Thesis Report

Detection and Antimicrobial Resistance Profiling Bacteria Isolated from Seawater, Major Bathing Beaches of Cox's Bazar, Bangladesh.

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of B.Sc. in Microbiology.

Department of Mathematics and Natural Sciences
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Declaration

It is hereby declared that

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

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Approval

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Thesis report has been accepted as satisfactory in partial fulfillment of the requirement for the degree of B.Sc. in Microbiology.

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Ethics Statement

This research on antibacterial resistance in water sources of Bangladesh was ethically conducted so that the study would be as sound and the environment unharmed. The study was mainly concerned with environmental samples, but all necessary precautions were taken to minimize any potential impact on local communities and ecosystems. Data collected will be treated in the respect of confidentiality and integrity so that honest reporting of results can be made even without fabrication and manipulation. Thus, fulfilling the ethical obligations, this research is likely to provide some invaluable insights into antibiotic resistance without infringing on the rights of either humans or the environment.

Acknowledgment

I would like to express my profound gratitude to my father for his constant encouragement and belief in me. His unwavering support was invaluable in helping me complete this project successfully. Thank you, Abbu.

Abstract

Coastal bathing waters are potential reservoirs of pathogenic bacteria, posing serious risks to public health. Cox's Bazar, the world's longest natural beach and a highly visited tourist destination in Bangladesh, lacks updated data on microbial safety. This study aimed to assess the bacteriological quality of seawater from major bathing sites in Cox's Bazar, focusing on two key pathogens and their antimicrobial resistance patterns. Seawater samples were systematically collected from four popular beaches—Sugandha, Laboni, Him Chori, and Inani. Bacterial isolation was carried out using selective media: MacConkey agar for *Escherichia Coli*, TCBS agar for *Vibrio cholerae*. Presumptive isolates were purified, confirmed by PCR, and subjected to antimicrobial susceptibility testing (AST) against 14 commonly used antibiotics following standard disc diffusion methods. A total of 260 bacterial isolates were confirmed, comprising 114 *E.coli*, and 146 *V. cholerae*. AST results showed varied susceptibility patterns. Overall, most isolates exhibited high susceptibility to imipenem, doxycycline, and chloramphenicol, while notable resistance was observed against vancomycin and ampicillin. *V. cholerae* isolates demonstrated the highest proportion of susceptibility, whereas *E.coli* showed more diverse resistance profiles. The detection of pathogenic bacteria and their multidrug-resistant traits in bathing waters of Cox's Bazar highlights a significant public health concern. Continuous monitoring and proper management strategies are urgently needed to ensure safe recreational water quality and prevent waterborne disease outbreaks.

Keywords

Antibacterial Resistance, Antibiotic Susceptibility Testing (AST), Polymerase Chain Reaction (PCR), Gel electrophoresis, *Escherichia Coli(E.coli)*, *Vibrio cholerae.*

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List of Acronyms

PCR	Polymerase Chain Reaction
TCBS	Thiosulfate-citrate-bile salts-sucrose agar
AST	Antibiotic Susceptibility Test

Glossary

- **Antibiotic Resistance:** The ability of bacteria to resist the effects of drugs that once killed them or inhibited their growth, making infections harder to treat.
- ***Escherichia Coli*(*E.coli*):** A common bacterium found in the intestines of humans and animals; some strains can cause serious food poisoning.
- ***Vibrio*:** A genus of bacteria, some species of which can cause gastrointestinal illness and are often associated with seafood.
- **Polymerase Chain Reaction (PCR):** A molecular biology technique used to amplify specific DNA sequences, allowing for the detection and identification of bacterial species.
- **Gel Electrophoresis:** A method used to separate DNA fragments based on their size, often used in conjunction with PCR for analysis.
- **Antibiotic Susceptibility Testing (AST):** Laboratory tests that determine the effectiveness of antibiotics against specific bacteria.
- **Selective Media:** Growth media designed to favor the growth of certain types of organisms while inhibiting others, used for isolating specific bacterial species.

Chapter 01: Introduction

Cox's Bazaar is the world's longest natural unbreakable sea beach; Length is over 120 km located in Bangladesh along with the Bay of Bengal. It is one of the country's most popular tourist destinations for its glittery sand, waves, and scenic beauty. This unbreakable sea beach attracts both local and international tourists every year. Popular attractions include Himchori National Park, Inani Beach with its coral stones and Laboni Beach for sunset, Sugandha and Kolatoli Beach, popular for various restaurants and activities. Cox's Bazaar also plays a significant role in Bangladesh's fishing industry. It offers a variety of seafood, making it a favorite spot for relaxation.

Figure 01 : Cox's Bazar Sunset.

1.1 Importance of Marine and Coastal Waters Recreation and Tourism.

Marine and coastal tourism is a major driver of global development, contributing significantly to revenue, foreign exchange, and employment, particularly in developing countries. Beyond economics, it supports conservation initiatives, fosters public awareness of marine ecosystems, and provides alternative livelihoods that reduce pressure on natural resources (UNEP, 2022). Socially, it enhances community development, preserves cultural heritage, and improves quality of life through infrastructure and recreational opportunities.
However, challenges such as overtourism, pollution, and climate change threaten long-term sustainability. To maintain its benefits, effective management is essential to balance growth with ecological protection (UNWTO, 2023).

1.2 Water quality of Cox's Bazar

Cox's Bazar, is experiencing increasing stress on its water resources due to groundwater depletion, salinity intrusion, and the demands of hosting one of the world's largest refugee populations.

Groundwater Scarcity and Salinization: Nearly one-fourth of tube wells longer often producing saline or turbid water. Seasonal salinity also intensifies during the dry months, with one-third of wells in Chakaria Upazila exceeding safe sodium thresholds (Global Voices, 2025). In contrast, the Dupi Tila aquifer in Ukhiya remains relatively stable, offering less saline groundwater and demonstrating stronger recharge potential (Rohingya Response, 2025).

Contamination Risks**:** Water quality monitoring indicates that contamination is a persistent threat. Water Security Network (2023) reported that 28% of source samples contained fecal contamination, which rose to 74% once stored at the household level. Similar concerns have been raised about poor source quality, long collection queues, and unsafe storage practices (WASH Cluster, 2022).

Hydrogeological and Water Quality Studies: Hydrochemical and isotopic research has identified seawater intrusion and groundwater vulnerability zones in the coastal belt (TU Darmstadt, 2025). Groundwater samples from refugee camps were found to be moderately hard with acceptable pH and free from arsenic; however, elevated concentrations of iron and manganese in some locations exceeded recommended limits (ResearchGate, 2025).

1.3 Rising Concern of Microbial Contamination in Bathing Place

The growing popularity of coastal recreation in Cox's Bazar has led to an increase in microbial contamination of bathing waters—a matter of serious public health concern. A study investigating recreational beach and offshore waters around Saint Martin's Island, part of the Cox's Bazar region, recorded alarming levels of bacterial indicators. Total coliform counts ranged from 15.33 to 657.22 CFU/100 mL, while fecal coliform levels fluctuated between 1.37 and 272.31 CFU/100 ml. The study further noted that bacterial loads spiked by approximately 63% during peak tourist seasons (Kashem et al., 2022). Moreover, broader microbial community analyses in Cox's Bazar coastal waters have identified significant bacterial diversity. Dominant phyla include Proteobacteria (71.7%), Bacteroidetes (17.4%), and Actinobacteria (4.7%), with genera such as Alteromonas, Vibrio, and Marivita among the most prevalent (Akter et al., 2023). The presence of Vibrio species, known opportunistic pathogens, is especially concerning in recreational waters where human exposure is frequent. Taken together, these findings emphasize the pressing need for continual microbial monitoring, stronger wastewater regulation, and improved sanitation infrastructure around Cox's Bazar's bathing sites. Without timely intervention, elevated microbial loads risk adverse health outcomes ranging from gastroenteritis to skin infections among locals and tourists alike. Additionally, dumping of raw sewage from hotel, resort, restaurant, and local household drains directly into the sea as there are no wastewater plants, especially near popular beaches like Sugandha, Laboni, Kolatoli, and Inani. Lastly, the most visible forms of human pollution at Cox's Bazar is litter left by visitors every day. Thousands of tourists, especially during peak season, left-over food, wrappers, plastics, bottles, etc. These items are often dumped directly onto the sand or water with minimal administration of disposal regulations.

1.4 Pathogenic bacteria in Bathing water - public health concern

Bathing waters are vital for recreation and tourism but face increasing risks from microbial contamination. According to the World Health Organization (2021), untreated sewage, stormwater, and agricultural runoff introduce fecal indicator bacteria such as *Escherichia coli* and intestinal enterococci, signaling the presence of pathogens. Studies in Cox's Bazar identified high coliform counts, particularly during peak tourist seasons, and detected *Vibrio* species of public health concern (Kashem et al., 2022; Akter et al., 2023). Such contamination is linked to gastrointestinal, skin, and respiratory illnesses (Ashbolt, 2022). Addressing these risks requires strict wastewater management, monitoring, and improved sanitation infrastructure.

1.4.1 Waterborne Diseases linked to Contaminated Seawater.

Microbial contamination of seawater by fecal matter is a major public health concern, particularly in coastal bathing areas. *Escherichia coli* is widely used as an indicator of fecal pollution; its elevated presence reflects potential contamination with enteric pathogens (WHO, 2021). High *E. coli* levels are associated with gastrointestinal illnesses, diarrhea, and urinary tract infections (Ashbolt, 2022). Similarly, *Vibrio cholerae*, the causative agent of cholera, is naturally present in coastal ecosystems and thrives in warm, nutrient-rich waters. Outbreaks of cholera linked to *V. cholerae* in bathing waters remain a pressing issue in South Asia, including Bangladesh (Kashem et al., 2022; Akter et al., 2023). Typhoid, Giardia or Hepatitis A is serious waterborne disease as well.

Figure 02 : Common Waterborne Disease.

1.4.2 Transmission of Enteric Pathogens

Bathing beaches are critical recreational spaces but are also vulnerable to microbial contamination, creating significant public health risks. Enteric pathogens such as *Escherichia coli* and *Vibrio cholerae* are among the most frequently detected microorganisms in coastal waters, and both are strongly associated with fecal contamination. Popular beach water always used for swimming or bathing by tourists can become contaminated by untreated sewage, animal waste or human waste or runoff carrying harmful microbes. Untreated or poorly treated sewage introduces high loads of fecal bacteria into coastal and freshwater bathing areas. Addiitionally, Livestock, wildlife, and pets contribute bacteria that wash into water bodies after rainfall. Heavy rains flush contaminants including fecal material from streets, drains, and land surfaces into recreational waters. Most importantly, People themselves release microbes into the water through skin, hair, or accidental fecal contamination. Although sea water is saline water, but Escherichia coli, Klebsiella *Vibrio cholera*, Salmonella, Shigella are commonly found and may cause gastrointestinal disease, skin infection, or life-

threatening diseases like cholera or typhoid. *E. coli* is widely used as an indicator of fecal pollution, as high concentrations often coincide with the presence of other enteric pathogens that cause gastrointestinal illness, skin infections, and urinary tract diseases (Ashbolt, 2022). In Bangladesh, studies around Cox's Bazar and Saint Martin's Island have documented *E. coli* and fecal coliform levels exceeding international safety guidelines, particularly during peak tourist seasons, when bacterial counts increased by more than 60% due to intensified human activity and insufficient waste management (Kashem et al., 2022).
Similarly, *Vibrio cholerae*, the causative agent of cholera, is naturally present in warm, saline coastal waters and proliferates under nutrient-rich conditions. As reported by Akter et al. (2023), Vibrio species are abundant in Cox's Bazar's marine environment, raising concerns of cholera outbreaks where bathing waters intersect with inadequate sanitation in nearby communities. Without proper wastewater treatment and monitoring, recreational beaches risk becoming transmission hotspots, threatening both local populations and visiting tourists (UNEP, 2022).

