysis of the bacterial isolates (Sanders, 2012).

2.9.1 Spread Plate

The diluted samples of bacteria were inoculated by the spread plate method on selective culture media to isolate and count viable microorganisms. An aliquot of the sample after performing serial dilution was plated on agar plates, and spread thoroughly with sterilized glass spreader to ensure equal spreading across the medium. Spreading the samples like this also avoided too many colonies growing in one area which meant it was easier to count distinct bacterial growth after incubating. These inoculated plates were incubated at 37°C for 18–24 h before visible

colonies were analyzed. This technique was particularly helpful for estimating microbial load and securing separate colonies from the water sample.

Figure 1: Colonies observed on TCBS agar, EMB agar and, SS agar after the spread plate method

2.9.2 Streak Plate Method

The streak plate method for isolating pure strains from mixed cultures was accomplished after spread plating. A separate individual colony from the culture plate was selected using a sterilised inoculation stick, and streaked uniformly across a new agar plate. In each of the three sequential streaks, the bacterial density was lowered sufficiently to allow individual cells to separate from one another and develop into separately observable colonies upon incubation. Well-separated colonies were selected for purification and subsequent identification after incubating the plates at 37°C for 18–24 hr. This procedure was crucial for the isolation of pure bacterial cultures needed for morphological observation and other microbiological assays.

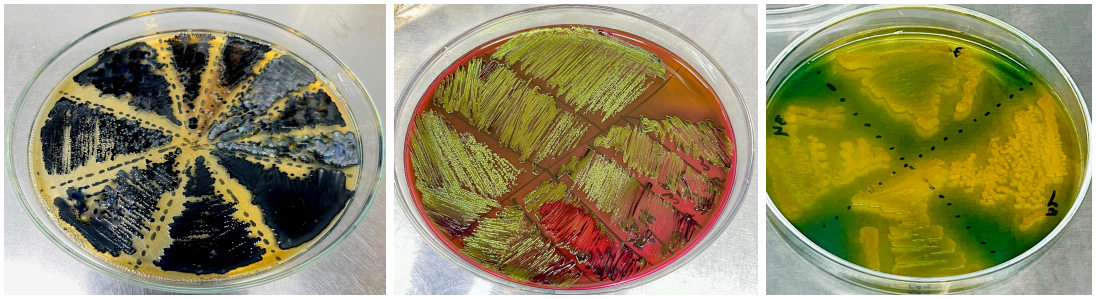

Figure 2: Colonies observed on SS agar, EMB agar and, TCBS agar after the streak plate method

2.10 Stocking of Samples

Sample stocking was carried out to preserve the isolated bacterial cultures for later use in sub-culturing and further laboratory analysis. Proper stocking is important because it helps maintain the viability of the isolates while reducing the risk of contamination, repeated handling, and unwanted changes in culture quality over time. For this purpose, soft agar medium with a lower agar concentration was prepared so that the bacteria could remain viable in a semi-solid environment. The medium was sterilized by autoclaving and, after cooling to a suitable temperature, was transferred aseptically into sterile microcentrifuge tubes and allowed to solidify. Well-isolated colonies from fresh culture plates were then introduced into the soft agar by the stab inoculation technique using a sterile inoculating needle. The needle was inserted carefully into the agar to create stab lines, allowing the bacteria to grow within the medium in a controlled way. Each stocked tube was labeled properly for traceability and kept under appropriate storage conditions until needed for further analysis. This method was useful for preserving pure bacterial cultures in a simple and reliable manner.

2.11 Morphological Identification of cultured bacteria

As one of the first steps to identify the bacterial isolates cultured from the selective media, a morphological identification was also performed. The cultured colonies were then scrutinized based on their macroscopic characteristics (color, size, shape, margin, elevation, texture and the growth of some colonies on solid medium) after incubation. The respective features were matched with standard references and HiMedia guidelines to presumptively identify the isolates. Colonies grown on EMB, SS and TCBS agar were observed to pay close attention since the media indicate the potential bacteria based on their growth characteristics as well as color change. Using this observation-based method, it was possible to preliminarily group the isolates and proceed with confirmatory analysis.

Bacteria	*Escherichia coli*	*Salmonella spp*	*Vibrio cholerae*
Selective Growth Media	Eosin Methylene Blue (EMB)	Thiosulfate-Citrate Bile-Sucrose(TCBS)	Salmonella Shigella (SS)
Colour	Green sheen	Black	Yellow
Cause Behind Colour	Lactose fermentation	Hydrogen sulfide (H2S) production	sucrose fermentation
Identification	Positive for *E.coli*	Positive for *Salmonella*	Positive for *Vibrio cholerae*

Table 1: HiMedia Analysis

2.12 Isolation of DNA (Boiling Method)

Genomic DNA was isolated from the bacterial colonies using the boiling method, a simple and rapid approach that is widely used for routine molecular identification.

- ➢ Streaked and inoculated bacterial colonies for 18-24 hours at 37°C.
- ➢ Colonies added in 500µl TE buffer or sterile distilled water in eppendorf tubes.
- ➢ The samples were then centrifuged at 13000 rpm for five minutes.
- ➢ After discarding the supernatant, the pellet was re-suspended in TE buffer or sterile distilled water.
- ➢ Centrifuge the eppendorf tubes at speed of 13000 for 5 minutes.
- ➢ The supernatant was removed and resuspended in 500µl TE buffer or sterile distilled water.
- ➢ The eppendorfs were given boiling water at 100°C, for 10 minutes. Lysis of the cell membrane by disrupting it due to heat. The heating step from about 75 °C needed for breaking open a bacterial cell may have ruptured the outer membrane, triggering lysis and allowing DNA to spill into solution.

- ➢ The samples were placed in an ice box to be cold shocked for 10 minutes and then stored at -20°C in the fridge after they were boiled.
- ➢ Centrifugation of eppendorf tubes (5 minutes, 13000 rpm) was performed to sediment cell debris.
- ➢ Supernatant containing DNA was transferred to new microcentrifuge tubes .

This DNA will be used for PCR and gel electrophoresis to verify the existence of the desired bacteria.

2.13 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is a molecular technology for synthesizing and amplifying specified sections of DNA in order to detect and analyze them more easily. Despite the presence of tiny quantities of genetic material, PCR generates millions of copies of the desired DNA sequence in a short time period. The technique operates in a series of heating and cooling cycles to separate the DNA strands, bind primers at the target region, and synthesize new DNA chains via DNA polymerase. PCR is commonly used in microbiology, biotechnology and molecular diagnostics such as identification and confirmation of microorganisms due to its high sensitivity and specificity. In the present study, PCR was used as a confirmatory method after culture-based isolation to verify the detection of your target bacterial species at the molecular level.

2.13.1 Steps of PCR

A reaction mixture is prepared consisting of template DNA, forward and reverse primers, PCR master mix or DNA polymerase, deoxynucleotide triphosphates (dNTPs), reaction buffer, magnesium ions and nuclease-free water . After the reaction mixture is ready, it is added to a thermal cycler that allows for amplification through multiple rounds of heating and cooling.

Initial denaturation

It is the first step which usually involves heating the reaction mixture to above the melting temperature 94–95 °C for 5 minutes. The fact that this high temperature essentially breaks the hydrogen bonds between the two strands of a double-stranded DNA and they are now

single-stranded. This is a crucial step as it makes the target DNA sequence accessible for primer binding in the next stage.

Denaturation

It takes place at the start of each amplification cycle. At this step the temperature is approximately 94–95°C, typically for a period of 30 seconds. The goal is identical as the first step, to denature the newly created double-stranded DNA, providing two single strands of DNA that will each guide further amplification.

Annealing step

The temperature is decreased to something between 50-65° C for this stage, depending on the melting temperature of the primers being used. The forward and reverse primers attach or anneal to their complementary sequences on the single-stranded template DNA at this lower temperature. The primers also are crucial as they determine which piece of DNA will get amplified. If the annealing temperature is excessively high, the primers may fail to bind correctly. Conversely, if it is excessively low, non-specific binding may take place. In this study, annealing was done at 57°C for E. coli, 60°C for Vibrio cholerae , and 53.2°C for Salmonella.

Extension/Elongation

In this step, the temperature increased to about 72°C which is the ideal working temperature of Taq DNA polymerase. The polymerase uses free nucleotides to create complementary new DNA strands starting from the bound primers. That is why the destination area is duplicated. For short DNA fragments, this step typically lasts anywhere from 30 seconds to 1 minute but the time varies with the size of the DNA fragment being amplified. The polymerase adds nucleotides in the 5′→3′ direction and makes the amplified DNA product.

Final Extension

Typically, a final extension step is performed after the last cycle at 72°C for 5 minutes. This brings the DNA polymerase to finish uncompleted DNA strands and guarantees that the amplicons are fully lengthened. It is intended to prevent formation of non-specific products that would negatively affect quality and yield of final PCR products.

Final Hold

After amplification is finished however, the thermal cycler transitions to a final hold temperature, typically 4°C and stable temperature, where it holds the amplified template until brought out of the machine after PCR. The PCR products are stored at -20°C for further analysis.

2.13.2 Components of PCR

Template DNA

Template DNA is the extracted genetic material that is obtained from the sample and serves as a template for amplification. It contains the particular target region that the PCR is denoted to identify. For studies with bacteria, this DNA is generally obtained from cultured colonies and serves as the template for the reaction. Low quality or presence of inhibitory substances in the template DNA make PCR non-functional.

Primers

Primers are short single-stranded DNA sequences that are designed to complement the ends of the target region. In a PCR reaction, there are two primers: one primer is forward and the other is reverse. These primers anneal to complementary sequences on the template DNA and direct the DNA polymerase to the specific region of interest that one wishes to amplify. Thus, by nature primers are very effective in establishing the specificity of the reaction.

DNA Polymerase

During PCR, new strands of DNA are synthesized by the use of an enzyme called DNA polymerase. The enzyme used most frequently in PCR is Taq polymerase, which is a thermophilic bacterial DNA replication enzyme that can endure the elevated temperatures required in the denaturation phase. After that, the polymerase attaches to the primer, extending from the primer and adding nucleotides to create a new complementary DNA strand.

dNTPs (Deoxynucleotide Triphosphates)

dNTPs are the raw material from which newly synthesized DNA is made by the DNA polymerase. Four of these are nucleotides: dATP, dTTP, dCTP and dGTP. Extension step- the polymerase adds all these nucleotides in one by one manner to a growing DNA strand based on the sequence of template DNA.

Buffer

The PCR buffer creates a chemical environment that facilitates the efficient performance of the reaction. It prevents a pH shock and guarantees an ideal ionic strength, which ensures the enzyme works correctly during the amplification process. Lack of proper buffer systems will allow a reaction to be pushed out of equilibrium leading to amplification failure.

Magnesium Ions (Mg^{2+})

One of the main requirements for DNA polymerase activity is magnesium ions, which are typically provided as magnesium chloride. They assist in the proper interaction of the enzyme with primer, template DNA and dNTPs during DNA synthesis. For example, the final concentration of magnesium is very critical as too low may decrease amplification and too high may yield non-specific products.

2.13.3 PCR Equipments

Thermal Cycler Machine

To perform the temperature cycling changes needed for PCR, a thermal cycler (or polymerase chain reaction machine) is used. This makes the steps in DNA amplification precise and repetitive, as automatically cycling the reaction through denaturation, annealing, and extension. The machine has a heated block (where the PCR tubes sit) and when the program is set, it handles everything automatically without intervention.

