A DISSERTATION ON

OCCURRENCE OF AMOXICILLIN RESISTANT BACTERIA IN THE GOMTI RIVER WATER

SUBMITTED TO

THE DEPARTMENT OF BIOSCIENCES

INTERGAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT FOR THE DEGREE

OF

MASTERS OF SCIENCE IN

MICROBIOLOGY

BY

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M.Sc. Microbiology (IV Semester)

Department of Bioscience

Integral University, Lucknow

Under the supervision of

Dr. Mohd Ikram Ansari

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DECLARATION

I hereby declare that the present work on **"Occurrence of Amoxicillin resistant bacteria in the Gomti river water"** is a record of original work done by me under guidance of **Dr. Mohd Ikram Ansari**, Assistant Professor, Department of Biosciences, Integral University, Lucknow during Feb, 2022 to June, 2022. I also declare not part of this thesis has previously been submitted to my Institution or any examining body for acquiring any diploma or degree.

Place: **Gulabsha Siddiqui**

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TO WHOM IT MAY CONCERN

This is to certify that **Gulabsha Siddiqui,** student of M.Sc. Microbiology (IV semester) Integral University has completed her four-month dissertation entitled "**Occurrence of Amoxicillin resistant bacteria in the Gomti river water"**. She has completed this work from 20th March - 15th June under the guidance of **Dr. Mohd Ikram Ansari**. The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and a bright future.

Prof. (Dr.) Snobar S. Mir

Head

Department of Biosciences

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Gulabsha Siddiqui** during the month of March-June 2022, reported in the present thesis, was under my guidance and supervision. The results she reported are genuine, and the candidate herself has written the thesis script. The thesis entitled "**Occurrence of Amoxicillin resistant bacteria in the Gomti river water"** is, therefore, being forwarded for the acceptance in partial fulfillment of the requirements for the award of the degree of Master of Science in Microbiology, Department of Biosciences, Integral University, Lucknow, and (UP).

Dr. Mohd.Ikram Ansari
Assistant professor
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Gulabsha Siddiqui

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CHAPTER 1 – INTRODUCTION

The usage of antibiotics is used to treat and prevent bacterial infections. The increase in life expectancy that was observed in the second half of the 20th century was greatly influenced by antibiotics. Modern agriculture and livestock sectors were drastically changed by antibiotics, which were employed in the latter for prophylaxis, metaprophylaxis, infection treatment, and as a growth stimulant to increase feed efficiency in healthy livestock. The body's natural defenses, such as the immune system, can eradicate microorganisms due to antibiotics because they are either cytotoxic or cytostatic to them. Most of our natural goods, which are low molecular weight chemicals created by bacteria or derived from natural products, are active against other microorganisms at low concentrations. However, other antibiotics, such as sulfa medications and oxazolidinones, are synthetic. They frequently work by preventing the development of a bacterial cell, protein synthesis, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), membrane disorganisation, or other particular processes. One of the biggest current worldwide health issues is antibiotic resistance. Antibiotic resistance mechanisms frequently develop due to an overuse of antibiotics in agriculture and medicine, posing a threat to modern medicine by reducing the efficacy of therapeutically relevant antibiotics.Due to treatment failures and rising healthcare expenses, antimicrobial agent resistance (AMR) has increased morbidity and death. Veterinarians have traditionally chosen antimicrobials to treat infectious bacterial infections based mostly on prior clinical experiences. However, it has become more challenging for doctors to empirically choose an appropriate antimicrobial drug due to the rise in bacterial resistance to commonly used antimicrobials. Antimicrobial resistance (AMR) is a serious concern to both humans and animals that has threatened decades of progress in healthcare outcomes. It threatens contemporary veterinary and human medicine and jeopardises food safety. Since they directly assess bacterial growth on solid or liquid medium in the presence of antibiotics, traditional techniques for phenotypic antibiotic susceptibility tests continue to be crucial in routine diagnostics.It takes at least 18 to 22 hours for bacteria to grow clearly on agar plates for solid media-based antibiotic susceptibility assays, like the disc diffusion assay or E-test, to be able to detect growth inhibition with a naked eye. An antibiotic resistance gene's existence can be checked through molecular analysis. Antibiotic resistance can be minimized by properly using antibiotics, immunization, education, research, the creation of novel antibiotics, legislation, regulations, antimicrobial resistance surveillance, and antibiotic use. The penicillin group is one of the most significant classes of antibiotics used in human and veterinary medicine as feed additives.They make up 70% of the antibiotics that are taken in several nations. Amoxicillin, ampicillin, bacampicillin, epicillin, and metampicillin are examples of the semi-synthetic antibiotics known as aminopenicillins with an additional amine group. They are among the antibiotics that are used the most. Amoxicillin and cephalosporin antibiotics have been found at g/L levels in drainage because beta-lactams are used later and released continually. The beta-lactam class of antibiotics includes Amoxicillin. It is effective against a variety of gram-positive and gram-negative bacteria. It is used to treat bacterial infections of the skin, urinary system, gastrointestinal tract, and respiratory system. Amoxicillin is used to treat and prevent animal infections as well as to promote growth in a variety of veterinary animals, such as fish and cattle.Amoxicillin is extremely unstable and rapidly breaks down into a variety of degradation products by hydrolysis. Numerous biotic and abiotic mechanisms have the potential to cleave the beta-lactam ring. The most often recommended drugs in European nations are Amoxicillin alone or in combination with clavulanic acid. It is an antibiotic that is semi-synthetic and developed from amino-penicillin. It works by preventing the bacterial cell wall from being produced. Bacteria have become resistant to Amoxicillin as a result of its widespread use. Amoxicillin is combined with the salt clavulanic acid to increase the antibiotic's potency. A beta-lactamase inhibitor is clavulanic acid. Amoxicillin and its breakdown products were found at a concentration of 62 ng/L in the effluent of sewage treatment facilities (STPs) in India. Salmonella, Klebsiella, Shigella, Escherichia coli, Clostridium difficile, and Klebsiella, Shigella, Salmonella, and Proteus are bacterial genera susceptible to Amoxicillin.An extended-spectrum antibiotic called Amoxicillin works by preventing the crosslinking of peptidoglycan polymers, which are crucial parts of both Gram-negative and Gram-positive bacteria. There are various strategies by which the bacteria combat the amoxicillin stress. The most crucial mechanism in Gram-negative bacteria is the synthesis of beta-lactamase. Gram-negative bacteria produce a Metallo beta-lactamase with a wide range of activity. It is used to increase the activity of Amoxicillin, particularly to increase the amoxicillin spectrum in organisms that produce the beta-lactamase enzyme. Beta-lactamase inhibitors include sulbactam and clavulanic acid. The Meng et al. study aimed to investigate the proliferation of ARGs in the wastewater system as well as the impact of Amoxicillin on the microbial population. An expanded granular sludge bed (EGSB) reactor treating the artificial amoxicillin manufacture wastewater was run for 241 days. The functional microbes analysis was done using 454 pyrosequencing.Class I integrons, TEM-1, CTX-M-1, OXA-1, OXA-2, and OXA-10 were just a few of the ARGs that were investigated in sludge samples. The breakdown of amines and sugars was facilitated by some species, including Aminobacterium, Spirochaeta, and Cloacibacillus. Additionally, it was noted that the relative quantification of beta-lactam resistance genes changed with increasing amoxicillin concentration. In the artificial amoxicillin production wastewater system, vertical gene transfer is thought to be the primary driver of ARGs rather than HGTs. Beta-lactams are effective against Gram-positive bacteria but ineffective against Gram-negative bacteria. Pharmaceuticals are a new class of contaminants that have been found in freshwater, marine, and soil sediments. Rivers, groundwater, lakes, drinking water, and treatment facilities are just a few of the environmental matrices where antibiotics have been found.They can be found in hospital waste and sewage in concentrations ranging from ng/L to g/L. Since aminopenicillins are the most often used antibiotics, they are abundant in wastewater, and surface water yet is the most challenging to identify. Considering the above challenges, the following objectives were designed in the study.

