A DISSERTATION ON

Multiple antibiotic-resistant bacteria in the Gomti river water in Lucknow

SUBMITTED TO THE

DEPARTMENT OF BIOSCIENCES

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT

FOR THE

DEGREE OF MASTER OF SCIENCE

IN MICROBIOLOGY

BY

VAISHALI MISHRA

M.Sc. Microbiology (IV semester)

Department of Biosciences

Integral University, Lucknow

UNDER THE SUPERVISION OF

Dr. Mohd Ikram Ansari

Department of Biosciences

Integral University, Lucknow

INTEGRAL UNIVERSITY

Established Under U.P. Act No 09 of 2004 by
State LegislationApproved by
University Grants Commission
Phone No.: +91 (0552) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026, Uttar Pradesh (INDIA)

TO WHOM IT MAY CONCERN

This is to certify that **Vaishali Mishra,** student of M.Sc. Microbiology (IV semester),Integral University has completed her four-month dissertation entitled "**Multiple antibiotic-resistant bacteria in the Gomti river water in Lucknow"**. She has completed this work from March - June 2022 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.

Dr. Snober S. Mir
Head,
Department of Biosciences,
Integral University, Lucknow

INTEGRAL UNIVERSITY

Established Under U.P. Act No 09 of 2004 by
State LegislationApproved by
University Grants Commission
Phone No.: +91 (0552) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026, Uttar Pradesh (INDIA)

CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Vaishali Mishra** 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 "**Multiple antibiotic-resistant bacteria in the Gomti river water in Lucknow"** 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 (UP).

Dr. Mohd.Ikram Ansari
Assistant professor
Department of Bioscence

DECLARATION

I hereby declare that the present work on "**Multiple antibiotic-resistant bacteria in the Gomti river water in Lucknow "** is record of original work done by me under the guidance of **Dr. Mohd. Ikram Ansari, (Assistant Professor Dept. of Biosciences)**, during the month of March - June 2022, at Integral University, Lucknow. All the data which were provided in this were through my own original work.

I also declare that not any part of this thesis has previously been submitted to my University or any examining body for acquiring any diploma or degree.

Place: Integral University, Lucknow

Date:27-06-2022 **Vaishali Mishra**

ACKNOWLEDGEMENT

First of all, I bow in reverence to the Almighty for blessing me with solid will power, patience, and confidence, which helped me complete the present work. I want to express my special thanks to **Dr. Snober S. Mir** (Head, Department of Biosciences) for allowing me to join the department laboratory and providing all the necessary facilities since I started my work.

I would like to express my deep sense of gratitude **to Dr.Mohd.Ikram Ansari (Assistant Professor, Dept. of Biosciences)** for his invaluable guidance throughout my dissertation work and academic session. It would have been impossible to complete this work in so short time without his constant guidance. I wish every trainee and research student were fortunate enough to have such an affectionate guide.

I am particularly grateful to **Dr. Manzar Aslam** for giving me an opportunity to join the department laboratory and providing all the necessary facilities ever since I started my work.

I gratefully acknowledge to **Ms. Fahmi Naznine (Ph.D Scholar)** who inspired and encouraged me during various steps of my work.

My acknowledgment will be incomplete if I do not mention my parents, with whose blessing I was able to achieve my goal successfully. There are no words to express my feelings toward them. I silently acknowledge my debt to them.

Researcher
VAISHALI MISHRA
(Dept. of Biosciences)
Integral University, Lucknow

List of Figures

Table of abbreviations

Table of Content

INTRODUCTION

Antibiotic resistance is a condition that allows bacteria to reproduce and survive in the presence of antibiotics inside the target areas. It makes common ailments challenging to cure. It also raises the cost burden of therapy. Antibiotic residues, antibiotic resistance genes, and antibiotic resistance microorganisms constitute a novel class of water pollutants owing to their negative impact on aquatic ecosystems and human health. According to the World Health Organization (WHO), antibiotic resistance is quickly rising, and treatment options are swiftly running out. According to studies, high levels of antibiotic resistance in environmental microorganisms are caused by human activities, which releases antibiotic resistance genes (ARGs) into the environment. The emergence of antibiotic-resistant bacteria (ARB) is connected to the kind of antibiotic used and the bacterial species. As a result, assessing antibiotic concentrations in wastewater, WWTP effluent, and natural water is critical. One of the most important sources of antibiotic-resistant microorganisms and their antibiotic-resistance genes in municipal wastewater. Recently, excessive levels of ARB and antibacterial compounds have been detected in rivers such as the Gomati and Ganga, posing a severe problem. The effluents of wastewater treatment plants are utilised for irrigation purposes, either directly or indirectly. As a result, there is widespread worry about the spread of antibiotic-resistant bacteria and soil contamination, as well as the release of antibiotic-resistant bacteria and antibiotic-resistant genes into drinking water.

Wastewater provides a favourable habitat for various microorganisms, including bacteria, viruses, and protozoa, and can serve as a reservoir for antibiotic-resistant pathogens. It transfers resistant bacteria into the sewage system from human excretions, domestic waste, agricultural and commercial sectors, medications, and hospitals (Houndt&Ochman et al., 2000). Bacteria are anticipated to experience distinct antibiotic resistance selection pressures in different habitats, resulting in diverse antibiotic resistance acquisition and evolution patterns. Antibiotic resistance determinants and/or organisms survive in the final effluent and are discharged into the environment by urban wastewater treatment facilities, which serve as important reservoirs of human and animal commensal bacteria. (Reinthaler et al., 2003; Tennstedt et al., 2003).

Antimicrobial resistance (AMR) is a worldwide health concern that is increasing the prevalence of both debilitating and deadly illnesses. Understanding how microorganisms acquire and transmit AMR can aid in developing novel anti-AMR therapies. Antibiotic-resistant microbes offer a health risk owing to a lack of therapeutic choices, particularly in developing countries where access to high-quality medications is restricted, and infections remain a major cause of morbidity and mortality. Antibiotic-resistant bacteria and unabsorbed antibiotic residues are excreted in urine and faeces and eventually reach wastewater treatment facilities via household sewage systems. Antibiotic residues including -lactam, macrolides, lincosamide, tetracyclines, sulphonamides, and fluoroquinolones have all been discovered in recovered urban wastewater. (Martins et al.2016).

The ongoing rise in illnesses caused by a variety of antibiotic-resistant pathogenic bacteria emphasises the importance of better understanding the environmental aspect of antibiotic resistance. Because antibiotic resistance in pathogenic bacteria is typically associated with a plasmid, plasmids carrying antibiotic resistance genes may offer a unique public health risk. Furthermore, because these plasmids are regularly mobilised, they are susceptible to acquiring antibiotic resistance genecassettes while in transit. Plasmid mobility is essential for the evolution and dissemination of antibiotic resistance in bacteria from varied environments. (Teddie et al.2014).Water contains many bacterial species; it is believed that 50% of all known bacterial genera contain species that can be classified as water bacteria. Antibiotics have been widely used for clinical and veterinary purposes since the late 1960s, so it's no surprise that antibiotic-resistant bacteria have grown common (Ansari et al.2007).

The quality of wastewater effluents causes the degradation of receiving water bodies. This is because untreated or inadequately treated wastewater effluent can produce eutrophication in receiving water bodies and create circumstances that stimulate the growth of toxin-producing cyanobacteria pathogens in the water. Anyone who comes into contact with infectious water, including recreational water users, is in danger.

Although many microorganisms play a range of important roles in wastewater systems, many are considered significant contributors to a variety of waterborne diseases. Careful planning, adequate and suitable treatment, regular monitoring, and proper laws are necessary to accomplish unpolluted wastewater discharge into recipient water bodies. (Akpor et al.2013). Considering the above problems of the antibiotic resistance, the current study was designed to address the following objectives.

Objectives:

1. Evaluation of the chloramphenicol-resistant bacterial population in the Gomtiriver water near the wastewater disposal site of Lucknow.
2. Isolation of the chloramphenicol-resistant bacteria from the Gomti river waterof Lucknow.
3. Determination of multiple antibiotic resistance patterns in the bacteria isolatedfrom the Gomti river water of Lucknow

REVIEW OF LITERATURE

The antibiotic chloramphenicol is bacteriostatic. It was developed from the bacterium Streptomyces venezuela, identified by David Gottlieb, and first used in clinical therapy in 1949. It was the first synthetic antibiotic to be mass-produced in large quantities. Chloramphenicol is a broad-spectrum antibiotic that is effective against gram-positive and gram-negative microorganisms. However, it is usually reserved for the treatment of serious and life-threatening infections in humans due to serious side effects (e.g., damage to the bone marrow, including aplastic anaemia).Chloromycetin was the initial name for chloramphenicol. The Parke–Davis team published the structure of the compound in July 1949, and it was given the generic name chloramphenicol; Parke–Davis used the brand name Chloromycetin.

