Prevalence of antimicrobial resistance enteropathogenic *Vibrio* sp. along with antibiotic-resistant genes and status of diarrhea in the fisherpersons of Sundarbans.

Dr. Sanjib Saha[1,2]

[1]Department of Zoology, Vidyasagar College for Women 39, Sankar Ghosh Lane, Kolkata – 700009, West Bengal, India.
[2]Department of Life Science and Biotechnology, Adamas University District-24 Parganas (North), Kolkata – 700126, West Bengal

Abstract:
In India, diarrhoeal illness is an unresolved issue. The Sundarbans region is also a diarrhea-endemic zone, with significant annual incidences that mostly afflict the Sundarbans residents during the monsoon season. Vibrio sp. and other halophilic, opportunistic, non-cholerae vibrios are encouraged to proliferate in the marine and coastal ecology. Along with fish and shellfish, Vibrio sp. is abundant in coastal and estuarine environments. Due to the presence of virulent or toxic genes (tox-R, trh, and trl) in Vibrios that are rarely found in the environment, approximately 20 Vibrio sp. have been linked to human disease (12 species have been identified for zoonotic infection). The development of antibiotic resistance by antibiotic-resistant genes (AMR & AMRGs) among these bacteria will make the situation more complicated. These vibrios invade as opportunistic pathogens directly or are spread through zoonotic transmission from fish and shellfish to fishermen, resulting in massive gastrointestinal diseases, including diarrhoea. According to recent research, aquatic environments like estuaries and coastal regions are rich potential reservoirs of resistance genes (ARGs) and antibiotic-resistant bacteria (ARBs). Therefore, the main way that resistance genes are transferred to human diseases is through aquatic environments. Vibrio sp. is the most common bacteria in marine creatures, according to the National Antimicrobial Resistance Monitoring System, which has already identified them as suitable candidates for tracking AMR. Ampicillin, Ceftazidime, Ceftriaxone, Tetracycline, Erythromycin, Azithromycin, Co-trimoxazole, Norfloxacin, Chloramphenicol, Pefloxacin, Kanamycin, and Vancomycin are only a few of the drugs to which bacteria identified from sediment and soil samples of the Sundarbans have demonstrated resistance. The proliferation of gastropathogenic bacteria and their antimicrobial genes throughout the Indian Sundarbans mangrove soil and water puts Sundarbans' lives and livelihoods at the highest risk. The pathogenic Vibrio species (*V. cholerae, V. vulnificus, V. fluvialis, V. parahaemolyticus, and V. fluvialis*) that are linked to coastal floods and the influx of seawater farther inland are initially encountered by fishermen. Therefore, more research is needed to determine the long-term impacts of microbial dysbiosis on disease susceptibility, antibiotic resistance, and the economic viability of aquaculture as well as the quality of life of Sundarban fishermen.

Keywords: *Vibrio* diversity, enteropathogenic diseases, antibiotic resistance, Fisherpersons, Sundarbans.

1. Introduction:

The world's largest mangrove, the Sundarbans, is home to a rich biodiversity and supports the employment of thousands of people, including fisherpersons who depend on its aquatic resources (fish and shellfish).[55] The Sundarbans mangrove ecosystem is a dynamic, productive, and diversified ecosystem facing several environmental stresses of human activities, commonly excessive non-renewable fuel combustion, causing a rise in CO_2 in nature, which alters the global carbon cycle and climate pattern, and natural origin.[5,22] Deforestation, oil spills, industries, coal mining, refineries, over-exploitation of natural resources, soil erosion, deforestation, the discharge of industrial and agricultural waste, deposition of heavy metals and metalloids, pesticides, extension of population, discharge of untreated sewage, and several other human activities have all been demonstrated to have a substantial impact on the quick deterioration of mangrove ecosystems.[47,55] Antibiotics and antibiotic resistance genes (ARGs) are regarded as man-made pollutants that affect the sustainability of the coastal environment, alongside traditional toxins such as pesticides, polyaromatic hydrocarbons, and heavy metals.[3,44] Indiscriminate and unmonitored disposal of various antibiotics from hospital use, nursing homes, veterinary, and aquaculture industries spreads antimicrobial resistance into the natural environment.[39] Antimicrobial resistance is highly prevalent in coastal and pristine estuaries worldwide, in addition to urban and hospital sewage disposal sites. Fluvial urban, industrial, and clinical contaminants, including heavy metals, PAHs, oil spills, antibiotics, and others, have been shown to affect the niche structure both geographically and temporally, and these pollutants have a significant impact on community metabolism, which eventually leads to changes in the microbial ecosystems' structure, function, and spatiotemporal dynamics.[12] The influence of the aforementioned toxins naturally builds up in the northern portion of the Sundarban estuary since it is close to important industrial belts, smaller ports, and urban interphases of big cities. Therefore, the potential increase in waterborne *Vibrio* sp. may cause a specific variety of illnesses within the public, including cholera, vibriosis, and septicemia, due to antibacterial resistance developing in Vibrio; they become insensitive towards antibiotics, and this causes a more complicated situation for public health, especially fisherpersons of Sundarbans.[35] People like Sundarbans fisherpersons are closer to coastal regions and face an increased risk of natural disasters along with waterborne diseases, including zoonotic bacterial diseases due to increasing anomalies of climate[50]. The Sundarbans region has the potential for an increase in waterborne *Vibrio* sp., a group of bacteria that can cause a range of illnesses in people, including cholera, vibriosis, and septicemia.[35] *Vibrio parahaemolyticus* causes gastroenteritis by horizontal transfer of genetic materials, ultimately creating a genetic deletion.[16] Natural disasters like floods cause an epidemic of watery diarrhoea and spread through

an outbreak of diarrhea (with or without blood in stool) caused by *Vibrio* sp. in Gosaba block in Sundarbans, in the coastal area, and aquatic animals of West Bengal.[13,10,44] The world's biggest public health concern today is bacterial resistance due to the frequent and unintentional use of antimicrobials, and less effective in treating serious infections, which leads to the arising and spreading of antimicrobial resistance (AMR).[43] It has been demonstrated that a variety of antibiotic-resistant microbes can be found in the environment, including soil, water bodies, ecosystems linked with plants or animals, and man-made environments, and these microorganisms can spread antimicrobial-resistant genes (ARGs) by horizontal microbial gene transfer.[3,37] As a result of antibiotics' declining effectiveness, infectious diseases are spreading, common infections are getting harder to cure, treatments are becoming more costly, and the economy is being burdened.[66] According to Sivam *et al.* (2017),[60] of eating seafood or drinking tainted water can either directly or indirectly spread antibiotic-resistant germs from animals to people. By 2050, AMR is predicted to cause 10 million annual fatalities and $100 trillion in lost productivity worldwide. This study aims to investigate the present status of prevalence of waterborne *Vibrio* sp. and antimicrobial resistance in the Indian Sundarbans mangrove ecosystem related to the public health of fisherfolk and to develop awareness among the fisherfolk community for adopting management practices.

2. ENTEROPATHOGENIC VIBRIO POPULATION AND PUBLIC HEALTH:

Due to long-term sea surface warming, there have been indications of increased seasonal duration, concentration, and geographic expansion of vibrios infections in the United States, Israel, and coastal Chile.[23] After an exceptionally warm summer in 1994, reports of *Vibrio vulnificus* were made throughout the German coast. There is evidence linking the Baltic Sea's long-term warming and temperature anomalies to an increase in *Vibrio vulnificus* infections. According to climate modeling methods, for every degree Celsius that the annual maximum water temperature rises, the prevalence of Vibrio infections in the Baltic region may almost quadruple.[7,31] Numerous vector-borne diseases, including vibriosis, may benefit from longer warm seasons, earlier spring seasons, milder and shorter winters, and hotter summers.[64, 65] Climate change refers to any long-lasting alteration to the average environment or climate variability. It represents a significant, long-term change in the planet's average temperature and weather patterns. Among halophilic vibrios, *V. parahaemolyticus, V. vulnificus, V. fluvialis, and V. alginolyticus* are the major leading seafood-associated human gastrointestinal damage bacteria present in almost all saline waters (Figure 1).[24] Temperature and salinity are the two main environmental factors or conditions that may help to multiply the growth of vibrios. On the other hand, the main impact of climate anomalies or alterations is global warming (Saha and Dash, 2021).[53] Therefore, the increased temperature can increase the population of vibrios that easily contaminate the saltwater as well as the surrounding freshwater during natural calamities or disasters.[57] So, anomalies in saltwater temperature can also describe the unexpected occurrences of vibrios in new locations.[33,29] Extreme weather events are occurring more frequently, temperatures are rising, and precipitation patterns are changing are creating the perfect environment for Vibrio growth. Important elements affecting Vibrio populations are as follows.[57] There are 12 *Vibrio* species known to cause infections in people who are directly exposed to that water, especially fisherpersons.[56,57] These Vibrio species can cause diseases typically acquired as wound infections from exposure to seawater or gastrointestinal illnesses associated with consuming raw or undercooked seafood.[30] Numerous clinical indications can result from vibriosis, which is caused by these non-cholera Vibrio species, mainly *Vibrio parahaemolyticus*, *Vibrio fluvialis, Vibrio alginolyticus*, *Vibrio vulnificus,* and *Vibrio mimicus*.[4,52] Although mild and self-limiting gastroenteritis is the most prevalent symptom, exposure to seawater from an open wound can also result in skin infections in fisherpersons directly or through zoonotic transmission. In many cases, it was found in isolated areas where there had been no previous cases of the disease and where the environmental circumstances were thought to be unfavorable for pathogenic Vibrios (Table I). Diarrhea is a major public health problem in the Indian Sundarbans, and diarrhea includes vibrios infection (Cholera, gastrointestinal infection, stomachache, septicemia, etc.).[35,54] The prevalence of enteropathogenic vibrios is closely linked to environmental factors like water surface warming, increases in sea temperature, climate change, and higher concentrations of Vibrio infections (Table I).

2a. *Vibrio Cholerae:*

A serious water-borne diarrheal illness that is common in developing nations is cholera. It is brought on by the gram-negative, curved bacillus bacterium Vibrio cholerae, which is primarily spread via tainted food, drink, etc. In the small intestine, V. cholerae proliferate and generate the enterotoxin choleragen, which attaches itself irreversibly to the intestinal epithelial cells and causes the mucosal cells to release cyclic AMP. Cyclic AMP activation prevents sodium ion absorption and causes an excessive amount of water and electrolytes (chlorine ions) to be secreted into the colon. The illness is characterized by extreme dehydration, diarrhea with "rice water stools," nausea, vomiting, and abdominal pain. Rapid fluid loss or dehydration results in shock, and if the infection is left untreated, the patient may die. Cholera has been endemic throughout Asia for centuries, primarily in Bangladesh, India, and the Ganges delta of the Bay of Bengal 15. Asiatic cholera has caused waves of worldwide pandemics, spread quickly, and burst multiple times.[54]

2b. *Vibrio parahaemolyticus*:

Around the world, Vibrio parahaemolyticus is a common resident of coastal regions that are both temperate and tropical. Although wound exposure to contaminated water can also result in infection, the epidemiology of *V. parahaemolyticus* is characterized by occasional occurrences of infection along coastal areas, primarily linked to the ingestion of raw or undercooked contaminated seafood during the warmer months. Although cross-contamination of cooked food (for instance, with contaminated water) may have been a secondary vehicle, the most likely cause of *V. parahaemolyticus* infections was the intake of raw or incorrectly prepared contaminated food. *V. parahaemolyticus* became a significant seafood-borne disease and a worldwide public health problem as a result of its occasional instances and outbreaks that were documented in Europe, Africa, New Zealand,

and the majority of Asian nations. Although certain groups predominated in each region, infections were linked to distinct strains in heterogeneous groupings. In Calcutta, India, a sharp rise in gastroenteritis cases was noted. The isolates from these individuals all clustered in a single homogenous group, a variation of the serotype with the same virulence features, unlike the majority of the preceding outbreaks. Bacterial infectious diarrhea is primarily caused by *V. parahaemolyticus.*[54] Along with two virulent components, TDH and TRH, and many combinations of somatic (o) and capsule (k) antigens, Vibrio parahaemolyticus has lately drawn international attention for its pandemic spread due to the clone O3:K6.[17] The pandemic parahaemolyticus O3:K6 strain was initially identified in Italy in the summer of 2007 after being isolated from the stool of a hospitalized patient who had diarrhea .[39]

2c. *Vibrio vulnificus:*

Common in estuarine waters, *Vibrio vulnificus* has been identified from a variety of environmental sources, such as sediment, seawater, and seafood items. Almost all instances of *V. vulnificus* occur in people who already have an underlying illness, making it an opportunistic pathogen in contrast to *V. cholerae* and *V. parahaemolyticus*. Diabetes mellitus, cancer, and liver illnesses (such as cirrhosis or hepatitis) are the most prevalent risk factors. According to earlier research, the risk of developing V. vulnificus-associated primary septicemia is up to 80 times higher in people with chronic liver disease, such as cirrhosis, than in healthy people.[54] Watery diarrhea, abdominal pain, nausea, vomiting, fever, and soft tissue lesions are the symptoms associated with the gastrointestinal tract.[69] Wound infection by *V. vulnificus* most commonly occurs as septicemia, and the capsulated form is much more virulent than the non-capsulated form. During its life cycle, it can spontaneously switch between capsulated and non-capsulated morphotypes, and endotoxin (LPS) is responsible for the infection factor in *V. vulnificus*. [68]