PCR Tubes

PCR tubes are thin walled small plastic tubes used to contain PCR reaction mixture during the amplification. This permits rapid, uniform heat transfer from the thermal cycler to the sample, which is necessary for accurate DNA amplification. Tightly capped tubes during the entire PCR run to avoid evaporation and contamination.

PCR Reaction Formulation

Master mix: 10µl

Isolated DNA : 5µl

Forward primer(FP): 2µl

Reverse primer (RP) : 2µl

Nuclease free water (NFW): 1µl

In total 20µl .

2.13.4 PCR Machine Run Mechanism

1. The thermal cycler was turned on and completed a full warmup.
2. PCR reaction with all components needed was prepared and PCR tubes were labeled
3. PCR tubes were tightly capped to minimize evaporation or contamination.
4. The tubes were gently dropped in the wells of the thermal cycler block.
5. The lid down was closed tightly so that heat can transfer properly.
6. PCR programs were selected depending on the necessary conditions
7. The 35 cycles option was selected
8. The F1 button was pressed to start the procedure.

2.13.5 Thermal cycle

Initial denaturation	95^0 (5 min)
Denaturation	95^0 (30 sec)
Annealing	E. coli 57^0 (1min) Vibrio 60^0 (1min) Salmonella 53.2^0 (1min)
Elongation	72^0 (1min)
Final elongation	72^0 (5min)
Storage	4^0C (short) and -20^0C (long)

Table 2: Steps of Thermal Cycle

2.13.6 Primer Condition

Organism	**Primer**
E.coli	F: 5'-GACCTCGGTTTAGTTCACAGA-3' R: 5'-CACACGCTGACGCTGACCA-3'
Salmonella	F: 5'-GTGAATTATCGCCACGTTCGGGCAA-3' R: 5'-TCATCGCACCGTCAAAGGAACC-3'
Vibrio	F: 5'-ATAATGGCTCACCAAGAAGG-3' R: 5'-TTAGAACTTATAACCACC-3'

Table 3: Primer condition

2.14 Agarose Gel Electrophoresis

The amplified DNA was detected by running a small fraction on an agarose gel and exhibiting their presence in positive fluorescence and PCR reactions. This method allows DNA fragments to be separated based on their size, and determines if a band is present where it should ideally appear. The presence of the expected band indicates that DNA using the target has been amplified. The absence of a band or presence of faint, smeared and/or non-size precisely sized bands may indicate problems including limited amplification, contamination, degraded DNA or nonspecific products. As DNA is negatively charged, it moves towards the positive electrode and smaller fragments travel faster in the gel than larger ones. For this study, gel electrophoresis was relevant as a confirmation step for post PCR. This allowed for observation of clear bands corresponding to the amplified products of target bacteria, thus confirming the presence of a desired DNA segment.

2.14.1 Gel Preparation

- ➔ 1g of agarose powder was measured in the digital weight machine and transferred to a conical flask.
- ➔ 98 mL of distilled water and 2mL of 50X TAE buffer was added to it
- ➔ This solution was also placed inside a microwave until the agarose dissolved, and resulting in a clear solution.
- ➔ When the solution cooled down a little, 4 µl EtBr was supplemented to it
- ➔ The comb was then inserted into the gel casting tray.
- ➔ The liquid gel was cast into the gel casting tray to cool down at room temperature for 20 to 30 minutes to solidify. Also, it was ensured that there was no air bubble anywhere in the gel.

2.14.2 Gel Electrophoresis

- ➢ After the solidification of the gel, the comb was carefully removed and the gel tray was placed in the gel chamber.
- ➢ 1X running buffer was poured into it until the walls were drowned.
- ➢ Using a micropipette, prepared pcr products were loaded into the wells of the gel.

- ➢ A DNA ladder in one well was loaded in order to compare the size of DNA fragments by comparing their size to the bands in the ladder.
- ➢ The electrophoresis chamber was correctly connected with the power supply.
- ➢ The gel run was set at 100V for 40 minutes.
- ➢ After the gel run was completed, DNA band was observed under UV light.

2.15 Antibiotic Susceptibility Test (AST)

Antibiotic susceptibility testing (AST) was performed to assess the response of the isolated bacteria to antibiotics under controlled laboratory conditions . The Kirby–Bauer disk diffusion method was used in this study because it is a standard and well-accepted method for assessing bacterial susceptibility patterns. A bacterial lawn was generated by spreading a uniform suspension of fresh colonies over the surface of Mueller-Hinton agar using sterile cotton swabs. Antibiotic discs with some selected antimicrobial agents were carefully placed on the inoculated agar surface and the plates incubated at 37°C for 18–24 h. Following incubation, the diameter of inhibition surrounding each disc was measured and isolates were classified as susceptible, intermediate or resistant. All operations were performed in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2024) to ensure the reliability, repeatability, and correctness of the results. This approach enabled the analysis of susceptibility patterns in isolated bacterial strains and characterization of their sensitivity to clinically utilized antibiotics.

2.15.1 Bacterial Suspension Preparation

- ➔ Bacterial isolates were collected from freshly growing agar plate incubated at 37ºC for 18–24 hrs.
- ➔ with the help of a sterile inoculating loop Colonies with similar morphologies were transferred into sterile 2–3 mL 0.9% NaCl or saline solution.
- ➔ For ensuring the equal distribution of the bacterial cells, the suspension was mixed with a vortex mixer .
- ➔ The turbidity of the suspension was adjusted at a figure and compared to a 0.5 McFarland turbidity standard to yield a uniform bacteria concentration .

➔ The suspension was used within 15 minutes of making it so that the cell density remained unchanged and all the test outcomes lasted consistent.

2.15.2 Mueller-Hinton Agar (MHA)

Mueller-Hinton Agar (MHA) is a non-deferential yet selective media. It was selected as the standard medium for antibiotic susceptibility testing since it produces consistent, reproducible results and supports the growth of most non-fastidious bacteria. It is commonly used in disc diffusion assays owing to its stable formulation and ability to permit equivalent effusion of antimicrobial agents, which yields distinct and quantifiable zones of inhibition. The starch in the medium also absorbs bacterial toxins that may otherwise interfere with antibiotic activity, leading to more accurate test results. The preparation was done by dissolving the 38 g of MHA powder in one liter distilled water and heated to achieve complete dissolution followed by sterilization via autoclaving. The medium was aseptically poured into sterile Petri dishes to solidify. Plates were checked for surface moisture prior to inoculation and dried if required. The agar surface was then inoculated with a standard bacterial suspension using a sterile swab to create a thin bacterial lawn, and the plates were left for 5 minutes before applying antibiotic discs.

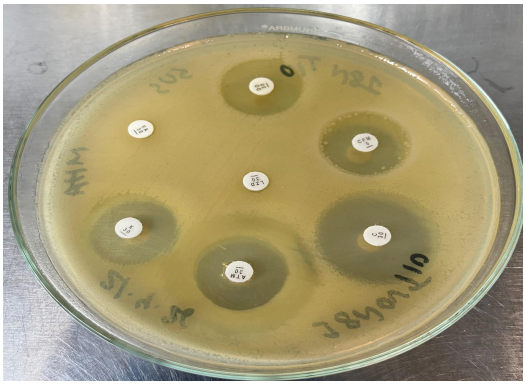

Figure 3: MHA plate incubating with antibiotics for 18-24 hours

2.15.3 Antibiotics List

Antibiotic group	Antibiotic Name	Code	Disc Concentration (mcg)	Diameter of zone inhibition		
				Susceptible (S) (mm)	Intermediate (I) (mm)	Resistant (R) (mm)
Penicillins	Ampicillin	AMP	2	≥17	14-16	≤13
Monobactams	Aztreonam	ATM	30	≥22	16-21	≤15
Amphenicols	Chloramphenicol	C	30	≥18	13-17	≤12
Cephalosporin	Cefixime	CFM	30	≥19	16-18	≤15
Aminoglycosides	Gentamicin	CN	10	≥15	13-14	≤12
Tetracyclines	Doxycycline	DO	30	≥16	13-15	≤12
Macrolides	Erythromycin	E	15	≥23	14-22	≤13
Cephalosporins (4th gen)	Cefepime	FEP	30	≥26	21-25	≤20
Carbapenems	Imipenem	IPM	10	≥23	20-22	≤19
Aminoglycosides	Kanamycin	K	30	≥18	14-17	≤13
Fluroquinolones	Levofloxacin	LEV	5	≥17	14-16	≤13
Oxazolidinones	Linezolid	LZD	30	≥23	21-22	≤20
Glycopeptides	Vancomycin	VA	30	≥17 (for gram +)	N/A	N/A

Table 4: Antibiotic List with their diameter zone of inhibition

Chapter 3

Result

3.1 Sample Labelling

The water sample was collected fromAftabnagar, Dhaka. The sample was taken 2 times each month which is 12 in total within the period of 6 months. The bacterial colony stocks were labeled containing bacterial species name, month name and sample number. The sample stocks names goes like this:

For the month names: August (Aug), September (Sep), October (Oct), November (Nov), December (Dec), January (Jan).

For bacterial species name : *Escherichia coli* (E), *Salmonella spp.* (Ss), *Vibrio cholerae* (V)

For sample number : First sample of the month (S1), Second sample of the month (S2).

Example: AugES1 = sample 1 of *E.coli* isolated in August, first sample.

3.2 Verification of PCR Products through Agarose Gel Electrophoresis and Confirmation

3.2.1 Gel Electrophoresis Result (*Escherichia coli*)

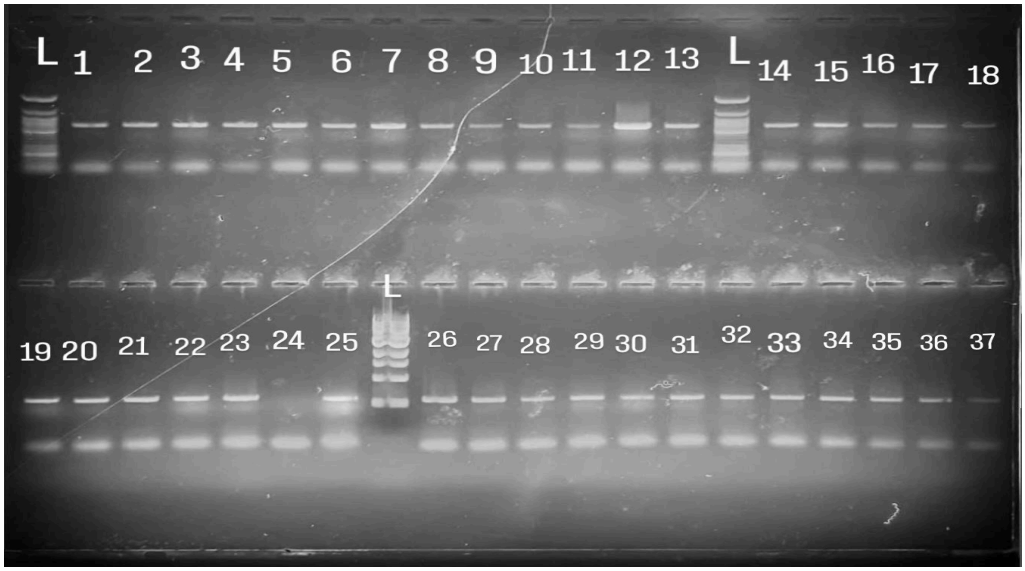

Figure 4: PCR result via Agarose gel-electrophoresis method of genomic DNA of the *E.coli* isolated colonies

The wells marked as L represent the ladder (50 bp upper row, 100bp lower row). Ladder bands are acute and clear, indicating a stable reference for estimating DNA fragments. The target DNA band of *E. coli* would be approximately 500-600 base pairs. In this gel, bold and profound bands are noticed in every well of E. coli genomic DNA sample except from well number 24. This well contains frail or negative amplification as this does not show the desired band . Remaining all well shows strong positive results and affirms the target DNA fragment of predicted size.

