A DISSERTATION ON

Erythromycin resistant bacteria from gomti river water show multiple antibiotic

resistance

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FOR THE

DEGREE OF MASTERS OF SCIENCE

IN MICROBIOLOGY

BY

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M. Sc. Microbiology (IV Semester)

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UNDER THE SUPERVISION OF

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TO WHOM IT MAY CONCERN

This is to certify that **Mansi Dey**, a student of M.Sc Microbiology (VI semester), Integral University has completed his/her three months dissertation work entitled "**Erythromycin resistant bacteria from gomti river water show multiple antibiotic resistance**" successfully. She has completed this work from March –June from Department of Biosciences, Integral University, 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 bright future.

(**Dr. Snobar S. Mir**)
Head Department of Biosciences,
Integral University, Lucknow

CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **MANSI DEY** during the month March - June, 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and script of the thesis has been written by the candidate himself. The thesis entitled is **Erythromycin resistant bacteria from gomti river water show multiple antibiotic resistance** 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 (U.P).

Dr. Mohd. Ikram Ansari
 Assistant professor
 Department of Bioscience

DECLARATION

I hereby declare that the present work on "High prevalence of Sulfonamide resistant bacteria in the Gomti river water" is a record of original work done by me under the guidance of Dr. Mohd. Ikram Ansari, Assistant Professor,Integral University, during the month of March -june 2022, at Integral University, Lucknow. All the data which were provided in this were through our 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   **Mansi Dey**

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Mansi Dey

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AR: Antimicrobial Resistance

ARB: Antibiotic Resistance Bacteria

ARG: Antibiotic Resistance Gene

COD: Chemical Oxygen Demand

DNA: Deoxyribonucleic Acid

DO: Dissolved Oxygen

DEA: Drug Enforcement Administration

EPA: Environmental Protection Agency

FDA: Food and Drug Administration

GI: Gastrointestinal Tract

HWW: Hospital Waste Water

MDR: Multiple Drug Resistance

PCR: Polymerase Chain Reaction

SCBA: Self- Contained Breathing Apparatus

WWTP: Wate Water Treatment Plant

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INTRODUCTION

Since ancient times, humans have randomly disposed of waste into the environment, such as in rivers and cesspits. The industrial revolution of the late eighteenth and early 19th centuries was a period that saw increased disposal of toxic organic chemicals by direct release into the environment. Many of these toxic molecules had antimicrobial activity, and it can be assumed that microbes resistant to these toxins multiplied in such environments. As a modern example, one can cite the concentrations of heavy oils dumped near detection stations in the distant early warning line at the end of the Second World War. These sites are now excellent sources of bacteria with enhanced biodegradation capacities and have been extensively studied in recent years. In the past 50 years, we have seen the rapid evolution of a new plague—that of worldwide antibiotic resistance. Though not a disease, antimicrobial resistance (AR) fails to effectively prevent and treat many diseases, leading to widespread untreatable microbial infections and greatly increased morbidity and mortality, a plague of resistance genes (Davies and Davies, 2010). The global use of antibiotics at low cost, auto medication, and short duration of treatment has accelerated, extended, and expanded the spectra of resistance worldwide.

The amounts of antibiotics and waste disposed of in this way cannot be accurately identified. However, according to recent estimates by the Union of Concerned Scientists in the United States, antibiotic use for nontherapeutic purposes in three major livestock sectors (chickens, cattle, and swine) was about eight times more than the consumption for human medicine (Mellon et al., 2001). Aquatic ecosystems have been identified as hotspots of resistance mechanisms (Rizzo et al., 2013). This is due to the large diversity of pathogenic and commensal microorganisms and the continuous discharge of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) into these environments. Over the past 15 years, increasing attention has shifted toward the identification and removal mechanisms of micropollutants from wastewater and sludge. Micropollutants are persistent organic or mineral substances such as pharmaceuticals and personal care products, detergents, and pesticides whose discharge, even at very low concentrations, is a constant growing environmental contamination (Luo et al., 2014).