2d. *Vibrio alginolyticus:*

Like other vibrios, *V. alginolyticus* is becoming more widely acknowledged as a new human disease, and summertime is when infection rates rise the most. Seawater is home to *V. alginolyticus*, which typically causes ear infections and superficial wounds. The majority of *V. alginolyticus* wound infection reports are caused by cuts or abrasions that are exposed to tainted saltwater. In addition, 131 cases—nearly 20% of all infections with vibriosis – *V. alginolyticus* was found to be a substantial cause of infection in a vibriosis investigation. Furthermore, new epidemiological data point to a sharp rise in the incidence of *V. alginolyticus* infections in the USA. Like *V. parahaemolyticus*, *V. vulnificus* infections can come from two different sources: either wounds are exposed to seawater or seafood products, which can cause wound infections and secondary septicemia, or eating contaminated seafood, especially molluscan shellfish, which can cause gastroenteritis or primary septicemia. Eating oysters is also frequently linked to the bacterium's high concentration. More than 95% of seafood-related deaths in the US are caused by *V. vulnificus*, a highly lethal human disease, in contrast to *V. parahaemolyticus*. [49,54]

2e. *Vibrio fluvialis:*

V. fluvialis is generally transmitted through oral and anal thought to be the mode of transmission for *V. fluvialis*. It results in diarrheal outbreaks and isolated instances. Warm, brackish, and salty water is the ideal habitat for this organism. It can thrive in temperatures ranging from 9 to 31^{0}C and it multiplies best in water that is 18^{0}C or higher. Most clinical diseases caused by *V. fluvialis* infections have a seasonal trend. The parameters of salinity and temperature may have some bearing on the growth of the bacteria. The method of transmission for *V. fluvialis* is believed to be oral-fecal. It happens in single cases and diarrheal epidemics. For this creature, warm, brackish, and salty water is the perfect environment. It grows best in water at 18°C or higher, but it can survive in temperatures ranging from 9 to 31^{0}C. The majority of clinical conditions brought on by *V. fluvialis* infections exhibit a seasonal pattern. The growth of the bacteria may be somewhat influenced by temperature and salt.[54] Worldwide, there is a notable rate of enteric infection-related morbidity and mortality, particularly among children in underdeveloped nations. *V. fluvialis* infections are common in areas where fecal contamination of water, food supplies, and consumption of raw seafood or seafood products is significant. Furthermore, the highest infection rates are found in places with poor or insufficient water supply, sanitary conditions, and overall standards of living. The biggest and most immediate health risk is still microbiological pollution of water, and the range of activities on the watershed causes surface water quality to fluctuate dramatically regularly. [13]

The prevalence of *Vibrio* is primarily determined through molecular biology methods by the extraction of DNA from cultured Vibrio samples. 100ng of genomic DNA was used as a template for the PCR reaction. 200 µM dNTPs, 0.2 U of PhusionTM high fidelity DNA polymerase, forward and reverse primers with final concentrations of 0.5 µM each, template DNA, and Phusion reaction buffer with 1X final concentration were all included in each reaction mix. The literature that is currently available is used to choose primers (Table II).[48,35] A heat cycler (Gene Amp 9700, ABI) was used to carry out the amplification. Universal primer targeting the 16s rDNA of Vibrio was first used to amplify the DNA purified from the bacterial colonies grown in a TCBS agar plate to validate the presence of bacteria in the collected samples. Then, species-specific oligonucleotide primers were used to amplify the same genomic DNA for the determination of specific Vibrio species (Table II). The sequence of universal primer, Vibrio genus primer, *Vibrio*s species-specific primers (*V. cholerae, V. parahaemolyticus, V. vulnificus, V. alginolyticus, V. mimicus, V. harveyi*), along with the primer annealing temperature (Tm) and amplicon size, are given in Table II. Images were captured in the iBright gel documentation system for Vibrio identification. [44]

3. DIAGNOSIS OF WATER-BORNE VIBRIO INFECTION:

A bacterial identification bottle kit was filled with the collected farm water, and the bottle was incubated at 35^{0}C for 24 to 48 hours. Different bacteria in farm water can be identified by the color of the bacterial colonies, which range from green to red, pink, blue, and black. Alkaline Peptone water (APW) was used to boost development in at least 0.1 milliliters of the water samples that were taken from the farm. From the APW, prepared microbiological culture media (TCBS and CV) by the

spread plate method. Observe bacterial colonies within a bacteriological incubator after 24 hr. incubation at room temperature (35^0C). Bacterial contamination of water with bacterial species is indicated by the presence of color or bacterial colonies (droplets) on plates. The average number of colonies per plate was multiplied by the reciprocal of the dilution factor to determine the counts of colonies per milliliter. Colony-forming units (CFU) per milliliter of sample are the unit of measurement used to express the computed results. Colony Forming Units (CFU) per 100 µl of blood (CFU/100 µl) were counted three times for each colony, sorted by size and color, and then averaged. For purification and characterisation, distinct members of each colony type were then chosen at random .[53,56,51,11] Selected colonies from the TCBS were moved to tryptone soy agar and then purified in TSB following a 24-hour incubation period at 35°C. Cultured Vibrio samples were centrifuged and moved into a pellet at the bottom of a micro centrifuge tube following final purification and growth in TSB (TCBS – TSA – TSB). Following the manufacturer's instructions in the laminar flow cabinet, the bacterial pellet was subjected to genomic DNA isolation using a bacterial genomic DNA isolation kit (Tissue Spin, Germany). Following centrifugation and a 70% ethanol wash, the DNA pellet was dissolved in TE buffer and kept for further use at -20 °C. A NanoDropTM spectrophotometer was used to measure and compute the ratio of optical densities at 260 nm and 280 nm wavelengths, respectively, in order to assess the quality and amount of isolated genomic DNA for each sample. Purified DNA was present in the aqueous phase, which was utilized straight away in the following tests.

Selected colonies from the TCBS were moved to tryptone soy agar and then purified in TSB following a 24-hour incubation period at 35°C. Cultured Vibrio samples were centrifuged and moved into a pellet at the bottom of a microcentrifuge tube following final purification and growth in TSB (TCBS – TSA – TSB). Following the manufacturer's instructions in the laminar flow cabinet, the bacterial pellet was subjected to genomic DNA isolation using a bacterial genomic DNA isolation kit (Tissue Spin, Germany). Following centrifugation and a 70% ethanol wash, the DNA pellet was dissolved in TE buffer and kept for further use at -20 °C. A Nano-Drop spectrophotometer was used to measure and compute the ratio of optical densities at 260 nm and 280 nm wavelengths, respectively, in order to assess the quality and amount of isolated genomic DNA for each sample. Purified DNA was present in the aqueous phase, which was utilized straight away in the following tests. 100ng of genomic DNA was used as a template for the PCR reaction. 200 µM dNTPs, 0.2 U of PhusionTM high fidelity DNA polymerase, forward and reverse primers with final concentrations of 0.5 µM each, template DNA, and Phusion reaction buffer with 1X final concentration were all included in each reaction mix. Primers are chosen from the body of existing literature.[35,44,48] A heat cycler (Gene Amp 9700, ABI) was used to carry out the amplification. To confirm that there were bacteria in the samples, the DNA extracted from the bacterial colonies cultured on a TCBS agar plate was first amplified using a universal primer that targets the 16s rDNA of Vibrio. The same genomic DNA was then amplified using species-specific oligonucleotide primers to identify particular Vibrio species. Table 2 provides the primer annealing temperature (Tm), amplicon size, and sequences for the universal primer, Vibrio genus primer, and Vibrios species-specific primers (*V. parahaemolyticus, V. cholerae, V. vulnificus, V. mimicus, V. alginolyticus*, and *V. harveyi*). The amplified products were examined on 1%agarose gels containing 0.5 µg/ml ethidium bromide in 1X Tris-acetate-EDTA (TAE) solution following the completion of the PCR amplification stages. The gel was then photographed following electrophoresis. Pictures were taken using a Gel Doc. At Barcode Biosciences in Bangalore, India, the DNA products amplified by PCR using the 8F/1492R primer pairs and one DNA product using the SWP 2F/2R primer pairs were purified and sequenced in both orientations. Sequence chromatograms were analyzed, and a consensus sequence was produced using the DNA Baser Assembler v5. GenBank matched sequences were found using BLAST (NCBI). Once received, the rRNA sequences were uploaded to the NCBI GenBank database. Clustal-W was used for data analysis and multiple alignments (MEGA-X). At http://www.ncbi.nlm.nih.gov/, the BLAST program was used to match the sequences to the GenBank database. The phylogenetic analysis and distance calculations were performed using the Kimura-2 adjustment for evolutionary rate. According to Felsenstein (1985),[28] 1,000 bootstrap analyses were used to determine the internal rod's confidence levels. The Neighbour-Joining approach was used to generate an evolutionary tree for nucleotide sequences. [59]

4. ANTIMICROBIAL RESISTANCE AND PUBLIC HEALTH:

Microorganisms, including bacteria, can acquire defense mechanisms against treatments intended to kill them, a phenomenon known as antibacterial resistance, or antimicrobial resistance (AMR). AMR is a global threat. Therefore, using conventional drugs to treat illnesses brought on by these resistant germs becomes increasingly challenging or even impossible. When bacteria stop responding to antimicrobial medications, it's known as antimicrobial resistance, or AMR. Because drug resistance renders antibiotics and other antimicrobial treatments ineffective and makes treating infections more difficult or impossible, it increases the risk of disease transmission, severe illness, disability, and death. By 2050, AMR is predicted to cause 10 million annual fatalities and $100 trillion in lost productivity worldwide.[17] According to WHO estimations, bacterial AMR contributed to 4.95 million fatalities worldwide in 2019 and was directly responsible for 1.27 million deaths. Over 2.8 million drug-resistant illnesses happen annually in the United States, and more than 35000 people die from this cause. It is predicted that there are over 3 million cases of all resistant diseases, including Clostridium difficile (CDI), which results in about 48,000 fatalities.[15] Infectious or parasitic disorders accounted for 3.8 million ED visits in 2021. Infectious and parasitic disease visits to doctors' offices totaled 39.5 million in that year (National Ambulatory Medical Care Survey, 2021).[40] The median reported rates of 42% for third-generation cephalosporin-resistant E. coli and 35% for methicillin-resistant Staphylococcus aureus in 76 countries are concerning. One out of five E. coli UTI infections in 2020 had reduced resistance to popular antibiotics such as ampicillin, co-trimoxazole, and fluoroquinolones. As a result, common infections are becoming harder to treat. High levels of resistance to significant medications were also shown by the common gut bacteria Klebsiella pneumoniae. Increased use of last-resort drugs like carbapenems, for which resistance is also observed in different geographic areas, may be the consequence of elevated

resistance levels. As these last-resort drugs become less effective, the risk of infections that cannot be cured increases.[67] In aquaculture industry, disease, especially bacterial infections, was identified as the primary cause of financial loss. Various *Vibrio* species cause vibriosis, a condition common worldwide that results in significant mortality and substantial financial losses for the crab industry.[57] A few species of *Vibrio* are considered pathogenic, although the majority are considered non-pathogenic commensals. In crab farms, antibiotics are used indiscriminately to prevent and control bacterial infections in hatcheries, especially when the development of the larvae is impeded.[49] As a result of antibiotics' declining effectiveness, infectious diseases are spreading, common infections are getting harder to cure, treatments are becoming more costly, and the economy is being burdened.[66]

By eating seafood or drinking tainted water, antimicrobial-resistant bacteria can spread from animals to people directly or indirectly.[56,52] According to estimates, AMR would cause 10 million annual fatalities and $100 trillion in lost output worldwide by 2050. By investigating the presence of antibiotic-resistant bacteria (ARB) in the microbial milieu of the Sundarbans mangroves and their environs, a recent study elucidates the potential influence of human interventions on the distribution of ARG and bacteria in the mangrove sediments. The study also revealed that the release of antibiotics from the animal feed industry, aquaculture feed industry, fertilizer industry, pharmaceutical industry, aquacultural farm, and agricultural fields directly contaminates water bodies, directly and indirectly (rivers and streams that join the Sundarbans estuary), and is finally connected to antibiotic resistance. Commonly used antibiotics in different industries and farms are ampicillin, kanamycin, vancomycin, tetracyclines, and clarithromycin. Bacteria can acquire genes from their environment to cope with their stress conditions (horizontal gene transfer), and some of these acquired genes may confer antibiotic resistance.[12] These strains proliferate and spread the resistance when they reproduce. Eighteen bacterial strains in the Sundarbans are resistant to ampicillin, kanamycin, tetracycline, and vancomycin; some of these strains are also resistant to more than one medication. With significant increases in salt concentrations ranging from 12 to 21 percent, it was discovered that all of the multidrug-resistant bacteria were extremely salt-tolerant. *Bacillus*, *Thalassocella*, *Nitratireductor*, *Halobacillus, Oceanobacillus*, and *Marinobacter* are marked as multidrug-resistant bacteria in the present study .[12]

There are several methods and ways to classify antibiotics; however, common classifications are based on the mechanism of action (Table III). Treatment of infections has become more difficult due to the development of antimicrobial resistance in Vibrio species. This resistance arises from the bacteria's ability to acquire resistance genes through horizontal gene transfer and create mutations. Because they can cause gastroenteritis and other infections through the consumption of bacteria-contaminated marine food or exposure to contaminated water, Vibrio bacteria—in particular, Vibrio parahaemolyticus—are frequently found in the coastal and brackish waters of the Sundarbans and can be extremely dangerous to fishermen's health. *Vibrio* species' antimicrobial resistance (AMR) is a developing worry since it can make treatment more difficult and make illnesses worse. Although studies from other places demonstrate significant rates of resistance to popular antibiotics like ampicillin and other beta-lactams, the search results do not specifically indicate the frequency of AMR in Vibrio isolates from the Sundarbans area. Given that fishermen in the Sundarbans are probably subject to the same environmental stresses that promote the development of AMR, this raises the possibility of danger for them. The main goal of the Bhattacharyya and colleagues' study was to use the marker antibiotic gene blaTEM in the estuary sand to evaluate the distribution of ARBs and ARGs[12].