Because of its broad antibacterial range, the oral route of administration, efficient penetration into multiple body compartments, low cost, and apparent safety, chloramphenicol has achieved widespread popularity. Unfortunately, substantial side effects were discovered within the first ten years of its usage, including non-dose-related deadly aplastic anaemia (Rich et aI., 1950). It is listed as an essential medicine by the World Health Organization. It's a drug that's available as a generic.

Chloramphenicol is bacteriostatic but can be bactericidal at high quantities or when employed against susceptible organisms.For systemic treatment, three common forms of chloramphenicol are used: a free base form, chloramphenicol palmitate, and chloramphenicol succinate depending on the route of administration. There are many other topical formulations available. It is used in treating *Salmonella typhi* (typhoid) and other types of salmonellosis in humans, as well as other potentially fatal infections of the central nervous system and respiratory tract (Parfitt, 1999).

Chloramphenicol is a veterinary antibiotic used to treat various animal infections, notably those caused by anaerobic bacteria or those resistant to other antibiotics. Chloramphenicol is absorbed in animals via both the oral and parenteral routes (Plumb, 2002). In humans, chloramphenicol has been demonstrated to have a haemotoxic effect, with two forms of toxicity identified. The first is a common reversible dose-related bone-marrow depression that occurs during therapy and can be reversed if the medication is discontinued. The second condition is severe aplastic anaemia, which is usually persistent and not dose-related.

STRUCTURE

Chloramphenicol is a dichloro-substituted acetamide with a nitrobenzene ring, an amide link, and two alcohol functionalities, an organochlorine molecule. The chloramphenicol molecule is made up of three parts: The p-nitrobenzene moiety is the first, while the dichloracetyl moiety is the second. A moiety of 2-amino-propanediol (III) The aromatic ring system is represented by part I, and the aliphatic haloacteylside chain is represented by part II. Two asymmetric carbon atoms make up the propanedoilmoiety.

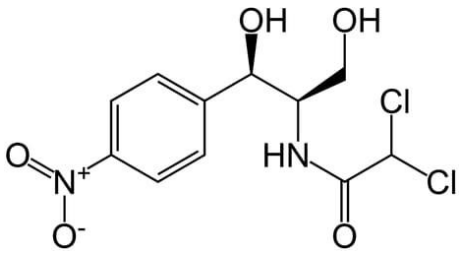


Figure 1: STRUCUTRE OF CHLORAMPHENICOL

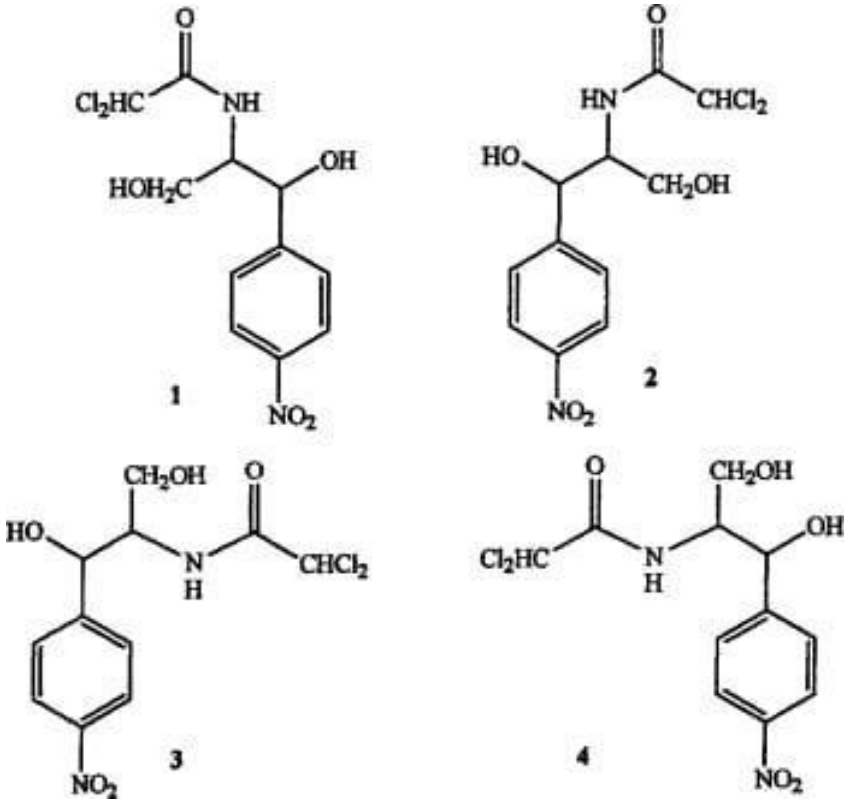


Figure 2: STEREOISOMERS OF CHLORAMPHENICOL

Theoretically, four stereoisomers of chloramphenicol exist. Figure 2 depicts the Newman projections of these four stereoisomers. The amide side-chain and the hydroxyl on carbon oneare carried by the D-threo and L-threo enantiomers on

opposing sides of the plane of the two asymmetric centers. The two substituents on the same side of the plane of the two asymmetric centers are carried by the other two stereoisomers, the D-erythro and L-erythro enantiomers (Fig. 2). The molecule has one of the most basic structures of any antibiotic (REBSTOCK etal., 1949; DUNITZ, 1952). It is one of the few naturally occurring chemicals with a nitro group. A vast number of chloramphenicol derivatives were synthesised, totaling over 500 substances(KOLOSOVetal.,1961).

Chemical and physical data

Molecular Formula – C11H12Cl2N2O5

Molecular Weight-445.2

Formal Charge – 0

Melting point - 150.1degree Celsius

Physical Description- Solid

Color/Form – White to greyish-white or yellowish-white, fine crystalline powder or fine crystals, needles, or elongated plates.

Taste- Bitter (Rebstocketal 1949) Brand name:- CHLOROMYCETIN

DOSING:-

Infants 1 week old: 25mg/kg every 24 hours Infants aged 1 to 4 weeks: 25mg/kg every 12 hours 50mg/kg/day split every 6 hours in older children/adults Adults with severe infections: 100mg/kg/day split every 6 hours (max dose 4g/day). Dosing based on disease state: Dosing changes are not required in the case of renal failure (including hemodialysis and peritoneal dialysis) Hepatic failure: There are no formal recommendations, however dose changes depending on serum levels may be required.

ANTIBIOTIC RESISTANCE MECHANISM:-

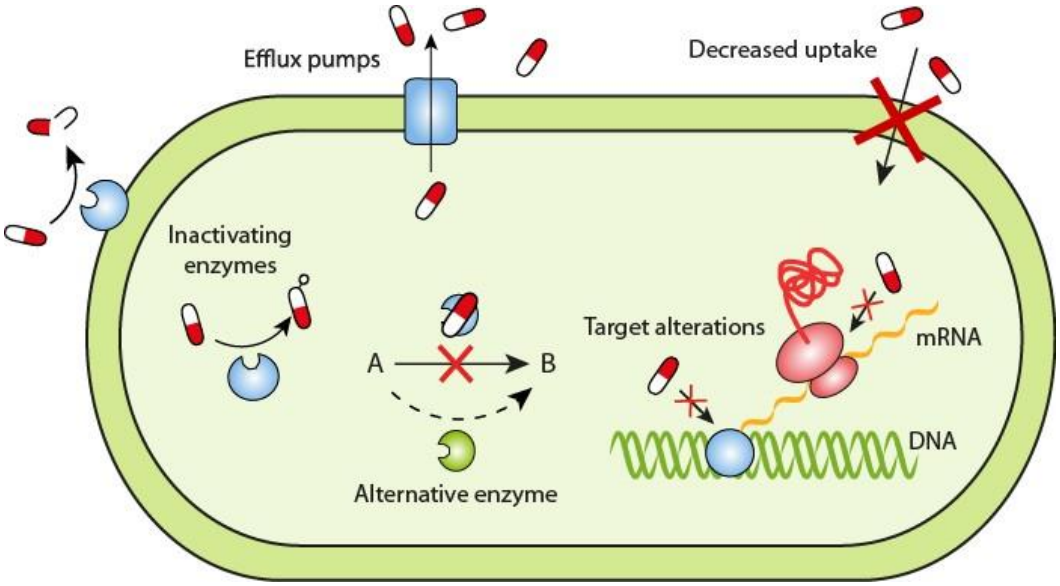


Figure 3: ANTIBIOTIC RESISTANCE MECHANISM:-

The selection procedure is quite simple. When antibiotic tackles a group of bacteria, cells particularly vulnerable to the medication die. Cells resistant from the start or acquire it later (through mutation or gene exchange) may survive, especially if too little medicine is administered to overwhelm the cells there. Those cells will then grow as they face less competition from vulnerable bacteria. When exposed to an antibiotic, the more resistant cells in a bunch will always outcompete all others. Antibiotics' only self-defeating activity is the promotion of resistance to recognised infections. When the drugs tackle disease-causing bacteria, they also impact benign microorganisms in their path, which are innocent bystanders. They destroy drug- susceptible bystanders who would otherwise limit disease spread while simultaneously encouraging the establishment of resistant bystanders. The growth of resistant, nonpathogenic bacteria expands the reservoir of resistance traits in the bacterial population, increasing the likelihood that such traits may spread to pathogens.