Objectives:

1. To evaluate the prevalence of amoxicillin-resistant bacterial population in the Gomti river water of Lucknow.
2. To isolate the amoxicillin-resistant bacteria from the Gomti river water of Lucknow.
3. To analyze the multiple antibiotic resistance in the bacteria isolated from the Gomti river water of Lucknow

CHAPTER - 2

REVIEW OF LITERATURE

AMOXICILLIN:

In 1972, researchers at Beecham Research Laboratories discovered Amoxicillin. The penicillins' limited antibacterial effectiveness prompted the search for penicillin derivatives that could cure a wider range of infections. The invention of ampicillin represented the first significant advancement. 6-[D()-amino-p-hydroxyphenyl-acetamido.] penicillanic acid is the chemical name for Amoxicillin. Its systematic name is given by the International Union of Pure and Applied Chemistry (IUPAC) as (2S,5R,6R) -6-[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl.]amino.] -3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0.] acid carboxylic of heptane-2. Amoxicillin generally works against Citrobacter, Klebsiella, and Pseudomonas aeruginosa, but not against Streptococcus, Bacillus subtilis, Enterococcus, Haemophilus, Helicobacter, or Moraxella. Most clinical strains of Staphylococcus aureus and some E. coli have varying degrees of amoxicillin resistance. Amoxicillin influences how bacteria make their cell walls. Chromosome changes in the so-called penicillin-binding proteins, which synthesise cell walls, cause streptococci, including S. pneumoniae, to develop resistance to -lactams (PBPs). Such changes in PBPs result from an ongoing mutation process that results in varying degrees of resistance, from reduced susceptibility to low-level resistance—typically referred to as intermediate or non-susceptibility—to full clinical resistance.

Classification: Amoxicillin is a broad-spectrum semisynthetic antibiotics that belongs to a class of penicillin named aminopenicillins. The aminopenicillins were the first class of penicillins, which were discovered as active agents against several gram (-) bacteria (e.g. H. influenzae and E. coli).

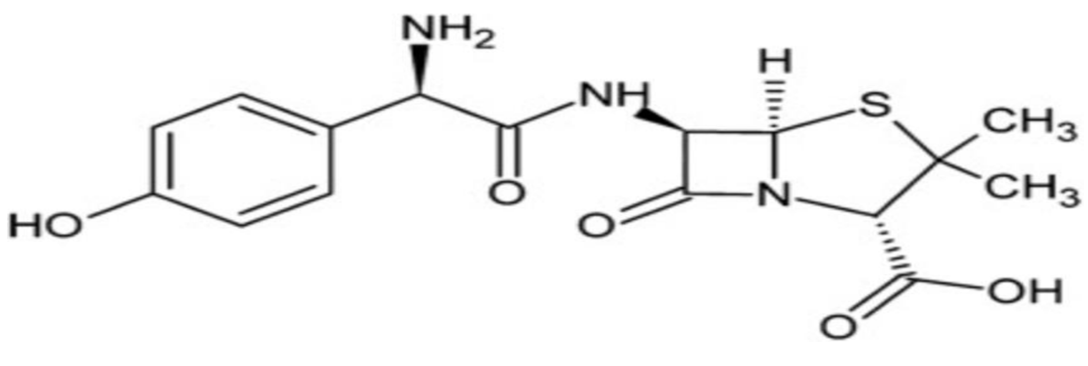

FIGURE 1 : Amoxicillin Structure

Molecular weight	365.4
Solubility	4,000mg /L
pKa1	2.8
pKa2	7.2
Log KOW	0.87
KOC	865.5
Vapor pressure	4.69 E_14 (mmHg)
Half life in environment	30% degradation after 3 months in laying hen
Feces	34% degradation after eight day broiler feces

Mechanism of action

Peptidoglycan, a crucial element of the cell walls of Gram-positive bacteria, is disrupted by b-Lactams. Transpeptidases assist in the final transpeptidation step of the synthesis of the peptidoglycan layer (penicillinbinding proteins). The final crosslinking (transpeptidation) between the linear peptidoglycan polymer chains is prevented by the -lactam nucleus's persistent attachment to penicillin-binding proteins, which changes the structure of the cell wall. In order to stop -lactamase from inactivating -lactam antibiotics, CA attaches to it irreversibly. As a result, when linked, it expands AMX activity to include bacterial strains that are both AMX sensitive and -lactamase-producing. The ability to produce -lactamase has become increasingly important for respiratory infections, most notably H. influenzae and M. catarrhalis.

Pharmacokinetic

Following administration of oral solution or tablet formulations, pharmacokinetic AMX and CA are both efficiently absorbed in the GI tract. However, there are differences in the amount of AMX absorbed in various regions of the GI tract, with high absorbed amounts in the upper small intestine and low absorbed amounts in the colon. Maximum serum concentrations of AMX happen 60 to 90 minutes after dose, with an oral bioavailability of 70 to 90%. Oral bioavailability of CA varies greatly (60.0 23.1 percent on average, ranging from 31.4 to 98.8 percent). CA is subjected to more extensive hepatic metabolism, likely by hydrolysis followed by Both drugs are well distributed into body tissues and extracellular fluids, with low binding to plasma proteins.Both drugs are well distributed into body tissues and extracellular fluids, with low binding to plasma proteins.

Clinical use

AMX is frequently used in out-patient settings, particularly for the treatment of community-acquired RTIs. It is also the first-line antibotic for the treatment of Streptococcus pyogenes infections because AMX is easily absorbed after oral administration. In order to treat Helicobacter pylori, it is also used in combination with other medications. Gram-negative infections brought on by -lactamase-producing strains of H. influenzae, N. gonorrhoeae, E. coli, M. catarrhalis, Proteus, Klebsiella, and Bacteroides are advised to be treated with AMC. In clinical settings where -lactamase-producing organisms are on the rise, such as for the treatment of otitis media, sinusitis, bronchitis, urinary tract infections, and skin and soft tissue illnesses, it is also a first-line antibiotic. Pseudomonas or methicillin-resistant Staphylococcus aureus because to its limited in vitro efficacy.