Furthermore, an attempt is made to investigate the connection between the environmental pollutants (heavy metals and PAHs) and the distribution of ARBs and ARGs in the sediment samples. They employed biochemical and biomolecular (analysis of 16S rRNA gene) methods to isolate and characterize 18 multidrug-resistant bacterial strains (resistant to ampicillin, vancomycin, kanamycin, and tetracycline) from various locations within the Indian Sundarban estuary. Through PCR techniques, they isolate bacteria that are resistant to ampicillin, tetracycline, kanamycin, and vancomycin, as well as antimicrobial resistance genes such as blaTEM (ampicillin), blaROB (ampicillin), aph (kanamycin), tetQ (tetracycline), tetM (tetracycline), tetW (tetracycline), vanA (vancomycin gene from Staphylococcus and Enterococcus), and (AMG) in the Sundarbans sediment.[12] Most common antibiotics like Oxytetracycline, Chlor-tetracycline, Amoxicillin, Co-trimoxazole, Sulphadiazine, and Sulphamethoxazole are used in aquaculture, which are the common contributors to AMR. Some common.

The Kirby-Bauer disc diffusion method was used to determine the Vibrio species' antimicrobial susceptibility profile (Figure 4). In short, the 5.0 ml Mueller–Hinton Broth was used as an inoculation medium for the purified isolates, and the antimicrobial susceptibility profile of the vibrios isolates was determined. The disc diffusion method of Kirby-Bauer was used to determine the antimicrobial susceptibility and resistance profile of the *Vibrio* sp. (Figure 4).[19] In conclusion, 5 milliliters of Mueller-Hinton Broth/Tryptone Soya Broth were used to plate the purified isolates. After that, these tubes were incubated for 20 to 24 hours to create a moderately cloudy bacterial suspension. Using a cotton swab, the bacterial broth suspension was uniformly scattered onto the medium's surface in three planes. Flamed forceps were used to insert the antibiotic discs (Pasteur pharmaceuticals) on the agar after the inoculum had dried, and they were gently pressed down to make sure they made contact (Figure 2). The zone of inhibition was measured to ascertain the sensitivity, intermediate, and resistance pattern of the isolates to the antibiotics. Generally, a total of 12 antibiotics belonging to 8 classes were selected based on CLSI guidelines that are to be tested for Vibrios (Table III). Different classes of antibiotics used in the study are penicillin and beta lactamase inhibitor combinations (Ampicillin10µg; Amoxycillin Clavulanic acid 30µg); Cephems (Cefepime 30µg; Cefotaxime30µg; Cefoxitin 30µg; Ceftazidime 30µg); Carbapenems (Meropenem 10µg); Aminoglycosides (Gentamicin 10µg); Tetracyclines (Tetracycline30µg); Fluoroquinolones (Ciprofloxacin 5µg); Folate pathway inhibitors (Trimethoprim sulfamethoxazole 25µg); Phenicol (Chloramphenicol 30µg) (Table IV).

5. EMERGENCE AND MECHANISM OF ANTIMICROBIAL RESISTANCE AGAINST VIBRIOS:

Pathogens have an innate ability to defend themselves against risks to their survival.

They are equipped with five defense systems to (Figure 3):

1. Create new cell functions that do not use the target of the antibiotic.
2. Use proteins and enzymes that break down antibiotics to alter or eliminate them.
3. Limit or alter the entryways to restrict access.
4. Modify the target of the antibiotic such that it no longer fits and performs its function.
5. Use pumps to get rid of antibiotics

In addition to becoming resistant to antibiotics, individual infections can transfer their resistant DNA to other bacteria. Pathogens have more opportunities to impose their innate resistance mechanisms when we use a particular antibiotic more frequently, which can render the drug ineffective on a larger scale. Antibiotics can occasionally also destroy beneficial bacteria that keep us safe, which increases the likelihood that resistant infections will proliferate and spread. Multidrug-resistant organisms (MDROs), or bacteria that are no longer susceptible to at least one (or more) classes of antibiotics, have also emerged. In the end, antimicrobials may not always be successful in treating infections, which is what caused the current AMR health issue.

Initially, cholera was successfully treated with tetracycline, streptomycin, and chloramphenicol. *V. cholerae* was previously killed by several antibiotics, including tetracycline, azithromycin, and fluoroquinolones, while V. vulnificus was found to be susceptible to tetracyclines, aminoglycosides, third-generation cephalosporins, chloramphenicol, and more recent fluoroquinolones.[6] However, many bacterial genera, including Vibrios, have developed antibiotic resistance as a result of the overuse and abuse of antibiotics in human, agricultural, and aquaculture settings. The mechanism of action of antibiotics against *V. vulnificus* and *V. parahaemolyticus* is still poorly understood.[25]

Numerous studies conducted recently have demonstrated that isolates of *V. vulnificus* have developed resistance to a variety of antibiotics, including ampicillin, tetracycline, aztreonam, streptomycin, gentamicin, and tobramycin. Regardless of the country, the most commonly observed antibiotic resistance for both *V. parahaemolyticus* and *V. vulnificus* is toward ampicillin, penicillin, and tetracycline.

According to several other investigations, the majority of *V. parahaemolyticus* strains that were isolated from clinical, seafood, and environmental samples exhibited high levels of resistance to several antibiotics, including bacitracin, carbenicillin, cefazolin, ceftazidime, cephalothin, colistin, gentamicin, penicillin, spectinomycin, tobramycin, and amoxicillin.[25] 22 distinct medications from nine different classes were used for this investigation, and it was shown that nearly 99% of V. cholerae isolates were resistant to 2 antibiotics, 17.2% to 10 antibiotics, and 7.5% to 14 antibiotics. Neomycin resistance was found to be the lowest (4.0%), and sulfamethoxazole resistance was the greatest (99.8%). Furthermore, there was a significant level of resistance to streptomycin, trimethoprim, and nalidixic acid.[45] We conducted a second investigation of the pattern of antibiotic susceptibility of *V. cholerae* in Bangladesh between 2000 and 2018. They discovered that between 2012 and 2017, *V. cholerae's* susceptibility to tetracycline rapidly dropped from almost 100% to less than 6%, before rising to 76% in 2018. During the study period, ciprofloxacin and azithromycin susceptibility were nearly 100%. The profile of antibiotic resistance in V. cholerae (n = 159) isolated during the 2012–2015 cholera outbreaks in Mozambique.[21] demonstrated that every isolate was sensitive to ciprofloxacin, 13% were also resistant to azithromycin, and all were resistant to ampicillin and nalidixic acid. Potential sources of antibiotic resistance genes for Vibrios include commensal gut bacteria and horizontal gene transmission from other pathogens via various mobile genetic elements.[20] Genes that confer antibiotic resistance are also found in the environment, and wastewater treatment plant effluents and aquatic bodies may function as reservoirs for various antibiotic resistance genes.[25] Similar to other bacteria, Vibrios' fundamental mechanisms of antibiotic resistance can be categorized as (Figure 3)[25] -

A) Intrinsic Resistance Mechanisms B) Acquired Resistance Mechanisms (vertical/horizontal transfer)

5a. Intrinsic Resistance Mechanisms include –

A significant permeability barrier is formed by the bacterial outer membrane, and antibiotic entrance may be impeded by modifications in the expression of outer membrane porins. Full-length lipopolysaccharide (LPS)-expressing strains are inherently resistant to hydrophobic antibiotics like aminoglycosides and macrolides. By reducing the net negative charge of lipid A from -1.5 to 1 or from -1.5 to 0, respectively, other common LPS changes prevent the binding of certain cationic antibiotics, such as polymyxins, leading to resistance. Modifications to the membrane lipid barrier can reduce the fluidity of lipopolysaccharide (LPS) and the permeability of antibiotics (tetracycline, carbapenems, aminoglycosides, fluoroquinolones, and chloramphenicol). Vibrios can either hydrolyze the primary drug structure with enzymes to remove antibiotic activity or chemically alter the antibiotic by adding a chemical group to the scaffolds. For example, metallo-β-lactamases (class B) or serine-β-lactamases (classes A, C, and D) hydrolyze β-lactams. Esterases of erythromycin with the exception of telithromycin, macrolides like erythromycin have their macro lactone rings hydrolyzed by EreA and EreB. Enzymatic modification of antibiotics includes various processes such as O-phosphorylation (fosfomycin), O-nucleotidylylation [nucleotidyl transferases (ANT)] (a variety of aminoglycosides), O-glycosylation (macrolide and rifampin), O-and N-acetylation (chloramphenicol, fluoroquinolone, streptothricin, and other aminoglycosides), O-ribosylation, hydroxylation, etc. Trimethoprim and sulfamethoxazole block the bacterial folate production pathway, but they become resistant to each other when sul2 and dfrA1 genes are acquired. Tolerance or reduced susceptibility can be attributed to the organism's innate cellular characteristics or regulatory circuit. For example, a change in the nutrient pools within a polymicrobial community may result in a change in the innate susceptibility of specific organisms to a particular antibiotic by changing bacterial metabolism.[25]

- Efflux Pumps = Vibrio species possess efflux pumps that actively remove antibiotics and other toxic compounds from the

cell, reducing their effectiveness.

- Outer Membrane Permeability = The outer membrane of Vibrio species can limit the entry of certain antibiotics, contributing to intrinsic resistance.
- Enzymatic Degradation = Some Vibrio species produce enzymes that can degrade or modify antibiotics, rendering them ineffective.
- Target Modification = Vibrio species can have modified target sites for antibiotics, reducing the binding affinity and effectiveness of these drugs.

5b. Acquired Resistance Mechanisms include –

Spontaneous mutation, which can be inherited by the progenitor cells by vertical transfer and manifest as non-similar point mutations or insertion elements, is a relatively slow process that can change the transcription of specific genes, modify target enzymes, or circumvent antibiotic activity. Recent research indicates that the genetic basis of the emergence of multidrug-resistant (MDR) and extensively drug-resistant (ExDR) Vibrios and other enteric pathogenic bacteria is primarily due to horizontal gene transfer (HGT) via various highly dynamic mobile genetic elements, such as plasmids, integrating conjugative elements, super-integron, transposable elements, and insertion sequences. These components can transfer from one bacterium to another, whether they are closely or distantly related. Drug-resistant Vibrios are eventually produced by Vibrios' natural capacity to take in and chromosomally integrate exogenous DNA from commensal gut bacteria that are resistant to numerous medications and other sources. HGT routes, which include conjugation, transduction, transformation, and fusion resistance in bacteria, including Vibrios, are the main methods through which antibiotic resistance genes are passed from one Vibrio to another. Comprehensive whole genome sequencing investigations have revealed that drug-resistant Vibrios may contain mobile genetic elements, including plasmids, integrating conjugative elements like SXT, gene cassettes, and integrons. The production of Type III, TDH-related hemolysin (TRH), and thermostable direct hemolysin (TDH) (tdh gene) in food-borne V. parahaemolyticus can be regulated by the expression of the tox R gene. Since the intrinsic secretion systems T3SS1 and T3SS2 can still cause damage from a distance even in the absence of direct pathogens, they are rendered useless in the presence of antibiotics. However, certain clinical strains of *V. parahaemolyticus* remain harmful despite lacking the virulence factors listed above, suggesting the presence of potential factors and the possibility that different strains may use different tactics to acquire pathogenicity (Figure 3).[25]

- Horizontal gene transfer = Through the exchange of mobile genetic elements such as integrons, transposons, and plasmids, a process known as horizontal gene transfer, Vibrio species can acquire antibiotic resistance genes from other bacteria.
- Mobile Genetic Elements = Mobile genetic elements, including integrons, plasmids, and transposons, are crucial for the transmission of antibiotic resistance genes among Vibrio species. These substances may carry several resistance genes, which could lead to multidrug resistance.
- Antibiotic Resistance Genes = Vibrio species can acquire some antibiotic resistance genes, such as those for beta-lactamase, aminoglycosides, fluoroquinolones, tetracyclines, and chloramphenicol.