Since 1890 with the building of the first biological wastewater treatment plant (WWTP) in Worcester, Massachusetts, advances in wastewater treatment technology have been improving the efficient removal of biodegradable organic pollutants. Currently,

enhanced biological phosphorus removal processes have not only enabled the removal of traditional carbonaceous contaminants but also reduced phosphorus concentrations to very low levels (‹0.1 mg/L) in the effluent discharge (Zuthi et al., 2013).

The occurrence of ARB and ARGs in the two main by-products of wastewater treatment systems (biosolids and effluent discharge) has been reported frequently. Currently, effluent water quality standards, prior to discharge, are limited to controlling the concentrations of carbonaceous biochemical oxygen-demanding matter, suspended solids, total residual chlorine, and un-ionized ammonia. No regulatory guidelines exist to monitor and control the levels of ARGs in bacteria and extracellular DNA from lysed microbial cells in the effluent discharge. Accordingly, studies have reported that Antimicrobial Resistance Genes and Wastewater Treatment 3 antibiotic resistance determinants and MDR pathogens are transported from the effluent to the receiving water (Iwane et al., 2001; Galvin et al., 2010; Goñi-Urriza et al., 2000). For example, LaPara et al. (2011) showed that the quantities of three tetracycline resistance genes were significantly higher in a tertiary treated effluent discharge than in receiving water samples in the St. Louis River, Duluth-Superior Harbor, and Lake Superior, USA.

Hospital wastewater is probably a major contributor to the spread of pathogenic MDR bacteria in WWTPs (Brown et al., 2006). Due to the presence of constant subinhibitory levels of broad-spectrum antimicrobials, hospital sewage creates a perfect situation for exchanging ARGs and their combinations between clinical pathogens and environmental bacteria (Amador et al., 2015; Santoro et al., 2015).

In this respect, the ratios of influent wastewater from institutions (including hospitals), blackwater (excreta, urine, and fecal sludge), graywater (kitchen and bathing wastewater), storm water, and other urban runoff sources are important determinants of the input quality, the frequency of detection of ARGs and pathogenic ARB, and the dissemination of antibiotics and AR from treatment plants (Harris et al., 2013). Over the past few years, some European countries have constructed specialized WWTPs to provide separate treatment of hospital wastewater (HWW).

With membrane bioreactors as a pretreatment, ozonation and powdered and granulated activated carbon have been proposed as the most attractive options to

remove micropollutants from HWW (Beier et al., 2010; Beier et al., 2012; Kovalova et al., 2013). Very recently, Chonova and coworkers (2016) published a comparative study on the efficiency of the removal of antibiotics from parallel wastewater systems providing separate treatment of hospital and urban wastewater.

Despite the higher concentration of antibiotics in the hospital influent and treated effluent, the results indicated increased removal efficiency of antibiotics during the separate treatment of HWW. It was also demonstrated that biofilm communities receiving hospital treated effluent had lower bacterial diversity and less developed biomass. Observations from this study confirm the adaptations of wastewater bacterial communities receiving HWW. With respect to the dedicated treatment of hospital waste, more studies are needed to reveal the mechanisms by which adapted biofilm microbial communities can be transferred to aquatic environments.

Urban water supply networks are susceptible to intentional, accidental chemical, and biological pollution, which threatens consumers' health. In recent years, drinking-water pollution incidents have occurred frequently, seriously endangering social stability and security. Real-time water quality monitoring can be effectively implemented by placing sensors in the water supply network. However, locating the source of pollution through the data detection obtained by water quality sensors is a challenging problem. The difficulty lies in the limited number of sensors, a large number of water supply network nodes, and dynamic user demand for water. This leads the pollution source localization problem to an uncertainty, large-scale, and dynamic optimization problem. In this paper, we mainly study the dynamics of the pollution source localization problem. Previous studies of pollution source localization assume that hydraulic inputs (e.g., water demand of consumers) are known. However, because of the inherent variability of urban water demand, the problem is a fluctuating dynamic problem of consumer's water demand.