1.4.3 Transmission Pathways of Pathogenic Bacteria in Bathing Beaches

According to WHO (2021), pathogenic bacteria such as *Escherichia coli* and *Vibrio cholerae* can reach recreational waters through multiple pathways. Once present, they can infect humans mainly through direct contact, ingestion, and inhalation during bathing activities.Once in the water, bacteria survive and disperse depending on the environmental transport system : Currents and tides are responsible in coastal waters. In addition, Temperature and sunlight, which influence bacterial survival and growth. Sediment resuspension, which can release pathogens back into the water column after being trapped in sand or mud. Pathways of Human Exposure is also included, Ingestion: Swallowing small amounts of contaminated water while swimming. Dermal contact: Pathogens entering through cuts, abrasions, or mucous membranes (eyes, ears, nose). And , Inhalation: Breathing in aerosols or droplets containing bacteria, particularly in surf zones or while diving.

These exposure pathways can lead to gastrointestinal infections, ear and eye infections, respiratory illnesses, and skin conditions.

1.4.4 WHO Guidelines on Recreational Water Quality

According to the World Health Organization (WHO, 2021), the Guidelines on Recreational Water Quality of Coastal and Fresh Waters are designed to protect public health by addressing hazards related to water quality. The focus is specifically on microbial and chemical risks. WHO emphasizes that while recreational waters like lakes, rivers, and beaches provide significant mental and physical health benefits, they can also be sources of exposure to harmful microorganisms and toxic substances if polluted. Protecting water users from such risks is therefore a central public health goal.
To achieve safe recreational water use, WHO recommends: Establish health-based targets for microbial and chemical indicators of contamination, such as fecal microbes and cyanotoxins. Implement Recreational Water Safety Plans which involve site-specific assessments and proactive risk management. Conduct regular monitoring and communicate risks promptly to the public whenever contamination levels are high. Rather than testing directly for all possible pathogens, WHO advises the use of indicator organisms—particularly Escherichia coli and enterococci—as reliable markers of fecal contamination. Their presence indicates an increased likelihood of health risks. The guidelines classify recreational water quality into categories

ranging from low risk to high risk, typically using the 95th percentile of enterococci concentrations as the benchmark for predicting the probability of illness among swimmers**.**

1.5 Target Pathogens in this Study

Among the various microorganisms that may be present in the saline water, two pathogenic bacteria were chosen of particular concern are Escherichia coli and Vibrio cholerae. E. coli is recognized widely as an indicator of fecal contamination and is associated with gastrointestinal illness and urinary tract infection. Secondly, Vibrio cholerae is a bacterium that is responsible for cholera, a serious disease that leads to watery diarrhea. It is commonly found in coastal areas and can grow well in slightly salty or salty water. They can survive in difficult environments, including salty water. Since the diseases mentioned are all waterborne, these three bacteria make the tourists a risk who swim or come into contact with seawater. As the bacteria can spread and cause infection.

1.5.1 Escherichia Coli

E. coli is a gram-negative, rod-shaped, facultative, anaerobic, coliform bacteria. These bacteria are harmless. Most E. coli strains are part of the normal microbiota of the gut. For example, some strains of E. coli benefit their hosts by producing vitamin K2. However, a few strains are potential foodborne and waterborne pathogens and produce potent enterotoxins. The most common E. coli strains cause disease by secreting Shiga toxin. This toxin causes mainly food contamination.

Escherichia coli as an Indicator of Pathogenic Microorganisms in Bathing Water

The presence of *Escherichia coli* in bathing waters is widely recognized as a key indicator of fecal contamination and, by extension, the possible presence of pathogenic microorganisms. According to the World Health Organization (2021), *E. coli* is not only a commensal organism in the human gut but also a reliable signal of fecal pollution, reflecting inputs from untreated sewage, animal waste, or stormwater runoff. Elevated *E. coli* concentrations correlate with increased risks of exposure to other enteric pathogens such as *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio cholerae* (Ashbolt, 2022). In coastal environments like Cox's Bazar, high *E. coli* levels during peak tourist activity further suggest inadequate waste management and rising health hazards (Kashem et al., 2022). Thus, monitoring *E. coli* in recreational waters is critical for early warning of microbial risks and for guiding effective public health interventions.

Beach or recreational water can be contaminated with *E. coli* and pose a risk of GastroIntestinal illness in general. For instance, a study in July 2025 found that children have a "high sensitivity to swimming-associated gastrointestinal illness response," with beach water polluted by sewage point sources carrying untreated human waste.

Gastrointestinal Illness from *E. coli* in Bathing Water

In Bangladesh, researchers have long suspected that unpolluted ponds and surface waters harbor pathogens such as *E. coli*—turning everyday bathing spots into lurking health hazards. A detailed study in rural Bangladesh found that many ponds—used for bathing, fishing, or daily chores—contained *E. coli* levels soaring up to 10,000 times above the U.S. EPA's safety threshold for recreational water (126 MPN/100 mL).These alarmingly high bacterial counts were strongly associated with nearby unsanitary latrines and population density, implying that human waste is a major contamination source. While the study didn't directly trace *E. coli*

infections back to specific bathing events, it strongly implies a credible risk—if the water looks like wastewater, you've got to expect trouble.

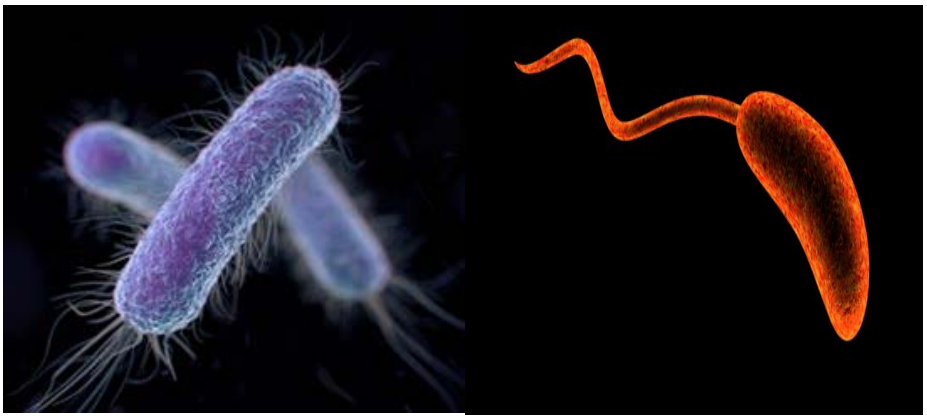
In the coastal or beach context, epidemiological studies elsewhere consistently link fecal contamination indicators—like *E. coli*—to higher rates of gastrointestinal illness among swimmers. One systematic review concluded that swimmers are more prone to gastrointestinal disease symptoms if exposed to seawater polluted with such indicators. In Canada, *E. coli* is even used as a go-to marker for bathing water quality, because its presence dramatically increases the likelihood of disease-causing pathogens being in the water.

1.5.2 Vibrio Cholera

Cholera is a severe gastrointestinal diarrheal disease caused by contaminated water containing vibrio cholera, a gram-negative proteobacteria. Cholera is also transmitted from contaminated food, especially not perfectly cooked shellfish. Cholera enterotoxin causes severe diarrhea and hydration that can result in death.

Vibrio cholerae as a Waterborne Disease

The pathogen thrives in aquatic environments such as rivers, coastal waters, especially in warm conditions. This makes recreational waters and bathing beaches potential transmission sites, particularly in areas with poor sewage treatment or during flooding.
Humans typically become infected by drinking contaminated water or swallowing seawater while swimming. The bacteria release cholera toxin in the small intestine, causing massive fluid loss that leads to profuse watery diarrhea, dehydration, and in severe cases, death within hours if untreated.
Outbreaks are often associated with inadequate water, sanitation, and hygiene conditions, which allow the bacteria to spread rapidly through communities. In coastal regions like Cox's Bazar, Bangladesh, the risk increases due to population density, heavy human activity at beaches, and the natural presence of *Vibrio* species in warm seawater.



Escherichia Coli *Vibrio Cholerae*

Figure 03 : Pathogenic Bacteria

1.6 Selection Criteria for Studied Bacteria

The selection of E. coli and V. cholera is important because of the relation with public health surveillance, particularly in recreational beach environments. Monitoring the microbial quality of this bathing water is essential because a large number of tourists and locals come throughout the year can lead to outbreaks of waterborne disease that chosen bacteria are widely accepted by public health and environmental authorities as key indicators of water quality. The presence of these bacteria can reveal both the sewage contamination condition and the hygienic condition of the water.

1.6.1 Selection Criteria of *E. coli*

E. coli is the most widely used indicator organism for fecal contamination in recreational water quality studies because:

1. Fecal Origin: *E. coli* is abundant in the intestines of humans and warm-blooded animals. Its presence signals recent fecal contamination, a strong proxy for pathogenic organisms.
2. Correlation with Health Outcomes: Numerous epidemiological studies show a direct correlation between *E. coli* counts and gastrointestinal illness risk in swimmers.

1.6.2 Selection Criteria of *Vibrio cholerae*

Vibrio cholerae is not just an indicator but a specific pathogen of public health concern, particularly in Bangladesh's coastal region:

1. Disease Relevance: It causes cholera, a severe, sometimes fatal diarrheal disease. Endemic and epidemic in South Asia, including Cox's Bazar and Bay of Bengal coastlines (Taylor-Brown et al., 2023).
2. Environmental Reservoir: Unlike *E. coli*, *V. cholerae* is a natural inhabitant of brackish and coastal waters. Its ecology is tied to plankton, sediments, and warm seawater — making it relevant for beach water studies.
3. Epidemic Potential : The Seventh Cholera Pandemic was caused by the Vibrio cholerae O1 biotype El Tor, a strain of the cholera bacterium that emerged in Indonesia in 1961 Even low levels in water can cause outbreaks if sanitation is poor.
4. Regional Priority: Bangladesh has a long history of cholera outbreaks linked to water. Selecting *V. cholerae* is crucial for region-specific risk assessment and early warning surveillance.

1.7 Antibacterial Resistance in Bangladesh

Bangladesh has witnessed a troubling increase in antibiotic resistance, with recent data indicating an 11% rise in resistance rates over the past five years (Institute of Epidemiology Disease Control and Research [IEDCR], 2023). The effectiveness of commonly used antibiotics has decreased significantly, with resistance rates reaching as high as 84% for critical drugs like carbapenems (IEDCR, 2023). This alarming trend is attributed to various factors, including the indiscriminate use of antibiotics in both healthcare and agriculture, where approximately 40% of antibiotics used in poultry were found to be ineffective against prevalent bacterial strains like E. coli. Dhaka's rivers, heavily polluted by untreated industrial waste, pesticides, and pharmaceuticals, have become untreatable (Chemicals, industrial waste contamination turn 6 Bangladesh rivers untreatable, n.d.). Major sources of pollution include untreated municipal sewage and industrial waste, with highly contaminated areas including

Buriganga River, Savar, Ashulia, Tongi, and Gazipur, as well as rivers near Khulna and Chittagong (Water resources, n.d.). Pharmaceutical pollution, particularly antibiotics released into water bodies, poses a severe threat to the environment and public health (Parvin et al., 2022).

1.8 Antibiotic Resistance in Marine Bacteria

Antibiotic resistance (AR) in marine bacteria occurs when bacteria living in the sea or coastal waters develop the ability to survive and grow despite the presence of antibiotics that would normally kill them or stop their growth. This happens when bacteria acquire resistance genes either through mutations or by exchanging genetic material (horizontal gene transfer).

1. Coastal Waters as Hotspots
A recent microbial study found 54 antibiotic and metal resistance genes in the coastal waters of Cox's Bazar—significantly higher than in nearby Saint Martin. Notably, resistance included phenicol, tetracycline, quinolone, macrolide, and sulfonamide types.

2. Worldwide, marine bacteria—like *Vibrio*, *Prochlorococcus*, and *Pelagibacter*—show widespread resistance: *Vibrio*: roughly 19% resistant to ampicillin.