Antibiotics are typically modified or degraded by enzymes; their entry into cells is restricted to prevent accumulation, metabolic pathways are altered, binding sites such as ribosomes are modified to decrease drug efficacy, and efflux pumps are activated to remove antibiotics from cells before their levels can reach sufficient levels. Additionally, bacteria can form surface-bound communities called biofilms, which have variable nutritional levels and little resistance to antibiotics. A summary of these resistance mechanisms can be found in Figure 3. The bacteria are further protected by these biofilms. Furthermore, bacteria are adept at horizontal gene transfer, which is made possible by plasmids and other mobile genetic elements, and can acquire resistance genes from neighboring cells or even different species. The rapid spread of MDR among microbial populations is made possible by the fact that these acquired genes usually contain many complex resistance mechanisms within a single unit. Microorganisms possess a diverse range of resistance methods due to their ability to transfer genetic information efficiently horizontally. These techniques can be modified as needed to maintain survival against continual breakthroughs and the use of antimicrobial treatments by the medical industry.

6. ANTIMICROBIAL-RESISTANT AND ANTIBIOTICS AGAINST *VIBRIO* SP:

Studies on the Sundarbans have shown that Vibrio species, particularly *V. parahaemolyticus*, exhibit high levels of antimicrobial resistance, including resistance to multiple antibiotics, for example, ampicillin, cephalexin, kanamycin, azithromycin, and polymyxin B (Figure 4). In the Sundarbans, 60% or more of *Vibrio* sp. exhibit 2-3 antibiotic resistance, including multidrug-resistant strains. Sundarbans may serve as a reservoir for germs that are resistant to antibiotics, which might then spread through the environment and possibly into human bodies. *V. parahaemolyticus* isolates from oysters in coastal West Bengal, India, were found to be resistant to cefpodoxime (100%) and ampicillin and cefotaxime (90%), ceftizoxime (60%), tetracycline (50%), ceftriaxone (40%), ciprofloxacin, and nalidixic acid (10% each) in another study. In Sundarbans mudcrab, Uddin *et al.* (2013)[63] isolated *Vibrio alginolyticus*, *V. cholerae*, *V. harveyi*, *V. fluvialis*, *V. parahaemolyticus*, and *V. mimicus*. All of the bacterial isolates demonstrated resistance to ampicillin, erythromycin, kanamycin, nalidixic acid, neomycin, oxytetracycline, penicillin, streptomycin, tetracycline, ampicillin, erythromycin, kanamycin, chloramphenicol, and norfloxacin (83.78%). The diversity of ARGs in Sundarbans mangroves is higher than in other ecosystems, with at least 42 ARGs detected in bacterial communities, and research suggests that the abundance of antimicrobial resistance genes (ARGs) in Sundarbans sediments is linked to the presence of heavy metals and polyaromatic hydrocarbons (PAHs) from industrial and agricultural runoff. Antimicrobials are frequently employed in the aquaculture industry to treat infectious diseases; nevertheless, their widespread use has rendered many known antimicrobials ineffective and caused microorganisms in aquatic products to develop resistance to them. During

shellfish farming, the most common bacterial infections are caused by *Vibrio* sp., which result in mass mortality (Figure 5).[57] Tox-R, trh, and tdh genes are the common genes that are causative agents of gastrointestinal diseases in humans, resulting from the consumption and rough handling (wounds). Indiscriminate and unscientific use of antibiotics in aquaculture and agriculture ultimately (mainly during the monsoon) contaminates the natural water bodies, including rivers, marine, and estuarine water. These antibiotic residues develop antibacterial resistance due to the AMR gene becoming active in the infectious bacteria and killing the beneficial bacteria. Finally, the emergence of potentially pathogenic multidrug-resistant bacteria within aquatic bodies that cause gastrointestinal diseases in humans who are very close to nature, like fisherfolk. In a study published in The Lancet in 2022, over 1 million people had already died from bacterial AMR in 2019, and the excess burden due to bacterial AMR is just the tip of the iceberg.[2] According to a study, isolates of *V. parahaemolyticus* from shellfish in West Bengal showed resistance to ciprofloxacin (10%), nalidixic acid (10%), ampicillin (90%), cefpodoxime (100%) and ampicillin (90%), cefotaxime (90%), ceftizoxime (60%), tetracycline (50%), and ceftriaxone (40%). According to a different study, antibiotic sensitivity tests showed that all Vibrio species were more than 90% resistant to ampicillin and that over 80% of the isolates were sensitive to ciprofloxacin, chloramphenicol, and norfloxacin, with corresponding sensitivity rates of 77.8%, 89%, 22.22%, and 77.77% to erythromycin, ciprofloxacin, chloramphenicol, and norfloxacin. Chloramphenicol (75%), ciprofloxacin (83.33%), norfloxacin (75%), erythromycin (66.66%), kanamycin (41.66%), oxytetracycline (75%), polymyxin B (58.33%), streptomycin (75%), and tetracycline (50%) were found to be effective against *V. parahaemolyticus*.[63] In Kerala, Vibrio cholera and *V. parahaemolyticus* were shown to be 100% resistant to ampicillin, 100% susceptible to chloramphenicol, and 96% resistant to ceftazidime when isolated from the retail markets of shrimp, shellfish, and crabs.[61] Fluoroquinolones, carbapenems, amikacin, gentamicin, netilmicin, tetracyclines, chloramphenicol, azithromycin, amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole were all 100% effective against 36 strains of V. parahaemolyticus, according to experimental results in seafood.[18] The susceptibility rate was 83% for streptomycin, 92% for sulfisoxazole, and 97% for cefoxitin, cefotaxime, ceftazidime, ceftriaxone, and cefepime.

An important proportion of intermediate susceptibility to cefuroxime was observed (81%). While cefuroxime and sulfisoxazole (8%) and cefotaxime, ceftazidime, ceftriaxone, and cefepime (3%) demonstrated a moderate resistance rate, ampicillin (83%) and colistin (100%) displayed a significant resistance pattern.[18] Antimicrobial resistance (AMR) and possible pathogenicity of Vibrio species isolated from inland saline shrimp culture farms were assessed in a study. An analysis of the antibiotic resistance patterns of all 200 Vibrio isolates revealed that cefotaxime was the most resistant antibiotic (93.0%), followed by amoxiclav (90.3%), ampicillin (88.2%), and ceftazidime (73.7%). The most prevalent antibiotic to which all Vibrio isolates were susceptible was chloramphenicol (99.5%), which was followed by gentamicin (53.2%), co-trimoxazole (88.2%), tetracycline (98.4%), and sulfisoxazole (83.9%). A further analysis of AST data for 105 *V. parahaemolyticus* revealed the highest ampicillin resistance (94.1%), followed by ceftazidime (78.2%), amoxiclav, and cefotaxime (93.1%). Tetracycline and chloramphenicol were shown to be the most effective treatments for all isolates of *V. parahaemolyticus* (99%), with imipenem (61.4%), sulfisoxazole (81.2%), and cotrimoxazole (92.1%) coming in close second and third, respectively.[62] The recent development and spread of antibiotic resistance in *V. parahaemolyticus* is, without a doubt, the most significant alteration to this dangerous bacterium (Ahmed *et al.*, 2012).[1] There is increasing concern about the emergence of multidrug-resistant *V. parahaemolyticus* phenotypes in many countries.[26] Despite the concerning increase in multidrug-resistant *V. parahaemolyticus* and the rise in reports of *V. parahaemolyticus* food-borne infections worldwide, little is known about the prevalence and characteristics of pathogenic *V. parahaemolyticus* in Indian retail markets.[46,44,42] V. parahaemolyticus has been reported to be resistant to tetracycline, ampicillin, streptomycin, kanamycin, and ciprofloxacin. Due to its direct connection to the management and control of disease, antimicrobial resistance, especially multidrug resistance, is one of the most significant public health issues. *Vibrio* sp. isolates from shellfish, which showed resistance to cefpodoxime, ceftazidime, aztreonam, and cefotaxime, tetracycline, cotrimoxazole, chloramphenicol, ciprofloxacin, were tested for genes like, TEM, CTXM*1,* CTXM*2,* CTXM*9,* CTX8/*25,* OXA, *tetA and tetB*, *sul1, sul2,* and *dfrA1, dfrA18, catA1*and *catA2, catA3* and *catB3,* qnr (Table 4).[36,32] The tetA and tetB genes were examined in isolates exhibiting phenotypic resistance to tetracycline, while the sul1, sul2, and dfrA1 and dfrA18 genes were examined in isolates exhibiting phenotypic resistance to cotrimoxazole for resistance to folate pathway inhibitors.[36,32] Qnr genes were examined in isolates exhibiting phenotypic ciprofloxacin resistance, while isolates exhibiting phenotypic chloramphenicol resistance were examined for catA1 and catA2, catA3, and catB3 genes. PCR for Vibrio antibiotic-resistant has been determined by using gene-specific primers in detail (Table II).

7. ANTIMICROBIAL RESISTANCE (AMR) STATUS IN SUNDARBANS AND ITS EFFECT:

Multiple studies have already reported that antibacterial-resistant bacteria and a high diversity of antibiotic resistance genes in the water and soil of Sundarbans rivers and estuaries, and researchers have already isolated multi-drug resistant (MDR) bacteria from the Sundarbans estuaries, which are resilient to multiple antibiotics (Figures 5, 6, and 7).[12] The primary controller of antimicrobial resistance in the Sundarbans is pollution from anthropogenic activities, i.e., wastewater discharge, agricultural runoff, untreated waste from houses, and waste from pharmaceutical industries, which introduce pollutants and antibiotics into the water system, selecting for resistant bacteria. The daily influx of microplastics into the environment provides a surface for microbes to proliferate, accelerating the spread of ARGs (Figure 6).

The numerous studies that have demonstrated the role of polycyclic aromatic hydrocarbons and potentially toxic elements in influencing the antibiotic resistance pattern in nature, and also shown that sediments between fresh and marine water typically have the largest concentrations of potentially toxic elements among the different settings because of their high pH and turbidity.[12]

Similarly, in estuary environments, the partitioning of hydrophobic contaminants like PAHs from the aquatic environment to the surrounding soil is determined by salinity and dissolved organic matter. Therefore, the estuarine environment provides an ideal ecological setting for evaluating the impact of PAHs and PTEs on antibiotic resistance. The biotic components in the Sundarbans are continuously facing challenges in an antibiotic environment. A research team surveyed 5 islands (Godkhali, Kalash, Sushnir Char, Lothian Island, and Bonnie Camp) and gathered sediment samples and discovered that the largest concentration of bacteria that break down PAHs, including Marinobacter and Rhizobium, was identified in Godkhali sediments. Sushnir Char had the highest concentration of bacteria that break down PAHs, including Arthrobacter and Cycloclasticus.[37] In these bacteria, they found 42 ARGs at different levels. Of these, resistance genes were either decreased or elevated in 17 and 25, respectively. Numerous studies have shown that anthropogenic effects are vital in establishing the microbiome of this estuary, and the Sundarban is one ecotype where bacteria are active in the biogeochemical cycling of nutrients (Figure 7). In mangrove environments, microbial communities play a significant role in the detritus formation process. Heavy metals, PAHs, oil spills, and antibiotic contaminants are examples of fluvial urban, industrial, and clinical contaminants that have been shown to significantly affect community metabolism. This, in turn, causes the structural, functional, and spatiotemporal alteration of microbial ecosystems (Figure 7). Because the northern part of the Sundarban estuary is situated near the urban interface of big towns, smaller ports, and significant industrial belts, the influence of the aforementioned toxins naturally builds up there. It is now well acknowledged that a variety of ARGs can be found in the natural environment, and that certain bacterial strains can even use antibiotics as their only carbon source. The acquisition and spread of ARGs are most facilitated by aquatic environments. The increasing distribution of ARGs in aquatic settings may be caused by mechanisms involving genetic mutation, recombination, horizontal gene transfer (HGT), and selection pressure caused by different contaminants (biocides, antibiotics, metals, and PAH). Interestingly, it has been proposed that unidentified evolutionary mechanisms are responsible for the spread of ARGs in habitats that appear to be antibiotic-free. River estuaries were among the many environmental niches where ARGs were found. Determining the variables that shape the variety and abundance of ARGs in estuaries and comprehending the antibiotic resistome may therefore yield valuable knowledge that aids in evaluating the possible ecological and human risks. In the present study, 18 multidrug-resistant (ampicillin, kanamycin, vancomycin, and tetracycline resistant) bacterial strains, from different parts of the Indian Sundarban estuary, were isolated and characterized (Figure 7).[12] Therefore, understanding the antibiotic resistome and identifying the factors that influence the diversity and abundance of ARGs in estuaries may provide important information that helps assess the potential dangers to humans and the environment. Eighteen multidrug-resistant (tetracycline, ampicillin, kanamycin, and vancomycin) bacterial strains were isolated and described in this study from various locations within the Indian Sundarban estuary (Figure 7).[12] In Sundarbans zones of high polyaromatic hydrocarbon (PAH) and heavy metal contamination (particularly Cu, Pb, and Ni) overlap with zones of enhanced Vibrio harboring multidrug resistance determinants such blaTEM, sul1, aadA1, and qnrS, according to metagenomic and sediment investigations conducted in 2022–2024.[12,54] MDR Vibrio exhibits seasonal maxima in the late monsoon season (August–October).