3.2.2 Gel Electrophoresis Result (*Salmonella spp.*)

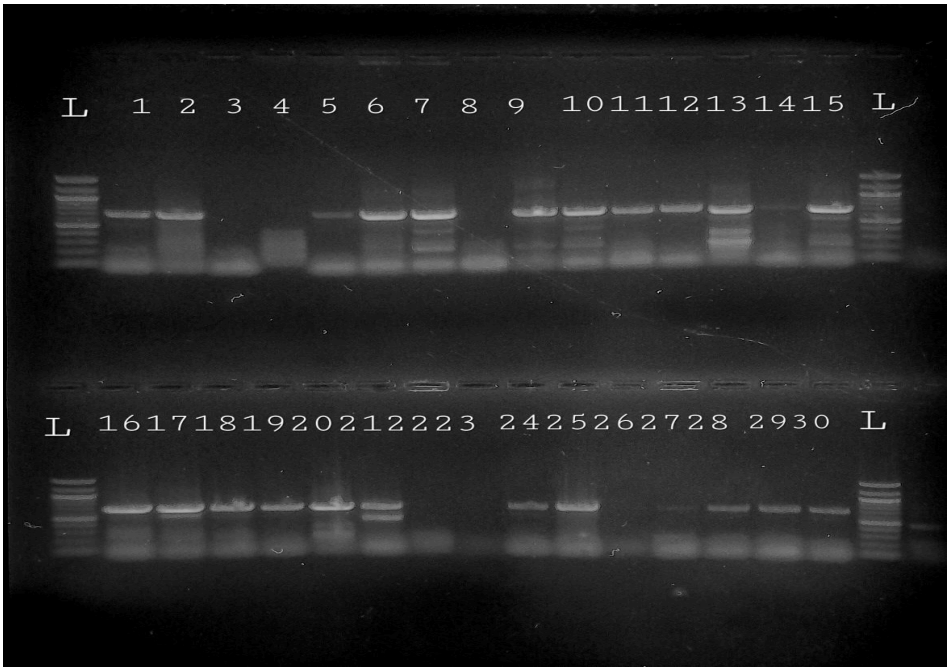

Figure 5: PCR result via Agarose gel-electrophoresis method of genomic DNA of the *Salmonella spp.* isolated colonies

For *Salmonella spp.* DNA amplification 100 bp ladder was used. Ladder bands are acute and clear, indicating a stable reference for estimating DNA fragments. The target DNA band of *Salmonella spp*. would be approximately 200-300 base pairs. Well number 1,2,5, 6,7, 9,10,11,12,13, 15, 16,17,18,19,20,21, 24, 25, 28, 29,30 shows vivid and strong bands which gives the validation for positive identification of *Salmonella spp.* genomic DNA . Well number

3,4,8,14,22,23,26,27 contains frail or negative amplification as they do not show the desired band .

3.2.3 Gel Electrophoresis Result (*Vibrio cholerae*)

Figure 6: PCR result via Agarose gel-electrophoresis method of genomic DNA of the *Vibrio cholerae* isolated colonies

For *Vibrio cholerae* DNA amplification 100 bp ladder was used. Ladder bands are acute and clear, indicating a stable reference for estimating DNA fragments. The target DNA band of *Vibrio cholerae* would be approximately 200-300 base pairs. All the wells here show vivid and strong bands which gives the confirmation for positive identification of *Vibrio cholerae* genomic DNA . No weak or negative amplification is found here.

3.3 Antibiotic Susceptibility Test (AST) Results

3.3.1 *E.coli* AST Results

3.3.1 (a) August

There are 7 *E.coli* isolates in total from the month August from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
AugES1 (1)	14 (I)	28 (S)	26 (S)	23 (S)	21.5 (S)	11 (R)	0(R)	36 (S)	29(S)	23 (S)	31.5 (S)	0(R)	0(R)
AugES1 (2)	21 (S)	29.5 (S)	23.5 (S)	24 (S)	22.5 (S)	21 (S)	20 (S)	30(S)	28(S)	21 (S)	34(S)	0(R)	0(R)
AugES1(3)	0(R)	29.5 (S)	31.5 (S)	16 (I)	24 (S)	21.5 (S)	0(R)	34(S)	26(S)	23 (S)	32(S)	0(R)	0(R)
AugES1(4)	0(R)	17 (I)	24.5 (S)	16 (I)	23 (S)	22 (S)	0(R)	27(S)	28.5 (S)	22 (S)	27.5 (S)	0(R)	0(R)
AugES2(1)	0(R)	29 (S)	25 (S)	26 (S)	19.5 (S)	23 (S)	0(R)	29(S)	26.5 (S)	21 (S)	28.5 (S)	0(R)	0(R)
AugES2(2)	0(R)	30 (S)	26.5 (S)	24.5 (S)	20 (S)	16 (S)	11.5 (R)	32.5 (S)	32.5 (S)	21 (S)	27.5 (S)	0(R)	0(R)
AugES2(3)	0(R)	26 (S)	24 (S)	23 (S)	22 (S)	19 (S)	0(R)	29(S)	30(S)	23 (S)	29(S)	0(R)	0(R)

Table 5 **:** *E.coli* isolates were collected in August and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

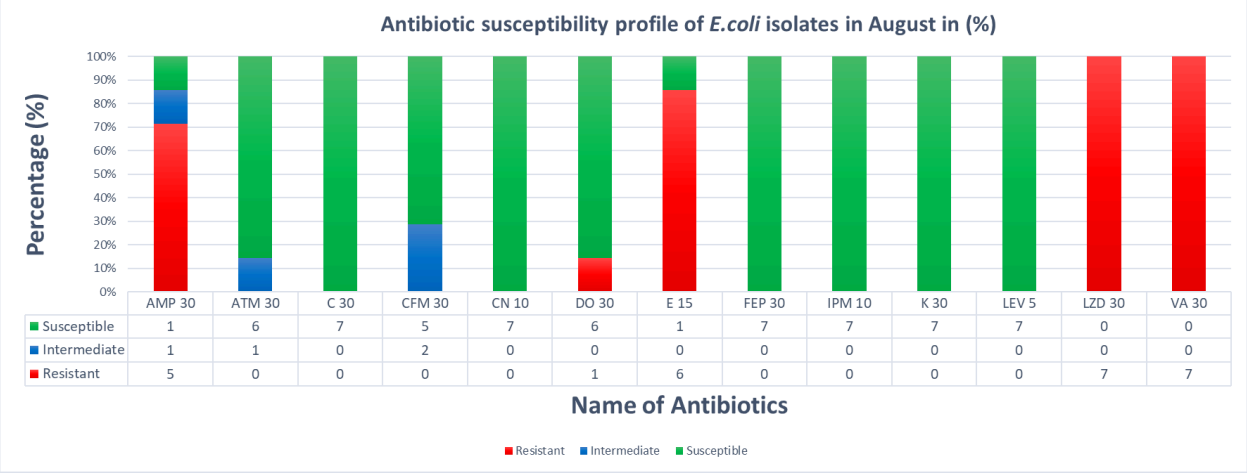

Figure 7 : Antibiotic susceptibility profile of *E.coli* isolates in August in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure is demonstrating the *E.coli* isolates from August shows high sensitivity to Chloramphenicol (100%), Gentamicin (100%), Cefepime (100%), Imipenem (100%), Kanamycin (100%), Levofloxacin (100%), Aztreonam (~85%), Cefixime (~70%), Doxycycline (~85%). Ampicillin , Aztreonam and Chloramphenicol exhibit some percentage ranging from 12

to almost 30 % intermediate . A high percent of resistance is shown in Ampicillin (72%) and Erythromycin, Linezolid, Vancomycin were not considered to the resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria.

3.3.1 (b) September

There are 3 *E.coli* isolates in total from the month September from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
SepES1(1)	23 (S)	36(S)	18(S)	27.6 (S)	20 (S)	21 (S)	10.5 (R)	38 (S)	26(S)	16(I)	33(S)	0(R)	0(R)
SepES2(1)	22(S)	29(S)	20(S)	27(S)	22 (S)	19 (S)	0(R)	36(S)	24(S)	21 (S)	29(S)	0(R)	0(R)
SepES2(2)	24(S)	34(S)	21(S)	25(S)	21 (S)	21 (S)	0(R)	39(S)	27(S)	19 (S)	28(S)	0(R)	0(R)

Table 6 : *E.coli* isolates were collected in September and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

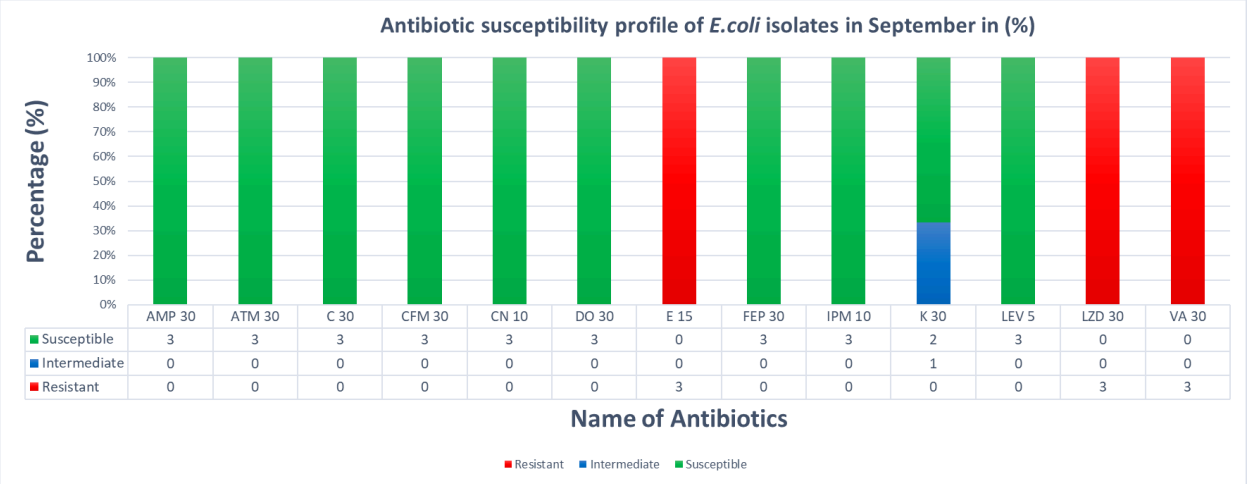

Figure 8: Antibiotic susceptibility profile of *E.coli* isolates in September in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *E.coli* isolate from September shows highest (100%) sensitivity to almost all the antibiotics (AMP,ATM, C, CFM,CN,DO, FEP, IPM, LEV) except Kanamycin

(~33% intermediate with ~67% sensitivity) . Erythromycin, Linezolid, Vancomycin were not considered for resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria.