3. A study from Germany's North Sea revealed staggering levels of resistance:

- 70% of marine bacteria were resistant to ofloxacin
- 95% resistant to clindamycin
- 58% to clarithromycin
- 100% to novobiocin

These findings emphasize how surface waters can accumulate and amplify resistance—and that even antibiotic micron-level exposure can promote resistance. (CIDRAP, 2024).

1.8.1 Resistance Reaches Marine Environments by :

Wastewater discharge: Untreated sewage, hospital waste, and industrial effluents often carry both antibiotics and resistant bacteria into rivers and beaches.

Aquaculture and fisheries: Antibiotics are sometimes used to prevent disease in farmed fish and shrimp, leading to resistant strains in coastal waters.

Agricultural runoff: Use of antibiotics in livestock farming contributes to resistant bacteria and genes entering water bodies via runoff.

1.9 AMR and AST

1.9.1 AMR

Antimicrobial resistance (AMR) refers to the ability of microorganisms, such as bacteria, fungi, viruses, and parasites, to resist the effects of medications that once effectively treated them. This resistance occurs when these pathogens evolve mechanisms to survive exposure to

antimicrobials, including antibiotics, leading to treatment failures and increased health risks. The misuse and overuse of antibiotics in humans, animals, and agriculture are significant contributors to the rise of AMR. For instance, inappropriate prescribing practices and the availability of antibiotics without prescriptions have accelerated resistance development (World Health Organization [WHO], 2023). Additionally, the agricultural sector's reliance on antibiotics for growth promotion in livestock further exacerbates this issue. The consequences of AMR are dire; it is estimated that AMR was directly responsible for 1.27 million deaths globally in 2019 and contributed to nearly 5 million deaths overall (WHO, 2023). Without concerted efforts to address this challenge, projections suggest that AMR could lead to 10 million deaths annually by 2050, highlighting an urgent need for effective strategies to combat this growing threat.

1.9.2 Antimicrobials Susceptible Test

Antibiotics are medicines that kill bacteria or inhibit their growth. Mankind uses antibiotics to treat infections caused by bacteria. But often use of antibiotics or used in the wrong way bacteria can change and learn how to fight back. That results in antibiotics stopping working and this is called antibiotic resistance. If the bacteria become resistant, it is difficult to treat infection. Nowadays in humans, animals, the environment, in plants, everywhere microbial resistant microorganisms are found. Thus, our ability to cure common disease is under great threat because of the spread of pathogenic bacteria that develop new resistant mechanisms against antibiotics. The increase of multi-resistant bacteria also superbugs which cause illness cannot be treated with many existing antibiotics. So, the Antimicrobial Susceptibility Test, AST, is a lab method used to find out which antibiotic can kill or stop the growth of a specific type of bacteria. It helps patients to know which antibiotics will work best against a bacterial infection based on the size of zones.

- **Sensitive:** Antibiotics work well against the bacteria.
- **Intermediate:** Antibiotics work as little as the growth of bacteria, not fully inhibited.
- **Resistant:** Antibiotics do not work against the bacteria.

This test is important because it helps choose the right medicine for treating disease. It also helps track antibiotic resistance in bacteria which is a very common public health issue. Most common antibiotics are chemical compounds produced by living organisms but harmful to other organisms. Bacteria and fungi can produce antibiotics. Some antibiotics work against specific bacteria. Some work against different types of bacteria.

1.10 Common Antibiotics used for treatments in Bangladesh

1. Ampicillin

    - Target Bacteria: effective against many Gram-negative bacteria like *E. coli*, *H. influenzae*.

- Mechanism: Inhibits bacterial cell wall synthesis by binding to PBPs, disrupting peptidoglycan cross-linking, causing cell lysis.

2. Aztreonam

- Target Bacteria: Aerobic Gram-negative bacteria only; *Pseudomonas*, *Enterobacteriaceae* etc. No activity against Gram-positive or anaerobic bacteria.
- Mechanism: Inhibits cell wall synthesis by binding PBPs, leading to cell death.

3. Cefepime (4th generation cephalosporin)

- Target Bacteria: Work against pneumonia, and skin, urinary tract, and kidney infections.
- Mechanism: Inhibits cell wall synthesis by binding PBPs, disrupting peptidoglycan cross-linking.

4. Chloramphenicol

- Target Bacteria: Broad spectrum against Gram-positive, Gram-negative both.
- Mechanism: Binds to the 50S ribosomal subunit and inhibits peptide bond formation, thus blocking protein synthesis.

5. Vancomycin

- Target Bacteria: Gram-positive bacteria, especially MRSA, *Clostridium difficile*, and other resistant strains.
- Mechanism: Binds to D-Ala-D-Ala terminus of peptidoglycan precursors, blocking cell wall synthesis.

6. Doxycycline

- Target Bacteria: Works against Chlamydia pneumoniae, and Mycoplasma pneumoniae.
- Mechanism: Binds to the 30S ribosomal subunit, blocking tRNA attachment and protein synthesis.

7. Erythromycin

- Target Bacteria: Mainly Gram-positive bacteria and some atypicals.
- Mechanism: Binds to the 50S ribosomal subunit, inhibiting protein elongation.

8. Gentamicin

- Target Bacteria: Primarily aerobic Gram-negative bacteria; sometimes used synergistically for Gram-positive infections.
- Mechanism: Binds irreversibly to the 30S ribosomal subunit, causing misreading of mRNA and defective proteins.

9. Kanamycin
   - Target Bacteria: Similar to gentamicin; effective against aerobic Gram-negative bacteria and some Gram-positive.
   - Mechanism: Same as gentamicin — inhibits protein synthesis by binding 30S subunit.
10. Levofloxacin
    - Target Bacteria: Broad spectrum including Gram-positive and Gram-negative bacteria.
    - Mechanism: Preventing DNA replication and transcription.
11. Linezolid
    - Target Bacteria: Gram-positive bacteria, including resistant strains like MRSA and VRE.
    - Mechanism: Binds to the 50S ribosomal subunit, preventing formation of the initiation complex in protein synthesis.
12. Imipenem
    - Targets: Very broad spectrum covering Gram-positive, Gram-negative, and anaerobic bacteria; resistant to many beta-lactamases.
    - Mechanism: Inhibits cell wall synthesis by binding PBPs.
13. Ceftriaxone (3rd generation Cephalosporin)
    - Target Bacteria: Broad spectrum with good activity against Gram-negative bacteria and some Gram-positive bacteria.
    - Mechanism: Inhibits cell wall synthesis by binding PBPs.
14. Cefixime (3rd generation Cephalosporin)
    - Target Bacteria: Mainly Gram-negative bacteria (e.g., Neisseria, E. coli), limited Gram-positive coverage.
    - Mechanism: Inhibits bacterial cell wall synthesis.

1.11 Research Gap

Existing studies in Bangladesh have highlighted significant concerns regarding gastrointestinal infections in Cox's Bazar, particularly among Rohingya refugee populations where inadequate water and sanitation conditions have contributed to frequent diarrheal diseases (World Health Organization, 2021; Taylor-Brown et al., 2023). However, these findings are largely limited to camp-based water and sanitation systems rather than the coastal recreational waters of Cox's Bazar beach. To date, there are no documented cases of gastrointestinal illness or urinary tract infections (UTIs) caused by *Escherichia coli* directly linked to bathing at Cox's Bazar beach**.**

Furthermore, studies directly associating UTIs with recreational beach exposure in Cox's Bazar—or in other Bangladeshi beaches—are absent. One qualitative report from the country's southwestern coastal region noted that women standing in saline water in fisheries often experienced UTIs and skin problems, but this evidence serves only as an indirect proxy and does not specifically address recreational beach activities (Global Health Now, 2024). Similarly, a broader investigation in Bandarban reported a 35.5% prevalence of self-reported UTIs among adolescent girls due to poor menstrual hygiene and inadequate water quality, yet this was in the context of inland water scarcity and is not directly related to Cox's Bazar beach (Rahman et al., 2024).

In addition, while the presence of epidemic *Vibrio cholerae* lineages has been documented in Cox's Bazar and surrounding communities (Taylor-Brown et al., 2023), there is no clear epidemiological evidence connecting environmental isolates from coastal bathing waters to clinical cholera cases or infections among beach users or tourists**.** Most available research has focused on drinking water in refugee camps or broader marine bacterial ecology, not on the specific risks associated with recreational bathing waters. Moreover, systematic monitoring of Cox's Bazar coastal sites remains sparse, with limited data on microbial loads, seasonal variation, and direct links to health outcomes (World Health Organization, 2021).

Lastly, The national AMR surveillance by IEDCR includes Cox's Bazar Medical College and Hospital. They isolate pathogens and perform AST in clinic/hospital settings. (IEDCR, 2016). And There is a work "Determinants of indiscriminate antimicrobial use in commercial chicken farms in Bangladesh" that uses Cox's Bazar as one of the pilot areas but no studies found that perform AST on *Escherichia coli* or *Vibrio cholerae* isolated from beach/coastal recreational waters of Cox's Bazar. Surveillance data is mostly clinical (hospital, drinking water, faecal sludge) or veterinary/poultry/environment related — but not bathing water. Though clinical and agricultural settings in Cox's Bazar have been studied for AMR, antibiotic susceptibility of bacteria from beach/coastal waters remains undocumented. This means we don't know how resistant environmental *E. coli* or *V. cholerae* are in bathing sites — a critical piece for assessing public health risk.

1.12 Aim of the Study

i. The aim of this research is to isolate and detect three important waterborne diseases causing pathogenic bacteria, E. coli, Vibrio cholera, from selected bathing sites, Coxs bazar water samples.
ii. Furthermore, the research includes an antibiotic susceptibility test to understand the resistance profiles of the isolated bacteria.

iii. These will be valuable for assessing the public health risks associated with bathing in this water and for implementing better water quality control measures in coastal areas of Bangladesh.

Chapter 02: Metarials and Method

2.1 Study Area

The study was conducted in four prominent beach sites of cox's bazar - Sugandha, Laboni, Kakra ghat near Himchori and Inani beach, most frequently visited by both local and international tourists. Highly visited beaches like Sugandha and Laboni have big problems during the peak season because too many tourists visit at once. On the other hand, Kakra ghat near Himchori and Inani is comparatively less crowded, but this beach also gets polluted from tourists as well.

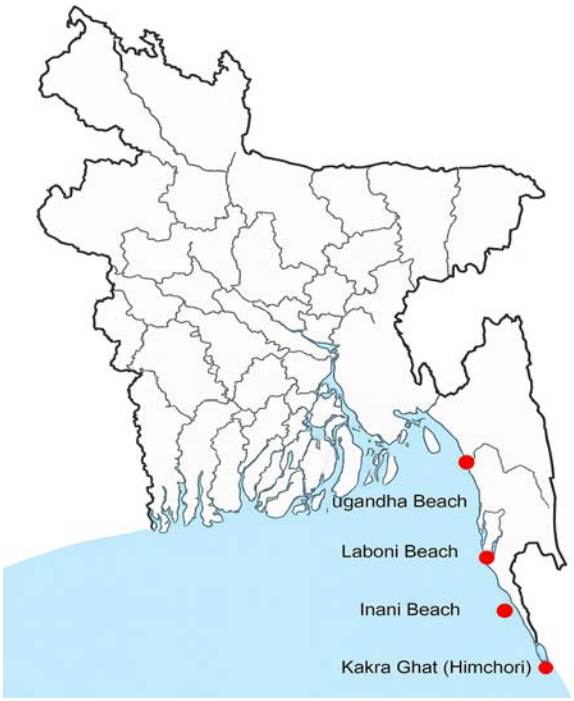


Figure 04 : Sample Collection Areas

2.2 Water sample collection

Water samples were collected from November to April. Every month we collected water samples two times from each beach. In six months, there were 12 samples we collected in total. Approximately 15 ml of water was aseptically collected using sterilized screw capped polypropylene falcon. All falcons were labeled, stored at 4'C in an icebox and transported to the Laboratory within 8 hours.