8. PREVENTION AND CONTROL STRATEGIES FOR ANTIBIOTIC RESISTANCE:

Public health and environmental security are seriously threatened by antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) in the environment. Techniques to precisely and successfully reduce environmental contamination or pathogen infection linked to ARGs and ARBs are desperately needed.[34] Species-specific technologies, including CRISPR-Cas, photosensitizers, nanoparticles, and phage-related technologies, can be used to eradicate a particular class of ARGs or ARBs at the species level. This can be used in conjunction with low-dose antibiotics to improve removal efficiency. Furthermore, the combination of antibiotics can be utilized to reverse microbial resistance and treat recurrent infections brought on by antibiotic-resistant bacteria (Figure 8). Community-specific methods such as biochar, hyperthermophilic compost, and fecal microbiota transplantation can eradicate most ARGs or ARBs in a single attack, reducing the chance of resistance developing. [54]

- Antibiotic Stewardship: To lessen selection pressure, encourage the prudent use of antibiotic-based medicines in agriculture and human medicine.
- Infection Control: To stop the spread of microorganisms that are resistant to treatment, use stringent infection control procedures in hospital environments.
- Surveillance: Set up surveillance systems to keep an eye on trends in antibiotic resistance and trace the transmission of microorganisms that are resistant to antibiotics.
- New Antibiotic Development: Promote the creation of novel antibiotics and complementary therapies.
- Raising Public Awareness: Inform people about the dangers of antibiotic resistance and the significance of using antibiotics responsibly.
- Control of Antibiotic Use: Put laws into place to limit the use of antibiotics in veterinary care and agriculture.
- Research and Development: Provide funding for studies on the causes underlying antibiotic resistance as well as the creation of novel diagnostic and therapeutic approaches.
- One Health Approach: To minimize antibiotic resistance, embrace a One Health strategy that unifies environmental, animal, and human health.
- Genetic Research: To better understand the processes and create focused therapies, investigate the genetics of antibiotic resistance.
- Alternative medicines: To lessen dependency on conventional antibiotics, investigate alternative medicines such as bacteriophage therapy and antimicrobial peptides.

- Appropriate antibiotic prescribing: Appropriate initial antibiotic therapy should be used to reduce the mortality of the patients.

9. Discussion

The Indian Sundarbans, a rich and vast mangrove ecosystem, is a house to a diverse range of aquatic life and a niche of fisherfolk. However, this region is also a hotspot for waterborne pathogens, including Vibrio species. The study reveals a detectable load of Vibrio species, including *V. cholerae, V. vulnificus, V. parahaemolyticus*, and *V. fluvialis* in the water bodies of the Indian Sundarbans. These bacteria pose a substantial risk to the health of fisherfolk and local communities who are directly dependent on the water resources for their livelihood[13] (Figures 5 and 6). The prevalence of these vibrios can cause gastrointestinal disease (diarrhea, dysentery, gastric dysbiosis, etc.) due to poor sanitation, inadequate waste management, water pollution, and climate change, etc. Carriers of non-cholera Vibrio species who are asymptomatic Vibrio species can inadvertently spread bacteria to other people through a variety of means, such as direct contact or contaminating food or water supplies.[38]

This study also reported that vibriosis and diarrhea are a serious but underreported public health concern in Southeast Asia, including India, and an alarming situation of antibacterial resistance among Vibrios. These bacteria have developed resistance to various antibiotics[16]. This resistance pattern poses a significant challenge to the treatment and management of Vibrio-based diseases, especially diarrhoea, making it essential to develop effective antimicrobial stewardship programs. The study highlights the necessity of a comprehensive strategy to tackle the problems of antibiotic resistance and waterborne Vibrio infections in the Indian Sundarbans[17]. This entails enhancing the infrastructure for sanitation, encouraging stewardship of antibiotics, and increasing knowledge of the dangers of Vibrio infections. Together, we can lessen the impact of waterborne illnesses and safeguard the well-being and means of subsistence of local populations in this ecologically delicate area[13,18].

In 2015, the World Health Organization launched the Global Antimicrobial Resistance Surveillance System (GLASS) to assist the global action plan on antimicrobial resistance, and all nations should actively engage[19]. A unified action plan is necessary for taking proactive measures along with preventing the spread of food-borne pathogens, even though several programs have been put in place in nations like the US, EU, Japan, Sweden, and Denmark, as well as more recently in nations like India, Thailand, China, and South Africa.[25,21] The development of novel antibiotics or contemporary alternative therapies is desperately needed to combat illnesses brought on by multiple drug-resistant and extensively drug-resistant bacteria, including vibrios, but sadly, progress has slowed significantly in recent decades. During this health crisis, vaccination, infection control in medical institutions, access to clean water and sanitation, and improved hygiene can all help prevent infections and possibly reduce the need for antibiotics. Probiotic treatment and re-sensitization of drug-resistant microorganisms require further investigation[22]. Interest in reviving the practice of phage therapy through cocktail treatment, the use of quorum-sensing inhibitors, and anti-secretory medicines has increased in the age of multi-drug resistance due to the general decline in antibiotic effectiveness[23]. Guidelines have been released by the World Health Organization (WHO) to encourage farmers and the food sector to stop routinely using antibiotics to promote the growth of healthy animals. EU Regulation 2019/6 emphasizes how legislative measures to limit the use of antibiotics have been spurred by the increase in multidrug-resistant bacteria. To create methods for controlling antibiotic resistance in aquaculture, stakeholders must work together. Around the world, microbial diseases provide a serious threat to aquaculture operations. Diseases caused by microbial pathogens have emerged as a major cause of production loss, impacting both human health and farmed species, as production has expanded to fulfill the demand for seafood worldwide[27]. In high-density farming settings, these diseases multiply, especially opportunistic bacteria like Aeromonas and Vibrio.[25]

The study concludes by highlighting the critical and intricate dual threat that environmental degradation poses to the Indian Sundarbans: an increase in waterborne Vibrio infections exacerbated by a severe antimicrobial resistance (AMR) crisis. A global hotspot for multi-drug-resistant pathogens, the distinctive coastal environment is under stress from both anthropogenic antibiotic dumping and climatic anomalies. According to the study, the ecosystem is the main way that ARGs are transferred to human pathogens, which makes treating infections in the local fishermen more challenging and expensive. The findings serve as a crucial warning that local environmental contamination poses a threat of international significance, as AMR is expected to cause 10 million annual deaths worldwide by 2050. Thus, to protect the Sundarbans' livelihoods and public health, a swift, multi-sectoral response is needed, with a focus on strong wastewater management, the prudent use of antibiotics in aquaculture, and focused community education initiatives to reduce microbial dysbiosis and guarantee the long-term financial and ecological viability of this essential mangrove ecosystem.

10. DISCLAIMER (ARTIFICIAL INTELLIGENCE):

We hereby declare that NO generative AI technologies, such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators, have been used during the writing or editing of this manuscript.

11. ACKNOWLEDGEMENT AND FUNDING:

Authors are grateful to the West Bengal Department of Science and Technology and Biotechnology (WBDSTBT) for funding support of the Project entitled “Epidemiological surveillance of waterborne *Vibrio* sp. diversity following climate change on gastrointestinal dysbiosis in Sundarbans fisherpersons” in the category of Animal Science and Fishery for Research Development Program [Sanction no. 1240(27)/9/2024-ST SEC, dated 07/02/2025].

12. CONFLICT OF INTEREST

The authors have no conflict of interest.

13. ETHICS STATEMENT
No ethical clearance needed.

14. AUTHORS' CONTRIBUTION
Author has contributed fully to this work.

15. References

1. Ahmed ZM, Dosoki MI, Nasr SAA. Occupational Hazards in Fish Industry. *World Journal of Fish and Marine Sciences.*2012; **4(2):** 201-210.
2. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022 Feb 12;399(10325):629-655. doi: 10.1016/S0140-6736(21)02724-0. Epub 2022 Jan 19. Erratum in: Lancet. 2022 Oct 1. 2019;**400(10358):**1102.
3. Asadgol Z, Badirzadeh A, Niazi S, Mokhayeri Y, Kermani M, Mohammadi H and Gholami M. How can climate change affect cholera incidence and prevalence? A systematic review. 2020; *Environ Sci Pollut Res Int.***, 27(28)**: 34906-34926. doi: 10.1007/s11356-020-09992-7. Epub 2020 Jul 14. PMID: 32661979.
4. Austin A. Vibrios as causal agents of zoonoses. *Vet Microbiol.*2010; **140 (3-4)**: 310 – 317
5. Baag S, and Mandal S. The influence of ocean acidification and warming on responses of *Scylla serrata* to oil pollution: An integrated biomarker approach. Comparative biochemistry and physiology Part B. *Biochemistry and molecular biology*. 2023; 266.110847.
6. Baker-Austin C, Oliver JD, Alam M. *Vibrio* spp. infections. *Nat Rev Dis Primers*. 2018; **4**, 1–19. https://doi.org/10.1038/s41572-018-0005-8
7. Baker-Austin C, Trinanes JA, Taylor NGH, Hartnell R, Siitonen A, & Martinez-Urtaza J. Emerging Vibrio risk at high latitudes in response to ocean warming. *Nature Climate Change*. 2012; **3(1),** 73–77. https://doi.org/10.1038/nclimate1628.
8. Batabyal P, Mookerjee S, Einsporn MH, Lara RJ, Palit A. High prevalence of toxin-producing enteropathogenic *Vibrios* among estuarine crab in Ganges delta of West Bengal, India. *Infect Genet Evol*. 2014; **26:** 359 – 361.
9. Batabyal P, Einsporn M H, Mookerjee S, Palit A, Neogi SB, Nair GB, Lara RJ. Influence of hydrologic and anthropogenic factors on the abundance variability of enteropathogens in the Ganges estuary, a cholera endemic region, *Science of The Total Environment*. 2014; **472:** 154-161.
10. Batabyal P, Mookerjee S, Einsporn MH, Lara RJ, Palit A. High prevalence of toxin-producing enteropathogenic Vibrios among estuarine crab in Ganges delta of West Bengal, India. *Infect Genet Evol.* 2014; **26:** 359 - 361. doi: 10.1016/j.meegid.2014.06.001.
11. Basak S, Choudhury J, Mandal P, Sarkar K, Parveen SM, Bhagat D, Saha S. Studies on pathogenic bacterial diversity in Subhas Sarobar, Kolkata, concerning public health. Ed. Ghosh, D. and Saha, S. Environment, Development and Sustainability. Bhumi Publisher, Mumbai, India. 2024; Pp. 95-106.
12. Bhattacharyya A, Haldar A, Bhattacharyya M, and Ghosh A. Anthropogenic influence shapes the distribution of antibiotic-resistant bacteria (ARB) in the sediment of the Sundarban estuary in India. *Science of The Total Environment*. 2019; DOI*:* 10.1016/j.scitotenv.2018.08.038.
13. Bhattacharjee S, Bhattacharjee S, Bal B, Pal R, Niyogi SK, Sarkar K. Is *Vibrio fluvialis* emerging as a pathogen with epidemic potential in the coastal region of eastern India following cyclone Aila? *J Health Popul Nutr.* . 2010; **28(4):** 311-7. doi: 10.3329/jhpn.v28i4.6036.
14. Bhushan C, Khurana A, Sinha R and Nagaraju M. Antibiotic Resistance in Poultry Environment: Spread of Resistance from Poultry Farm to Agricultural Field, *Centre for Science and Environment*. 2017. New Delhi.
15. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States: 2019. U.S. Department of Health and Human Services. 2019. doi: 10.15620/cdc:82532.
16. Chimalapati S, De Souza Santos, M., Servage, K., De Nisco, N. J., Dalia, A.B., and Orth, K. Natural transformation in *Vibrio parahaemolyticus*: a rapid method to create genetic deletions. *J Bacteriol*. 2018; 200: e00032-18.
17. Chowdhury G, Ghosh S, Pazhani GP, Paul BK, Maji D, Mukhopadhyay AK, Ramamurthy T. Isolation and characterization of pandemic and non-pandemic strains of *Vibrio parahaemolyticus* from an outbreak of diarrhea in North 24 Parganas, West Bengal, India. *Foodborne Pathog Dis.* 10(4):338-42. doi: 10.1089/fpd.2012.1340.
18. Changsen C, Likhitrattanapisal S, Lunha K, Chumpol W, Jiemsup S, Prachumwat A, Kongkasuriyachai D, Ingsriswang S, Chaturongakul S, Lamalee A, Yongkiettrakul S, Buates S. Incidence, genetic diversity, and antimicrobial resistance profiles of *Vibrio parahaemolyticus* in seafood in Bangkok and eastern Thailand. *Peer J.* 2023; 11:e15283 DOI 10.7717/peerj.15283.
19. Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. 2010; CLSI Document M100-S20. Wayne, Pennsylvania.
20. Das B, Verma J, Kumar P, Ghosh A, and Ramamurthy T. Antibiotic resistance in Vibrio cholerae: understanding the ecology of resistance genes and mechanisms. *Vaccine*. 2020; **38**: A83–A92. doi: 10.1016/j.vaccine.2019.06.031.
21. Dengo-Baloi LC, Semá-Baltazar CA, Manhique LV, Chitio JE, Inguane DL, Langa, J.P. Antibiotic resistance in El Tor *Vibrio cholerae* 01 isolated during cholera outbreaks in Mozambique from 2012 to 2015. *PLOS ONE*. 2017; **12(8):** e0181496. https://doi.org/10.1371/journal.pone.0181496
22. De Souza WM, Weaver SC. Effects of climate change and human activities on vector-borne diseases. *Nat Rev Microbiol*. 2024; **22(8):** 476 - 491. doi: 10.1038/s41579-024-01026-0. Epub 2024 Mar 14. PMID: 38486116.