3.3.1(c) October

There are 8 *E.coli* isolates in total from the month October from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
OctES1(1)	23 (S)	36(S)	18(S)	27.6 (S)	20(S)	21 (S)	10.5 (R)	32(S)	26(S)	16(I)	33.5 (S)	0(R)	0(R)
OctES1(2)	24(S)	34(S)	22(S)	27(S)	21(S)	19 (S)	0(R)	30(S)	28(S)	18 (S)	32(S)	0(R)	0(R)
OctES1(3)	19(S)	35.5 (S)	23(S)	29(S)	22(S)	22.5 (S)	0(R)	38(S)	33(S)	23 (S)	31.5 (S)	0(R)	0(R)
OctES1(4)	20.5 (S)	35(S)	20(S)	24.5 (S)	20.5 (S)	20.5 (S)	0(R)	34(S)	29(S)	20 (S)	31(S)	0(R)	0(R)
OctES2(1)	0(R)	41.5 (S)	22(S)	26(S)	25(S)	21 (S)	0(R)	36(S)	33.5 (S)	22 (S)	32(S)	0(R)	0(R)
OctES2(2)	0(R)	33.5 (S)	21.5 (S)	22.5 (S)	21(S)	0(R)	0(R)	41.5 (S)	24.5 (S)	21.5 (S)	24.5 (S)	0(R)	0(R)
OctES2(3)	21.5 (S)	34 (S)	23(S)	28(S)	21(S)	0(R)	0(R)	31(S)	29.5 (S)	23 (S)	29.5 (S)	0(R)	0(R)
OctES2 (4)	17.5 (S)	29(S)	20(S)	19.5 (S)	19(S)	19.5 (S)	0(R)	34(S)	17.5 (R)	20 (S)	33(S)	0(R)	0(R)

Table 7 : *E.coli* isolates were collected in October and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

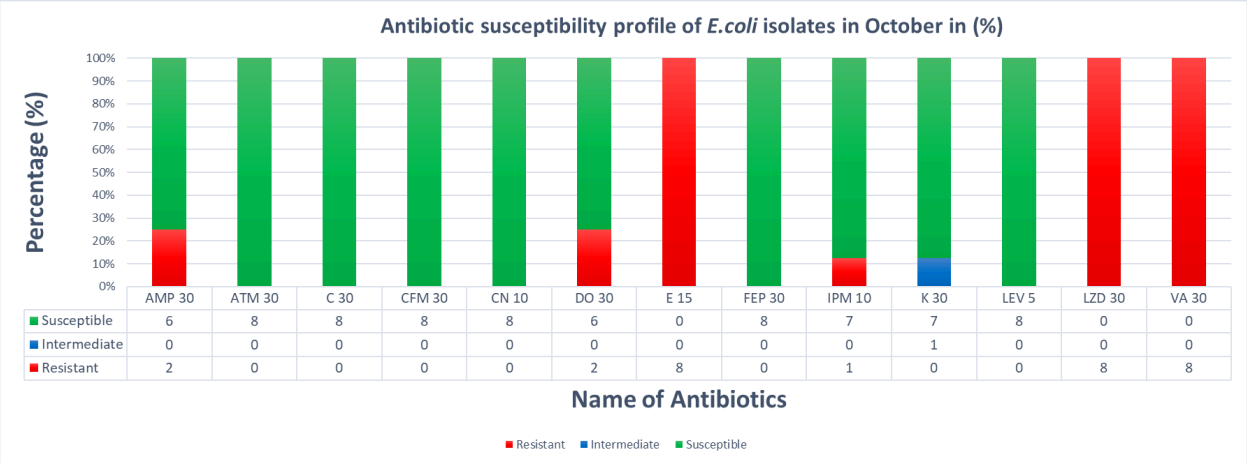

Figure 9: Antibiotic susceptibility profile of *E.coli* isolates in October in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *E.coli* isolate from September shows highest (100%) sensitivity to most of the antibiotics (ATM, C, CFM,CN, FEP,LEV) except Kanamycin (~12% intermediate with ~88% sensitivity), Ampicillin (25% resistance with 75% sensitivity), Doxycycline (25% resistance with 75% sensitivity) and Imipenem (~14% resistance with ~86% sensitivity). Erythromycin, Linezolid, Vancomycin were not considered for resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria.

3.3.1(d) November

There are 25 *E.coli* isolates in total from the month November from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
NovES1 (1)	18.5 (S)	33 (S)	24 (S)	24.5 (S)	19.5 (S)	23.5 (S)	0(R)	35 (S)	31(S)	19(S)	35(S)	0(R)	0(R)
NovES1 (2)	20 (S)	27(S)	26.5 (S)	24(S)	19(S)	13.5 (I)	0(R)	31.5 (S)	27.5 (S)	21(S)	29.5 (S)	0(R)	0(R)
NovES1 (3)	0(R)	31 (S)	25.5 (S)	25(S)	19.5 (S)	13(I)	0(R)	34(S)	27.5 (S)	19.5 (S)	30(S)	0(R)	0(R)
NovES1 (4)	0(R)	12(R)	26 (S)	0(R)	19(S)	21 (S)	0(R)	15 (R)	26.5 (S)	21(S)	30(S)	0(R)	0(R)

NovES1 (5)	20 (S)	32(S)	26.5 (S)	25.5 (S)	20(S)	15(I)	0(R)	34.5 (S)	28(S)	21(S)	27.5 (S)	0(R)	0(R)
NovES1 (6)	13 (R)	26.5 (S)	24(S)	0(R)	19(S)	13(I)	0(R)	30(S)	22 (I)	20(S)	26.5 (S)	0(R)	0(R)
NovES1 (7)	0(R)	15 (R)	25.5 (S)	0(R)	24(S)	13.5 (I)	0(R)	14 (R)	22.5 (I)	22(S)	30(S)	0(R)	0(R)
NovES1 (8)	19 (S)	28.5 (S)	28(S)	24.5 (S)	21.5 (S)	20.5 (S)	0(R)	28(S)	22.5 (I)	19.5 (S)	31(S)	0(R)	0(R)
NovES1 (9)	0(R)	29.5 (S)	0(R)	25(S)	19(S)	11 (R)	0(R)	30(S)	27(S)	19.5 (S)	25(S)	0(R)	0(R)
NovES1(10)	20 (S)	31 (S)	27(S)	22(S)	19(S)	0(R)	0(R)	34(S)	27(S)	20(S)	30.5 (S)	0(R)	0(R)
NovES1(11)	0(R)	28(S)	24.5 (S)	23.5 (S)	19(S)	13.5 (I)	0(R)	30(S)	23.5 (S)	21(S)	27(S)	0(R)	0(R)
NovES2(1)	0(R)	24.5 (S)	0(R)	23.5 (S)	21.5 (S)	12 (R)	0(R)	29.5 (S)	28(S)	0(R)	27.5 (S)	0(R)	0(R)
NovES2(2)	19 (S)	28.5 (S)	27.5 (S)	21.5 (S)	21.5 (S)	21.5 (S)	0(R)	29(S)	28(S)	22(S)	36(S)	0(R)	0(R)
NovES2(3)	19 (S)	29.5 (S)	22.5 (S)	18 (I)	19(S)	21 (S)	0(R)	33(S)	25(S)	22(S)	27(S)	0(R)	0(R)
NovES2(4)	0(R)	32 (S)	19(S)	26(S)	21(S)	16.5 (S)	0(R)	33(S)	30(S)	0(R)	25.5 (S)	0(R)	0(R)
NovES2(5)	18.5 (S)	27.5 (S)	0(R)	22.5 (S)	19.5 (S)	0(R)	0(R)	27(S)	26.5 (S)	0(R)	0(R)	0(R)	0(R)
NovES2(6)	0(R)	34 (S)	22(S)	24(S)	20(S)	13(I)	0(R)	32(S)	32(S)	0(R)	26(S)	0(R)	0(R)
NovES2(7)	0(R)	27 (S)	22(S)	22(S)	0(R)	0(R)	0(R)	27.5 (S)	27.5 (S)	0(R)	11(R)	0(R)	0(R)
NovES2(8)	0(R)	36 (S)	30(S)	24(S)	18(S)	22 (S)	0(R)	36(S)	25(S)	22(S)	24(S)	0(R)	0(R)
NovES2(9)	0(R)	33.5 (S)	26(S)	22(S)	17(S)	13(I)	0(R)	31.5 (S)	26.5 (S)	19.5 (S)	31(S)	0(R)	0(R)
NovES2(10)	0(R)	28 (S)	26(S)	23(S)	19.5 (S)	15.5 (I)	0(R)	28 (S)	26(S)	20(S)	26(S)	0(R)	0(R)
NovES2(11)	22 (S)	34.5 (S)	27(S)	26(S)	21.5 (S)	24.5 (S)	0(R)	36(S)	27.5 (S)	24.5 (S)	30.5 (S)	0(R)	0(R)
NovES2(12)	0(R)	32 (S)	26.5 (S)	25(S)	21.5 (S)	17 (S)	0(R)	32(S)	27(S)	20.5 (S)	29.5 (S)	0(R)	0(R)
NovES2(13)	16 (I)	26 (S)	26(S)	20.5 (S)	19.5 (S)	20 (S)	0(R)	29.5 (S)	25.5 (S)	20.5 (S)	31.5 (S)	0(R)	0(R)

NovES2(14)	0(R)	16 (I)	27.5 (S)	0(R)	21.5 (S)	21 (S)	0(R)	15.5 (R)	30(S)	21(S)	29(S)	0(R)	0(R)

Table 8 : *E.coli* isolates were collected in November and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

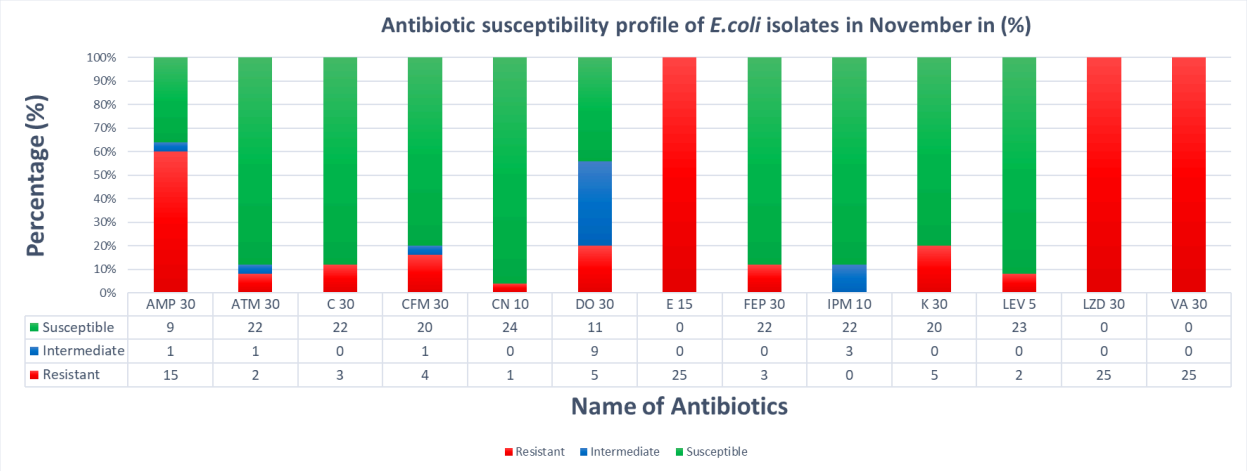

Figure 10 : Antibiotic susceptibility profile of *E.coli* isolates in November in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that none of the *E.coli* isolate from September is 100% sensitive to ant antibiotics. All of them show approximately 4% to 60% resistance range with sensitivity. Ampicillin, Aztreonam, Chloramphenicol, Cefixime , Gentamicin, Doxycycline exhibit intermediate characteristics along with both sensitivity and resistance whereas Imipenem shows approximately 13% intermediate and 87% sensitivity without resistance. Erythromycin, Linezolid, Vancomycin were not considered for resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria.