AMC does not increase the effectiveness of the use of AMX alone, which has minimal in vitro activity, as the Streptococcus pneumoniae mechanism of resistance is not caused by -lactamase production. AMC does not increase the efficacy of the administration of AMX alone, and it is crucial to keep in mind that the Streptococcus pneumoniae mechanism of resistance is not caused by -lactamase production.

Amoxicillin inactivation

Amoxicillin, a beta-lactam antibiotic, forms adducts on serum and cellular proteins as its target. This procedure may have a significant role in the emergence of negative reactions to this antibiotic, particularly allergic ones. We found that reducing agents affected the amount of amoxicillin-protein adducts formed in research investigating the haptenation of proteins by Amoxicillin. Therefore, we demonstrate that a number of thiol-containing substances, such as dithiothreitol, N-acetyl-L-cysteine, and glutathione, carry out a nucleophilic assault on the amoxicillin molecule, followed by an internal rearrangement to produce amoxicillin diketopiperazine, a recognized antibiotic metabolise with residual activity. However, L-cysteine primarily creates the amoxicilloyl amide but does not exhibit increased diketopiperazine conversion. Thiols have a catalytic effect that can completely convert Amoxicillin. Interestingly, the inclusion of an amino group in the lateral antibiotic chain, such as Amoxicillin and ampicillin, is necessary for this process to occur. Additionally, it doesn't happen when using other -lactam antibiotics, such as cefaclor or benzylpenicillin. Reduced bacteriostatic activity and a decreased ability of thiol-treated Amoxicillin to produce protein adducts are two examples of the biological effects of thiol-mediated amoxicillin transformation. Finally, inhibiting glutathione synthesis altered the intracellular redox status, affecting how much Amoxicillin formed adducts with cellular proteins. These findings provide fresh insights into the metabolism and effects of Amoxicillin, including the development of adducts linked to allergic responses.

ARGs in water bodies:

Antibiotic resistance is an issue that is progressively affecting nations all over the world. Since they are utilised in veterinary care, animal husbandry, agriculture, aquaculture, and human medicine, antibiotics typically play a crucial part in treating infectious diseases. Antibiotic resistance genes eventually spread quickly due to the massive amount of antibiotics released into surface water, groundwater, and the ocean (ARGs). Most prior research has focused on the idea that selection pressure brought on by antibiotic pollution is primarily responsible for the high levels of clinically significant ARGs; however, the most recent research demonstrates that faecal contamination, not antibiotic pollution, is largely responsible for the existence of resistance genes. The pharmaceutical industry regularly created new antibiotics between the late 1960s and the early 1980s to combat antibiotic resistance, which also led to the current global phenomenon of antibiotic abuse. After that, the development of new antibiotics slowed, and the number of existing antibiotics has steadily declined. Antibiotics still threaten people, and one study estimates that by 2050 if humans don't take the necessary steps, there might be 10 million annual deaths brought on by antibiotic resistance. Bacterial illnesses still pose a threat to humans. ARGs are currently acknowledged as environmental hazards that should be constantly avoided due to their role in the spread of antibiotic resistance. In related research on ARGs, their transport and distribution are a hot issue in environmental science. For instance, applying fertilizer to fields can act as a conduit for the spread of germs resistant to antibiotics from livestock to crops, animals, and people. The regional ARG's abundance will be significantly impacted by rainfall.

Additionally, it has been noted that microbes exhibit strong taxonomic level specificity, which is directly related to the transmission of ARGs. In addition to diffusing in water bodies due to their fluidity, ARGs can also be distributed widely by horizontal gene transfer between bacteria. Due to their long-term absorption of different contaminants, water bodies serve as important ARG reservoirs. Investigating the distribution, abundance, and variation of ARGs in natural environments is therefore crucial for determining the water quality and reducing pollution. The distribution and diversity of ARGs in various bodies of water, including drinking water, coastal water, and tap water, have been the subject of numerous studies in recent years. Although some seasonal sensitive elements, such as water flow, human activity, and bird migration, may have an impact on the abundance of ARGs, these studies of ARGs in water often take place throughout the same season. despite the fact that seasonal influences are occasionally studied.

Amoxicillin induced resistance in bacteria

When bacteria are exposed to antibiotics and continue to grow, this is known as antimicrobial resistance. It can also occur when bacteria cannot reproduce due to antibiotic exposure. Bacterium possesses resistance via a number of clinically significant ways. Antibiotic resistance is often acquired through genetic changes in microorganisms. However, non-genetic mechanisms might also be involved. A critical issue for world health is antibiotic resistance. Bacteria can develop resistance through antibiotic detoxification, intracellular buildup, antibiotic target substitution, and defence. The aquatic system has been found to contain Amoxicillin at a concentration of 6.94 g/L; nevertheless, the main issue is the development of amoxicillin resistance genes as a result of prolonged amoxicillin exposure. Amoxicillin is an antibiotic with a broad spectrum of activity that prevents the crosslinking of peptidoglycan polymers, which are crucial elements of both the Gram-negative and Gram-positive bacteria. There are various strategies by which the bacteria combat the amoxicillin stress. The most crucial mechanism in Gram-negative bacteria is the synthesis of beta-lactamase. Gram-negative bacteria produce a metallobeta-lactamase with a wide range of activity. It is used to increase the activity of Amoxicillin, particularly to increase the amoxicillin spectrum in organisms that produce the beta-lactamase enzyme. Beta-lactamase inhibitors include sulbactam and clavulanic acid. These beta-lactamase inhibitors attach permanently to the catalytic site of the bacteria's beta-lactamase, causing the latter to develop resistance to the beta-lactam ring of Amoxicillin. Extensive-spectrum beta-lactamases (ESBLs), blaTEM, blaSHV, blaCTXM (ESBL genes), and carbapenemases are some of the mechanisms by which beta lactams develop resistance. Because metallo beta-lactamases (MBL) L1 are present, Stenotrophomonas maltophila is resistant to carbapenems [53]. The reduced penetration of the medication in gram-positive bacteria is caused by changes in the penicillin-binding proteins (PBPs) and a decrease in the expression of the outer membrane proteins (OprD) [54]. Beta-lactamase, mainly mediated by plasmids in Staphylococcus aureus, is the cause of drug resistance, which decreases the efficacy of antibacterial agents. The PBPs' antibiotic binding sites combine to create the novel protein PBP 2a, which encourages the construction of the bacterial cell wall and aids in developing resistance. The expression of femX in S. aureus, which either directly or indirectly controls peptidoglycan structure and metabolism, results in abnormal cell wall thickening, which confers resistance to Amoxicillin. Numerous unidentified genes contribute to the emergence of antibiotic resistance when the body is under stress. Gastric ulcers and cancer are linked to Helicobacter pylori. Numerous antibiotics, such as rifampin [58] and Amoxicillin, are ineffective against H. pylori. The PBP1 protein's decreased affinity for Amoxicillin and reduced uptake of beta-lactame are two mechanisms for amoxicillin resistance in H. pylori. Beta-lactamase inhibitors like Augmentin, a brand name for Amoxicillin, clavulanic acid, and sulbactam are also created. However, bacteria immune to Augmentin develop beta-lactamase, displacing Augmentin's clavulanic acid. Another beta-lactamase inhibitor is sulbactam, however it is less effective against staphylococci than augmentin. Tazobactam, a sulbactam derivative, has shown to be just as efficient as a clavulanate for beta lactams inhibition