2.3 Bacterial Isolation

To Isolate bacteria from water first filtered with Whatman filter paper. After the filtration 8 ml autoclaved Luria Broth and 2 ml filtered water are mixed in a sterilized falcon for enrichment and put the falcon in the shaker incubator at 37C for 4 hours for optimum growth of bacteria. After culture period samples were serially diluted up to 10^-3 using sterile saline. Lastly from the diluted sample 0.1 ml sample spread plate onto selective media. 10 bacteria were isolated from each beach water. To isolate pure culture and obtain well isolated colonies of bacteria from an array population we perform streak plate method. And finally, the pure isolated colonies we stored in Soft Luria agar in room temperature for further use.

2.4 Media

As this lab work is searching for two specific bacteria - Escherichia Coli and Vibrio Cholerae so two selective media we used consecutively MacConkey Agar and TCBS agar. Additionally, we use Luria Agar and Miller-Hunton Agar for Streaking plate and antimicrobial susceptibility tests.

2.4.1 **MacConkey Agar**

MacConkey agar is a selective and differentiate culture medium used to isolate and differentiate gram negative bacteria.

- Selective properties:

MacConkey agar has components named bile salt and crystal violet that inhibit the growth of gram-positive bacteria, function as a selective agent.

- Differentiate properties:

Lactose is the differentiating sugar in MacConkey agar. Lactose fermenting bacteria can produce acid, causing the pH indicator “neutral red” in the agar to turn red. Non lactose fermenter bacteria result in colorlessness. E. coli, a common gram-negative bacterium, lactose fermenter that produces colonies on MacConkey agar. (Aryal, 2023)

2.4.2 TCBS agar (Thiosulfate Citrate Bile salt Sucrose agar)

TCBS agar is also a selective and differential agar medium that also inhibits the growth of most other bacteria and allows vibrio species to be easily identified.

- Selective properties

TCBS agar has a component Bile salt that inhibits the growth of gram-positive bacteria. that makes it selective for vibrio species.

- Differential properties

Sucrose is a type of a sugar that helps tell different vibrio species apart by checking if they can ferment sucrose. If the bacteria can ferment sucrose, they form yellow colonies and if they cannot ferment, they form green colonies. (Aryal, 2022)

2.4.3 Miller-Hunton agar (MHA)

MH agar has the ability to support the growth of many types of non-fastidious bacteria. MH agar also allows the antibiotics for the diffusion which is important for testing which antibiotics work best against the bacteria. MH agar is the recommended media for antimicrobial test especially the Kirby-Bauer disk diffusion method. This is the method we applied in our research work. MH agar contains beef extract, starch, acid hydrolysate of casein and agar. (Aryal, 2023)

Figure 05: Different Types of Agar Media

2.5 Hi-Media Analysis

Streaking method was conducted on culture medium plates after reviewing the microscopic results. To identify species, colony development patterns, and colors were examined following the Hi-Media publication's recommendations. These criteria were used to identify the colonies on MacConkey and TCBS agar by looking at their distinctive colors and morphologies, *E. coli*, and *Vibrio cholerae* could all be accurately identified using this approach (Hi-Media Laboratories, n.d.).

Selective Agar Name	Name of the Bacteria	Morphology (Color of the colony)	Preparation (For 1liter)	Incubation Condition
MacConkey Agar	*Escherichia Coli*	Bright pink to Red	52 grams	24 hours at 37°C
TCBS Agar	Vibrio cholerae	Yellow	89.08 gram	24 hours at 37°C
MH Agar	Several types	Colorless	38 grams	16-18 hours at 37°C

Table 01: HiMedia Analysis (HiMedia Laboratories, n.d.)

2.6 Spread Plate Method

This is a laboratory technique used to find and isolate the number of living organisms from the liquid sample. This method allows the isolation of individual colonies. In addition, this is a well-grounded method for estimating the number of viable organisms from a sample. (Dahal ,2022)

Materials

1. Sterile Selective Agar Plate.
2. Spreader
1. Bunsen burner
2. Ethanol

Procedure

- 100 µ of diluted liquid sample is placed on the surface of a solidifying agar plate.
- For each plate sterile a spreader in Bunsen burner flame after dipping in ethanol.
- Wait to cool the spreader.
- After cooling the spreader used to spread the sample liquid evenly over the surface of the agar.
- After spreading the plate placed in the incubator at 37℃ for 24 hours.
- After incubation, the resulting colonies are isolated.
-

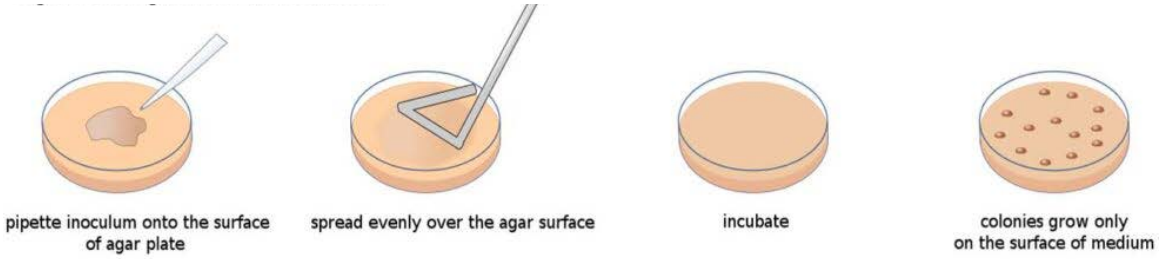


Figure 06 : Spreading Plate Method.

2.7 Streak Plate Method

Streak plate method is a common technique in microbiology labs to separate and grow individual types of bacteria from a mixer colony. The main goal is to get pure culture - meaning bacteria of just one type. After we isolated bacterial colonies using the spread plate method, we picked some of these colonies to perform the streak plate method.

Materials

1. Sterile Selective Agar Plate.
2. Bunsen burner.
3. Loop or Needle.

Procedure:

- Using a loop, sterilize it by Bunsen burner until red hot and let it cool. - Divide the agar plate into four quadrants.

Quadrant 01: streak the inoculum back and forth across the first quadrant.
Quadrant 02: Sterilize the loop, cool it, and drag the loop from quadrant 01 to the 2nd quadrant streaking back and forth.
Quadrant 03: Repeat the loop sterilization, cooling and streaking process, dragging from 2nd quadrant to the third quadrant.
Quadrant 04: After loop sterilizing and cooling, dragging from third to fourth quadrant and zigzag line streaking need to be done.

- Put the plate in the incubator at 37℃ for 24 hours.

After the isolation of the pure bacterial colony, we isolated the bacteria in the sterilized micro centrifugal tube filled with Soft Luria agar for further use like DNA extraction or antimicrobial susceptibility test. (Aryal,2023)

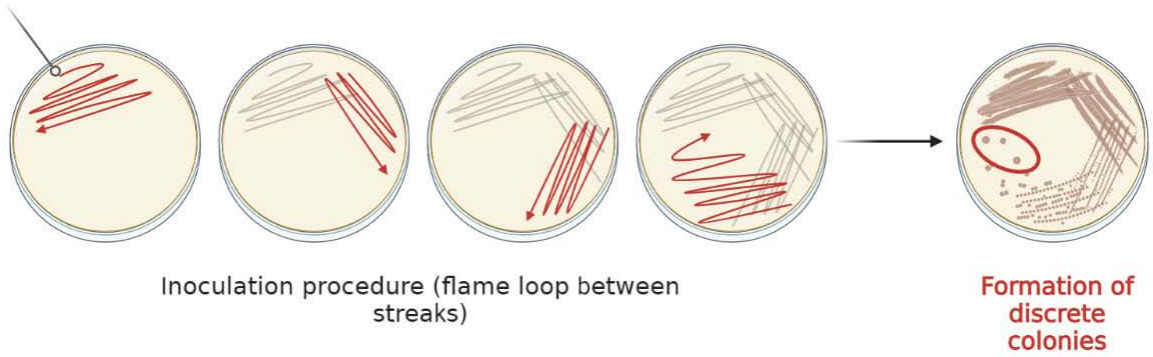


Figure 07: Straeking Plate Method.

2.8 DNA isolation by boiling method

Materials

1. Centrifuge Machine
2. Vortex Machine
3. Water bath

Method

- **Preparation of Bacterial Suspension**

Collect a loopful of bacterial colonies from a freshly subculture Plate. Suspend the cells in sterile distilled water in a microcentrifuge tube and vortex thoroughly to mix well.

- **Centrifugation**

Centrifuge the suspension at 1300 rpm for 10 minutes to pellet the bacterial cells. Discard the supernatant.

- **Cell Lysis (Boiling Method)**

Resuspend the pellet in 200 µl of TE buffer. Place the tube in a water bath at 95°C for 10 minutes to lyse the cells and release DNA.

- **Cold Shock**

Immediately transfer the tube onto ice and keep it for 10 minutes to stabilize the DNA and enhance cell debris precipitation.

- **Second Centrifugation**

Centrifuge again at 1300 rpm for 5 minutes. Carefully transfer the clear supernatant (which contains the DNA) into a fresh, sterile microcentrifuge tube.

- **Storage**

Store the DNA extract at −20°C until further use in PCR amplification.
(Gupta, 2019)

2.9 Polymerase Chain Reaction (PCR)

Materials

1. **Thermo-cycler machine:** This thermocycler equipment offers the ideal temperature for the steps of the PCR process. The accurate and controlled temperature control of PCR facilitates the denaturation, annealing, and extension processes. The fact that PCR is repeated depending on the size of the gene is how the term "cycler" for the device came to be used.
2. **PCR tubes:** These types of tubes are particularly effective to work with since they assist in improving outcomes by preventing evaporation during PCR.
3. **Template DNA:** It is basically sample DNA where the target sequence is present. To separate the two strands of the original double-stranded DNA molecule from one another, high temperatures are used to initiate the process.
4. **Primers:** A primer must hybridize with the DNA in the template to allow for the polymerase to attach to it and start the polymerization process. Primers generally have a length of 18–25 nucleotides.
5. **DNA Polymerase:** A specific sort of enzyme called DNA polymerase makes new DNA strands that are complementary to the target sequence.
6. **Mg^{2+}:** Magnesium ions are crucial cofactors especially for the DNA polymerase enzyme because they are necessary for the enzyme's correct operation and the best potential DNA synthesis.
7. **Buffer:** A buffer solution is used in the PCR reaction mixture to supply the DNA polymerase activity with the ideal pH and ionic circumstances. Any enzymatic reaction requires a buffer because it maintains the stability of the reaction's components and prevents DNA damage.
8. **dNTPs:** It helps as a building block, especially for new DNA strands. If dNTPS are not enough, then PCR stops in the midway.

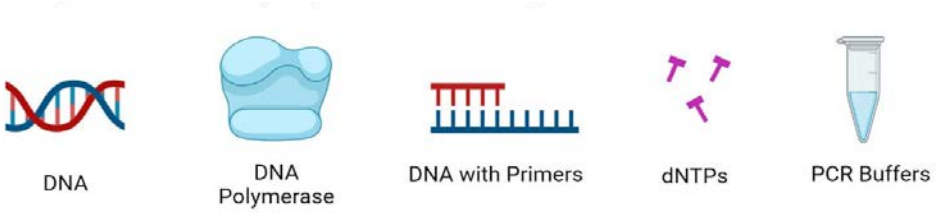


Figure 08: Enzymes Used in PCR.

Main Steps of PCR

- **Initial denaturation** –

In this step, heating is required for the DNA polymerase. This step consists of heating the reaction to a temperature of 94-96°C for 5 minutes. If extremely thermostable polymerase is used, heat should be 98 degrees Celsius.

- **Denaturation** -

This step is the first cycle and consists of heating the reaction at 94-98 °C around for 30 seconds. Heating causes DNA melting of the DNA template by disrupting the hydrogen bond between the two DNA strands and separating them into a single strand.