Furthermore, rising populations of bystanders can become disease agents themselves. The widespread use of cephalosporin antibiotics, for example, has aided in spreading the previously harmless gut bacterium E. faecalis, which is inherently resistant to those treatments. Most people's immune systems can inhibit the growth of even multidrug-resistant E. faecalis, preventing disease. However, in

hospitalized individuals with weakened immune systems,, the enterococcus can spread to the heart valves and other organs and establish deadly systemic disease.

ROUTE OF ADMINISTRATION

Chloramphenicol can be applied topically as eye or ear drops or as an eye ointment. It can be given orally as capsules or parenterally as an intravenous injection or infusion because of the high danger of toxicity and serious effects. It should be administered in 6-hourly split dosages at therapeutic levels of no more than 50 mg/kg/day. This dose may need to be increased to 100 mg/kg/day for severe infections caused by moderately resistant pathogens. Close monitoring is essential if a dose increase is required, with dose decreases to 50 mg/kg/day as soon as possible..Dosage reductions of 25 mg/kg/day may be recommended for babies and persons with impaired hepatic or renal function. If given intravenously, it must be given intermittently and diluted in either 0.9 percent sodium chloride or 5% glucose solutions. Clinicians should avoid using chloramphenicol for an extended period.

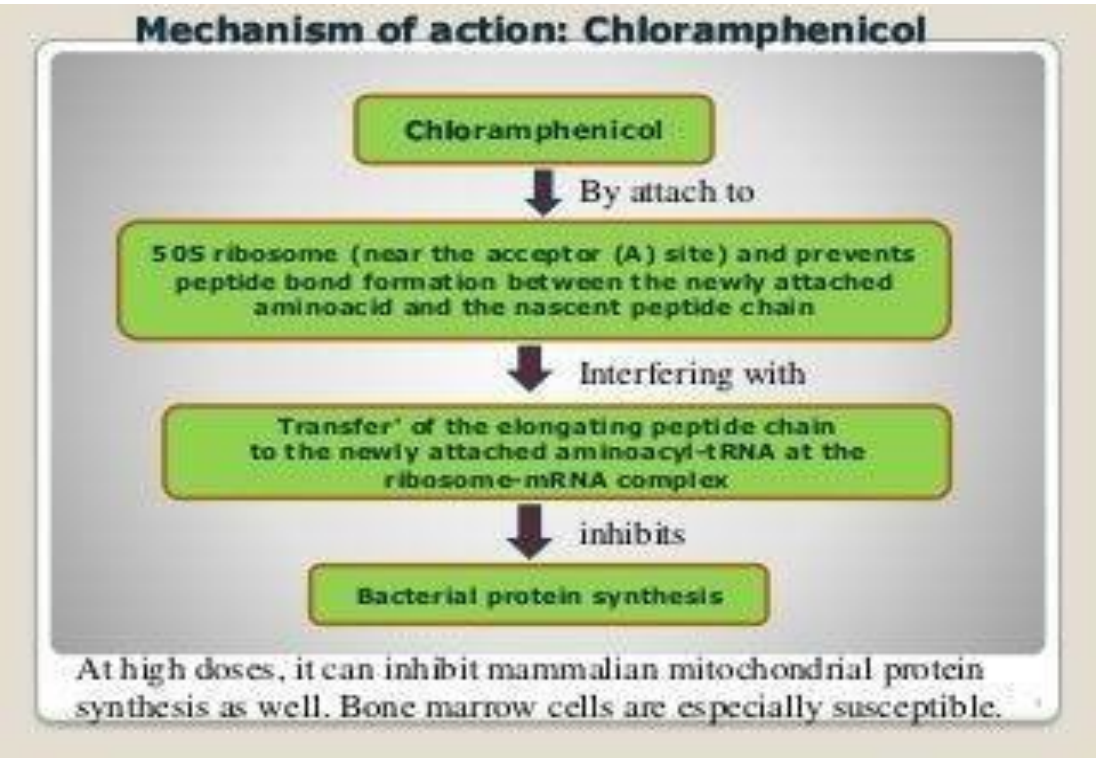


Figure 4:MECHANISM OF CHLORAMPHENICOL

RESISTANCE OF BACTERIA TO CHLORAMPHENICOL

Chloramphenicol appears to be sensitive to all bacteria species and inhibits them fully at doses ranging from 1 to 10 ug per ml. Many articles have reported the emergence of chloramphenicol resistance, which is of genetic and biochemical interest in addition to its clinical implications. Cavalli and Maccacaron1950 conducted considerable research on the genetic basis of chloramphenicol resistance. These researchers used Escherichia coli K-12 strains to perform genetic crosses via mating. The strains employed needed a variety of growth factors, as well as markers for fermentation, phage resistance, and drug resistance. Originally, these strains were susceptible to 5 to 10 g/ml chloramphenicol. One-step resistance mutants could be isolated by plating large populations of cells on agar containing 20 to 49 per ml of the antibiotic.

By exposing mutants to increasing amounts of chloramphenicol (up to 1000 jig per ml), these researchers could isolate mutants resistant to the medication. The rise of high-level resistance was always gradual. These resistant strains were subsequently crossed with sensitive strains, and recombinants based on nutritional indicators were chosen. When these recombinants were examined for resistance, all resistance levels were found, including some strains that were completely susceptible. Fully sensitive recombinants could be found by crossing two highly resistant strains. The authors interpret these findings to mean that many genes at distinct loci give low levels of chloramphenicol resistance and that these loci can interact positively or negatively, resulting in stronger resistance or even vulnerability. It's possible that repeated selection in isolating high-level resistant strains builds up a polygenic system with many positive interactions and that recombination will break down such positively interacting systems, revealing negative interactions by combining loci that don't interact positively in one genome. Cross-resistance to various antibiotics has been investigated in strains resistant to chloramphenicol. Most workers have noticed that enteric bacteria resistant to chloramphenicol are also resistant to tetracyclines, although bacteria from other families do not display this cross-resistance. Cavalli 1950 conducted preliminary genetic research of the cross-resistance between chloramphenicol and oxytetracycline (Terramycin). He

discovered that high levels of oxytetracycline resistance conferred high levels of chloramphenicol resistance, but high levels of chloramphenicol resistance conferred relatively low levels of oxytetracycline resistance. In crosses between oxytetracycline-resistant and sensitive strains, it was discovered that oxytetracycline-resistant recombinants were always chloramphenicol-resistant. In contrast, oxytetracycline-sensitive recombinants were always chloramphenicol-conferred resistance to chloramphenicol and oxytetracycline. In contrast, another group of genes in the methionines, threonines, leucine (M-TL) region had little effect on oxytetracycline resistance but did confer chloramphenicol resistance. The lack of cross-resistance between chloramphenicol and tetracyclines in organisms other than enteric bacteria is thought to be due to distinct genetic backgrounds and resistance mechanisms in various organisms. It would be fascinating to investigate the genetics of chloramphenicol resistance in different organisms now that genetic recombination techniques are more widely available. Antibiotics with cross-resistance have frequently been stated in the literature to have similar mechanisms of action. The findings presented here demonstrate the fallacy of this thinking since it would lead one to believe that chloramphenicol and tetracyclines have identical modes of action in E. coli but different modes of action in Staphylococcus aureus. In truth, cross-resistance may simply suggest that the genetic loci for resistance in a given organism are the same, as Cavalli in 1950 demonstrated. Several researchers compared the resistant strains they recovered to the parent strain to see if there were any alterations in antigenic qualities, diagnostic biochemical values, or other physiological properties. The deletion or decrease of the H antigen in chloramphenicol-resistant enteric bacteria has been the most typical report. Increased or decreased growth factor needs, decreased growth rate, changes in respiratory activity, and variations in sensitivity to various inhibitors have all been described. Cavalli and Maccacaro 1950 identified mutants with high levels of resistance, which had slower growth rates and tended to produce mucoid colonies. Both of these features were separated from chloramphenicol resistance in recombination experiments, isolating slow-growing sensitives or mucoid sensitives and rare nonmucoid resistants. As a result of their impacts on chloramphenicol resistance, these modifier loci may impart other features to the organism. Chloramphenicol resistance has a metabolic basis that is unclear. Resistance is not owing to the increased synthesis of antibiotic-destroying enzymes, as most enzymes

are not produced in higher quantities in resistant cells than in sensitive cells. Another possibility is that permeability to the antibiotic has been lost; however, this has not been investigated due to the lack of radioactive chloramphenicol.