Molecular study of microorganisms on Amoxicillin

Penicillin, Amoxicillin, ampicillin, cefuroxime, and cloxacillin are examples of beta-lactams that work by preventing the formation of cell walls. The ade, bla, mec, cme, and mex genes are among those at play. The synthesis of beta-lactamases in Staphylococcus aureus confers resistance. The plasmids serve as a conduit for the creation of the enzyme. The beta-lactam structure is hydrolyzed, which prevents them from becoming bactericidal. While Gram-positive bacteria prefer penicillin-binding protein (PBP) based resistance mechanisms, Gram-negative bacteria confer amoxicillin resistance by producing beta-lactamase enzyme. Amoxicillin resistance in bacteria is significantly influenced by PBP 2B [62]. New Beta-lactamase enzymes are produced as a result of mutations in the beta-lactams' active site. For instance, the TEM-3 evolution that breaks down cephalosporins and Extended-spectrum Beta-lactamases (ESBLs). There are beta-lactamases. The Enterobacteriaceae family of bacteria has chromosomes that contain beta-lactamases, mostly in the form of silent genes. Some of these enzymes are also present on mobile genetic elements. One of Qureshi et alstudies .'s looked at the development of H. pylori's amoxicillin resistance as well as the mechanisms the bacteria used to fight off increasing in vitro amoxicillin concentrations. They came to the conclusion that decreased efflux and permeability, PBP changes, and Amoxicillin being rendered inactive by the enzyme beta-lactamases are all contributing factors to resistance in Gram-negative bacteria. The aim of the study by Meng et al. was to examine the impact of Amoxicillin on the microbial community and the dissemination of ARGs in the wastewater system. Treatment of artificial Amoxicillin in an expanded granular sludge bed (EGSB) reactor treating the artificial amoxicillin manufacture wastewater was run for 241 days. The functional microbes analysis was done using 454 pyrosequencing. Class I integrons, TEM-1, CTX-M-1, OXA-1, OXA-2, and OXA-10 were just a few of the ARGs that were investigated in sludge samples. The breakdown of amines and sugars was facilitated by some species, including Aminobacterium, Spirochaeta, and Cloacibacillus. Additionally, it was noted that the relative quantification of beta-lactam resistance genes changed with increasing amoxicillin concentration. In the artificial amoxicillin production wastewater system, vertical gene transfer is thought to be the primary driver of ARGs rather than HGTs. Beta-lactams make Gram-positive bacteria vulnerable, while Gram-negative bacteria are resistant to them. Gozlan et al.

[9] examined the breakdown products of Amoxicillin. They found only one degradation product, namely amoxicillin oxide when exposed to sunlight, amoxicillin acid, and 2,5-diketopiperazine when treated with photocatalysis.For the treatment of harmful chemicals, an alternative is to employ the Photo-Fenton method

Ecotoxicity of Amoxicillin and its impact on living organisms :

When antibiotics get into drinking water, they can trigger severe allergies in species that are susceptible, including people. ARBs may develop from polluted water and soil with low antibiotic concentrations. Approximately 75% of antibiotics consumed by animals are eliminated through faeces and urine. Antibiotics are excreted from the urine. Amoxicillin's breakdown products and metabolites are far more toxic and long-lasting than the original drug. Elimination rates for ampicillin and amoxicillin range from 60 to 90%. Antibiotics are created to target particular pathways in humans and animals to treat infectious diseases, but they also impact organisms that are not the intended targets. Although there is little information about the toxicity of Amoxicillin to living things, it poses a threat to both terrestrial and aquatic life.As a result of its widespread use in food animals, amoxicillin residues in these animals are a source of concern for people worldwide.

Amoxicillin is discovered to be a possible mutagen, carcinogen, and teratogen at higher doses. Amoxicillin is categorised as a high-risk chemical among the antimicrobials when the ecological risk of these compounds was evaluated. When antibiotics get into drinking water, they can trigger severe allergies in susceptible species, including people. Because beta-lactam is persistent in both water and soil and breaks down in the soil at various water potentials, it is a sign of resistant bacteria. Antibiotic overuse has increased the bacterial reluctance mechanism, which results in the development of multidrug-resistant microorganisms. Studies on bacteria, such as Clostridium, Lactobacillus, Escherichia coli, and Enterococcus, revealed that the metabolic activities of the bacteria are impacted by the stress of antibiotics, including penicillin, Amoxicillin, erythromycin, ciprofloxacin, and sulfamethoxazole. Antibiotics are persistent in the environment, affecting the biogeochemical cycles and having an adverse effect on non-target organisms. In aquatic environments, antibiotics produce toxic effects on aquatic organisms, alter plant growth, change the sex ratios of higher animals, and alter the anatomy of many organisms. Amoxicillin bioaccumulation in fish tissues, which are suitable for human consumption, can lead to immunoallergic reactions in people and can cause bacterial resistance genes to be induced in fish tissues.Amoxicillin is toxic to the fish Oryzias latipes and at 96 h the LC50 was recorded to be 1000 mg/L.

Biodegradation of Amoxicillin

Because Amoxicillin is not sufficiently eliminated in the WWTP, it enters groundwater and surface water, where it might cause chronic resistance in aquatic flora and animals due to repeated exposure to minute amounts of the antibiotic. Amoxicillin is removed from wastewater by several methods, including hydrolysis, adsorption, photolysis, and oxidation-reduction. Fig. 2 depicts a generalised pathway for the degradation of Amoxicillin. These mechanisms are not economical, and the processing runs into numerous issues. Amoxicillin is entirely broken down by antibiotic biodegradation in every environment, including sludge, groundwater, soil, and sediments. Temperature, pH, nitrogen and carbon sources, solid retention time (SRT), and microbial activity are only a few of the environmental factors that affect how quickly Amoxicillin degrades. Amoxicillin can be broken down by bacteria like Rhodococcus B30 and Microcystis aeruginosa. Based on the Zahn Wellens test, the biodegradation of 17 antibiotics was examined. Amoxicillin showed partial degradation with the formation of stable metabolites, while penicillin demonstrated 87 percent degradation. Amoxicillin resistance exists in many bacteria, including Staphylococcus aureus, Escherichia coli, Acinetobacter and Helicobacter pylori, making its removal from the environment a difficult task. For amoxicillin removal, one might take advantage of the bacteria that can endure and proliferate in its presence. Amoxicillin was removed from M9 minimal media by bacteria from the genus Stenotrophomonas and Alcaligenes in 14 days. LC-MS/MS was used to confirm the degradation products and metabolites, including benzoic acid and penicillic acid, were detected. Acinetobacter lwoffi (74.3 percent), Pseudomonas aeruginosa (77.8 percent), Bacillus subtilis (76.7 percent), Corynebacterium sp. (43 percent), Enterobacter sp. (76.7 percent), Flavobacterium sp. (41 percent), and Bacillus subtilis (76.7 percent) can all break down 60 ppm of Amoxicillin. It has also been demonstrated that numerous other bacterial species, including Escherichia coli, Achromobacter sp., Vibrio sp., and Micrococcus sp., break down Amoxicillin.