- **Annealing** –

In this step, the temperature is lowered to allow the primers to bind to their complementary sequences on the single-stranded DNA. This temperature must be carefully chosen it should be low enough to enable hybridization of the primers, but high enough to ensure specificity. In other words, the primers should only bind to perfectly complementary regions of the DNA template.

- If the annealing temperature is too low, primers may bind non-specifically or imperfectly.
- Conversely, if the temperature is too high, the primers may not bind at all.

Therefore, the optimal annealing temperature varies depending on the primer sequence and is typically 3 to 5°C lower than the melting temperature of the primer.

In this study, the annealing temperatures used for the specific detection of the three target bacteria were as follows,

1. ***Escherichia coli:*** **57°C**
2. ***Vibrio cholerae:*** **60°C**

Stable DNA-RNA hydrogen bonds are only formed when the primer sequence closely matches with the template sequence, the polymerase binds to the primer-template hybrid and begins DNA formation.

- **Elongation** –

The temperature used during the elongation step of PCR depends on the type of DNA polymerase enzyme. For example, Taq polymerase has an optimal activity at around 75–80°C, but a standard temperature of 72°C is typically used. In this step, the

DNA polymerase synthesizes a new DNA strand by adding deoxynucleotide triphosphates (dNTPs) that are complementary to the template strand. This synthesis occurs in the 5' to 3' direction, where the 5' phosphate group of each incoming dNTP is joined to the 3' hydroxyl group of the growing DNA strand. The duration of the extension step depends on both the polymerase being used and the length of the DNA fragment being amplified. Generally, DNA polymerase can synthesize approximately 1,000 base pairs per minute under optimal conditions. If all necessary components such as dNTPs, primers, and buffers are available in sufficient amounts, the target DNA is doubled in each cycle during the extension step, resulting in exponential amplification of the specific DNA sequence.

- **Final elongation** –

This single step performs for 5-15 minutes after the last PCR cycle to ensure that any remaining single strand DNA is fully extended. The temperature is 70-74°C used normally as this is the suitable temperature for optimal activity for most polymerase used in PCR.

- **Final Hold** –

This step at 4°C for an indefinite time employed if needed for short term storage.

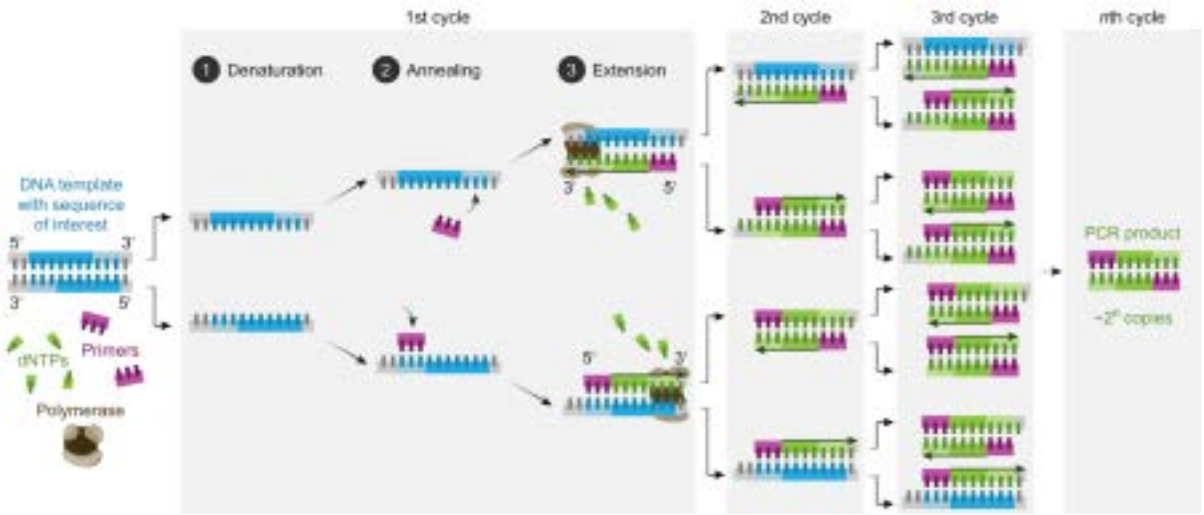


Figure 09 : Schematic drawing of a complete PCR cycle

PCR Reaction Preparation:

- Nuclease Free water
- Master Mix
- Template DNA
- Forward Primer
- Reverse primer

Component	Volume per sample
Nuclease-free water	1µl
Master mix	10µl
Isolated DNA	5µl
Forward primer	2µl
Reverse Primer	2µl
	Total volume: 20µl

Table 02 : PCR Component.

Procedure

- Using a micropipette, 1µL of Nuclease free water was transferred inside a sterilized PCR tube.
- 10µL of PCR master mix was then added in the tube.
- After that 2µL of forward and 2µL of reverse primer added depend on the bacteria are using.
- Finally, previously extracted DNA was added into the tube of 5 µL.

After that the PCR machine was programmed for maintaining the following conditions,

- 95 °C for 5 minutes for initial denaturation.
- 95 °C for 30 seconds for denaturation.
- Required temperature for 1 minute for primer annealing.
- 72 °C for elongation for 1 minute.
- 72 °C for final elongation for 5 minutes.
- Number of cycles - 35 cycles.
- 4 °C infinite hold.

Then the tube was placed inside the PCR machine and the lid was closed. After completing PCR, the tube containing amplified DNA was stored at -20 °C until further use such as agarose gel electrophoresis. (Gupta, 2019)

2.10 Agarose Gel Electrophoresis

Materials

1. PCR product (amplified DNA)
2. DNA ladder (100 bp)
3. Agarose gel
4. DNA strain (EtBr)
5. Running Buffer

Method

-**Agarose Gel Prepare**: Prepare agarose gel by weighting the agarose powder in 100 ml distilled water.

- **Buffer Addition:** Add 2 ml of 50X TAE buffer and swirl the flask for even distribution.

- **Boiling the Mixture:** The mixture needs to boil to make sure all agarose is dissolved in the buffer. Continue to boil until the solution is clear, translucent; no solid powder form should be visible.

- **Cooling and Staining:** While the agarose gel solution is lightly cooled, add 4 µL of ethidium bromide into the solution.

- **Gel Casting and Well creating:** Pour the mixture into a gel tray with a well comb, properly fixed.

- **Solidifying the Gel:** Once the gel has cooled completely and solidified, the comb is removed.

- **Gel Chamber Setup:** Gel tray inserted into gel chamber and filled the gel chamber with 1X TAE buffer. Pour enough buffer so the well is filled.

- **Sample Loading:** Into the well, pipette 10 µL of 100 base pair DNA ladder and 10 µL of samples serially.

- **Running and Visualizing the Gel:** Close the lid of the gel chamber for 40 minutes. Run the gel at 100 V, Under UV light, visualize the gel.

Visualization of DNA- When DNA becomes separated by size, they appear as bands in the gel. DNA cannot be seen within agarose gel. So, a fluorescent strain is added in the gel that binds with DNA and glows under UV or blue light. DNA ladder contains multiple bands in one lane. Each band represents a predetermined length of DNA. Ladder bands act like a reference, helping to estimate the size of DNA fragments from each PCR product by comparing their position to the ladder. And the primer-dimer is, as PCR reactions are set up with primers, some primers may bind to each other instead of DNA that creates primer-dimer. Excess primer as fast as it binds on the bottom of the gel.

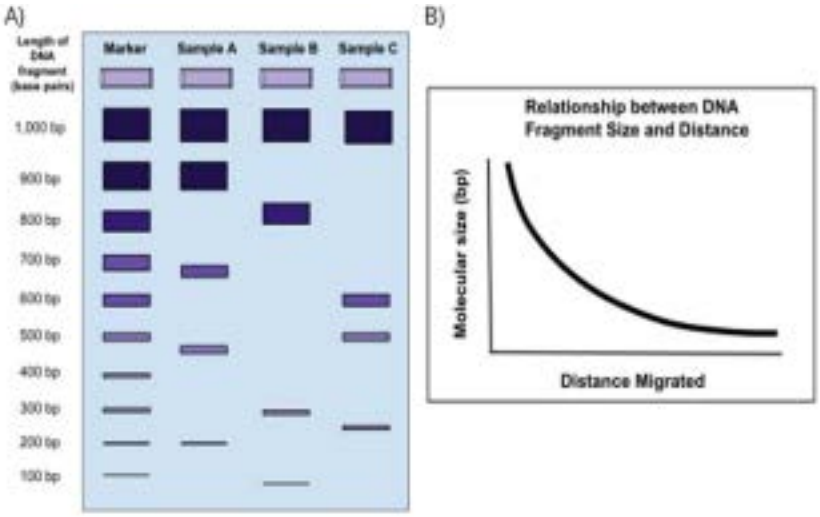


Figure 10: Gel electrophoresis allows for differentiation of DNA by size. Larger fragments do not move as fast as smaller fragments, and there is a nonlinear relationship between the size of the DNA fragments, and the distance migrated. A DNA ladder, a sample with known fragment sizes should always be run to identify the size of experimental bands.

2.11 Antibiotic Susceptible Test

Materials

1. MH Agar
2. Saline
3. Cotton Swab
4. Antibiotic Disk

Method

– **Preparation of Mueller-Hinton Agar plate.**

- Allow the MH agar plate to come to room temperature.
- For our research two plates were prepared for each organism to be tested.
- Plates placed in laminar flow hood until dry.
- Appropriately label each plate as necessary.

– **Preparation of inoculum**

- Using a sterile loop or needle, pick four to five isolated colonies.
- Suspend the colonies in 5ml of sterile saline in a sterile falcon.
- Vortex the tube.
- Adjust the turbidity of the suspension to a 0.5 McFarland standard by adding more colony if the suspension is light or dilute with saline if suspension is heavy.
- This suspension needs to be used within 15 minutes after preparation.

– **Inoculation of the MH plate**

- Dip a sterile cotton swab into the suspension.
- Streaking the swab four times over the entire agar surface: rotate the plate approximately 60 degree each time to ensure an even distribution of the inoculums.
- Discard the swab.
- Allow the plate to sit at room temperature at least 3-5 minutes for the surface of the agar plate to dry but not for more than 15 minutes.

– **Placement of Antibiotic Disks**

- Place the needed antibiotic disk on the surface of the agar using sterilized forceps.
- Use the forceps carefully to remove one disk from the cartridge.
- Once all the disks are in place, put the plate in the incubator at 37°C for 18 hours.

(Aryal, 2018)

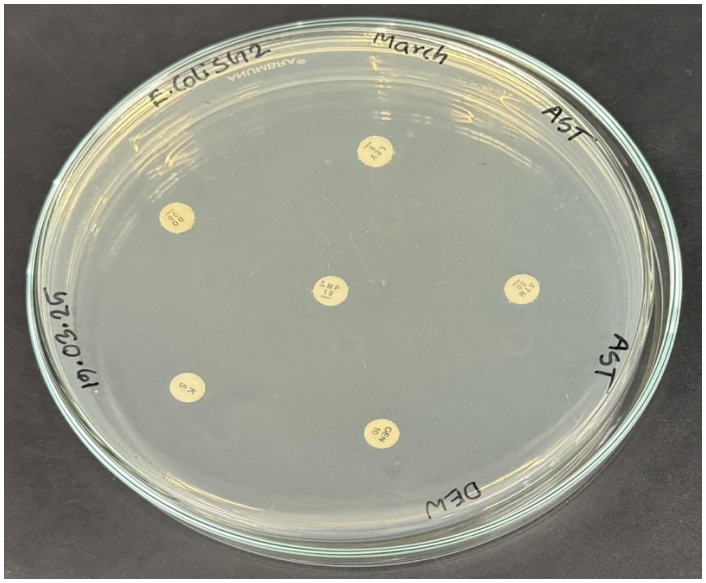


Figure 11 : Placement of Antibiotic Disk in Muller Hinton Agar Plate.