23. Deeb R, Tufford D, Scott GI, Moore JG and Dow K. Impact of Climate Change on *Vibrio vulnificus* Abundance and Exposure Risk. *Estuaries Coast.* 2018; **41(8)**: 2289–2303. doi:10.1007/s12237-018-0424-5.
24. Dietrich J, Hammerl JA, Johne A, Kappenstein O, Loeffler C, Nöckler K, Rosner B, Spielmeyer A, Szabo I, Richter MH, Impact of climate change on foodborne infections and intoxications. *J Health Monit.* 2023; **8(Suppl 3**): 78-92. doi: 10.25646/11403.
25. Dutta D, Kaushik A, Kumar D and Bag S. Foodborne Pathogenic Vibrios: Antimicrobial Resistance. *Front. Microbiol*. 2021; **12:**638331. doi: 10.3389/fmicb.2021.638331.
26. Elhadi, S. Occurrence of potentially human pathogenic Vibrio sp. in the coastal water of the Eastern province of Soudi Arabia. *Research J. Microbiology*. 2013; **8(1):** 1-12.
27. FAO and WHO. Risk assessment tools for Vibrio parahaemolyticus and Vibrio vulnificus associated with seafood. Microbiological Risk Assessment Series No. 20. 2020; Rome.
28. Felsenstein J. Phylogenies and the Comparative Method. *The American Naturalist*. 1985; **125:** 1-15. https://doi.org/10.1086/284325.
29. Filho WL, Ternova L, Parasnis S. A., Kovaleva, M., & Nagy, G. J. Climate Change and Zoonoses: A review of Concepts, Definitions, and bibliometrics. *International Journal of Environmental Research and Public Health*. 2022; **19(2)**: 893. https://doi.org/10.3390/ijerph19020893.
30. George PR and Harini E. Fish-borne Zoonotic Diseases Are Risk to Public Health. Present Status of Fish Disease Management Programmes in India. *Our Heritage*. 2019. **67(2):** 118 – 123.
31. Jayakumar JM, Martinez-Urtaza J, Brumfield KD, Jutla AS, Colwell RR, Cordero OX and Almagro-Morno S. Climate change and *Vibrio vulnificus* dynamics: A blueprint for infectious diseases. *PLoS Pathog*. 2024; **20(12):** e1012767. https://doi.org/10.1371/journal.ppat.1012767.
32. Kim C, Torres A, Smith W, Kulinczenko A, Pao S, Wildeus S, Ettinger M, Gruszynski, G, and Wynn C. Prevalence of Antimicrobial Resistance (AMR) in Bacteria Isolated from Farm Animals, Wildlife, and Food Samples in the Eastern United States between 2007 and 2013. *EC Nutrition*. 2017. **7(6):** 264-274.
33.Martinez-Urtaza J, Bowers JC, Trinanes J, DePaola A. Climate anomalies and the increasing risk of Vibrio parahaemolyticus and Vibrio vulnificus illnesses. *Food Research International*. 2010; **43:** 1780–1790.
34. Mishra A, Kim HS, Kumar, R and Srivastava V. Advances in *Vibrio*-related infection management: an integrated technology approach for aquaculture and human health. *Critical Reviews in Biotechnology*. 2024; **44(8):** 1610–1637. https://doi.org/10.1080/07388551.2024.2336526.
35. Mookerjee S, Batabyal P, Sarkar MH, Palit, A. Seasonal Prevalence of Enteropathogenic Vibrio and Their Phages in the Riverine Estuarine Ecosystem of South Bengal. *PLoS ONE*, 2015; 10(9): e0137338. doi:10.1371/journal.pone.0137338.
36. Momtaz H, Safarpoor Dehkordi F, Taktaz T, Rezvani A, Yarali S. Shiga toxin-producing Escherichia coli isolated from bovine mastitic milk: serogroups, virulence factors, and antibiotic resistance properties. Scientific World Journal. 2012; 618709. doi: 10.1100/2012/618709.
37. Mukherji S, Imchen M, Mondal S, Bhattacharyya A, Siddhardha B, Kumavath R, and Ghosh A. Anthropogenic impact accelerates antibiotic resistome diversity in the mangrove sediment of Indian Sundarban. *Chemosphere*. 2022; **309:** 136806. https://doi.org/10.1016/j.chemosphere.2022.136806.
38. Muzembo BA, Kitahara K, Hayashi C, Mashino S, Honda J, Ohno A, Khatiwada J, Dutta S, Miyoshi SI. Non-cholera Vibrio infections in Southeast Asia: A systematic review and meta-analysis. *J Infect Public Health.* 2024; **17(11):**102564. doi: 10.1016/j.jiph.2024.102564.
39. Ngasotter S, Mukherjee S, Singh SK, Bharati D, Haque R, Varshney S, Nanda C, Waikhom D, Devi MS, & Singh AS. Prevalence, Virulence, and Antibiotic Resistance of Vibrio parahaemolyticus from Seafood and its Environment: An Updated Review. Mediterranean Journal of Infection Microbes and Antimicrobials, 2022; 11(1), 1-1. https://doi.org/10.4274/mjima.galenos.2021.2021.1.
40. National Ambulatory Medical Care Survey. 2021 National Summary Tables, tables 10 and 25. Centers for Disease Control and Prevention.
41. National Ambulatory Medical Care Survey. 2021 National Summary Tables, tables 13. Centers for Disease Control and Prevention.
42. Narayanan SV, Joseph TC, Peeralil S, Mothadaka MP and Lalitha KV. Prevalence, Virulence Characterization, AMR Pattern and Genetic Relatedness of Vibrio parahaemolyticus Isolates From Retail Seafood of Kerala, India. Front. Microbiol. 2020; **11:**592. doi: 10.3389/fmicb.2020.00592
43. Nekouei O, Checkley S, Waldner C, Smith BA, Carson JIC, Avery B, Gow JSS. Exposure to antimicrobial-resistant Escherichia coli through the consumption of ground beef in Western Canada, *International Journal of Food Microbiology.* 2018; **272:** 41-48,
44. Parthasarathy S, Das SC, Kumar A, Chowdhury G, Miyoshi SI, Dutta S, Mukhopadhyay AK. Molecular characterization and antibiotic resistance of Vibrio parahaemolyticus from Indian oyster and their probable implication in the food chain. *World Journal of Microbiology and Biotechnology*. 2021; **37:**145. https://doi.org/10.1007/s11274-021-03113-3.
45. Parvin I, Shahunja KM, Khan SH, Alam T, Shahrin L, Ackhter MM, *et al*. Changing Susceptibility Pattern of *Vibrio cholerae* O1 Isolates to Commonly Used Antibiotics in the Largest Diarrheal Disease Hospital in Bangladesh during 2000-2018. *Am. J. Trop. Med. Hyg*. 2020; **103:** 652–658. doi: 10.4269/ajtmh.20-0058.
46. Pal D and Das N. Isolation, identification, and molecular characterization of Vibrio parahaemolyticus from fish samples in Kolkata. *European Review for Medical and Pharmacological Sciences*. 2010; **14(6):**545-549.

47. Ray S and Saha S. Arsenic toxicity of estuarine mudcrab. LAP LAMBERT Academic Publishing GmbH & Co. KG. 2011; Germany. ISBN: 978-3-8443-3035-9.
48. Raissy M, Moumeni M, Ansari M, Rahim E. Occurrence of *Vibrio* spp. In lobster and crab from the Persian Gulf. *J Food Safety.* 2012; **32(2):** 198-203. doi: 10.1111/j.1745- 4565.2012.00368.x
49. Saha S. Bacterial Diseases in Aquacultural Shellfish and Public Health. In Dr. D. S. Rathod and Dr. S. S. Pedge (Ed.) Current Research in Fisheries and Aquatic Science (Vol. - 1), 2024; pp. 43-64. Weser Books, Germany.
50. Saha S and Basu B. The impact of climate change on waterborne microbial diversity and Dysbiosis in carcinogenesis of Indian Sundarbans fisherpersons: A review. In Prof. Aysun Turmen (Ed.) Research Advances in Environment, Geography and Earth Science. B P International, Vol.2. 2024; pp. 150 -170.ISBN: 978-81-972756-7-8. DOI: 10.9734/bpi/raeges/v2.
51. Saha S and Basak S. Studies on Enteropathogenic Bacterial Diversity as the alarming indicator of freshwater lakes: Concerning public health in West Bengal. *Journal of XI'AN University of Architecture and Technology*. 2024; **16 (6):** 38-57. https://doi.org/10.37896/JXAT16.6/34104.
52. Saha S and Dash G. Climatic Stress on Cultured *Scylla* sp.: Impact on livelihood and Public Health in West Bengal. *International Journal of Research in Biosciences, Agriculture & Technology.* 2021; **S17:** 331 – 344.
53. Saha S and Dash G. Zoonotic *Vibrio* diversity and diseases in mudcrab fishery: impacts on public health in India. *Uttar Pradesh Journal of Zoology*. 2021; **42(19):** 48-58.
54. Saha S and Munian D. Impact of Climate Change on Prevalence of Waterborne Vibrio sp. in Fisherfolk Health of the Indian Sundarbans. *Uttar Pradesh Journal of Zoology*. 2025; **46 (18)**: 71-90
55. Saha S and Ray S. Sublethal effect of arsenic on oxidative stress and antioxidant status in the edible crab, *Scylla serrata,* in India. *Clean soil, air, water*. 2014; **42(9): 1216 – 1222.**
56. Saha S, Pradhan D, and Dash G. Studies on the diversity of bacterial diseases and occupational risks through mudcrab aquaculture in West Bengal. *Journal of Pure and Applied Microbiology*. 2023; **17(2):**722-731. doi-https://doi.org/10.22207/JPAM.17.2.23.
57. Saha S, Basu B, Dash G**.** Studies on climate change and its impact on the occupation and health of fisherpersons in West Bengal. *Indian Journal Environmental Protection*. 2024; **44(7): 637 – 648.**
58. Saha S, Pradhan D, Dash D. Studies on Bio-resource, Threats and Conservation of Aquacultural *Scylla serrata*. In Shashi, P. and Sashi, A. (Ed.) Management of natural resources and sustainable regional development. Akinik Publications, New Delhi, India. 2022. Chapter 10: pp. 111 – 141. DOI: https://doi.org/10.22271/ed.book.2032.
59. Saitou N and Nei M. The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution*. 1987, **4**: 406-425.
60. Sivam V, Murty LN, Pankyamma V, Sivaraman GK. Study on retail fish markets: Possible occurrence and transmission of emerging pathogens from faecal indicators. *Journal of Environmental Biology.* 2017; **38(3):**465-470
61. Sudha S, Mridula C, Silvester R, Hatha AAM. Prevalence and antibiotic resistance of pathogenic Vibrios in shellfish from Cochin market. *Indian J Geo Mar Sci*. 2014; **43:**815–824.
62. Sudan P, Tyagi A, Dar RA, Sharma C, Singh P, Naveen BTK, Chandra M, Arora AK. Prevalence and antimicrobial resistance of food safety-related *Vibrio* species in inland saline water shrimp culture farms. *International Microbiology*, 2023; https://doi.org/10.1007/s10123-023-00323-7.
63. Uddin SA, Sikder M NA, Rahman A, Zafor M. Antibiotic Resistance of *Vibrio* Bacteria Isolated From Mud Crab *Scylla Serrata* of Chakoria Coast, Bangladesh. *Research Journal of Pharmaceutical*, *Biological and Chemical Sciences*. 2013; **4(3):** 325 – 333.
64. Vezzulli L, Colwell RR, Pruzzo C. Ocean warming and spread of pathogenic vibrios in the aquatic environment. *Micro Ecol.* 2013; **65(4):** 817-825, 10.1007/s00248-012-0163-2.
65. World Health Organization. WHO Director-General summarizes the outcome of the emergency Committee regarding clusters of microcephaly and Guillain-Barré Syndrome. 2016.
66. World Health Organization. Global Antimicrobial Resistance Surveillance System (GLASS) Report: Early Implementation 2017–2018. 2018.
67. World Health Organization. Antibacterial agents in clinical and preclinical development: an overview and analysis. 2021.
68. Wright AC, Simpson LM, Oliver JD, Morris JG. Phenotypic evaluation of acapsular transposon mutants of Vibrio vulnificus. *Infection and Immunity*. 1990; **58** (6): 1769–1773. doi:10.1128/IAI.58.6.1769-1773.1990.
69. Yun NR and Kim DM. Vibrio vulnificus infection: a persistent threat to public health. *Korean J Intern Med.* 2018; **33(6):** 1070-1078. doi: 10.3904/kjim.2018.159. Epub 2018 Jun 15. PMID: 29898575; PMCID: PMC6234401.