Alcaligenes, Acinetobacter, Citrobacter, Pseudomonas, Klebsiella, Serratia, and Proteus are said to be resistant to amoxicillin

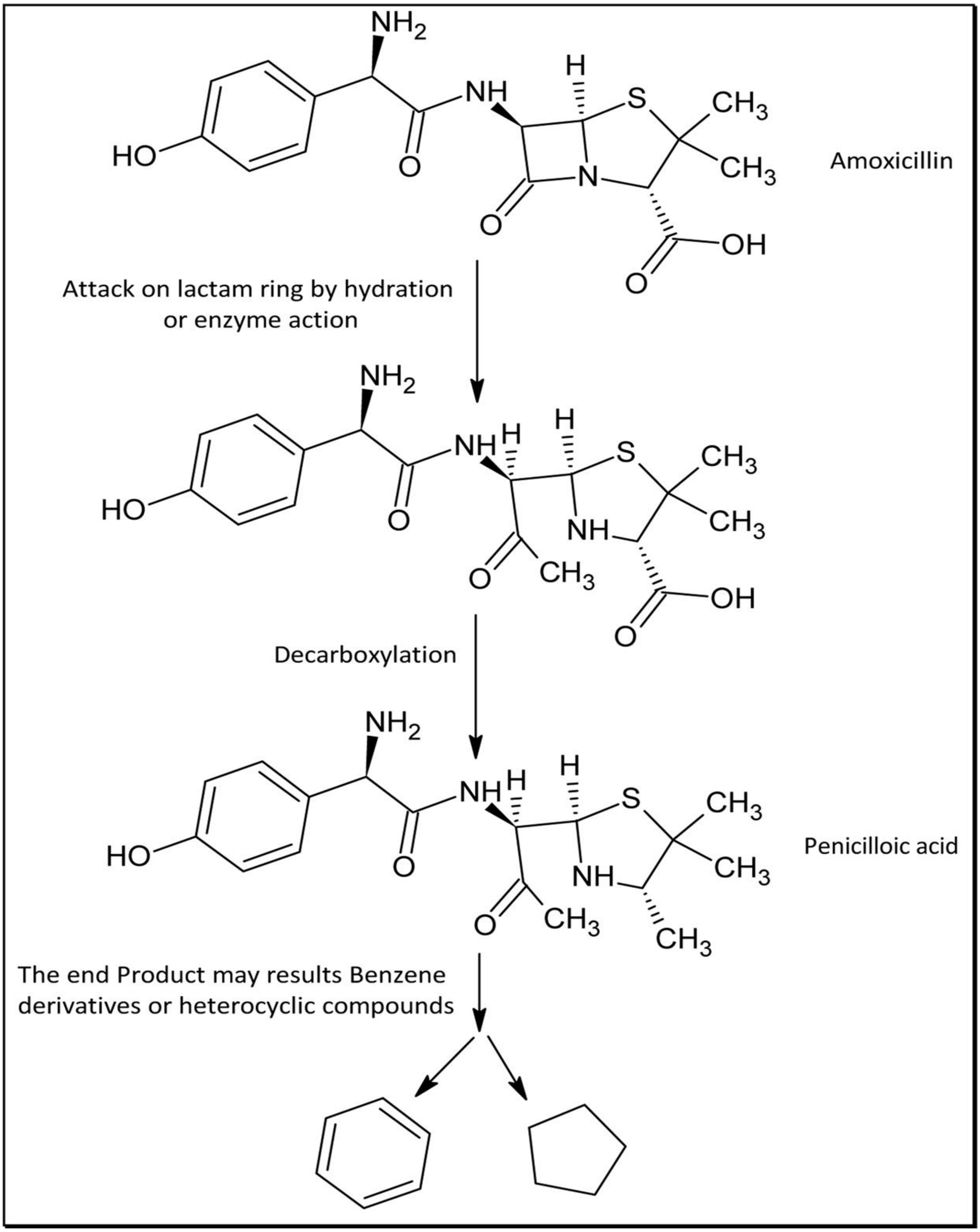

Figure:2 A generalised degradation of Amoxicillin along with its metabolites.

CHAPTER 3 - MATERIAL AND METHODS

SAMPLING SITE:

The Gomti river water in Lucknow, Uttar Pradesh, was chosen as the sampling location. The northernmost part of the middle Gangetic plain, between latitudes 26°30′ and 27°10′, and the easternmost part, between latitudes 80°30′ and 81°13′, is where Lucknow, the capital of Uttar Pradesh (India), is situated. The city experiences a hot, humid subtropical climate from April to June, with chilly, dry winters from December to February. Extreme temperatures ranged from 1.67 °C in the winter to 48.9 °C in the summer. The city receives roughly 900 mm of rain annually between July and September, primarily from the southwest monsoon. The city is between 100 and 130 meters above mean sea level and slopes primarily east. With a projected population increase from 2.8 million in 2011 to 4.7 million in 2031, Lucknow is one of the cities in the nation that is expanding the quickest. Rapid, unplanned urbanisation has produced many issues since it puts enormous strain on resources like land, water, housing, transportation, health, and education. The area's natural resources, particularly the amount and quality of water, are significantly impacted by this growing population. The most vital natural resource for life is fresh water, yet its quality has declined due to overuse and inappropriate consumption.

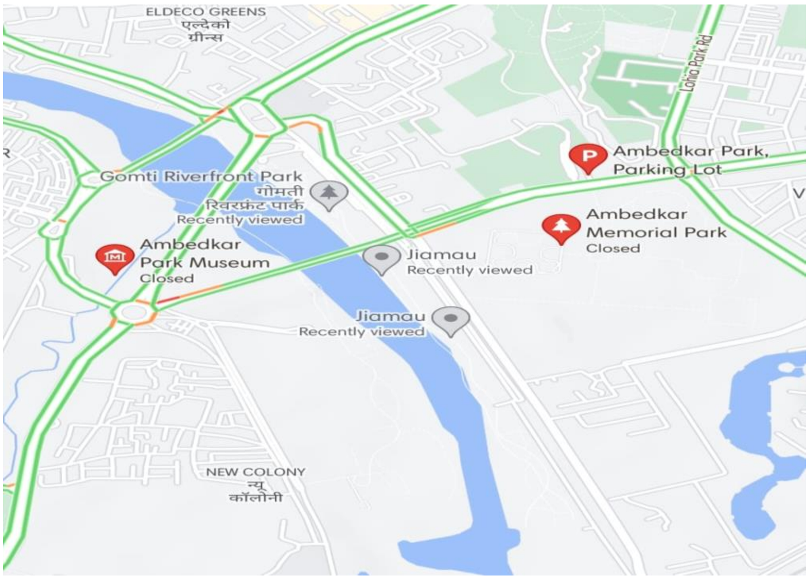

Figure 3: Map showing Gomti river showing sampling location

Numerous waterways traverse Lucknow. The main river, the Gomti, runs through the heart of the city from North to South. Along with groundwater, it serves as a significant source of the city's public water supply. The main issue in the city is the production of sewage and the proper treatment and disposal of this waste. The quality of river water has been significantly reduced due to poorly functioning sewage infrastructure and sewage treatment facilities.