Chapter 03: Result

3.1 Labeling the isolated colony

We have collected water samples from 4 different beaches of Cox's Bazar. In total 12 times we collected water samples in six months. So, the total number or sample was 48 from 4 different beaches. For the samples, isolated colonies of bacteria were labeled as,

- We have collected our sample two times in a month so 01 or 02 if the first sample or second.
- First two letters of beach name.
- The dilution serial.
- Colony number as we isolate 10 bacteria for each beach for each sample.
- Lastly, according to the first letter of the media name.

SO, SG10^-1 3T means, Sugandha beach dilutes 10^-1 bacteria number 3 from TCBS agar.

3.2 Presence of Bacteria by Colony Morphology

Water samples collected from four bathing sites—Sugandha, Laboni, Himchori, and Inani— were cultured on selective media. After getting pure Culture colonies we have collected 10 bacteria per sample per month. In total we have collected more than 400 single colony.

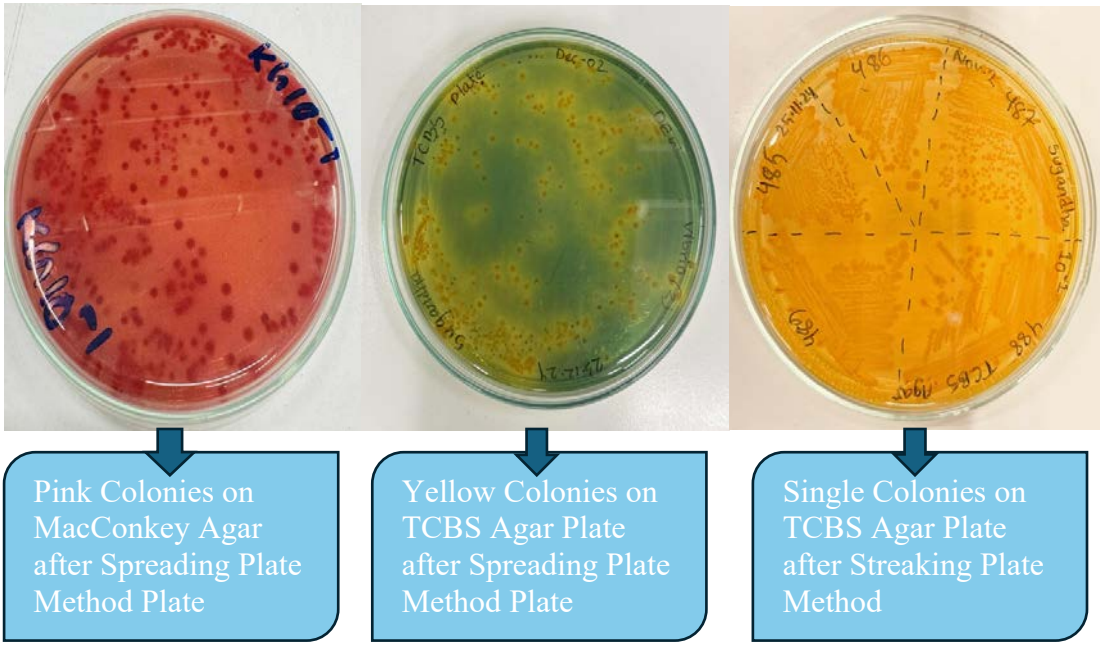


Figure 12 : Bacterial colonies observed from spreading and streaking plates.

Based on colony morphology:

1. ***E.coli*** colonies appeared **pink to red, matte with bile salt precipitate** on **MacConkey agar**, indicating lactose fermentation.
2. ***Vibrio cholerae*** formed **yellow colonies** on **TCBS agar**, indicating sucrose fermentation.

Month name ans sample number	Characteristics	Isolated Colony Number	Bacterial Species
November Sample 01	Pink to red	16	*Escherichia Coli*
November Sample 01	Yellow colony in TCBS Agar	39	*Vibrio cholerae*
November Sample 02	Yellow colony in TCBS Agar	20	*Vibrio cholerae*
December Sample 01	Pink to Red	14	*Escherichia Coli*
December Sample 01	Yellow colony in TCBS Agar	19	*Vibrio cholerae*
December Sample 02	Pink to Red in MAC Agar	11	*Escherichia Coli*
December Sample 02	Yellow colony in TCBS Agar	21	*Vibrio cholerae*
January Sample 01	Pink to Red in MAC Agar	12	*Escherichia Coli*
January Sample 01	Yellow colony in TCBS Agar	32	*Vibrio cholerae*
January Sample 02	Pink to Red in MAC Agar	30	*Escherichia Coli*
January Sample 02	Yellow colony on TCBS Agar	16	*Vibrio cholerae*
February Sample 01	Pink to Red in MAC Agar	10	*Escherichia Coli*
February Sample 02	Pink o Red in MAC Agar	14	*Escherichia Coli*
February Sample 02	Yellow colony in TCBS Agar	20	*Vibrio cholerae*
March Sample 01	Pink o Red in MAC Agar	16	*Escherichia Coli*
March Sample 01	Yellow colony in TCBS Agar	23	*Vibrio cholerae*
March Sample 02	Pink o Red in MAC Agar	21	*Escherichia Coli*
March Sample 02	Yellow colony in TCBS Agar	13	*Vibrio cholerae*
Aril Sample 01	Pink o Red in MAC Agar	10	*Escherichia Coli*
April Sample 02	Pink o Red in MAC Agar	20	*Escherichia Coli*

Table 03 - Colony count of isolated bacteria per site showing *E.coli* and *Vibrio cholerae* colony for each beach site.

Total isolated colony number is,

- *Escherichia Coli*- 174
- *Vibrio cholerae* - 203

Among the 377 isolated bacterial colonies, *Vibrio* cholerae was the most prevalent, accounting for approximately 203 colonies, followed by *Escherichia Coli* with about 174 colonies. These findings indicate a higher prevalence of *Vibrio cholerae* in the bathing waters, with *E.coli* also present in significant numbers, suggesting varying degrees of microbial contamination from different pathogenic sources.

Based on the colony observed on selective media—pink to red colonies on MacConkey agar for *E.coli*, and yellow colonies on TCBS agar for *Vibrio* approximate identification of the target bacteria was isolated based on morphology. However, morphological and cultural characteristics are not always sufficient for precise identification, as closely related species may share similar appearances. For example, in MacConkey Agar *Escherichia Coli*and Klebsiella spp. Both form pink to red colonies. This is why isolated colonies were subjected to genomic DNA extraction. The extracted DNA was used in PCR amplification with primers specific to *Escherichia Coli*, *Vibrio.* The resulting PCR products were analyzed by agarose gel electrophoresis to visualize the amplified DNA fragments.

3.3 DNA Extraction result

After centrifugation, the cells were lysed by applying heat and cold shock. After final centrifugation,

- The supernatant contains DNA since DNA is a lighter substance that can be measure in Nano-grams and does not precipitate.
- On the contrary, the cell debris or broken particles of cells are larger substances (macromolecules) and therefore settle at the bottom of the tubes as pellets.

For this reason, the supernatant was collected in a separate tube. The tubes containing DNA are stored at -20°C.

Figure 13: Extracted DNA

3.4 PCR Observation and result:

After amplification inside the PCR machine, the samples were collected. The PCR tubes are expected to contain the amplified DNA molecules. The samples were then preserved at -20˚C for further use.

Figure 13 : PCR product in tube

3.5 Interpretation of Agarose Gel Electrophoresis Results:

PCR amplification was performed using species-specific primers designed to detect *Escherichia Coli*, *Vibrio cholerae*. The expected amplicon sizes were determined using NCBI Primer-BLAST based on the primer sequences,

- ***Escherichia Coli***:

– Forward primer- 5'-GACCTCGGTTTAGTTCACAGA-3'
– Reverse primer- 5'-CACACGCTGACGCTGACCA-3’

Amplified band at 585 bp

- ***Vibrio cholerae***:

– Forward primer- 5’-CAAGCTCCGCATGTCCAGAAGC-3’
– Reverse primer- 5’-GGGGCGTGACGCGAATGATT-3’

Amplified band at 154 bp

Following amplification, the PCR products compared against a 100 bp DNA ladder. Clear and distinct bands were observed at ~585 bp, ~154 bp in the respective lanes, corresponding to *E.coli*, *V. cholerae*. The presence of bands at the expected positions confirmed successful amplification and indicated positive detection of these pathogens in the tested water samples. The results also validated the specificity of the primers and the efficiency of the PCR protocol. PCR was performed to confirm the presence of target bacteria using species-specific primers. The amplified products were visualized by gel electrophoresis.

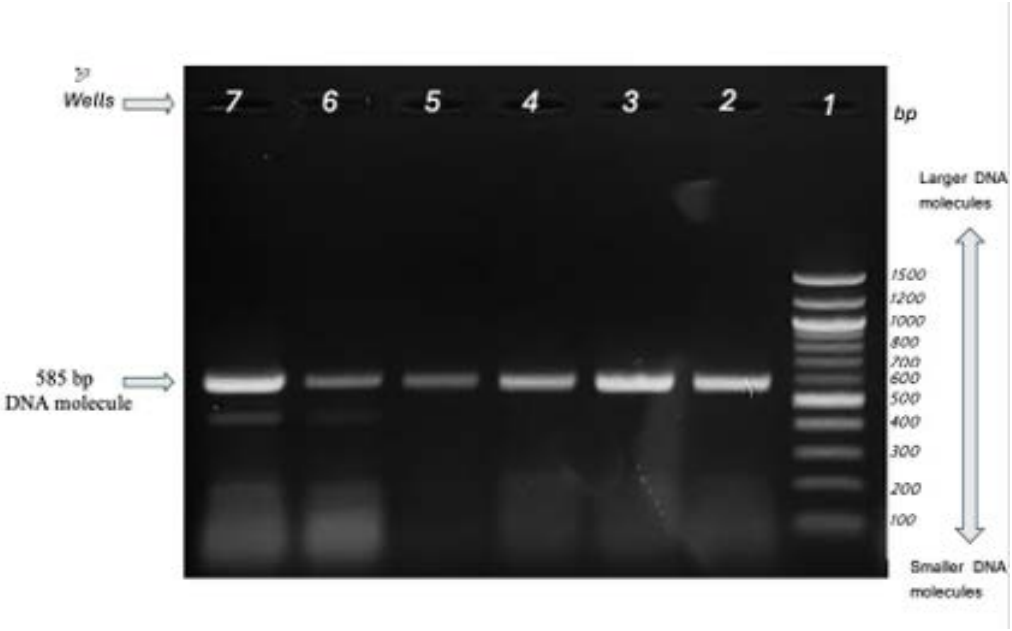


Figure 15 : Visualization of *Escherichia Coli* in gel electrophoresis

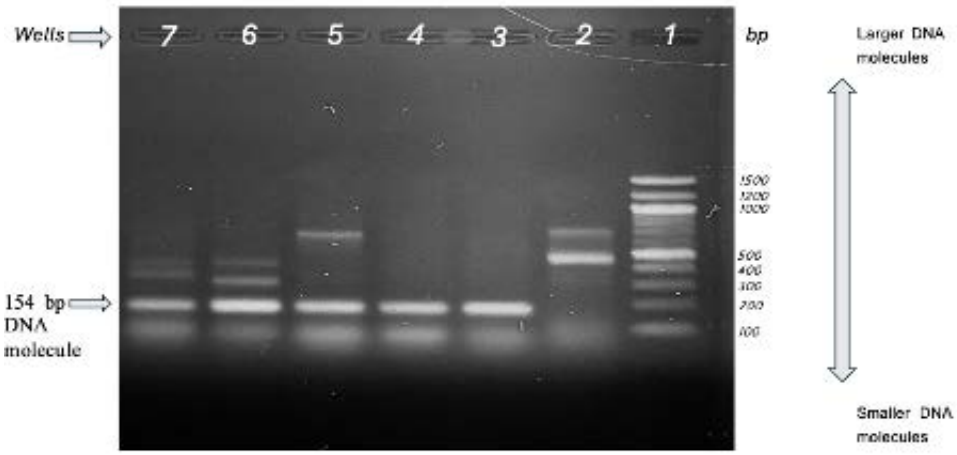


Figure 16: Visualization of *Vibrio* Cholera in gel electrophoresis

After Gel Electrophoresis confirmed number of bacteria are,

- ✓ ***Vibrio*** - 146 number of colony.
- ✓ ***Escherichia Coli***- 114 number of colony.