3.3.1(e) December

There is one *E.coli* isolate in total from the month December from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
DecES1(1)	0(R)	27(S)	27(S)	0(R)	20 (S)	12.5(R)	0(R)	30(S)	22(I)	21.5 (S)	24(S)	0(R)	0(R)

Table 9: *E.coli* isolates were collected in December and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

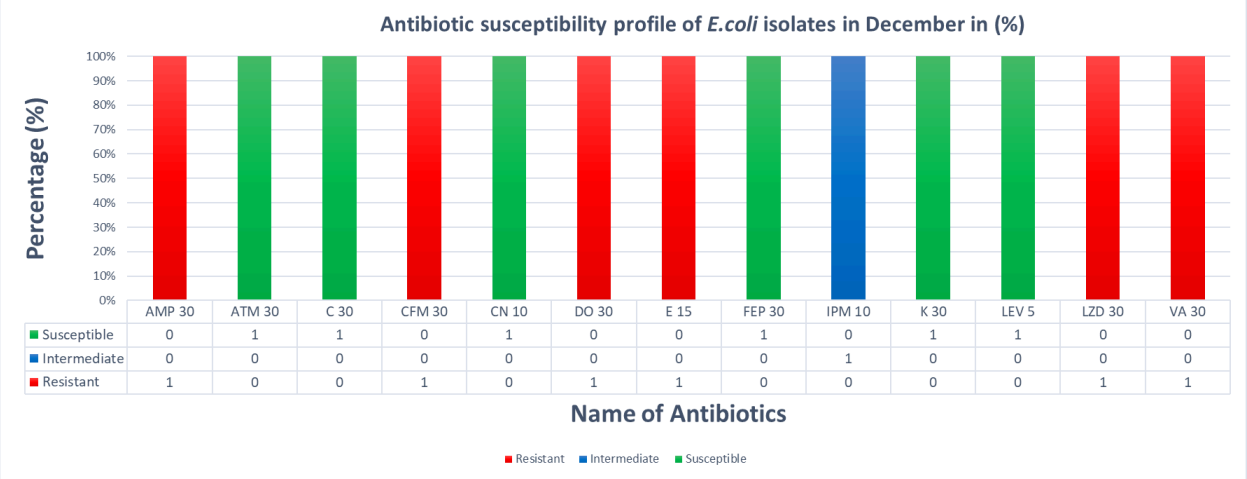

Figure 11 : Antibiotic susceptibility profile of *E.coli* isolates in December in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *E.coli* isolate from December exhibits high sensitivity toward Aztreonam, Chloramphenicol, Gentamicin, Cefepime, Kanamycin and Levofloxacin. It shows high resistance toward Ampicillin, Cefixime and Doxycycline. Erythromycin, Linezolid, Vancomycin were not considered for resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria.

3.3.1(f) January

There are 3 *E.coli* isolates in total from the month January from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
JanES2(1)	19(S)	31(S)	26(S)	24(S)	19.5 (S)	20.5 (S)	0(R)	31(S)	26.5 (S)	21 (S)	26.5 (S)	0(R)	0(R)
JanES2(2)	0(R)	30(S)	27.5 (S)	23.5 (S)	19.5 (S)	12.5 (R)	0(R)	29(S)	26(S)	19.5 (S)	26(S)	0(R)	0(R)
JanES2(3)	0(R)	32(S)	25(S)	24(S)	19 (S)	12 (R)	0(R)	30.5 (S)	29(S)	21 (S)	25.5 (S)	0(R)	0(R)

Table 10 : *E.coli* isolates were collected in January and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

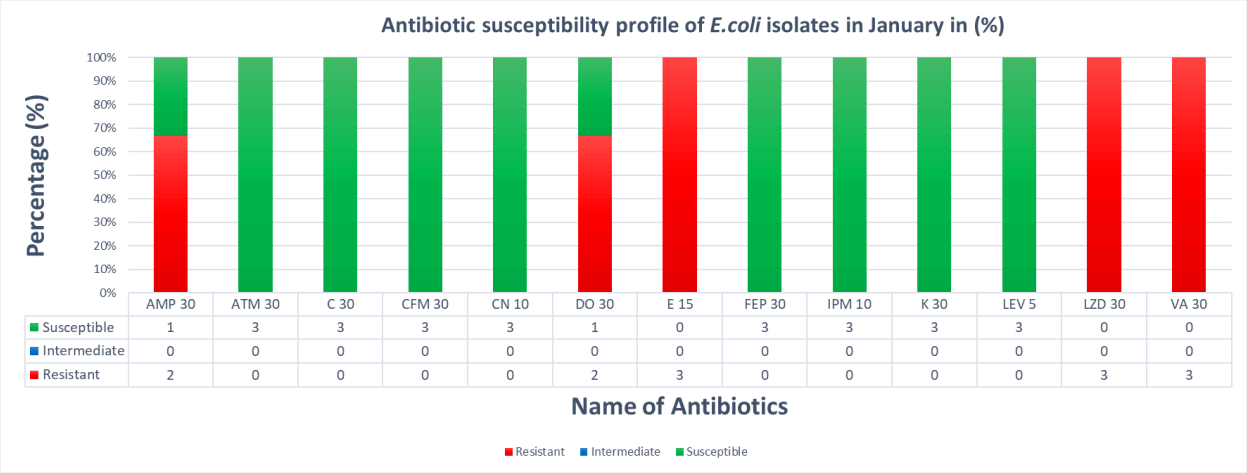

Figure 12 : Antibiotic susceptibility profile of *E.coli* isolates in January in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *E.coli* isolate from January exhibits high sensitivity (100%) toward Aztreonam, Chloramphenicol, Cefixime , Gentamicin, Cefepime,Imipenem Kanamycin and Levofloxacin. It shows resistance toward Ampicillin and Doxycycline (almost 70% with some sensitivity). Erythromycin, Linezolid, Vancomycin were not considered for resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria.

3.3.2 *Salmonella spp.* AST Results

3.3.2 (a) August

There are 8 *Salmonella spp.* isolates in total from the month August from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
AugSsS1(1)	0(R)	31 (S)	22.5 (S)	18.5(I)	16.5 (S)	18 (S)	0(R)	34.5 (S)	25.5 (S)	18 (S)	28(S)	0(R)	0(R)
AugSsS1(2)	0(R)	24(S)	25.5 (S)	19(S)	24.5 (S)	16 (S)	0(R)	32.5 (S)	25.5 (S)	18.5 (S)	23(S)	0(R)	0(R)
AugSsS1(3)	0(R)	28(S)	21.5 (S)	28(S)	17 (S)	17 (S)	0(R)	32(S)	23(S)	16.5 (I)	25(S)	0(R)	0(R)
AugSsS1(4)	0(R)	21(I)	22.5 (S)	19(S)	17 (S)	16 (S)	0(R)	32(S)	21(I)	16(I)	22(S)	0(R)	0(R)
AugSsS2(1)	0(R)	30.5 (S)	20(S)	19(S)	16 (S)	18.5 (S)	0(R)	30(S)	21(I)	19 (S)	24.5 (S)	0(R)	0(R)
AugSsS2(2)	0(R)	30(S)	23.5 (S)	18.5(I)	19 (S)	18 (S)	0(R)	34(S)	29(S)	18.5 (S)	27(S)	0(R)	0(R)
AugSsS2(3)	0(R)	27.5 (S)	22(S)	19(S)	16.5 (S)	18 (S)	0(R)	32(S)	25.5 (S)	18 (S)	22(S)	0(R)	0(R)
AugSsS2(4)	0(R)	29.5 (S)	21(S)	17.5(I)	18 (S)	19 (S)	0(R)	29.5 (S)	25(S)	18 (S)	26.5 (S)	0(R)	0(R)

Table 11 :*Salmonella spp.* isolates were collected in August and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

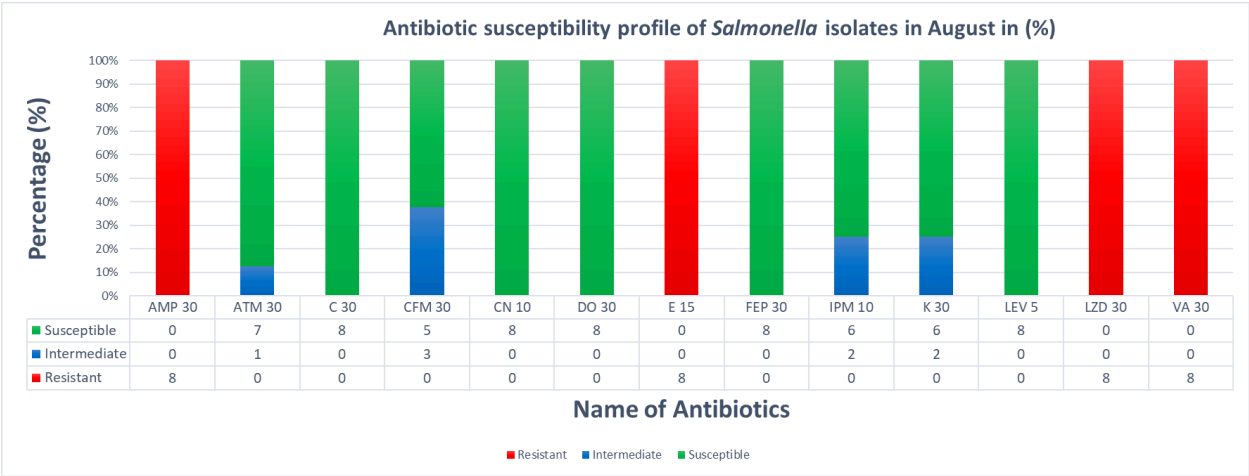

Figure 13: Antibiotic susceptibility profile of *Salmonella spp.* isolates in August in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *Salmonella spp.* isolate from August exhibits highest sensitivity (100%) toward Chloramphenicol, Gentamicin, Doxycycline, Cefepime . It shows higher sensitivity to Aztreonam (87.5% with 12.5% intermediate) , Cefixime (62.5% with 37.5% intermediate), Imipenem and Kanamycin with 75% sensitivity and 25 % intermediate. It shows 100% resistance toward Ampicillin. Erythromycin, Linezolid, Vancomycin were not considered for resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria.