COLLECTION OF WATER SAMPLE:

In February 2022, composite water samples were taken from the Gomti river in Lucknow, UP (India), at two distinct sampling locations. A location close to where effluents were dumped directly into open channels was chosen as the first sample site (site I). The second sampling site (site II), where additional wastewater is also fed to the river at various points, was chosen roughly a kilometre away from the first site. In order to create a 4 L composite sample, 2 L of water were collected at two distinct points at each sampling site. Samples were aseptically taken, appropriately labelled, and brought on ice to the lab for examination.

Figure 4: Gomti River site

Culture media used for enumeration of normal and Amoxicillin resistant bacterial population

The total numbers of culturable heterotrophic aerobic bacteria and colony-forming units (CFU) were determined by serial dilution and plating on Nutrient Agar. Both with and without the media, the nutrient agar plates were created. Amoxicillin was added to the nutrient agar at a final concentration of 100 g/ml to count the amoxicillin-resistant bacteria population. River water (10 mL) was serially diluted in 90 mL of sterile saline solution. After 3–5 days of incubation on nutritional agar (peptic digest of animal tissue, 5 g/L; sodium chloride, 5 g/L; beef extract, 1.5 g/L; agar, 15 g/L), bacteria were counted at 35°C. The population was assessed using data on the number of colonies by the formula:

CFU = Number of colonies X Dilution factor/ volume of culture plated

Isolation of Amoxicillin resistant bacteria from water

The water sample was prepared by vortex-mixing 90 ml of saline solution with 10 ml of water for 30 minutes (0.86 percent). Following serial dilutions (up to 10^7), the supernatant was plated on nutritional agar containing 100 g/ml of Amoxicillin, and incubated at 35°C for 24 hours. By repeatedly streaking on nutrient agar, ten different fast-growing bacterial isolates with distinctive colony morphologies were chosen and purified (Table 2).

Subculturing for pure culture preparation

The inoculating loop was sterilised in the Bunsen burner using aseptic technique by exposing the loop to the flame until it was red hot. It was given time to cool. From the agar plate culture, a single colony was chosen and applied to the first quadrant in near-parallel streaks before flaming once again and giving time to cool, returning to the edge of the first area, which had just crossed the second-half of the plate. Three to four more times, this method was performed. The striped plate was incubated for 24 hours at 37°C. On the plate, the developed colonies were carefully examined.

Preparation of agar slant:

The nutrient agar was weighed and dissolved in desired volume of double distilled water in conical flask and boiled to mix properly. The solution was mixed properly to make the uniform media solution and dispensed in the testubes to a volume of 5 ml and autoclaved at 121°C for 30 minutes. The agar was allowed to cool with the tube lying in a slant position resulting in a large surface area for inoculating a culture. After the slant agar tubes cooled, the bacteria were inoculated by the loop and the slant test tubes were incubated at 37°C overnight. This process was done aseptically in laminar airflow. Finally, it was used for storing pure cultures for a moderately long term and can be used to culture bacterial cells for other experiments.

Antibiotic sensitivity test

All the isolates were tested for sensitivity to antimicrobial agents using disc diffusion method (Bauer et al. 1966). The following antibiotics (all from Hi-media, Mumbai, India) were used. The concentration of the antibiotics used is given in μg ⁄ disc. The abbreviations and concentrations of the respective antibiotics are given in parentheses: ampicillin (A 25), chloramphenicol (C 25), ciprofloxacin (Cf 30), co-trimoxazole (Co 25), doxycycline (Do 30), gentamicin (G 30), kanamycin (K 30), nalidixic acid (Na 30), neomycin (N 30), streptomycin (S 10) and tetracycline (T 30).

CHAPTER 4

RESULT AND DISCUSSION

Enumeration of microbial population in the Gomti River water

Table 1. Heterotrophic bacterial population in the Gomti River water; (A) without added antibiotic; (B) with added DMSO in the medium.

Sample	Heterotrophic bacteria without Amoxicillin	Amoxicillin resistant heterotrophic bacteria
GRW2	$2.33\times10^{2}\pm1.88\times10^{2}$	$1.22\times10^{2}\pm6.35\times10^{1}$

Amoxicillin-resistant bacteria are prevalent in the water samples taken from the Gomti River in Lucknow. When no antibiotic was added to the growing media, the average heterotrophic bacterial count in the Gomti water was determined to be 3.61X109–4.45X109 CFU/ml (Table 1). Tetracycline was added to the growth media, though, and the bacterial population dropped to 9.07 X 109 1.25 X 102 CFU/ml (Table 1). When antibiotic was introduced to the medium, the overall number of bacteria decreased. The Gomti river water contains a sizable population of AMOXICILLIN-resistant bacteria, according to heterotrophic bacterial analysis on the growth media. The findings of the investigation showed that the percentage of amoxicillin-resistant bacteria in the gomti water is about 42%.

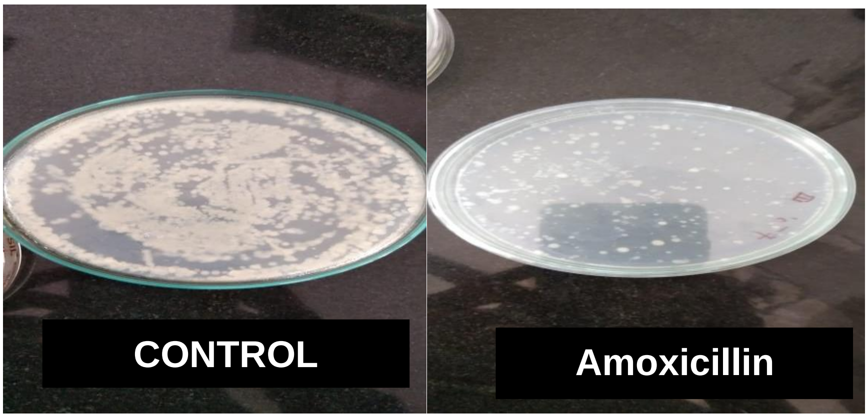

Figure 5 .simple microbial plate antibiotic microbial plate

The emergence of antibiotic resistance in microbes from humans and animals is a global concern. It is due to the overuse of antibiotics suggesting an input of large amounts of ARGs in the environment, including in surface and river waters. Inputs in these ecosystems mainly occur through livestock waste discharges effluents from hospitals and wastewater treatment plants. Wastewater treatment plants cannot eliminate antibiotics because they are not designed to remove them. Tao et al. (2010) reported multiple drug resistance in bacterial isolates from various Pearl River sites, possibly due to sewage discharge and input from other anthropogenic sources along the rivers. The presence of antibiotics in rivers may also negatively affect natural microbial communities, which are essential for key cycles/mechanisms/processes and the maintenance of water quality, compromising fundamental ecological processes .