TOXICITY

An overdose of chloramphenicol, which usually happens with intravenous drug administration and is more likely to impact newborns, can be fatal. Nausea and vomiting, abdominal distension, metabolic acidosis, hypotension, hypothermia, circulatory collapse, and coma are all poisoning symptoms. Gray baby syndrome will develop from the accumulation of chloramphenicol in the infant. The symptoms of grey baby syndrome differ based on the drug's serum levels in the body. Poor eating, agitation, stomach distension, vomiting, grey skin discolouration, and rapid collapse from cardiovascular and respiratory issues are all signs and symptoms of poisoning.

Bone Marrow Suppression

Chloramphenicol's damaging effect on the bone marrow is the most significant. There are two types of negative consequences. The first is reversible bone marrow suppression, a direct pharmacologic action of the antibiotic caused by mitochondrial synthesis inhibition. Chloramphenicol is thought to accomplish this via attaching to the 70S ribosomes in mammalian mitochondria and inhibiting ferrochelatase activity. Hemoglobin production is generally catalysed by ferrochelatase in the mitochondria of bone marrow erythroid cells.As a result, reticulocytopenia, anaemia, leukopenia, or thrombocytopenia might occur in any combination. Serum iron levels may also rise in tandem with a decrease in radioactive iron uptake by red blood cells, indicating haemoglobin deficiency. The erythroid and myeloid precursors are vacuolized within the bone marrow. These side effects are prevalent, dose-related, and occur during treatment. Patients consuming at least 4g/day or with blood levels greater than 25 mg/L are more prone to develop them. When chloramphenicol is stopped, the effect goes away. Hemolytic anaemia has also been reported in patients treated with chloramphenicol who have glucose6-phosphate dehydrogenase impairment

Gray Baby Syndrome

The grey baby syndrome is a circulatory collapse that can affect premature and newborn babies and is linked to high chloramphenicol levels in the blood.An ashen-gray colour, abdominal distention, vomiting, flaccidity, cyanosis, circulatory collapse, and death are all symptoms. It usually begins 2 to 9 days following the start of treatment. Chloramphenicol impairs cardiac contractility by interfering directly with myocardial tissue respiration and oxidative phosphorylation, resulting in the syndrome. It is thought to affect neonates more frequently because of their impaired ability to conjugate chloramphenicol and eliminate the active form in the urine. There have also been cases of accidental overdoses of the substance in tiny infants and adults. Chloramphenicol serum levels greater than 50 mg/L are usually linked with the condition, which can also be accompanied with unexplained metabolic acidosis. Exchange transfusion and charcoal hemoperfusion have been used to speed up drug elimination.

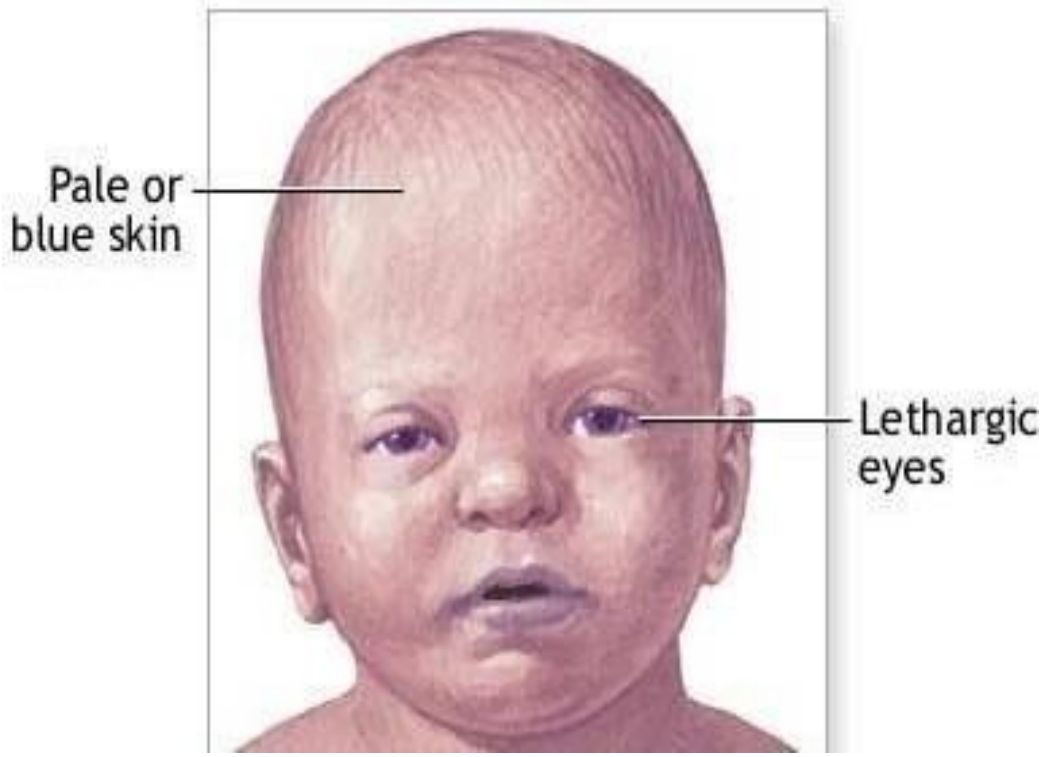


Figure 5:GREY BABY SYNDROME

Other Reactions

Nausea, vomiting, diarrhoea, glossitis, and stomatitis are some gastrointestinal side effects that can occur but are rarely severe. Rashes, medication fever, and anaphylaxis are all examples of hypersensitivity reactions. During the treatment of syphilis, brucellosis, and typhoid fever, Jarisch-Herxheimer's reactions have been seen. Chloramphenicol may cause bleeding if taken over an extended period. This could be due to bone marrow suppression or a reduction in gut flora, which would hinder vitamin K synthesis.Chloramphenicol has also been linked to acute porphyria attacks; hence it should be avoided by porphyria sufferers.Chloramphenicol may interfere with the development of immunity if taken during active immunization.

Absorption and Bioavailability

Because oral chloramphenicol capsules are readily absorbed from the intestinal tract, they are roughly 80% bioavailable. The oral palmitate solution produces lower serum levels than the capsule form. The palmitate ester is physiologically inactive and is absorbed after being degraded into free chloramphenicol by pancreatic lipases in the upper digestive tract. Even when compared to the intravenous succinate version, the oral palmitate ester can produce higher serum levels. The concentration of active chloramphenicol is determined by the rate of hydrolysis of the succinate in liver enzymes, which is why oral formulations can be more bioavailable. In the serum, 44 percent to 60 percent of the medication is bound to protein.Peak blood levels of 10 to 13 mg/L appear following a 1-g dosage in adults after 2 hours.

The half-life ranges from 1.6 to 3.3 hours, and therapeutic levels can last up to 8 hours following administration.In newborns, the half-lives of chloramphenicol succinate given intravenously vary greatly. The serum half-life in adults is around 1.2 hours, while the elimination half-life is about 4 hours.Because of variable hydrolysis and delayed absorption, intramuscular injection is not recommended, resulting in unpredictable serum concentrations.

Drug Elimination

Chloramphenicol is metabolized mainly in the liver and is conjugated to glucuronic acid. The inactive chloramphenicol glucuronide is the main metabolite. This is then excreted in the urine and other minor metabolites (accounting for about 75 percent to 90 percent of drug elimination). Through glomerular filtration, approximately 5% to 15% of the unaltered, active medication is removed. Bile excretes just about 3% of the total. In the faeces, less than 1% is removed.