3.3.2(b) September

There are 12 *Salmonella spp.* isolates in total from the month September from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
SepSsS1(1)	0(R)	29(S)	13(I)	18(I)	17 (S)	18 (S)	0(R)	29 (S)	24.5 (S)	18.5 (S)	29.5 (S)	0(R)	0(R)
SepSsS1(2)	0(R)	32(S)	21.5 (S)	16(I)	17.5 (S)	18 (S)	0(R)	32.5 (S)	25.5 (S)	18 (S)	27(S)	0(R)	0(R)
SepSsS1(3)	0(R)	28(S)	21(S)	14.5 (R)	19 (S)	17 (S)	0(R)	29(S)	26.5 (S)	18.5 (S)	23.5 (S)	0(R)	0(R)
SepSsS1(4)	0(R)	28(S)	21.5 (S)	16.5(I)	17.5 (S)	19 (S)	0(R)	31(S)	26.5 (S)	19 (S)	25(S)	0(R)	0(R)
SepSsS1(5)	0(R)	29(S)	25(S)	17(I)	0(R)	16 (S)	0(R)	31(S)	21(I)	16.5 (I)	27.5 (S)	0(R)	0(R)
SepSsS1(6)	0(R)	30.5 (S)	23.5 (S)	27(S)	18.5 (S)	19 (S)	0(R)	32.5 (S)	28.5 (S)	18.9 (S)	26.5 (S)	0(R)	0(R)
SepSsS1(7)	0(R)	30(S)	22(S)	17(I)	17 (S)	16 (S)	0(R)	32(S)	20.5 (I)	16 (I)	26 (S)	0(R)	0(R)
SepSsS1(8)	0(R)	29(S)	25.5 (S)	20.5 (S)	17 (S)	19 (S)	0(R)	33.5 (S)	25.5 (S)	17(I)	28(S)	0(R)	0(R)
SepSsS1(9)	0(R)	29(S)	10 (R)	28(S)	20 (S)	0(R)	0(R)	33(S)	26(S)	21.5 (S)	32(S)	0(R)	0(R)
SepSsS1 (10)	0(R)	29.5 (S)	20(S)	16.5(I)	16.5 (S)	17.5 (S)	0(R)	22.5(I)	26.5 (S)	18 (S)	24.5 (S)	0(R)	0(R)
SepSsS2(1)	0(R)	30(S)	17(I)	16(I)	19 (S)	0(R)	0(R)	33(S)	19.5 (I)	19.5 (S)	28(S)	0(R)	0(R)

SepSsS2(2)	0(R)	30(S)	22.5 (S)	20.5 (S)	18 (S)	18 (S)	0(R)	34(S)	21.5 (I)	18.5 (S)	23(S)	0(R)	0(R)

Table 12 :*Salmonella spp.* isolates were collected in September and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

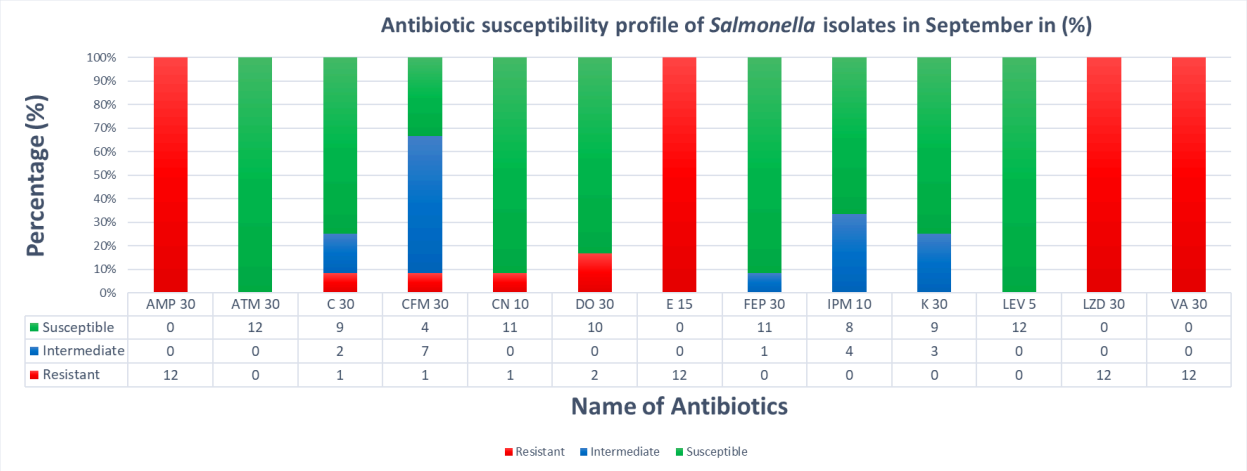

Figure 14 : Antibiotic susceptibility profile of *Salmonella spp.* isolates in September in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *Salmonella spp.* isolate from September exhibits high sensitivity toward Aztreonam (100%), Chloramphenicol and Kanamycin (75%), Gentamicin and Cefepime (91.6%) , Imipenem (66.6%). It shows 100% resistance toward Ampicillin. Erythromycin, Linezolid, Vancomycin were not considered for resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria. Exhibition of intermediate high in Cefixime (41.6%) along with C, FEP, IPM and K.

3.3.2 (c) October

There are 11 *Salmonella spp.* isolates in total from the month October from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
OctSsS1(1)	0(R)	37(S)	0(R)	18(I)	21 (S)	0(R)	0(R)	33 (S)	18 (R)	0(R)	29.5 (S)	0(R)	0(R)
OctSsS1(2)	0(R)	38.5 (S)	0(R)	31.5 (I)	23.5 (S)	11 (R)	0(R)	35 (S)	21 (I)	24.5 (S)	30.5 (S)	0(R)	0(R)
OctSsS1(3)	0(R)	35(S)	0(R)	19(S)	20 (S)	0(R)	0(R)	31.5 (S)	18.5 (R)	0(R)	28 (S)	0(R)	0(R)
OctSsS1(4)	0(R)	38(S)	0(R)	31(S)	21 (S)	11 (R)	0(R)	33 (S)	18 (R)	0(R)	27 (S)	0(R)	0(R)
OctSsS1(5)	0(R)	37(S)	23 (S)	22.5 (S)	21 (S)	11 (R)	0(R)	34 (S)	22(I)	21 (S)	0(R)	0(R)	0(R)
OctSsS1(6)	0(R)	33(S)	24.5 (S)	18(I)	19 (S)	20 (S)	0(R)	36 (S)	25.5 (S)	20.5 (S)	29 (S)	0(R)	0(R)
OctSsS1(7)	0(R)	27(S)	24(S)	18(I)	18.5 (S)	17 (S)	0(R)	30 (S)	22(I)	11 (R)	26 (S)	0(R)	0(R)
OctSsS2(1)	0(R)	38(S)	0(R)	0(R)	21 (S)	0(R)	0(R)	32 (S)	18(R)	18 (S)	31 (S)	0(R)	0(R)
OctSsS2(2)	0(R)	36.5 (S)	17(I)	0(R)	19 (S)	0(R)	0(R)	32 (S)	19(R)	18 (S)	30 (S)	0(R)	0(R)
OctSsS2(3)	0(R)	33.5 (S)	0(R)	0(R)	19.5 (S)	0(R)	0(R)	31.5 (S)	15(R)	18 (S)	30 (S)	0(R)	0(R)
OctSsS2(4)	0(R)	30(S)	16(I)	16(I)	18.5 (S)	0(R)	0(R)	31 (S)	20(I)	18 (S)	24 (S)	0(R)	0(R)

Table 13 :*Salmonella spp.* isolates were collected in October and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

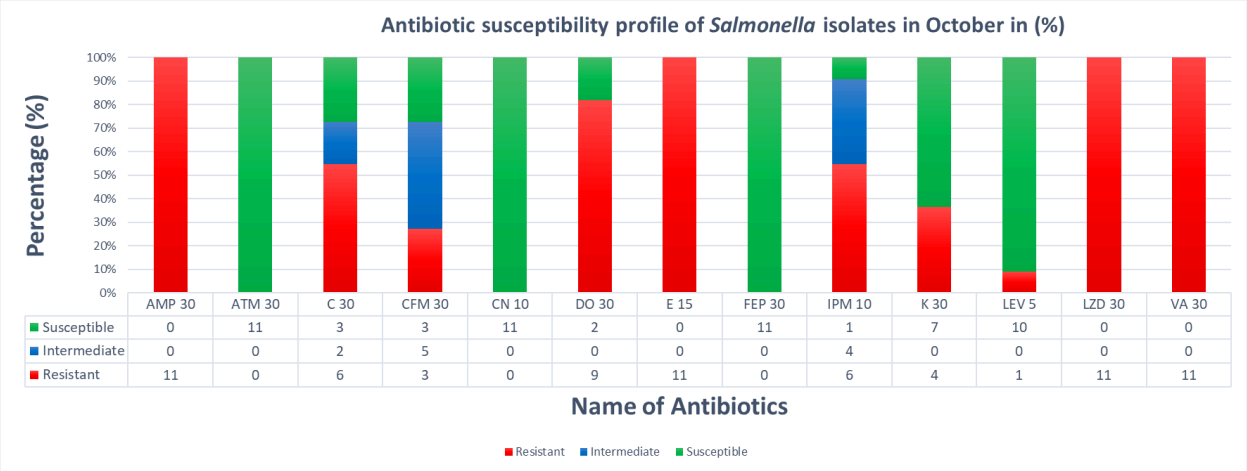

Figure 15 : Antibiotic susceptibility profile of *Salmonella spp.* isolates in October in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *Salmonella spp.* isolate from October exhibits high sensitivity toward Aztreonam (100%), Gentamicin (100%) and Kanamycin (63.3%). It shows high resistance toward Ampicillin (100%), Doxycycline (81.8%) , Chloramphenicol and Imipenem (54.5%). Erythromycin, Linezolid, Vancomycin were not considered for resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria. Exhibition of intermediate high in Cefixime (45.4%) along with CFM and IPM.

3.3.2 (d) November

There are 7 *Salmonella spp.* isolates in total from the month November from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
NovSsS1(1)	0(R)	29(S)	18.5 (S)	0(R)	20 (S)	0(R)	0(R)	33 (S)	26 (S)	18.5 (S)	27.5 (S)	0(R)	0(R)
NovSsS1(2)	0(R)	0(R)	18.5 (S)	0(R)	26.5 (S)	0(R)	0(R)	11 (R)	25 (S)	21 (S)	23 (S)	0(R)	0(R)
NovSsS1(3)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	20.5 (S)	22 (S)	22 (S)	0(R)	0(R)

NovSsS1(4)	0(R)	29(S)	17.5 (I)	0(R)	18.5 (S)	0(R)	0(R)	33 (S)	26.5 (S)	18.5 (S)	24 (S)	0(R)	0(R)
NovSsS2(1)	16(I)	29(S)	21(S)	22(S)	20 (S)	19 (S)	0(R)	34.5 (S)	26 (S)	21 (S)	31 (S)	0(R)	0(R)
NovSsS2(2)	21(S)	30(S)	25(S)	22(S)	18.5 (S)	22 (S)	0(R)	33.5 (S)	27.5 (S)	20 (S)	28 (S)	0(R)	0(R)
NovSsS2(3)	0(R)	30(S)	25.5 (S)	0(R)	21.5 (S)	23 (S)	0(R)	31.5 (S)	26(S)	20 (S)	27 (S)	0(R)	0(R)

Table 14:*Salmonella spp.* isolates were collected in November and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

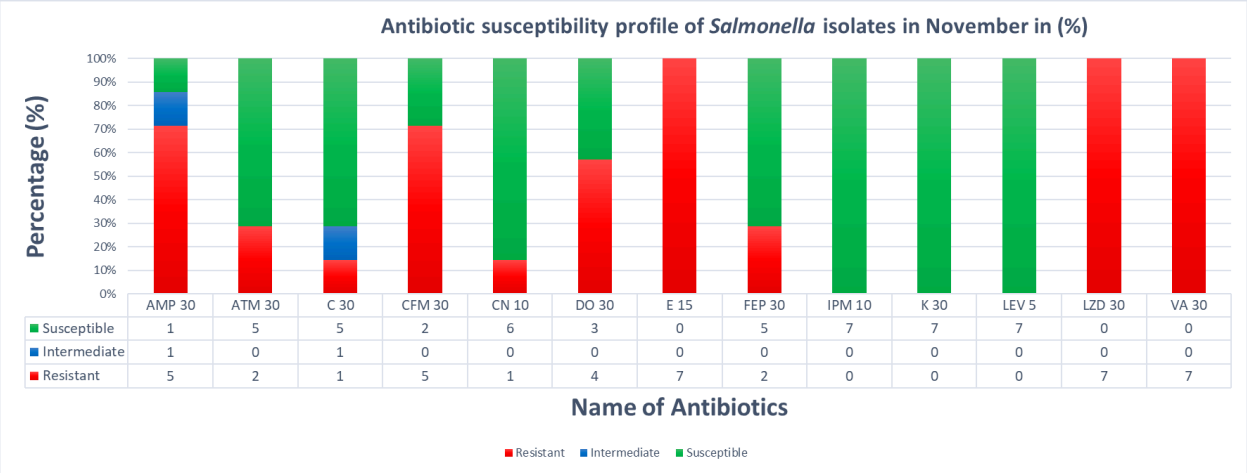

Figure 16 : Antibiotic susceptibility profile of *Salmonella spp.* isolates in November in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *Salmonella spp.* isolate from November exhibits high sensitivity toward Imipenem and Kanamycin (100%), Gentamicin (85.7%), Chloramphenicol and Aztreonam(71.4%). It exhibits higher resistance to Ampicillin and Cefixime (71.4%), Doxycycline (57.1%). AMP and C also exhibit intermediate. Erythromycin, Linezolid, Vancomycin were not considered for resistance type determination as intrinsic resistant antibiotics especially to the gram negative bacteria.