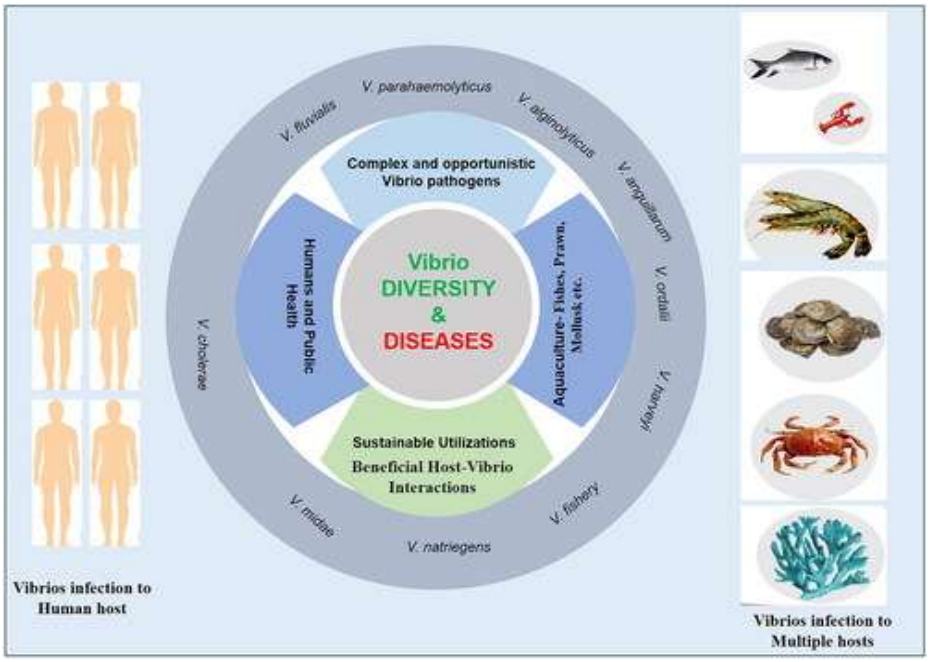

Figure 1. Enteropathogenic Vibrio sp. diversity that is transmitted through zoonotic transmission from aquatic animals, such as fish and shellfish, and affects human health directly or indirectly (after Mishra *et al.*, 2024)

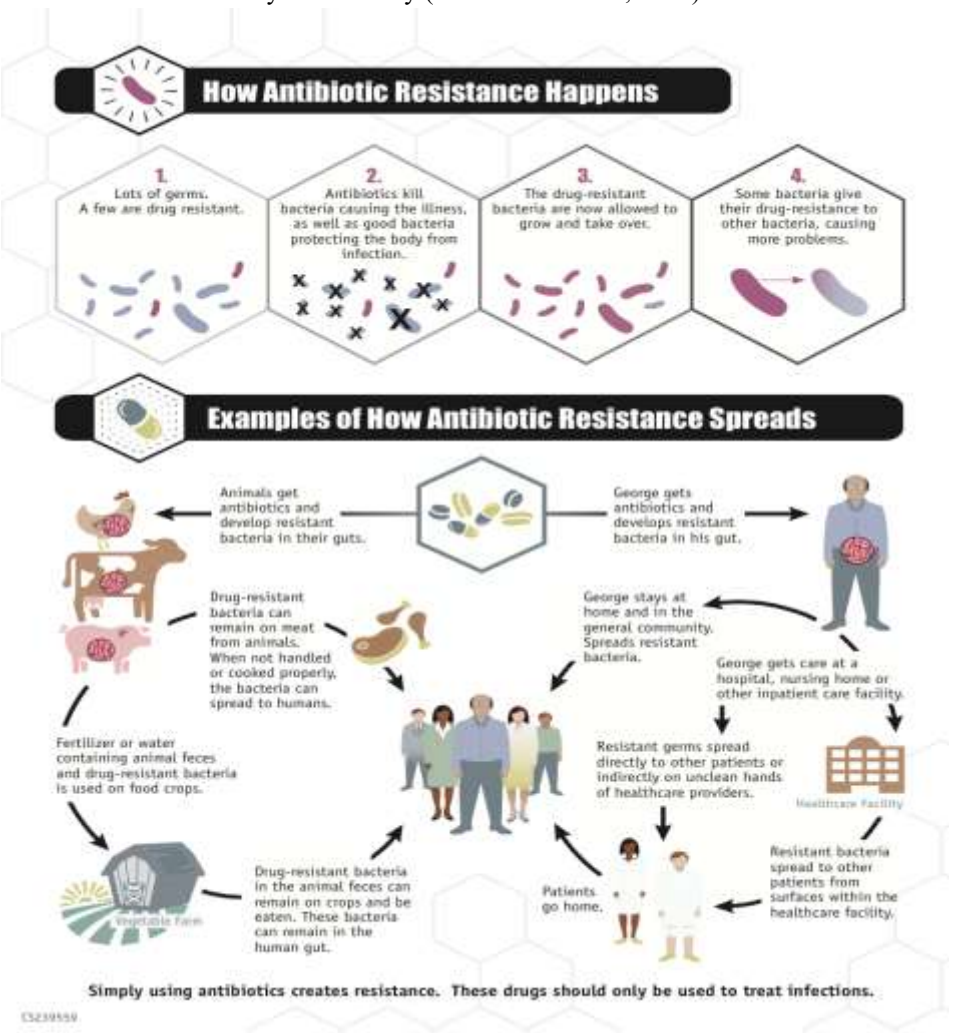

Figure 2. Antibacterial resistance patterns against antibiotics and spreading pattern (adapted from Bhattacharyya *et al*., 2019)

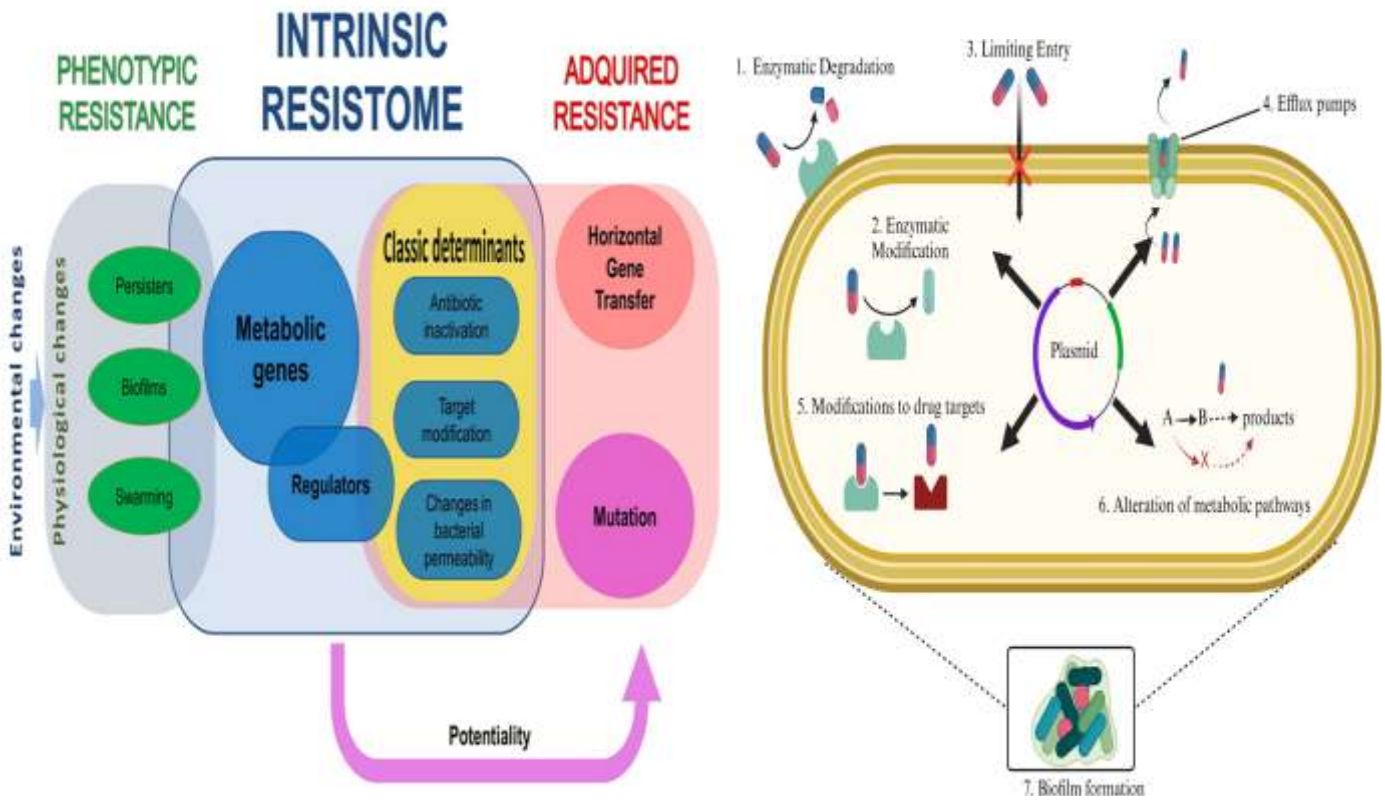

Figure 3. Mechanisms of antimicrobial resistance in bacteria (adapted from Wright, 2010)

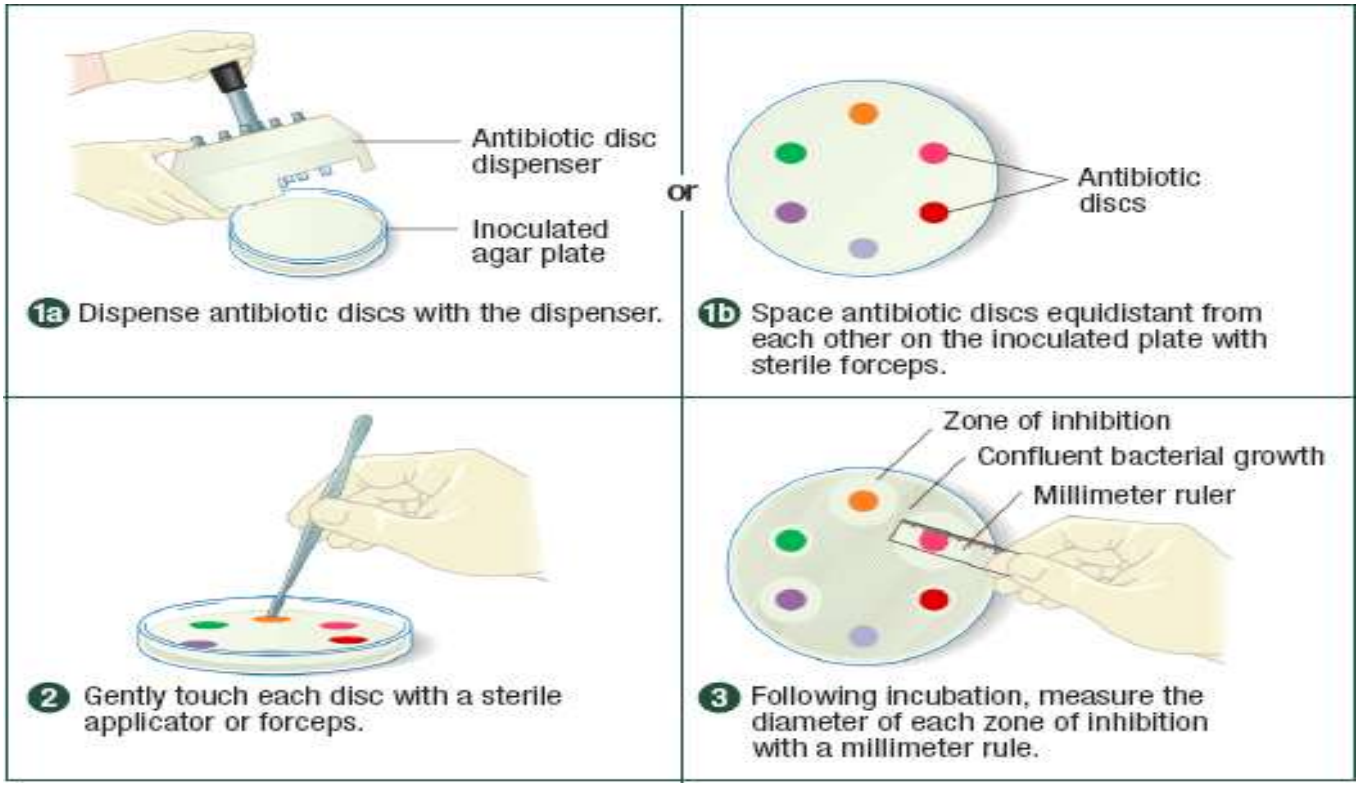

Figure 4. Method for disc diffusion test: inoculate and place the antibiotic disc on the agar plate, and measure the diameter of each zone of inhibition for determination of resistance (1 to 5).

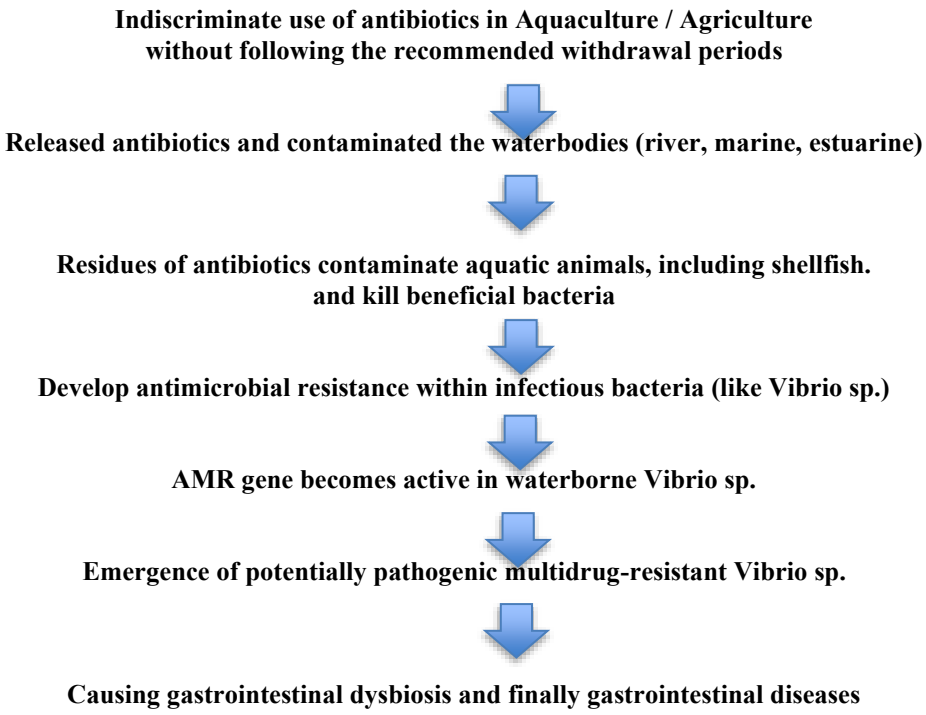

Figure 5. Development of AMR and multidrug-resistant strains in Vibrio, causing gastrointestinal dysbiosis and disease for chronic infection.