Antimicrobial Activity

Many gram-positive and gram-negative bacteria, anaerobes, rickettsiae, chlamydiae, and mycoplasmas are susceptible to chloramphenicol. Chloramphenicol is generally bacteriostatic, although it can be bactericidal against meningeal germs, especially at higher dosages.

Gram-Positive Bacteria

In vitro, chloramphenicol inhibits streptococci, staphylococci, and enterococci. Resistance is varied in different parts of the world. S. pneumoniae had greater rates of resistance in the Western Pacific Region and South Africa (17.1%), compared to Europe (12.7%), the United States (10.6%), Canada (4.5%), and Latin America (4.5%), according to surveillance studies from the late 1990s (4.3 percent). Penicillin-intermediate and penicillin-resistant strains are more likely to be resistant to chloramphenicol. Chloramphenicol was found to be effective against 81.6 percent of all S. aureus isolates examined in a recent North American surveillance survey. Another recent North American investigation found that both methicillin- sensitive (96%) and methicillin-resistant (81%) isolates have high susceptibility rates.

found to be 87 percent in North America.Another research of VRE isolates found that 28.6% of 56 E. faecalis isolates from North America were resistant to chloramphenicol, compared to 7.1 percent of 14 isolates from Europe. In contrast to E. faecium isolates, this was not the case. Resistance was found in 0.5 percent of 776 isolates in North America, compared to 15 percent of 40 isolates in Europe, the latter attributable to clonal occurrences. Certain gram-positive bacilli are sensitive to chloramphenicol. Corynebacterium diphtheriae, L. monocytogenes, and B. anthracis

are almost always susceptible, whereas Corynebacterium jeikeium and Nocardia spp. are usually resistant.

Gram-Negative Bacteria

Chloramphenicol's activity against gram-negative bacteria varies. For example, H. influenzae and Moraxella catarrhalis are particularly active against community-acquired organisms. Both N. meningitidis and N. gonorrhoeae are highly vulnerable.

E. coli activity varies across the globe. Chloramphenicol-resistant isolates were found in various countries, ranging from 8% in Curaçao to 82% in Ghana, according to a recent surveillance research. 346 Another study in the UK found that resistance rates decreased from 20.2 percent in 1991 to 7.9 percent in 2004. Some members of the Enterobacteriaceae, such as P. mirabilis, Salmonella spp., Shigella spp., and Yersinia spp., have good action against chloramphenicol, whilst others, such as Klebsiella spp., Serratia spp., Morganella spp., and Enterobacter spp., are usually more resistant.. P. aeruginosa is generally resistant to chloramphenicol using an active efflux pump. Acinetobacter spp. is also generally resistant, while S. maltophilia is usually susceptible. Chloramphenicol has activity against B. pseudomallei, whereas Burkholderiacepacia is usually resistant due to decreased drug permeability.

Anaerobic Bacteria

Chloramphenicol is effective against most gram-positive and gram-negative anaerobic bacteria. At feasible amounts, anaerobic gram-positive cocci, such as Peptostreptococcus spp., are all sensitive. Clostridium, Lactobacillus, and Propionibacterium spp. are among the anaerobic gram-positive bacilli that are vulnerable. *Clostridium perfringens* and *Clostridium difficile* resistant strains have been discovered. Chloramphenicol is one of the most effective antibacterial medicines against gram-negative anaerobic bacteria. Bacteroides spp., Fusobacterium spp., and Prevotella spp. are among the most vulnerable bacteria. In a recent investigation in the United States, B. fragilis group isolates were found to be susceptible to chloramphenicol.

INHIBITION OF PHYSIOLOGICAL PROCESSES

A. Energy-Generating Processes Action

Gale and Folke 1953 discovered that very high concentrations of chloramphenicol did not affect glucose fermentation or respiration. Hahn et al. discovered no suppression of bioluminescence or motility, both of which require energy. Also unaffected was phosphorylation in glucose dissimilation. In light of these findings, Kushner's findings that chloramphenicol strongly inhibits the oxidation of succinate, fumarate, malate, and a-ketoglutarate in *Pseudomonas fluorescens* may appear strange until it is realised that Kushner grew his cells in yeast extract-peptone medium and tested them for oxidation without adapting them to the desired substrate. Chloramphenicol did reduce the oxidation of these substrates, but it did so by reducing the induced manufacture of the required enzymes rather than through any direct action on oxidative reactions. These factors should serve as a caution about the complexities involved in data interpretation and highlight the need to employ a well-designed experiment to research the antibiotic mechanism of action. Because chloramphenicol does not inhibit respiration, it is an effective

inhibitor for a wide range of protein synthesis experiments. Traditional respiratory inhibitors including cyanide, azide, and dinitrophenol halt all cellular activities

.

Action on Permeation Processes

Chloramphenicol did not inhibit the accumulation of free glutamic acid in the cell. However,according to Gale and Paine, it did enormously hinder the conversion of glutamic acid to a mixed form. Other researchers have also demonstrated no suppression of free amino acid buildup. Because amino acid absorption is not impeded, whereas protein incorporation is, there is a rapid increase in the free amino acid pool size after the injection of chloramphenicol, and the rate of accumulation in the pool approaches the rate at which these amino acids were incorporated into protein. There is also no inhibition of 3-galactoside uptake.Presumably, all permeation processes will function normally in the presence of chloramphenicol, although there have been no studies on the uptake of ions

Action on Synthesis of Small Molecules

There is no direct evidence that chloramphenicol suppresses the synthesis of any of the tiny chemicals that serve as cell building blocks, and there is plenty of indirect evidence that it does not. Mandelstam demonstrated that when chloramphenicol is added to cultures of Escherichia coli growing in glucose-salts medium, where all essential amino acids must be synthesized, there is a significant increase in the amount of all free amino acids, presumably because the synthesis of these continues while incorporation into protein is blocked. This causes a rise in amino acid concentration within the cellular pool and amino acid excretion into the medium. Because the antibiotic does not block nucleic acid production (see below), the synthesis of purine and pyrimidine bases, ribonucleotides, and deoxyribonucleotides should be unaffected.

Inhibition of Synthesis of Large Molecules from Small Molecules

Inhibition of protein synthesis.

Protein synthesis is reduced by chloramphenicol at concentrations of 10 jug per ml and higher in developing cells, as assessed in various techniques. This was first demonstrated by Gale and Folkes for Staphylococcus aureus and Wisseman et al. for E. coli, and has since been validated by several researchers in a wide range of organisms. Because total protein synthesis is inhibited, it stands to reason that the

synthesis of individual enzymes or other specific proteins would be inhibited as well. A review of the literature reveals that chloramphenicol inhibits the synthesis of the following proteins: aldolase, alkaline phosphatase, amylase, 5-aminolevulinic acid dehydrase, aminolevulinic acid synthetase, carbamyl phosphate synthetase, catalase, flagella, f3-galactosidase, (3-galactoside per In all of these cases, chloramphenicol was found to limit protein synthesis rather than activity.

2. Nitrogen fixation.

Chloramphenicol does not prevent nitrogen fixation in Azotobacter, but it does limit protein synthesis, causing the acid-soluble intermediate products of nitrogen fixation to accumulate. As a result, the antibiotic could be beneficial in evaluating the earliest steps in nitrogen-fixing.

3. Effects on carbohydrate assimilation.

In growing E. coli, the antibiotic has little effect on carbohydrate assimilation. It also does not prevent the formation of an amylopectin-like polysaccharide in *Neisseria perflava* resting cells.

4.Effects on nucleic acid synthesis.

When nucleic acid synthesis is assessed using various techniques, chloramphenicol has minimal influence on the process. Gale and Folkes were the first to describe a stimulation of nucleic acid synthesis in S. aureus, and subsequent studies have found either a small stimulation or no substantial inhibition in E. coli. In the presence of chloramphenicol, developing cells may produce both RNA and DNA. Normally, DNA synthesis is suppressed somewhat, but the DNA that is generated appears to be physiologically active. This has been demonstrated in phage-infected E. coli, where DNA generated in the presence of chloramphenicol may be integrated into viable phage when the antibiotic is withdrawn or can be implicated in genetic recombination. Furthermore, mutations generated in E. coli cells in the presence of chloramphenicol can be expressed, implying that this DNA is genetically functional. DNA produced in human cells in the presence of chloramphenicol has also been shown to be physiologically useful. However, chloramphenicol-RNA appears to be unstable, as it is destroyed and expelled from the cell under nongrowing

circumstances, but conventional RNA remains stable. The electrophoretic mobility, ultracentrifugal sedimentation rate, and ease of dissociation of chloramphenicol-RNA differ from those of regular RNA. Its base ratio, on the other hand, is typical..This RNA's instability may not necessarily result in its expulsion since it may be maintained within the cell in some systems. This shift in RNA properties is mirrored in the makeup of the cell's ribonucleoprotein particles (ribosomes). Growing cell ribosomes contribute 80 to 90% of the cell's RNA and come in various sizes with sedimentation constants of 30, 50, 70, and 100 S, with the 70 S and 100 S components predominating. When chloramphenicol-treated cells are analysed, additional high-concentration peaks at 18 S and 14 S are discovered. When the antibiotic is eliminated, these peaks vanish, and the usual 29-30 S peak is increased.