Wastewater treatment plants (WWTPs) are important hotspots for spreading antibiotic resistance. However, the release and impact factors of antibiotic-resistant bacteria and the relevant genes in WWTPs have rarely been investigated over long periods. In WWTP effluent and biosolids, a high prevalence of heterotrophic bacteria resistant to vancomycin, cephalexin, sulfadiazine, and erythromycin were detected, each with a proportion of over 30%. The sampling season imposed considerable influence on the

release of all ARB. High release loads of most ARB were detected in the spring, while low release loads were generally found in the winter.

In comparison, the ARG loads changed only slightly over various seasons. No statistical relevance was found between all ARB abundances and their corresponding genes over the long-term investigation period. This inconsistent behavior indicates that bacteria and genes should be considered when exploring resistance characteristics in wastewater. This study assessed the fate of bacteria and antibiotic resistance to five commonly used antibiotics. Considering all these points, the following objectives were designed to address during the study period.

Objectives:
1. Evaluation of the erythromycin-resistant bacterial population in the Gomti river water near the wastewater disposal site of Lucknow.
2. Isolation of the erythromycin-resistant bacteria from the Gomti river water of Lucknow.
3. Determination of multiple antibiotic resistance patterns in the bacteria isolated from the Gomti river water of Lucknow.

REVIEW OF LITERATURE

Erythromycin nowadays is the most important vital macrolide antibiotics. It is produced by the actinomycete Streptomyces erythreus and was first introduced into clinical medicine in 1952 by McGuire and associates. Erythromycin has proved to be a safe, effective therapy for many commonly encountered infections, and specific indications for its use continue to increase. Erythromycin is composed of a macrocyclic lactone ring linked to two sugar moieties. Erythromycin base is bitter. The crystalline compound is poorly soluble in water and has a pk of 8.8, which is rapidly inactivated by the acid. Modifications of the drug and its pharmaceutical preparations have been made to improve absorption and subsequent serum levels. This is achieved either by providing an enteric coating or by "film" coating, which protects the erythromycin base from acid degradation, and by preparing salt and an ester, the salt of an ester to modify the chemical structure. Five oral preparations and two intravenous water-soluble salts are available. Intramuscular injection is painful and not recommended. Like the other macrolide antibiotics, which act at the level of the 50S ribosomal subunit. In susceptible microorganisms, erythromycin inhibits RNA-dependent protein synthesis by blockage of transpeptidation and/or translocation reactions without affecting the synthesis of nucleic acid. The antibacterial activity of lincomycin and chloramphenicol may be interfered by erythromycin because of competition at the same binding sites. The action of erythromycin may be bactericidal or bacteriostatic depending on microbial species, growth phase, the density of inoculum, and drug concentration. In addition, the activity of the drug increases markedly with increasing ph. (Marie J. et al. 1982)

Erythromycin is a macrolide antibiotic first isolated in 1952. By today's standards, it can no longer be considered a broad-spectrum drug, particularly when compared with the newer beta-lactam antibiotics, quinolones, or aminoglycosides. And yet, its modest spectrum is a very useful one and includes organisms against which many of the newer agents are inactive. Some indications for its use have been subtracted as the years have passed, such as gonorrhea and severe staphylococcal infections. However, new diseases and indications have continued to arise, including Legionnaire's disease, campylobacter enteritis, and chlamydia infections. The Medical Letter lists erythromycin as drug of first choice for nine organisms and as an alternative drug. Because of its useful spectrum and long track record of safety, erythromycin use is increasing in many institutions. Erythromycin is elaborated by the actinomycete

Streptomyces erythraeus. It was first isolated in 1952, and the first clinical paper appeared in the same year. It is the only macrolide antibiotic to have received substantial clinical attention in the United States. A macrocyclic lactone ring characterizes macrolides. Although sometimes discussed with clindamycin or chloramphenicol, erythromycin chemically is unrelated to these compounds. Erythromycin is only slightly soluble in water, is a weak base, and has a pKa of 8.8. The active form of the drug is erythromycin base. It is a bitter crystalline compound readily inactivated in an acidic medium such as that found in the human stomach. (David C. Brittain, MD 1987)