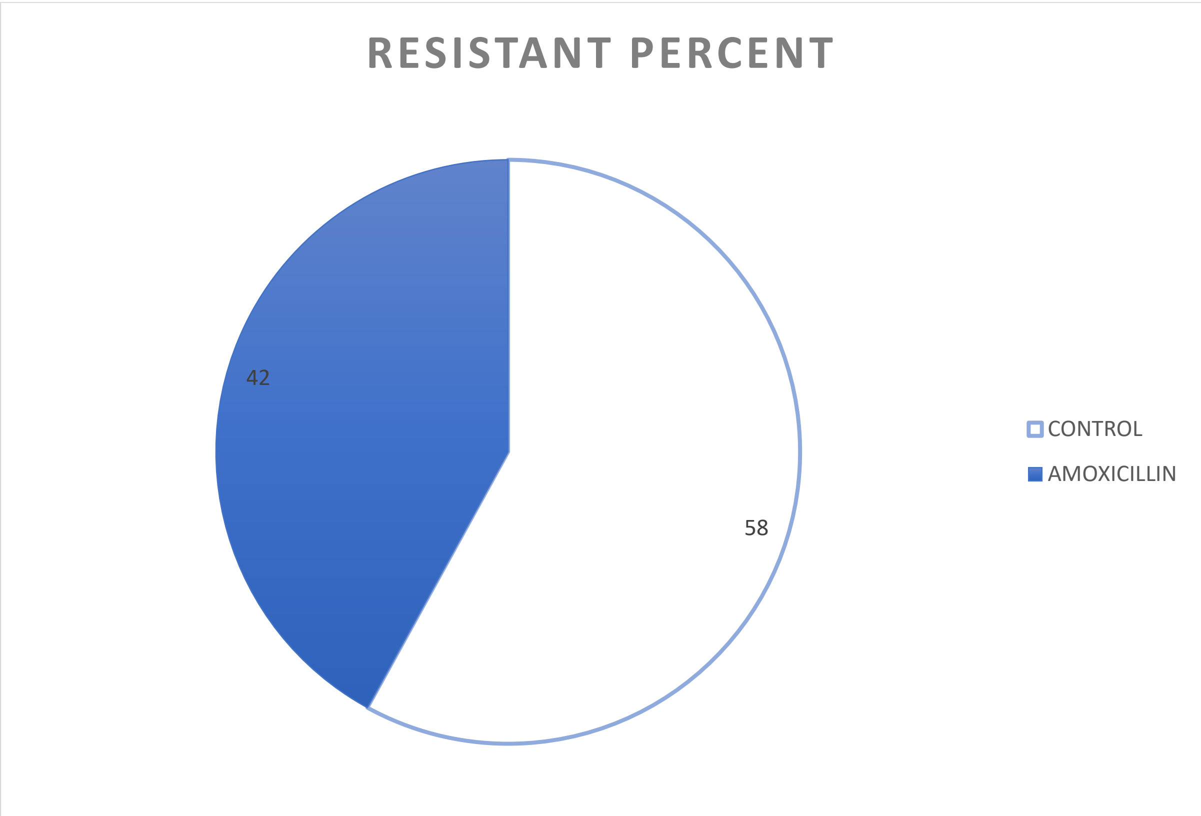

Figure 6. Percentage of Amoxicillin resistant bacteria in Gomti river water

Isolation of antibiotic resistance Bacteria

Amoxicillin-resistant bacteria were identified on the LB agar with Amoxicillin at a concentration of 100 g/ml. Ten bacterial isolates were chosen from this group based on their morphology and colour. By frequently re-streaking, the well-separated colonies were chosen and purified (Table 2). AMX-1, AMX-2, AMX-3, AMX-4, AMX-

5, AMX-6, AMX-7, AMX-8, AMX-9, and AMX-10 were the names given to the bacterial isolates. Slants were created from the ten streaked plates in ten test tubes to retain the culture.

Table 2. Morphological and physical appearance of isolated and purified isolates

Isolate	Colony morphology		
	Colour	Shape	Size
AMX-1	white	Round	Small
AMX-2	White	Round	Small
AMX-3	White	oval	Small
AMX-4	White	Oval	Large
AMX-5	White	Round	Large
AMX-6	White	Regular	Large
AMX-7	Cloudy	Round	Small
AMX-8	Cream	Regular	Large
AMX-9	White	Irregular	Small
AMX-10	White	Round	Small