The 14 S and 18 S peaks are more responsive to sonic oscillation than regular ribosomes and are disaggregated by Mg++ ions but not citrate. They are made up of49% RNA and 51% protein, which is more RNA and less protein than regular

ribosomes. These components, however, are significantly bigger than the soluble RNA. Because chloramphenicol inhibits all protein syntheses, it appears logical to assume that it inhibits ribosomal protein synthesis. This suppression may result in a secondary change in ribosome size, probably because no new protein is created to stabilise the newly generated RNA. At the same time, the 29-30 S particles degrade, and a new smaller 14-18 S particle is rich in RNA forms. There is no reason to suppose that chloramphenicol has a particular activity on these ribonucleoprotein particles.

MATERIAL AND METHODS

SAMPLING SITE:

The sampling site selected was the Gomti river water, located in Lucknow, Uttar Prades. Lucknow, the capital of Uttar Pradesh (India), is located in the part of the central Gangetic plain between North latitudes 26°30' and 27°10' and East longitudes 80°30'and 81°13' (Fig. 3). The city has a humid subtropical climate with a cool, dry winter from December to February and a hot summer from April to June. The temperature extremes varied from 48.9 °C in the summer to 1.67 °C in the winter. Between July and September, the city receives about 900 mm of annual rainfall, mostly from the southwest monsoon. The city's elevation varies from 100 to 130 m above mean sea level and generally slopes to the east. Lucknow is one of the fastest-growing cities in the country, with a population projection of 4.7 million in 2031 from 2.8 million in 2011. Rapid unplanned urbanization has created many problems as it places huge pressure on land, water, housing, transport, health, education etc. This rising population has a major impact on the area's natural resources, especially water quality and quantity. Fresh water is the most important natural resource for life, but overexploitation and unjustified use of water has led to the deterioration of water quality.

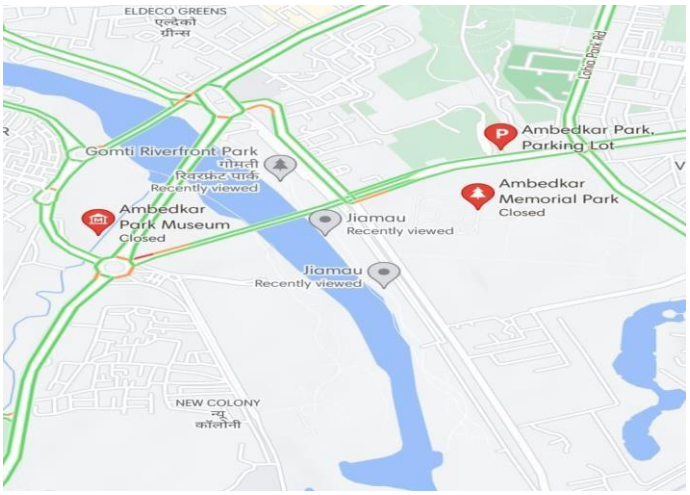


Figure 6: Map showing Gomti river showing sampling location

Several streams cut across Lucknow. Gomti, the major river, flows from North-West to South-East through the city's center. It is one of the city's major public water supply sources, along with groundwater. Generation of sewage and proper treatment and disposal of this waste is the major problem in the city. Poorly drained sewerage systems and lack of treatment capacity of sewage treatment units haveseverely degraded the quality of river water.

COLLECTION OF WATER SAMPLE:

Composited water samples were collected from two different sampling sites in the Gomti river of Lucknow, UP (India), in February 2022 (Fig. 5).The first sampling site (site I) was selected near the localities where effluents were poured directly into the open channel.The second sampling site (site II) was selected about 1 km from the first site, during which another wastewater is added to the river at several points. The sample was composited by mixing 2 L of water collected at two different points at each sampling site to make 4 L composite sample. Samples were aseptically collected, appropriately labeled, and transported on ice to the laboratory

Figure 7: Figure showing the actual site for collection of water sample

for analysis.

Culture media used for enumeration of normal and chloramphenicol-resistant bacterial population

Total numbers of culturable heterotrophic aerobic bacteria and colony-forming units (CFU) were determined by serial dilution and platingon Nutrient Agar. The nutrient

agar plates were prepared with and without chloramphenicol in the media. The Nutrient agar was amended with chloramphenicol to get a final concentration of 100 µg/ml to enumerate the chloramphenicol-resistant microbial population. Serial dilutions of river water(10 mL) were made in 90 mL of normal saline solution. Bacteria were counted at 35°C after 3–5 days of incubation on nutrient agar (peptic digest of animal tissue, 5 g/L; sodium chloride, 5 g/L; beef extract, 1.5 g/L; agar, 15 g/L). The number of colonies was counted and the population was evaluated using the formula

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

Isolation of chloramphenicol-resistant bacteria from water

The water sample was made by vortex mixing 10 ml of water for 30 min. in 90 ml saline solution (0.86%). The supernatant was then serially diluted (up to 10^7), plated on nutrient agar containing 100 µg/ml of chloramphenicol, and incubated at 35°C for 24 h. Ten fast-growing bacterial isolates with distinct colony morphology were picked and purified by repeated streaking on nutrient agar (Table 2).

Subculturing for pure culture preparation

Under the aseptic technique, the inoculating loop was sterilized in the Bunsen burner by putting the loop in the flame until it was red hot. It was allowed to cool. An isolated colony was picked from the agar plate culture and stroked over the first quadrant using close parallel streaks. The loop was flamed again and allowed to cool. It was returned to the edge of area one that just streaked over the second quarter of the plate. This process was continued three to four times. The streaked plate was incubated at 37°C for 24hrs. The colonies grown were observed on the plate carefully.

Preparation of agar slant:

The nutrient agar was weighed and dissolved in the desired volume of double distilled water in a conical flask and boiled to mix properly. The solution was mixed properly to make the uniform media solution, dispensed in the test tubes 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 antimicrobialagents using the 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 inparentheses: Ofloxacin (C5mcg), Streptomycin (C 10mcg), Sulfadiazine(C 100mcg), Amoxicillin (C 30mcg), ,nalidixic acid (c 30mcg), neomycin (N 30).

RESULT AND DISSCUSION

Enumeration of microbial population in the Gomti River water .

1.OBJECTIVE -Isolation of microbial population.

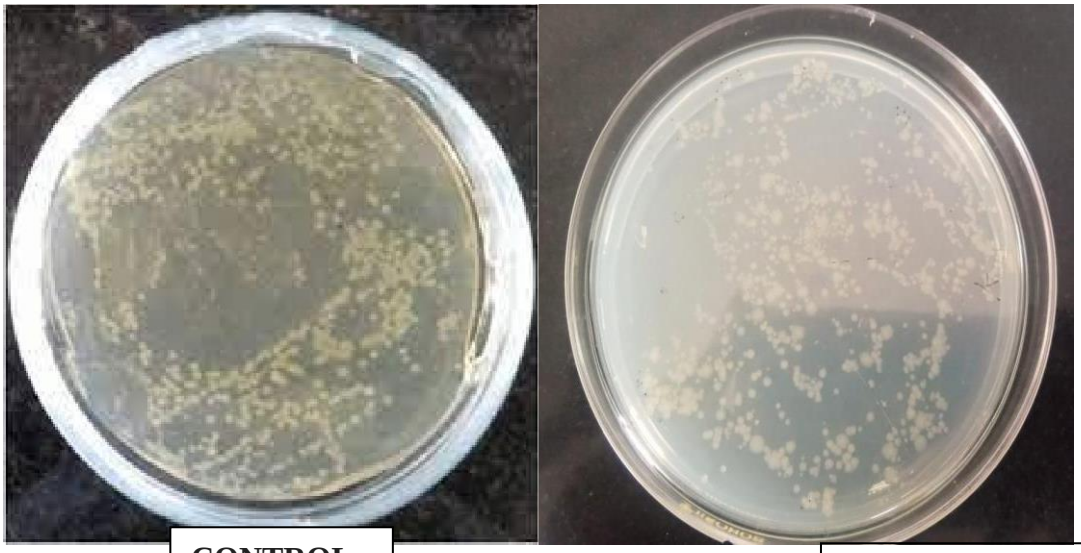
Table 1. The heterotrophic bacterial population in the Gomti River water; (A) without added antibiotic; (B) with added chloramphenicol in the medium