3.6 Antibiotic Susceptibility Testing (AST)

All confirmed isolates were subjected to AST using the Kirby-Bauer disc diffusion method with 14 antibiotics. Zones of inhibition were measured in mm. Zone of inhibition (mm) for each isolate against tested antibiotics were analyze and the result is,

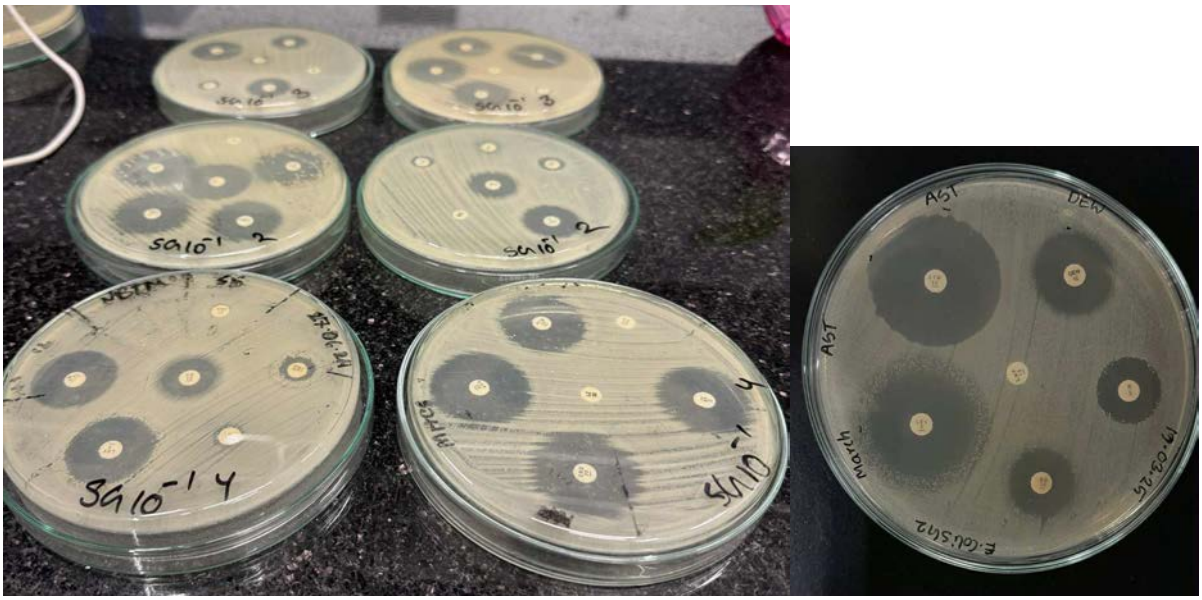


Figure 17 : Some Result MHA Plate of Antimicrobial Susceptibility Test

3.6.1 Susceptibility Test for *Escherichia Coli*

These findings demonstrate that while a majority of *E.coli* isolates remain responsive to antibiotics, a significant proportion show resistance, reflecting the ongoing challenge of antimicrobial resistance in this pathogen.

Antibiotic Susceptibility of *Escherichia Coli*

- **K (Kanamycin):** 106 susceptible, 2 intermediate, 6 resistant
- **AMP (Ampicillin):** 35 susceptible, 6 intermediate, 73 resistant
- **FEP (Cefepime):** 107 susceptible, 7 resistant.
- **DO (Doxycycline):** 84 susceptible, 15 intermediate, 15
- **CN (Gentamicin):** 107 susceptible, 4 intermediate, 3 resistant.
- **LEV (Levofloxacin):** 114 susceptible.
- **VA (Vancomycin):** 114 resistant .
- **C (Chloramphenicol):** 98 susceptible, 6 intermediate, 10 resistant.
- **ATM (Aztreonam):** 107 susceptible, 3 intermediate, 4 resistant.
- **IPM (Imipenem):** 114 susceptible.
- **CRO (Ceftriaxone):** 104 susceptible, 7 intermediate, 3 resistant
- **E (Erythromycin):** 2 susceptible, 23 intermediate, 89 resistant.
- **LZD (Linezolid):** 3 susceptible, 4 intermediate, 107 resistant.
- **CFM (Cefixime):** 98 susceptible, 2 intermediate, 14 resistant

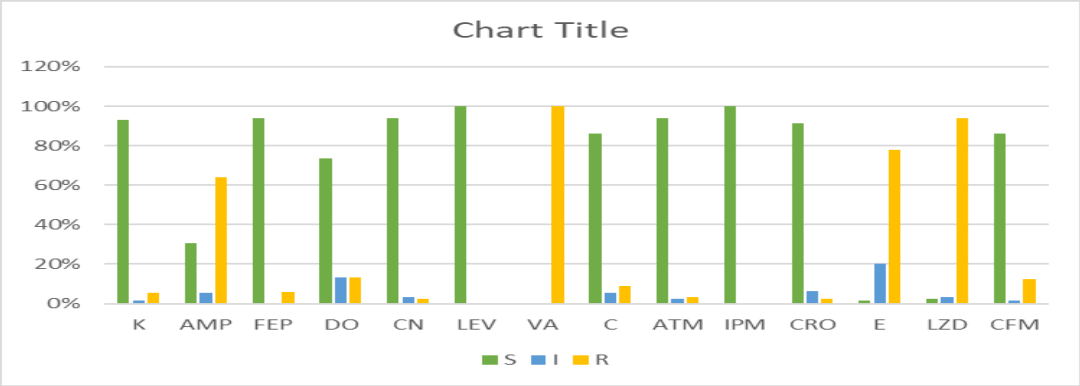


Figure 18: Column Chart of AST result for *Escherichia Coli*

3.6.2 Antibiotic Susceptibility Test for *Vibrio*,

In the antimicrobial susceptibility test (AST) for *Vibrio*, the majority of isolates were found to be susceptible to the tested antibiotics, indicating effective treatment options. A smaller proportion showed intermediate response, while many were resistant. This suggests that although most antibiotics remain effective, resistance is present and should be carefully monitored.

Antibiotic Susceptibility of *Vibrio*

- **FEP (Cefepime):** 139 susceptible, 7 intermediate
- **LEV (Levofloxacin):** 142 susceptible, 6 intermediate
- **CFM (Cefixime):** 106 susceptible, 15 intermediate, 25 resistant.
- **CN (Gentamicin):** 142 susceptible, 4 intermediate
- **IPM (Imipenem):** 146 susceptible.
- **K (Kanamycin):** 138 susceptible, 8 intermediate.
- **LZD (Linezolid):** 110 susceptible, 12 intermediate, 24 resistant.
- **CRO (Ceftriaxone):** 118 susceptible, 9 intermediate, 19 resistant .
- **DO (Doxycycline):** 146 susceptible .
- **ATM (Aztreonam):** 102 susceptible, 16 intermediate, 28 resistant.
- **AMP (Ampicillin):** 103 susceptible, 14 intermediate, 29 resistant .
- **C (Chloramphenicol):** 146 susceptible .
- **VA (Vancomycin):** 60 susceptible, 86 resistant
- **E (Erythromycin):** 78 susceptible, 32 intermediate, 36 resistant.

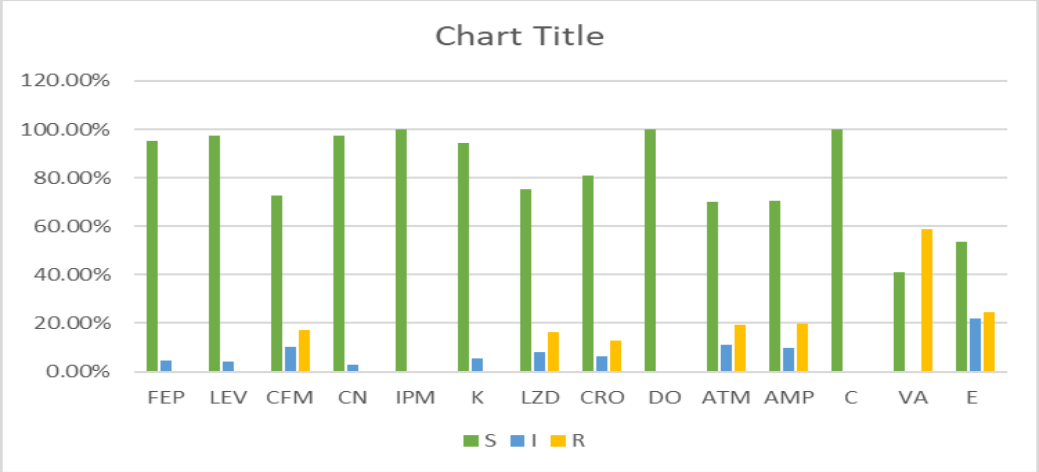


Figure 19: Column Chart of AST result for *Vibrio cholerae*.

Chapter 04: Discussion

Cox's Bazar, Bangladesh, is renowned as the world's longest natural sandy sea beach, extending over 120 kilometers along the Bay of Bengal. Known for its golden sand, scenic waves, and attractions like Himchori, Inani, Laboni, and Kolatoli, the area is a vital tourist hub and supports the nation's fishing and seafood industries. However, alongside its beauty lies a critical concern: deteriorating bathing water quality caused by untreated sewage, tourist litter, and inadequate waste management. Sewage from hotels and households is often discharged directly into the sea, while food scraps and plastics from visitors accumulate on beaches. This results in turbid, foul-smelling water with visible waste, particularly at crowded sites such as Shugandha, Laboni, and Kolatali. Beyond damaging the environment, these conditions expose beach goers to skin infections, diarrhea, stomach disorders, and other illnesses linked to microbial contamination.

Bathing water can harbor pathogenic microorganisms, especially of fecal origin. Among these, *Escherichia Coli*, *Vibrio cholerae* are critical indicators of microbial safety and significant threats to public health.

E.coli is a gram-negative bacterium commonly used as a marker of fecal pollution. While many strains are harmless, pathogenic types, such as enterohemorrhagic *E.coli*, can cause severe diarrhea and toxin-mediated illness. Its detection in bathing water signals direct fecal contamination. *Vibrio*, the agent of cholera, thrives in saline coastal environments like Cox's Bazar and can trigger outbreaks through contaminated water or seafood. It produces cholera toxin, leading to rapid dehydration and potentially fatal watery diarrhea. These pathogens were selected in this study because of their global significance and their ability to reflect water quality hazards.

To ensure reliable detection, both cultural and molecular methods were applied. DNA was extracted using the boiling method, where bacterial cells were lysed through heating and freeze–thaw cycles, with the DNA preserved in TE buffer for stability. Extracted DNA was subjected to polymerase chain reaction (PCR) for species-specific confirmation. PCR amplifies DNA through repeated cycles of denaturation, annealing, and elongation. Optimized annealing temperatures were used for precision: 57°C for *E.coli*, and 60°C for *V. cholerae*. Products were analyzed through agarose gel electrophoresis, which separates DNA fragments by size under an electric field. DNA bands were visualized with fluorescent dye under UV light and compared with a molecular marker to confirm results. This molecular approach ensured accurate identification of target bacteria.

Beyond identification, the study focused on antibiotic susceptibility testing (AST) to assess resistance patterns. The emergence of multidrug-resistant bacteria in aquatic environments is a growing global concern, with significant implications for treatment. The disc diffusion method was used to classify isolates as susceptible (S), intermediate (I), or resistant (R) depending on inhibition zone diameters. A broad panel of antibiotics was included, covering multiple classes and mechanisms of action.