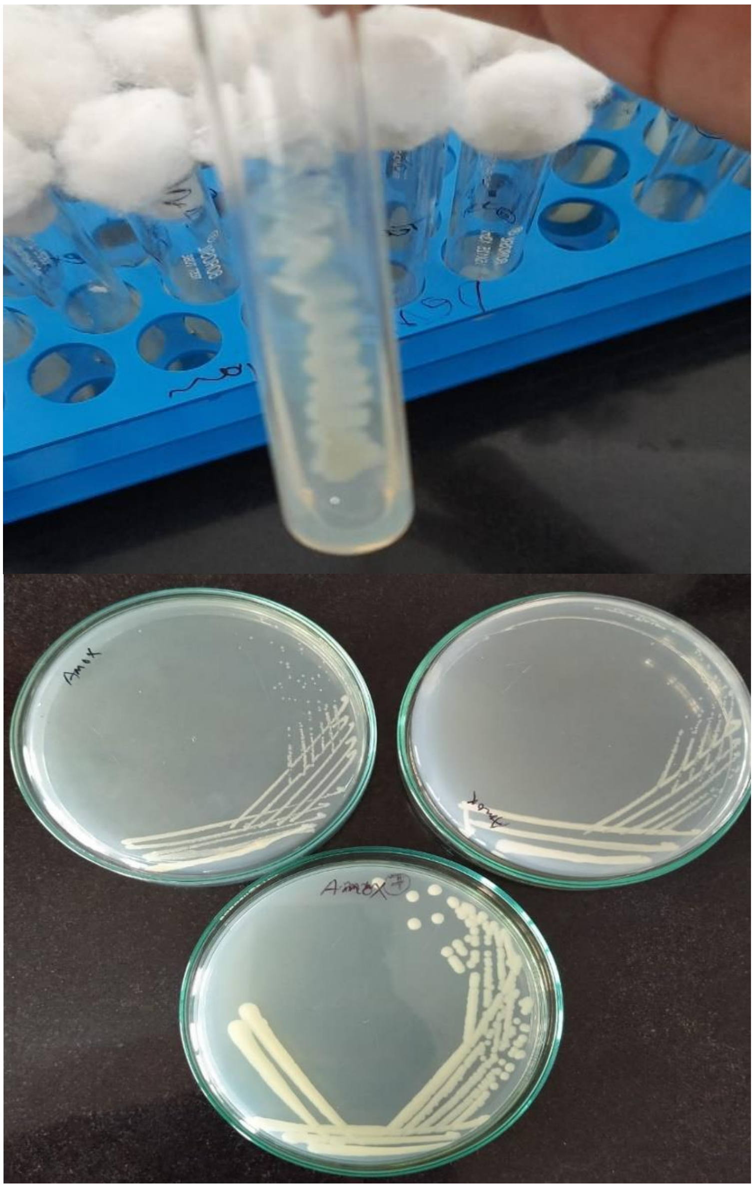

Figure 7: Amoxicillin : (i) slant culture (ii) Streak plate

Multiple antibiotic resistance in the isolated bacteria

The disc diffusion method was used to test each of the ten isolated bacteria for resistance to the other five types of antibiotics. To investigate the trend of antibiotic resistance, five antibiotic discs—Ofloxacin 30 mcg (AMX), Kanamycin 30 mcg (K), Amikacin 30 mcg (AK), Methacilin 5 mcg (MET), and Nalidixic acid 30 mcg (NA)—were utilised (Table 3)

Table 3 : Five antibiotic discs i.e., Ofloxacin 5mcg (OF), Kanamycin-30mcg (K), Amikacin-30mcg (AK), Methacilin-5mcg (MET), Nalidixic acid-30mcg (NA) were used to check the antibiotic resistance pattern

BACTERIA	ANTIBIOTIC				
	OFLOXACIN	METHACILIN	KANAMYCIN	AMIKACIN	NALIDIXIC ACID
Amx 1	R	R	R	S	R
Amx 2	S	R	S	S	S
Amx 3	S	R	S	R	S
Amx 4	R	R	R	R	R
Amx 5	S	R	S	S	S
Amx 6	R	R	S	S	S
Amx 7	S	R	S	S	S
Amx 8	R	R	S	S	S
Amx 9	S	R	S	S	S
Amx 10	S	R	R	S	S

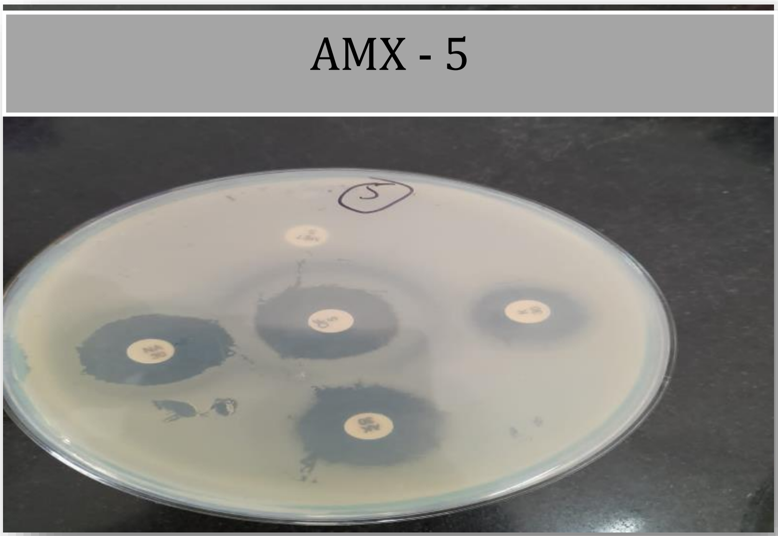

Figure 8. Antibiotic-resistance of the AMX – 5 isolate using the disc diffusion method

Table 4. Percent antibiotic-resistant bacteria in the Gomti river water

ANTIBIOTICS	CONCENTRATION	NO.OF ISOLATES	RESISTANCE ISOLATE (%)
Ofloxacin	5mcg	4	40
Kanamycin	30mcg	3	30
Amikacin	30mcg	2	20
Methicilin	5mcg	10	100
Nalidixic acid	30mcg	2	20

Table 4 displays the proportion of bacterial strains that are resistant to antibiotics found in Gomti river water. Out of the ten recovered bacteria, 20% exhibited resistance to Amikacin and Nalidixic acid, 30% exhibited resistance to Kanamycin, 40% exhibited resistance to Oflaxacin, and 100% of the bacteria exhibited resistance to Methicilin. (Table 4). The isolates were resistant to five distinct antibiotics in 60% of cases and four different antibiotics in 10% of cases. Moreover, 30% of the isolates exhibited resistance to three distinct antibiotics when used in two different combinations (Table 5).

High levels of antibiotic resistance were found in the bacterial strains isolated from Gomti River water receiving effluent. Our findings concur with those of past studies. Biswas et al. (2015) documented the presence of various antibiotic-resistant bacteria in the Ganga river in Serampore, West Bengal, India. Antibiotic contamination may be indicated by the presence of microorganisms that are resistant to antibiotics in a particular environment. The presence of anthropogenic wastes, particularly sewage, may be the cause of the high number of faecal coliform bacteria in this riverine water. Shafiani and Malik (2003) recovered 64 bacteria from wastewater-irrigated soil, including 40 Pseudomonas species, 12 Azotobacter species, and 12 Rhizobium species. All of the Pseudomonas isolates were tested for their antibiotic susceptibility to a variety of antibiotics, including nalidixic acid, cloxacillin, chloramphenicol, tetracycline, amoxycillin, methicillin, and doxycycline. They found that 100% of the isolates were resistant to cloxacillin and 57.7% to methicillin. Five different antibiotics were present in three different combinations in 7.5% of the isolates, whereas four different antibiotics were present in seven different combinations in 25% of the isolates.

NO. OF ANTIBIOTICS	NO.OF ISOLATES	RESISTANCE PATTERN
1	4(40%)	MET
2	1(10%) 2(20%) 1(10%)	MET,K MET,OFLOX MET,AK
4	1 (10%)	MET,K,NA,OFLOX
5	1(10%)	MET,K,NA,AK,OFLOX

Table 5. Antibiotic resistance pattern of the ten isolates from water of Gomti River

Conclusion

Highly multi-resistant bacteria have been recovered from the water of the Gomti River and are characterised by their multi-drug resistance profiles to five antibiotics, which display a variety of resistance combinations. The pathogenic bacteria in the river water are multi-drug resistant because the bacterium population isolated from the river water is resistant to several medications. The substantial problem of multi-drug resistance in pathogenic bacteria results in high morbidity and mortality. The faecal inputs at the sample collection sites are anthropogenic, and the site's proximity to the anthropogenic source may cause high antibiotic resistance. The multi-drug resistant microbial population can be controlled by limiting the use of antibacterial medications and educating people about their negative effects. Additionally, it's important to motivate patients to take their medications as prescribed in order to prevent drug-resistant gut bacteria. To further limit the pathogenic germs accessing the river, decentralised home wastewater treatment should be promoted.

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