Sample	**Heterotrophic bacteria (control)**	**Heterotrophic bacteria (Antibiotic)**
GRW3	$6.20X10^{8}\pm2.83X10^{7}$	$1.04X10^{5}\pm4.67X10^{3}$

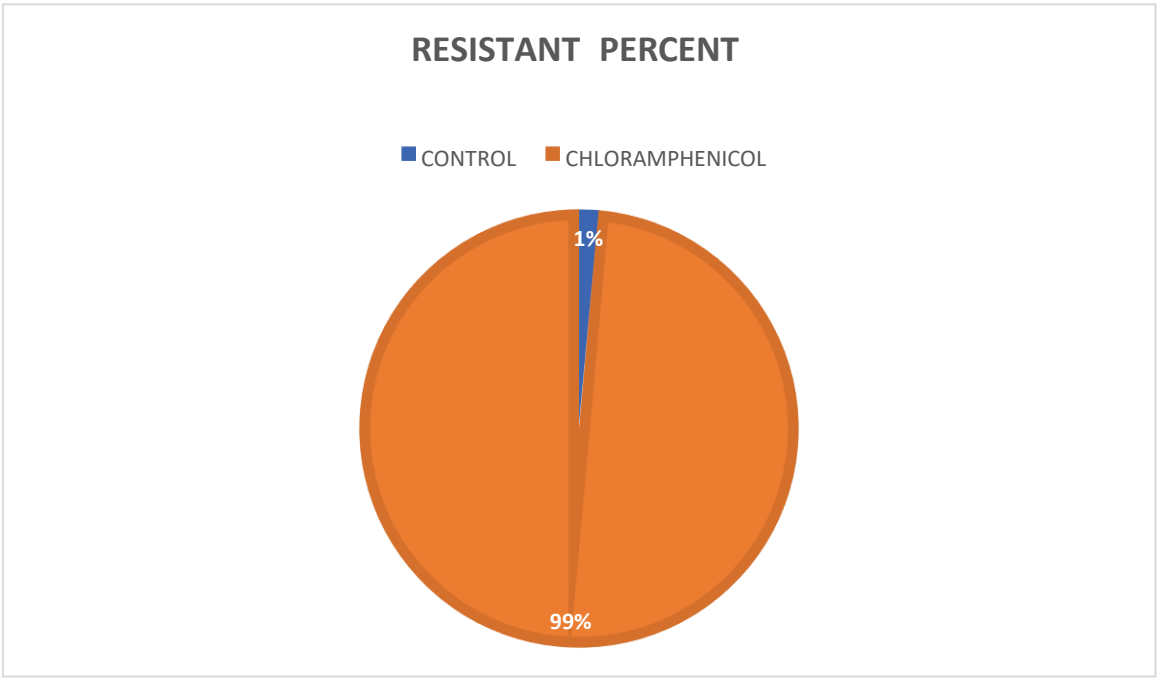
The water samples collected from the Gomti River, Lucknow, show a population of chloramphenicol resistance bacteria.The average heterotrophic bacterial count in the Gomti water was found to be $6.20X10^{8}\pm2.83X10^{7}$CFU/ml when no antibiotic was added to the growth media (Table 1). However, the bacterial population reduced to $1.04X10^{5}\pm4.67X10^{3}$ CFU/ml when chloramphenicol was added to the growth media (Table 1).There was a reduction in the total bacterial population when antibiotic The water samples collected from the Gomti River, Lucknow, show a population of chloramphenicol-resistant bacteria. The average heterotrophic bacterial count in the Gomti water was found to be $5.08X10^{8}\pm1.01X10^{8}$ CFU/ ml when no antibiotic was added to the growth media (Table 1). However, the bacterial population reduced to $1.24X10^{3}\pm7.07X10^{2}$CFU/ ml when chloramphenicol was added to the growth media (Table 1). There was a reduction in the total bacterial population when antibiotic was added to the medium. Heterotrophic bacterial analysis on the growth media shows that the Gomti river water contains a population of chloramphenicol-resistant bacteria. The analysis of the results revealed that Gomti water contains about 1% of chloramphenicol-resistant bacteria.

Antibiotic residues and antibiotic-resistant bacteria enter the aquatic environment, including river water, through various pathways such as discharge of industrial effluent, hospital and municipal wastewater, and agricultural runoff. Rivers appear to be a reservoir of antibiotic resistance and play an important role in the transportation of antibiotic resistance between various environmental compartments. River water might create possible pathways for antibiotic resistance transmission between the environment, humans, and animals. In this regard, similar patterns in antibiotic resistance of Escherichia coli (E. coli) isolates from humans, animals, and their water environment have been reported [18,19]. E.

coli is a useful indicator of fecal contamination and is considered a reservoir of antibiotic resistance in bacterial communities [20].



Percent Of resistant bacteria



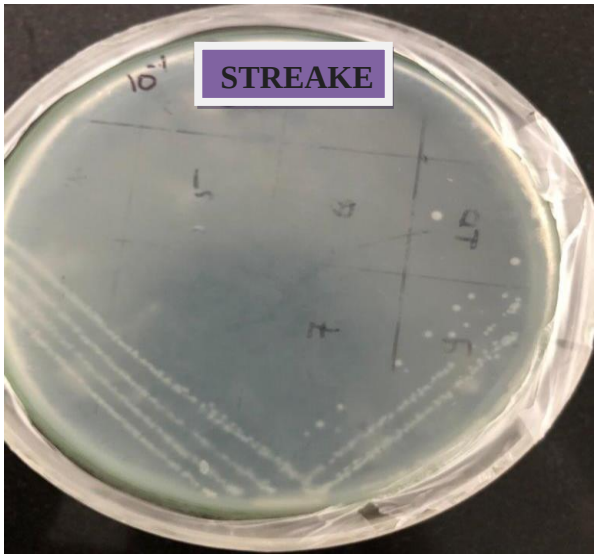
OBJECTIVE 2: Isolation of antibiotic resistant bacteria

Chloramphenicol-resistant bacteria were isolated on the LB agar containings chloramphenicol at a 100 µg/ml concentration. From which nine bacterial isolates

selected based on morphology and colour. The well-separated colonies were selected and purified by repeatedly re-streaking (Table 2). The bacterial isolates were named as GC1,GC2,GC3,GC4,GC5,GC6,GC7,GC8, and GC9.

Table 2. The morphological and physical appearance of isolated and purified isolates

Isolate	Colony morphology		
	Colour	**Shape**	**Size**
GCV-1	white	Round	Small
GCV-2	yellow	Irregular	Small
GCV-3	orange	Regular	Small
GCV-4	cloudy	Regular	Small
GCV-5	brown	Regular	Large
GCV-6	Yellow	Irregular	Small
GCV-7	Cloudy	Irregular	Large
GCV-8	White	Regular	Large
GCV-9	Orange	Irregular	Small


STREAKE

OBJECTIVE 3:-

(a) Multiple antibiotic resistance patternsof the isolated bacteria

Five antibiotic discs i.e., Ofloxacin-5mcg (OF),Sulfadiazine–(SZ)100mcg , Amoxicillin-30mcg (AMX), Streptomycin-10mcg (S), Nalidixic acid-30mcg (NA)were used to check the antibiotic resistance pattern.

Table 3. Antibiotic sensitivity test of the isolated bacteria

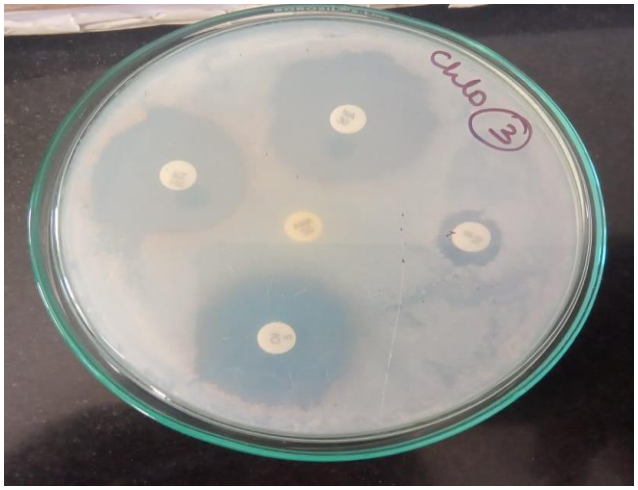
BACTERIA	ANTIBIOTICS				
	Of	Sz	Amx	S	Na
GCV 1	S	S	R	S	S
GCV 2	S	S	R	R	S
GCV 3	S	S	R	R	S
GCV 4	S	S	R	R	S
GCV 5	S	S	R	R	S
GCV 6	S	S	R	R	S
GCV 7	S	S	R	S	S
GCV 8	S	S	R	R	S
GCV 9	S	S	R	S	S
GCV 10	S	S	R	S	S

Table 4. Multiple antibiotic resistance among the isolated bacteria.