3.3.3 *Vibrio cholerae* AST Results

3.3.3(a) September

There are 3 *Vibrio cholerae* isolates in total from the month September from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
SepVS1(1)	0(R)	24.5 (S)	26 (S)	18 (I)	19.5 (S)	18.5 (S)	0(R)	26 (S)	19.5 (R)	15.5 (I)	24.5 (S)	0(R)	0(R)
SepVS2(1)	0(R)	30 (S)	27 (S)	15.5 (R)	18.5 (S)	0(R)	0(R)	29 (S)	15 (R)	19.5 (S)	25 (S)	0(R)	0(R)
SepVS2(2)	23.5 (S)	41 (S)	17 (I)	36 (S)	23 (S)	0(R)	0(R)	35 (S)	28.5 (S)	25 (S)	29 (S)	0(R)	0(R)

Table 15 : *Vibrio cholerae* isolates were collected in September and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

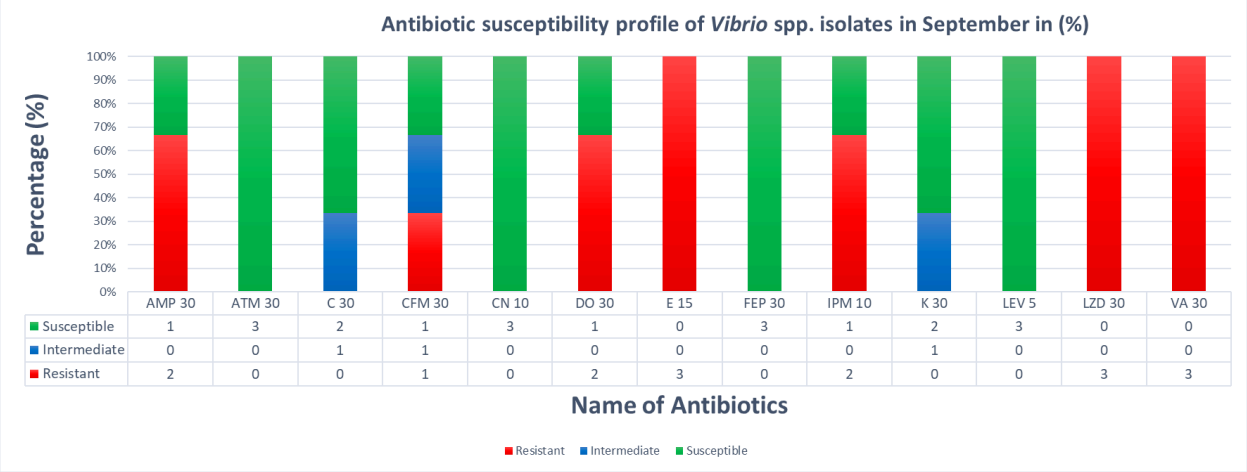

Figure 17 : Antibiotic susceptibility profile of *Vibrio cholerae* isolates in September in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *Vibrio cholerae* isolate from September shows complete sensitivity to Aztreonam, Cefepime and Levofloxacin . A high percent of sensitivity can also be noticed in Chloramphenicol and Kanamycin(66.66%). Higher resistance is exhibited towards

Ampicillin, Imipenem, Doxycycline(66.66%). C, CFM (shows resistance too), K also show an intermediate response as well. Linezolid, Vancomycin in itself are ineffective against Gram-negative bacteriophages .

3.3.3 (b) October

There are 5 *Vibrio cholerae* isolates in total from the month October from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
OctVS1(1)	10.5 (R)	25 (S)	30 (S)	27.5 (S)	18.5 (S)	25 (S)	14 (I)	25.5 (I)	13.5 (R)	21.5 (S)	25.5 (S)	19.5 (R)	0(R)
OctVS1(2)	13.5 (R)	25(S)	29.5 (S)	25 (S)	19 (S)	25.5 (S)	15 (I)	25 (I)	15 (R)	19.5 (S)	26 (S)	21.5 (I)	0(R)
OctVS2(1)	11.5 (R)	25 (S)	27.5 (S)	26 (S)	19.5 (S)	24 (S)	14 (I)	25.5 (I)	15.5 (R)	18.5 (S)	26 (S)	18.5 (R)	0(R)
OctVS2(2)	0(R)	22.5 (S)	29 (S)	25 (S)	18.5 (S)	23.5 (S)	15.5 (I)	26.5 (S)	15.5 (R)	18 (S)	24 (S)	20 (R)	0(R)
OctVS2(3)	0(R)	24.5 (S)	30.5 (S)	28.5 (S)	19 (S)	27 (S)	16 (I)	27.5 (S)	15 (R)	17.5 (I)	27 (S)	23 (S)	0(R)

Table 16 : *Vibrio cholerae* isolates were collected in October and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

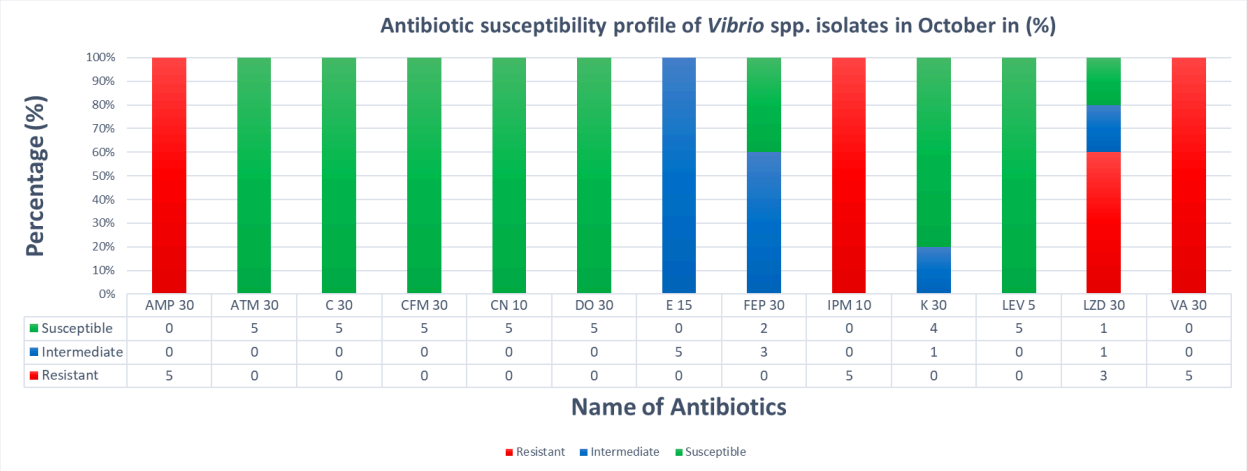

Figure 18 : Antibiotic susceptibility profile of *Vibrio cholerae* isolates in October in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *Vibrio cholerae* isolate from October shows complete sensitivity to Aztreonam, Chloramphenicol, Cefixime, Gentamicin, Doxycycline, Levofloxacin. Kanamycin shows great sensitivity (80%) along with intermediate response. It shows complete resistance towards Ampicillin and Imipenem and complete intermediate response towards Erythromycin. Cefepime shows great intermediate response(60%) along with sensitivity. Linezolid, Vancomycin in itself are ineffective against Gram-negative bacteriophages.

3.3.3(c) November

There are 17 *Vibrio cholerae* isolates in total from the month November from Aftabnagar.

Sample Name	AMP 30	ATM 30	C 30	CFM 30	CN 10	DO 30	E 15	FEP 30	IPM 10	K 30	LEV 5	LZD 30	VA 30
NovVS1(1)	0(R)	31.5 (S)	29 (S)	18 (I)	19 (S)	26 (S)	0(R)	31 (S)	15 (R)	20 (S)	26 (S)	0(R)	0(R)
NovVS1(2)	0(R)	35.5 (S)	25 (S)	26 (S)	15 (S)	28.5 (S)	0(R)	36 (S)	20 (I)	19 (S)	0(R)	20 (R)	0(R)
NovVS1(3)	0(R)	27.5 (S)	30 (S)	28.5 (S)	20 (S)	26.5 (S)	16 (I)	27.5 (S)	17.5 (R)	23.5 (S)	25 (S)	23.5 (S)	0(R)
NovVS1(4)	0(R)	28 (S)	31.5 (S)	31 (S)	18.5 (S)	29 (S)	13 (I)	32 (S)	18 (R)	22 (S)	23 (S)	21.5 (I)	0(R)
NovVS1(5)	0(R)	37 (S)	25 (S)	19.5 (S)	17 (S)	21 (S)	0(R)	34 (S)	26.5 (S)	19.5 (S)	30.5 (S)	0(R)	0(R)
NovVS1(6)	0(R)	26.5 (S)	24 (S)	19 (S)	16 (S)	17.5 (S)	0(R)	27.5 (S)	20.5 (I)	16.5 (I)	26 (S)	0(R)	0(R)
NovVS1(7)	0(R)	29 (S)	25 (S)	22 (S)	18 (S)	18 (S)	0(R)	27 (S)	21 (I)	19 (S)	0(R)	0(R)	0(R)
NovVS2(1)	0(R)	28 (S)	35 (S)	31 (S)	19 (S)	25 (S)	16(I)	27 (S)	15.5 (R)	20 (S)	25 (S)	0(R)	0(R)
NovVS2(2)	0(R)	29 (S)	27.5 (S)	24 (S)	22 (S)	22 (S)	0(R)	31.5 (S)	19 (R)	22.5 (S)	29.5 (S)	0(R)	0(R)
NovVS2(3)	0(R)	39 (S)	42 (S)	40 (S)	25 (S)	40 (S)	19.5 (I)	37.5 (S)	24.5 (S)	24 (S)	25 (S)	23.5 (S)	0(R)
NovVS2(4)	0(R)	25.5 (S)	25 (S)	16.5 (I)	14 (I)	22 (S)	0(R)	24.5 (S)	19.5 (R)	0(R)	23 (S)	0(R)	0(R)

NovVS2(5)	0(R)	25.5 (S)	29 (S)	23(S)	18 (S)	21.5 (S)	0(R)	27 (S)	15 (R)	21.5 (S)	22 (S)	0(R)	0(R)
NovVS2(6)	0(R)	27.5 (S)	28 (S)	22 (S)	22 (S)	23 (S)	0(R)	31 (S)	24 (S)	19 (S)	30 (S)	0(R)	0(R)
NovVS2(7)	0(R)	27 (S)	35 (S)	29.5 (S)	19.5 (S)	27.5 (S)	16.5 (I)	30 (S)	16.5 (R)	21 (S)	26 (S)	0(R)	0(R)
NovVS2(8)	0(R)	21 (I)	29 (S)	22 (S)	20.5 (S)	22 (S)	0(R)	28 (S)	19 (R)	25 (S)	28.5 (S)	0(R)	0(R)
NovVS2(9)	0(R)	31.5 (S)	25.5 (S)	24 (S)	16.5 (S)	21.5 (S)	15.5 (I)	29 (S)	30.5 (S)	19.5 (S)	23 (S)	0(R)	0(R)
NovVS2(10)	16 (I)	26.5 (S)	22.5 (S)	14.5 (R)	17 (S)	17 (S)	0(R)	26 (S)	22 (I)	17 (I)	25 (S)	0(R)	0(R)

Table 17 : *Vibrio cholerae* isolates were collected in November and subjected to antibiotic susceptibility tests, all of which were collected from Aftanagar.