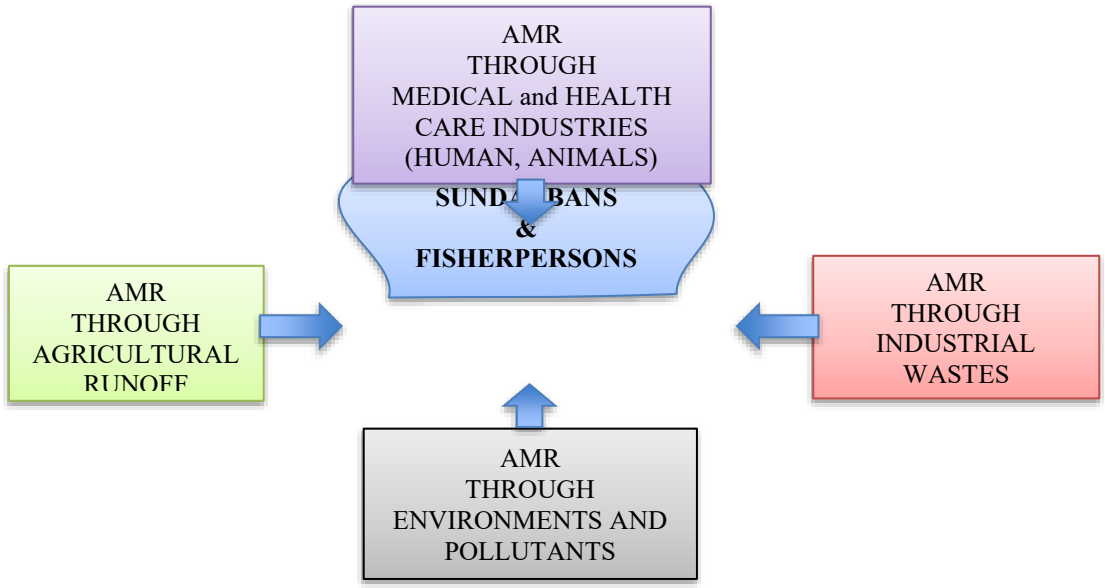

Figure 6. Epidemiology of Antimicrobial Resistance of fisherpersons in Sundarbans

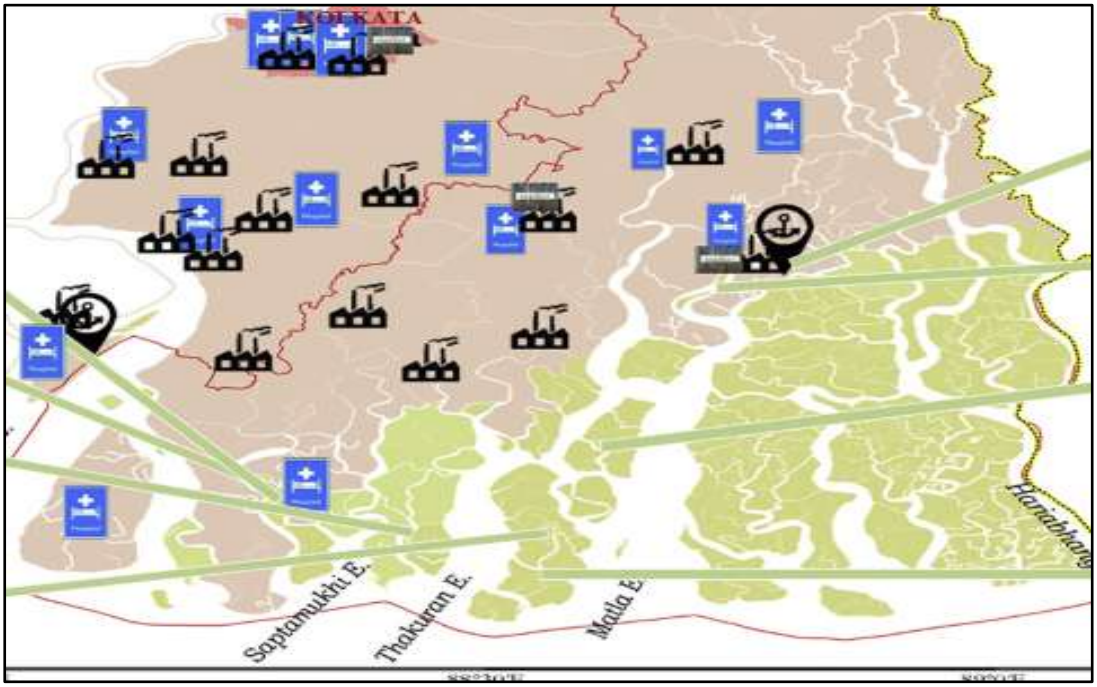

Figure 7. Sources of Antibiotic contamination in Sundarbans (adapted from Bhattacharyya *et al.*, 2019).

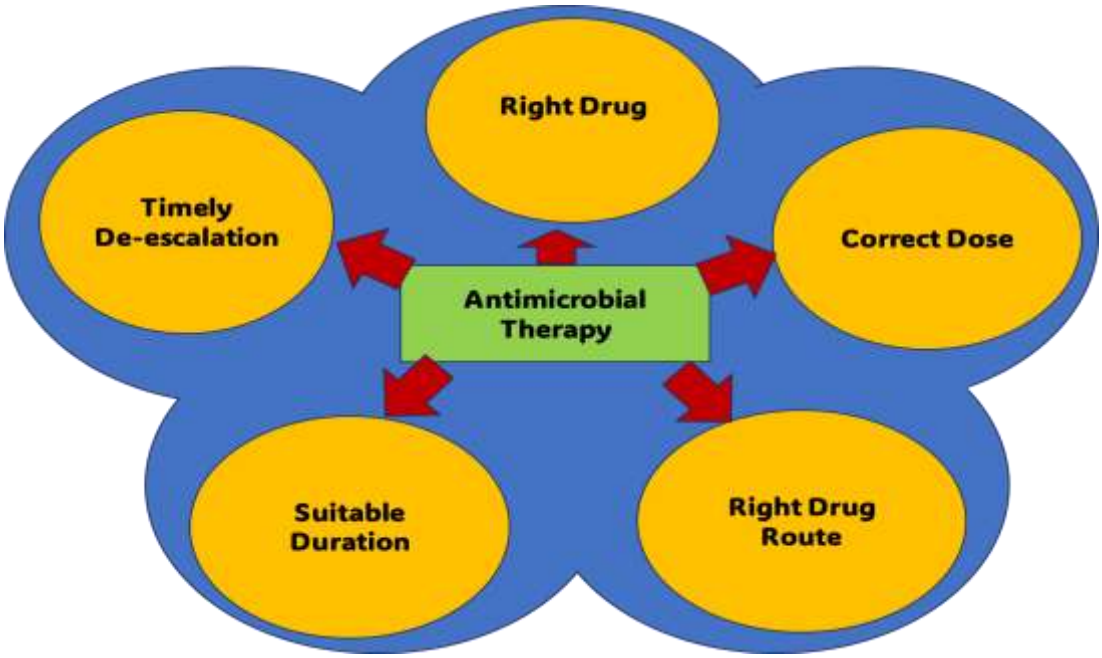

Figure: 8. 5‘D’s of antimicrobial therapy against AMR.

Table I. Climate change and Zoonotic *Vibrio* sp. Infections

Pathogenic Vibrio sp.	Diseases in Shellfish	Vibrio Zoonotic Diseases (Public Health)
Vibrio alginolyticus	Septicemia, Bacteremia (adult), Shell disease (Juvenile and Adult), wound infection.	Out of 1400 micro-organisms 200 infections are zoonotic in nature. 12 vibrios in no. • Gastroenteritis • Infection in wound skin • Food poisoning • Watery diarrhea • Abdominal cramping • Nausea • Vomiting • Fever • Chills # V. vulnificus may cause severe effect & life-threatening infections. # V. mimicus causes eye and ear infections
Vibrio parahaemolyticus	Vibriosis, Shell disease (Juvenile and Adult)	
Vibrio vulnificus	Bacteremia (adult), Shell disease (Juvenile and Adult), wound infection, cutaneous lesions.	
Vibrio harveyi	Luminescent Vibriosis (eggs and larvae), Shell disease (Juvenile and Adult), high mortality.	
Vibrio fluvialis	Shell disease (Juvenile and Adult)	
Vibrio mimicus	Shell disease (Juvenile and Adult)	
Vibrio cholerae	Vibriosis, Bacteremia (adult)	

Table II. Primer for target *Vibrio* sp. with amplicon size and amplicon size.

Target species	Sequence (5′ - 3′) (F and R)	Amplicon size (bp)	Targeting gene
8F 1492R	5'-AGAGTTTGATCCTGGCTCAG-3' 5'-GGTTACCTTGTTACGACTT-3'	**1500**	-
27F 1492R	AGAGTTTGATCMTGGCTCAG TACGGYTACCTTGTTACGACTT	**1500**	-
700F 1325R	5'-CGGTGAAATGCGTAGAGAT-3' 5'-TTACTAGCGATTCCGAGTTC-3'	**663**	*Vibrio* genus (16srRNA)
Vibrio parahaemolyticus	GCAGCTGATCAAAACGTT GAGT ATTATCGATCGTGCCACTCAC	**897**	flaE
Vibrio cholerae	AAGACCTCAACTGGCGGTA GAAGTGTTAGTGATCGCCAGAGT	**248**	sodB
Vibrio vulnificus	GTCTTAAAGCGGTTGCTGC CGCTTCAAGTGCTGGTAGAAG	**410**	hsp
Vibrio mimicus	CATTCGGTTCTTTCGCTGAT GAAGTGTTAGTGATTGCTAGAGAT	**121**	sodB
Vibrio alginolyticus	CGAGTACAGTCACTTGAAAGC CACAACAGAACTCGCGTTACC	**737**	collagenase
Vibrio harveyi	CTTCACGCTTGATGGCTACTG GTCACCCAATGCTACGACCT	**235**	vhh
Vibrio fluvialis	GACCAGGGCTTTGAGGTGGACGAC AGGATACGGCACTTGAGTAAGACTC	**217**	tox-R

Table III. Antibiotic Susceptibility Test [Antimicrobial Classification]

Places of sample collection and Vibrio sp.	Antibiotic Susceptibility Profile [ANTIMICROBIAL CLASS]						
	MACROLIDES	**TETRA CYCLINES**	**CEPHALOS PORINS**	**CHLORAM PHENICOL**	**AMINO GLYCOSIDES**	**FLUORO QUINOLONES**	**PENICILLINS / BETA-LACTAM**
	Example of each class						
	Azithromycin (AZM)	Tetracycline (TE)	Ceftazidime (CAZ)	Chloram-phenicol (C)	Streptomycin (S)	Norfloxacin (NX)	Ampicillin (AM)

Table IV. Antibiotic Sensitivity Pattern (in accordance with the NCCL

	Antibiotic	Abbreviation	Disc Potency (µg/ disc)	Interpretation of the diameter of the zone of inhibition		
				Resistant (R, mm or less)	Intermediate (I, mm)	Sensitive (S, mm or more)
1.	**Ampicillin**	**AMP**	10	≤13	14-16	≥17
2.	**Azithromycin**	**AZM**	15	≤13	14-17	≥18
3.	**Chloramphenicol**	**C**	30	≤12	13-17	≥18
4.	**Ceftriaxone**	**CTR**	30	≤19	20-22	≥23
5.	**Ciprofloxacin**	**CIP**	5	≤15	16-20	≥21
6.	**Cefpodoxime**	**CPD**	10	≤17	18-20	≥21
7.	**Streptomycin**	**HLS**	300	≤11	12-14	≥15
8.	**Erythromycin**	**E**	15	≤13	14-22	≥23
9.	**Pefloxacin**	**PF**	5	≤14	15-18	≥19
10.	**Norfloxacin**	**NX**	10	≤12	13-16	≥17
11.	**Levofloxacin**	**LE**	5	≤15	16-18	≥19
12.	**Tetracycline**	**TE**	30	≤17	18-20	≥21
13.	**Ceftazidime**	**CAZ**	30	≤17	18-20	≥21
14.	**Co-Trimoxazole**	**COT**	25	≤10	11-15	≥16