ANTIBIOTICS	CONCENTRATION	NO.OF ISOLATES	RESISTANCE ISOLATE(%)
Ofloxacin	5mcg	0	0
Sulfadiazine	100mcg	0	0
Amoxicillin	30mcg	10	100
Streptomycin	10mcg	6	60
Nalidixic acid	30mcg	0	0

Table 5. Antibiotic resistance pattern of 10 isolates from water of Gomti River

NO. OF ANTIBIOTIC	NO. OF ISOLATES	RESISTANCE PATTERN
2	6	Amx, S
1	4	Amx



From table 4, we can conclude that 100% of bacterial isolates were resistant to Amoxicillin; 60% were resistant to Streptomycin.

Table 5 shows that from the ten bacterial isolates, six isolates were resistant to 2 antibiotics (Amx, S). The other four isolates were resistant to oneantibiotics(Amx). Thus all the bacterial isolates show a different pattern of resistance. Correia et al. (2020) isolated 579 ampicillin-resistant bacteria and tested were resistance to 10 antibiotics. They found that 92.7% of the isolates were resistant to four or more antibiotic classes, indicating a high level of multi-resistance. They reported 143 resistance profiles among the isolated bacteria.Thus all the bacterial isolates show a different pattern of resistance. Correia et al. (2020) isolated 579 ampicillin-resistant bacteria and tested were resistance to 10 antibiotics. They found that 92.7% of the isolates were resistant to four or more antibiotic classes, indicating a high level of multi-resistance. They reported 143 resistance profiles among the isolated bacteria.

Conclusion

The culturable bacterial population from the Gomti river water contains highly-multi-resistant bacteria, some of which have been isolated and characterized for multi-drug resistance profiles to 5 antibiotics, which show a diversity of combinations of resistances. The bacterial population isolated from river water is resistant to multiple antibiotics, and it can be concluded that the river water contains pathogenic bacteria having multi-drug resistance. Multi-drug resistance in pathogenic bacteria is a significant challenge that leads to high morbidity and mortality. The sample collection sites have anthropogenic inputs of fecal origin, and the site's location near the anthropogenic source may be the reason for high antibiotic resistance. The microbial population with multi-drug resistance can be managed by restricting the usage of antibacterial drugs and making people aware of the ill effects. Also, the patients should be encouraged to complete the required dosage of the medicines so that the gut microbes cannot develop drug resistance. Further, decentralized domestic wastewater treatment should be encouraged to reduce the pathogenic bacteria reaching the river.

REFERENCE

Blondeau, J.M. and Yaschuk, Y., 1995. In vitro activities of ciprofloxacin, cefotaxime, ceftriaxone, chloramphenicol, and rifampin against fully susceptible and moderately penicillin-resistant Neisseria meningitidis. Antimicrobial agents and chemotherapy, 39(11), pp.2577-2579

Cavalli, L.L. and Maccacaro, G.A., 1950. Chloromycetin resistance in E. coli, a case of quantitative inheritance in bacteria. Nature, 166(4232), pp.991-992

Ehrlich, J., Bartz, Q.R., Smith, R.M., Joslyn, D.A. and Burkholder, P.R., 1947. Chloromycetin, a new antibiotic from a soil actinomycete. Science, 106(2757), pp.417-417.

Gale, E.F. and Paine, T.F., 1951. The assimilation of amino-acids by bacteria. 12. The action of inhibitors and antibiotics on the accumulation of free glutamic acid and the formation of combined glutamate in Staphylococcus aureus. Biochemical Journal, 48(3), p.298.

Gale, E.F. and Folkes, J.P., 1953. The assimilation of amino-acids by bacteria. 15. Actions of antibiotics on nucleic acid and protein synthesis in Staphylococcus aureus. Biochemical Journal, 53(3), p.493.

GREENBERG, P.A. and SANFORD, J.P., 1967. Removal and absorption of antibiotics in patients with renal failure undergoing peritoneal dialysis: tetracycline, chloramphenicol, kanamycin, and colistimethate. Annals of Internal Medicine, 66(3), pp.465-479.

Hammett-Stabler, C.A. and Johns, T., 1998. Laboratory guidelines for monitoring of antimicrobial drugs. Clinical chemistry, 44(5), pp.1129-1140..

Kauffman, R.E., Miceli, J.N., Strebel, L., Buckley, J.A., Done, A.K. and Dajani, A.S., 1981. Pharmacokinetics of chloramphenicol and chloramphenicol succinate in infants and children. The Journal of pediatrics, 98(2), pp.315-320.

Kolosov, M.N., Shemiakin, M.M., Khoklov, A.S. and Gurevich, A.I., 1961. Chloramphenicol. KhimiaAntibiotikow I. USSR: IedetstoAkademiiNavk

Koup, J.R., Lau, A.H., Brodsky, B. and Slaughter, R.L., 1979. Chloramphenicol pharmacokinetics in hospitalized patients. Antimicrobial Agents and Chemotherapy, 15(5), pp.651-657.

KUNIN, C.M., 1967. A guide to use of antibiotics in patients with renal disease: A table of recommended doses and factors governing serum levels. Annals of Internal Medicine, 67(1), pp.151-158.

Manyan, D.R., Arimura, G.K. and Yunis, A.A., 1972. Chloramphenicol-induced erythroid suppression and bone marrow ferrochelatase activity in dogs. The Journal of laboratory and clinical medicine, 79(1), pp.137-144.

Moffa, M. and Brook, I., 2015. 26—Tetracyclines, Glycylcyclines, and Chloramphenicol. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases,, pp.2607-2618.

Musa, A., Yakasai, I.A., Garba, M. and Mathias, B.K., 2010. In vitro availability of chloramphenicol in the presence of cadmium and lead salts. Journal of Basic and Clinical Pharmacy, 1(2), p.97.

Rebstock, M.C., Crooks, H.M., Controulis, J. and Bartz, Q.R., 1949. Chloramphenicol (Chloromycetin). 1 IV. 1a chemical studies. Journal of the American Chemical Society, 71(7), pp.2458-2462.

RICH, M.L., RITTERHOFF, R.J. and HOFFMANN, R.J., 1950. A fatal case of aplastic anemia following chloramphenicol (Chloromycetin) therapy. Annals of Internal Medicine, 33(6), pp.1459-1467.

Slaughter, R.L., Cerra, F.B. and Koup, J.R., 1980. Effect of hemodiaiysis on total body ciearance of chloramphenicol. American Journal of Hospital Pharmacy, 37(8), pp.1083-1086.

Smith, A.L. and Weber, A., 1983. Pharmacology of chloramphenicol. Pediatric Clinics of North America, 30(1), pp.209-236.

Suarez, C.R. and Phillip Ow, E., 1992. Chloramphenicol toxicity associated with severe cardiac dysfunction. Pediatric cardiology, 13(1), pp.48-51.

Sutherland, J.M., 1959. Fatal cardiovascular collapse of infants receiving large amounts of chloramphenicol. AMA journal of diseases of children, 97(6), pp.761-767.

Thompson, W.L., Anderson, S.E., Lipsky, J.J. and Lietman, P.S., 1975. Overdoses of chloramphenicol. JAMA, 234(2), pp.149-150.

Wisseman Jr, C.L., Smadel, J.E., Hahn, F.E. and Hopps, H.E., 1954. Mode of action of chloramphenicol I: action of chloramphenicol on assimilation of ammonia and on synthesis of proteins and nucleic acids in Escherichia coli. Journal of bacteriology, 67(6), pp.662-673.

Yogev, R., Kolling, W.M. and Williams, T., 1981. Pharmacokinetic comparison of intravenous and oral chloramphenicol in patients with Haemophilus in fluenzae Meningitis. Pediatrics, 67(5), pp.656-660.

Yunis, A.A., 1973, December. Chloramphenicol induced bone marrow suppression. In Seminars in hematology (Vol. 10, No. 3, pp. 225-234). WB Saunders Ltd.