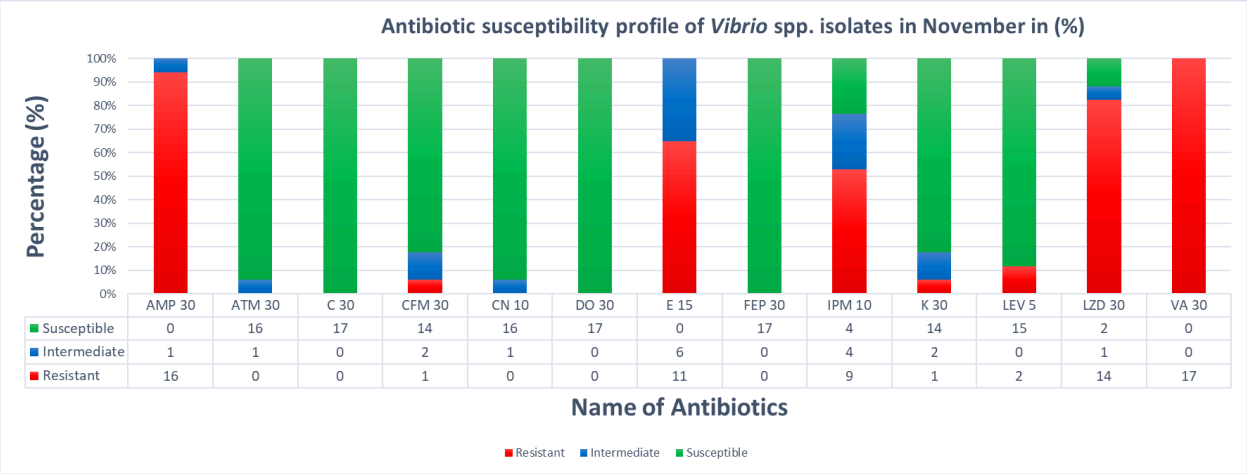

Figure 19 : Antibiotic susceptibility profile of *Vibrio cholerae* isolates in November in terms of resistant, intermediate and susceptible reactions to various antibiotics.

This figure demonstrates that the *Vibrio cholerae* isolate from October shows complete sensitivity to Chloramphenicol, Doxycycline, Cefepime. A high sensitivity can be seen towards Aztreonam and Gentamicin (94.1%), Levofloxacin (88.22%), kanamycin and Chloramphenicol (

82.33%). Almost 95% of resistance can be seen towards Ampicillin. A strong resistance can be noticed towards Erythromycin and Imipenem as well. ATM, CFM, CN, E, IPM, K show intermediate response as well. Linezolid, Vancomycin in itself are ineffective against Gram-negative bacteriophages.

Overall from the testing of antibiotic reactions, *Salmonella* exhibited the overall highest resistance burden 40.28% followed by overall resistant rates in *E. coli* at 32.41% and *Vibrio cholerae* at 32.31%. Of the tested organisms, this indicates that *Salmonella spp.* had the most phenotypic pattern resistance in Aftabnagar water sample.

Note

No bacterial growth was found for Salmonella spp in December and January. No bacterial growth was found for Vibrio cholera in August, December and January.

Chapter 4

Discussion

This study presents the evidence that antibiotic-resistant bacteria in this water sample collected from Aftabnagar, Dhaka has a clear public and environmental-health significance. Isolation of *Escherichia coli, Salmonella spp*. and *vibrio cholera* from this single urban source indicates that the water body is subject to sufficient contamination to support not only bacterial viability, but antibiotic resistant survival as well. The recovery of resistant enteric bacteria from this source is anticipated since Aftabnagar has high density anthropogenic activity, drainage pressure and urban waste pressure . The finding is significant because it is the same for months and across more than one bacteria group.

There were 110 isolates studied in this study. *Escherichia coli* was the most common organism isolated, accounting for 42.73% (47/110) of the total isolates recovered, followed by *Salmonella spp.* and *Vibrio cholera* occurs at 34.55% (38/110). at 22.73% (25/110). By month-wise distribution also, there was highly uneven bacterial recovery pattern. From the month of August until January, November was the peak month with 53% (25/47) of all *E. coli* counted during this time span being obtained in that month alone. In August, 14.89% (7/47), in October 17.02% (8/47), September and January each 6.38% (3/47), whereas December had the least recovery of 2.13% (1/47). *Salmonella spp.* were isolated from August to November, recovering 31.58% (12/38) in September, 28.95% (11/38) in October, 21.05% (8/38) in August and 18.42% (7/38) in November respectively. For *Vibrio cholera*, more isolates were recovered in November 68.00% (17/25), followed by October 20.00% (5/25) and September 12.00% (3/25). The variations indicate that the presence of bacteria in Aftabnagar source was not constant over the sampling period, and could probably be affected by seasonal or environmental factors like rainfall, temperature, waste input, movement of water or change in human activities around it.

Over the study months, *E. coli* showed a broadly distributed but sometimes unpredictable resistance pattern. AMP, E, LZD, and VA were the drugs against which repeated resistance was most regularly seen; all isolates remained susceptible to ATM, C, FEP, LEV and many strains in November but much lower proportions than in earlier months, also showed some level of intermediate or resistant responses with regard to CFM,CN, DO IPM and K. This implies that the

burden of resistance of *E. coli* was not homogenous in the Aftabnagar water source, possibly the temporal nature of the resistance pattern as well. One of these is that *E. coli* is a fecal indicator organism and an environmental reservoir of other resistance traits, and its high frequency and repeated non-susceptibility in this study raise particular concern. Among the respective group of bacteria *E. coli* demonstrated rather high-end resistance against other antibiotics, for instance 100% towards linezolid and vancomycin respectively and 97.87% towards erythromycin. Ampicillin exhibited a resistance level of 53.19%, whereas doxycycline had a resistance rate of 23.40%. On the contrary, lower resistance rates were observed against cefixime (10.64%), kanamycin 10.64%, chloramphenicol (6.38%), cefepime (6.38%), aztreonam (4.26), levofloxacin 4.26%, gentamicin and imipenem (2.13%). Cumulatively, these results indicate that while *E. coli* was susceptible to many of the antibiotics evaluated, it retained considerable resistance against some specific antibiotics.

The response profile for *Salmonella spp*. seemed slightly more homogeneous than that of *E. coli*, which also showed a similar pattern of significant reduction , The majority of the *Salmonella* isolates were susceptible to ATM, C, FEP, and LEV but resistance against AMP, E, LZD and VA was repeatedly documented during the follow-up period. In the month of September, four (10%) and in October fourteen (20%) isolates reacted intermediately or resistant against CFM, IPM and K while it had complicated resistance profiles restricted (not at all) on to three antibiotics throughout the remaining months. *Salmonella* is a ubiquitous enteric pathogen and the concurrent presence of reduced antibiotic susceptibility in the environment raises concerns about waterborne exposure to resistant strains. In some cases, we found an even stronger resistance pattern of the *Salmonella* isolates. Results Total resistance to erythromycin (100%), linezolid (100%) and vancomycin (100%).Very high levels of resistance were also observed for ampicillin (94.74%). Of moderate resistance had been observed against doxycycline (39.47%), cefixime (23.68%), chloramphenicol (21.05%)B, and imipenem antimicrobial agents (15.79%); Resistance percentages were lower for kanamycin (10.53%), aztreonam (5.26%), gentamicin (5.26%), cefepime (5.26%) and levofloxacin (2.63%). This trend is suggestive of Salmonella resultants from the Aftabnagar water sample exhibited a higher degree of antibiotic resistance than many of the *E. coli* isolates.

The resistance pattern in *Vibrio cholerae* isolates. AMP, E and VA had a high rate of repeat resistance with many isolates continuing to be susceptible for ATM, C, CFM, CN, DO and FEP. At the same time , a number of isolates were non-susceptible to IPM and occasionally showed decreased susceptibility towards K, LEV and LZD in September to November. The isolation of the highest number of *Vibrio* isolates in November, together with their mixed response, resistant to intermediate and susceptible indicates that this month may represent environmental conditions particularly favourable for survival or enrichment of resistant *Vibrio* populations into the water source. *Vibrio* species are naturally occurring in water, so their presence is not entirely surprising, but together with antibiotic resistance makes these bacteria one of the more consequential threats to public health. For *Vibrio cholerae*, more than two-thirds (67.00%) are resistant against vancomycin (100%), ampicillin (92.00%) linezolid (80.00%) and imipenem (64.00%). Erythromycin had a relatively high resistance rate (56.00%). No resistant isolates were detected against aztreonam, chloramphenicol, gentamicin or cefepime. In contrast to lower resistance observed against some antibiotics, cefixime (8.00%), doxycycline (8.00%), levofloxacin (8.00%), kanamycin (4.00%). These data indicate that while *Vibrio* isolates continued to exhibit susceptibility to several antibiotics, they demonstrated significant resistance to certain critical medications, ampicillin and imipenem.

This study indicates that the water sample from Aftabnagar, Dhaka can act as a reservoir for environmental antibacterial resistance. The most frequent pathogen was *E. coli* followed by *Salmonella spp*. and *Vibrio spp.*, along with the repeated resistance patterns seen within all three groups suggests that this waterway is of microbiological and public-health significance. These results suggest that urban water contamination and antibiotic resistant environmental bacteria could be a potential public health problem in Dhaka needing improved environmental monitoring, waste disposal practices, and community awareness.

Chapter 5

Conclusion

The bacteria present in the water sample from Aftabnagar that this study evaluated were found to be antibiotic resistant and of distinct environmental pollution importance. Escherichia coli (42.73%) was the most prevalent organism isolated among 110 isolates recovered, followed by Salmonella spp. (34.55%) and Vibrio spp. (22.73%) and remain significantly contaminated with microbes comparing these sampled water source. Enteric bacteria resistant to antimicrobials are repeatedly maintained in urban surface waters in and around Dhaka where anthropogenic pressure is high. This study provides localized evidence from Aftabnagar and will contribute to the increasing body of evidence suggesting that environmental AMR in Dhaka is a city-wide concern, not merely a site-specific anomaly. Only an impact on water quality should take measures to limit the environmental spread of resistant bacteria with potential epidemic consequences for the community .

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