Beta-lactams such as ampicillin, cefepime, and ceftriaxone disrupt bacterial cell wall synthesis, while aminoglycosides like gentamicin and kanamycin interfere with protein synthesis. Fluoroquinolones such as levofloxacin inhibit DNA replication, and

tetracyclines like doxycycline prevent protein elongation. Macrolides such as erythromycin target ribosomal function, while vancomycin and linezolid, often considered last-resort antibiotics, were included to detect emerging resistance. Aztreonam and imipenem provided further representation of clinically relevant agents. By testing this wide range, the study not only identified treatment options but also highlighted the degree of resistance circulating in environmental isolates.

The primary objectives were therefore threefold: first, to isolate and identify *E.coli* and V. cholerae. from bathing water at Cox's Bazar; second, to confirm their identity at the molecular level using DNA extraction, PCR, and gel electrophoresis; and third, to determine their antibiotic resistance profiles through susceptibility testing.

This combined approach provides a clearer understanding of the microbial risks in Cox's Bazar's bathing waters. The findings are vital for public health surveillance, guiding authorities in water safety management, and raising awareness of antibiotic resistance in environmental pathogens. Furthermore, they emphasize the urgent need for wastewater treatment facilities and responsible waste disposal practices in tourist and coastal regions. Cox's Bazar, while globally admired for its beauty, faces an unseen but significant health threat if such interventions are neglected.

This study was carried out at four coastal sites in Cox's Bazar, Bangladesh—Sugandha, Laboni, Kakra Ghat near Himchari, and Inani. Sugandha and Laboni are the busiest beaches, experiencing severe contamination during peak tourism, while Kakra Ghat and Inani, though less crowded, are also affected by tourist pollution. Together, these sites provided a representative overview of microbial water quality across different levels of human activity.

Water samples were collected twice monthly between November and April, yielding twelve samples in total. Roughly 15 ml of seawater was aseptically collected in sterile polypropylene falcons, labeled, stored at 4°C, and transported to the laboratory within eight hours to ensure minimal microbial change during transit.

Samples were filtered through Whatman filter paper and enriched by mixing 2 ml of filtered water with 8 ml of sterile Luria Broth, followed by incubation in a shaker at 37°C for four hours. The enriched cultures were serially diluted up to 10^{-3} with sterile saline, and 0.1 ml were spread on selective agar plates. Ten isolates were collected from each site, and pure cultures were obtained using streak plating. Isolates were preserved in soft Luria agar for further use Three selective and differential media targeted specific pathogens:

- **MacConkey agar** for *Escherichia Coli*. Bile salts and crystal violet inhibited gram positive bacteria, while lactose fermentation produced pink-red colonies.

- **TCBS agar** for *Vibrio*. Bile salts inhibited gram positives, and sucrose fermentation distinguished yellow colonies from non-fermenters. .

For routine culturing and antimicrobial tests, Luria agar and Mueller-Hinton agar (MHA) were used. MHA was particularly important for antibiotic diffusion in the Kirby-Bauer disk diffusion method. Spread plating allowed quantification of viable organisms and isolation of discrete colonies, while streak plating produced pure single

colonies by successive dilution across four quadrants. Pure isolates were stored for DNA extraction and antimicrobial testing. DNA was isolated from freshly subcultured bacteria using the boiling method. Colonies were suspended in sterile water, centrifuged, resuspended in TE buffer, boiled at 95°C for ten minutes, and rapidly cooled on ice. After a second centrifugation, the supernatant containing DNA was collected and stored at –20°C.PCR amplification was conducted using nuclease-free water, master mix, primers, and template DNA. The thermal cycle involved initial denaturation at 95°C for five minutes, followed by 35 cycles of denaturation (95°C, 30s), annealing (species specific temperature), elongation (72°C, 1 min), and a final extension at 72°C for five minutes. Amplified DNA was stored at –20°C until gel analysis.

PCR products were analyzed by agarose gel electrophoresis. Agarose was dissolved in TAE buffer, stained with ethidium bromide, and cast in trays with wells. DNA ladder (100 bp) and PCR products were loaded, and gels were run at 100V for 40 minutes. DNA bands were visualized under UV light and compared with molecular markers for confirmation.

Antibiotic resistance was evaluated using the Kirby-Bauer disk diffusion method on MHA. Isolates were suspended in sterile saline, adjusted to 0.5 McFarland turbidity, and spread evenly on MHA plates. Antibiotic disks were placed on the surface, and plates were incubated at 37°C for 18 hours. The antibiotic panel covered major classes including penicillins (ampicillin), monobactams (aztreonam), cephalosporins (cefepime, ceftriaxone, cefixime), carbapenems (imipenem), amphenicols (chloramphenicol), glycopeptides (vancomycin), tetracyclines (doxycycline), macrolides (erythromycin), aminoglycosides (gentamicin, kanamycin), fluoroquinolones (levofloxacin), and oxazolidinones (linezolid). Zone diameters were measured and interpreted as susceptible, intermediate, or resistant according to established breakpoints.

Water samples collected from four bathing sites—Sugandha, Laboni, Himchori, and Inani— were cultured on selective and differential media to identify bacterial colonies based on morphology. This preliminary screening provided a rapid picture of the contamination levels in the seawater and highlighted the presence of two target organisms: *Escherichia Coli*, *Vibrio cholerae*.

On MacConkey agar, *E.coli* colonies appeared pink to red with a matte surface and visible bile salt precipitate, indicating lactose fermentation. This colony type is a classical feature of fecal coliforms and served as an immediate indication of sewage-related contamination in the bathing water. On TCBS (Thiosulfate Citrate Bile Salt Sucrose) agar, *V. cholerae* produced bright yellow colonies, reflecting sucrose fermentation. This characteristic appearance is routinely used in environmental monitoring to distinguish cholera *Vibrio*s from other non fermenting *Vibrio* species.. These visual signatures enabled quick presumptive identification and efficient isolation of target organisms from mixed microbial populations.

The total number of colonies obtained through morphological identification was 174 for *E. coli* and 203 for *V. cholerae*. giving an overall count of 337 isolates. When expressed as proportions. These results demonstrate that *Vibrio*s were the most abundant bacterial group in the bathing waters, followed by *E.coli*. Such variation suggests differing levels of contamination across the studied sites, with the

predominance of *Vibrio*s highlighting the potential public health risks linked to marine bathing in Cox's Bazar.The strength of morphology-based identification lies in its ability to rapidly signal contamination and direct further investigation. The pink to red lactose-fermenting colonies of *E.coli* reliably indicate fecal pollution, the yellow sucrose-fermenting colonies of *V. cholerae* point toward pathogenic *Vibrio*s,. suggest the presence of enteric pathogens. While morphology alone cannot serve as absolute confirmation, it provides a crucial first step in narrowing down candidate isolates for molecular testing. After centrifugation, the bacterial cells were lysed through the application of heat followed by cold shock. This combination effectively disrupted the cells and released their intracellular contents. A second centrifugation step then separated the components based on size and density. The DNA, being a lighter and soluble substance that can be measured in nanograms, remained suspended in the supernatant rather than precipitating. In contrast, the larger macromolecules such as cell debris and broken cell particles settled at the bottom of the tubes as pellets. For this reason, only the clear supernatant was carefully collected in fresh sterile tubes, as it contained the extracted DNA. Finally, the tubes holding the DNA were stored at –20°C to maintain stability and prevent degradation. This ensured that the extracted genetic material would remain suitable for subsequent molecular analysis.

After the amplification process inside the PCR machine, the samples were collected for further analysis. At this stage, the PCR tubes were expected to contain the amplified DNA molecules, meaning that the targeted genetic regions had been successfully copied through repeated cycles of denaturation, annealing, and elongation. The presence of these amplified DNA fragments indicated that the PCR procedure had worked as intended.

To ensure the stability and integrity of the amplified DNA, the samples were preserved at – 20˚C. This low-temperature storage prevented enzymatic activity or degradation, thereby maintaining the DNA in a suitable condition for subsequent applications such as agarose gel electrophoresis or sequencing.

PCR amplification with species-specific primers successfully generated distinct bands corresponding to the three target pathogens. For *Escherichia Coli*, clear amplification was observed at ~585 bp, consistent with the expected product size. *Vibrio* produced bands at ~154 bp. The comparison against a 100 bp DNA ladder confirmed that the observed fragments aligned precisely with the predicted sizes.

The appearance of clear and specific bands indicated not only the presence of these pathogens in the collected water samples but also the reliability of the designed primers. The detection of amplified DNA confirmed that PCR was effective in identifying target species, complementing the initial colony morphology findings.

Quantitatively, gel electrophoresis revealed *Vibrio* as the most abundant, confirmed in 146 colonies, followed by *Escherichia Coli*in 114 colonies. These results reaffirm the dominance of *Vibrio* in the tested bathing waters, while also highlighting notable contamination by *E.coli*. The successful visualization of expected amplicon sizes provided strong evidence that PCR served as a reliable molecular tool for confirming bacterial identity in environmental samples.
For *Vibrio* (146 isolates), the majority of antibiotics demonstrated strong effectiveness, with high proportions of susceptible strains. Drugs such as imipenem (IPM), doxycycline (DO), chloramphenicol (C), and gentamicin (CN) exhibited near-

complete susceptibility, highlighting their continued efficacy. However, notable resistance was observed against vancomycin (VA), where almost all isolates were resistant, indicating limited or no utility of this drug against *V. cholerae*. Moderate resistance was also seen for ampicillin (AMP) and aztreonam (ATM), where resistant colonies were present alongside intermediate ones. Linezolid (LZD) and ceftriaxone (CRO) displayed mixed results, with susceptible isolates dominating but still showing a considerable number of resistant strains. Overall, the data suggest that while several antibiotics remain effective, certain frontline drugs face reduced activity due to emerging resistance.

For *Escherichia Coli*(114 isolates), the susceptibility pattern was more diverse, showing a clearer split between effective and ineffective drugs. Imipenem (IPM), levofloxacin (LEV), gentamicin (CN), and aztreonam (ATM) demonstrated high to complete susceptibility, confirming their effectiveness in treating *E.coli*. In contrast, vancomycin (VA) showed total resistance, mirroring the *V. cholerae* results and reinforcing its lack of therapeutic relevance for Gram-negative bacteria. Ampicillin (AMP), erythromycin (E), and linezolid (LZD) were associated with high resistance, reflecting their reduced clinical utility. Moderate resistance was observed for doxycycline (DO) and cefixime (CFM), where intermediate isolates suggested a gradual shift towards resistance.

Chapter 05: Conclusion

This study showed that the bathing waters of Cox's Bazar—Sugandha, Laboni, Himchori, and Inani—are contaminated with harmful bacteria, mainly *Vibrio*, *Escherichia Coli*. Among them, *Vibrio* was the most common, followed by *E.coli*. Laboratory culture, PCR confirmation, and antibiotic testing all proved their presence and reliability of the results. Worryingly, many of these bacteria were resistant to commonly used antibiotics such as ampicillin and erythromycin, although they remained sensitive to stronger drugs like imipenem and levofloxacin. This means that not only are tourists exposed to contaminated water, but treating possible infections could also be difficult. The findings highlight a clear risk to public health, especially during peak tourist seasons. To reduce this danger, regular monitoring of seawater, proper waste management, better sanitation near beaches, and public awareness campaigns are urgently needed. Continued surveillance of antibiotic resistance is also important for guiding treatment and supporting national health programs. In short, the waters of Cox's Bazar are not microbiologically safe. Without quick action, they may continue to spread dangerous pathogens, creating both health risks and economic losses. Protecting beach water quality is therefore essential for safe recreation, public health, and the future of coastal tourism in Bangladesh. In addition, This study was limited by a less time of sampling and only 4 beach of sampling. Future work should include year round monitoring, quantitative microbial risk assessment, and detection of additional pathogens.

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