Erythromycin is one of a group of antibiotics with a macrocyclic lactone nucleus, called a macrolide. 74 Of the six macrolide antibiotics that have been made available commercially, erythromycin, oleandomycin, and tylosin (agricultural use only) are available in the United States. Wileyl08 reported the structural formula of erythromycin. The propionyl ester of erythromycin was developed by Stephens and was further modified by the preparation of its lauryl sulfate salt. (R. S. GRIFFITH et. al 1970)

Resistance of Bacteria to Erythromycin

Variants with high degrees of resistance to erythromycin were not isolated from small volumes of cultures of erythromycin-sensitive bacteria which had not previously been exposed to that antibiotic. When cultures of erythromycin-sensitive staphylococci (which were completely inhibited by 0.4 µg/ml in the plate-dilution test) were grown from a 10% inoculum in broth in the presence of 16 mUg/ml of erythromycin, strains of varying resistance could be recovered after 48 hours; these required concentrations of erythromycin ranging from 0.2 to 25 mUg/ml for complete inhibition. By repeated subcultures of erythromycin-sensitive bacteria on blood agar containing graded concentrations of erythromycin it was possible to obtain strains of increased resistance to this antibiotic. The rate at which this resistance increased varied markedly with the strain. Several strains of Staphylococcus aureus, an enterococcus and a pneumococcus each increased more than 512-fold in from 3 to 12 such subcultures. Strains of only moderately increased resistance were obtained after 20 similar subcultures of a strain of hemolytic streptococcus and one of Streptococcus viridans. The strains of increased resistance resulting from repeated subcultures on the erythromycin-containing agar retained that resistance after 10 different subcultures in

antibiotic-free broth. Strains of increased resistance to erythromycin derived by repeated subcultures in that antibiotic were almost invariably similar to their respective parent strains in their sensitivity to 8 other antibiotics; however, an erythromycin-resistant strain of type 3 pneumococcus had apparently increased in sensitivity to neomycin following its repeated subcultures in erythromycin. Staphylococcal strains of markedly increased resistance to erythromycin were obtained from blood cultures of 2 patients with endocarditis following 7-10 days of treatment with this antibiotic. The colonial, morphologic and biochemical characteristics of the erythromycin-resistant strains obtained by repeated subcultures in erythromycin-containing media resembled those of the respective parent strains from which they were derived in almost every instance. Staphylococcus aureus strains lost their ability to produce coagulase and exhibited other changes in their biochemical properties following their repeated subcultures in the presence of erythromycin. Similar changes were not observed in the 2 strains of Staphylococcus aureus, which had become resistant during erythromycin therapy.

Mechanism of Action

Erythromycin does not bind to 80S ribosomes but instead binds to the 50S subunit of 70S ribosomes of susceptible microorganisms, an action that inhibits protein synthesis. Erythromycin does antagonize the binding of chloramphenicol to the 50S subunit; however, the mechanisms of action of these two antibiotics differ in that erythromycin inhibits the translocation reaction and chloramphenicol inhibits the peptidyl transferase reaction. (Edwards DI et al. 1980)

The Antibacterial Action of Erythromycin

The results of studies on some properties of erythromycin, which may be important in its clinical and laboratory applications have been reported. Erythromycin solutions retained their activity after prolonged storage in the cold or in the frozen state. However, they showed progressive deterioration after several days at room or incubator temperatures or after brief exposures to 60°C or higher. Filtration of erythromycin through bacterial filters entailed the loss of some activity. The antibacterial action of erythromycin increased progressively with increasing alkalinity of the culture medium, within the pH range of bacterial growth. Many substances that

may affect the action of other antimicrobial agents had no important effect on erythromycin. Erythromycin inhibiting substances could not be demonstrated in cultures of erythromycin-resistant bacteria. The size of the inoculum affected the results of sensitivity tests with erythromycin, but there was considerable tolerance within the range of inocula used in clinical testing. The broth-dilution and agar-plate dilution methods generally gave comparable results in tests for the sensitivity of bacteria to erythromycin, but the values obtained by the agar method often were higher, particularly with some coliform organisms. Tests done on more than 1000 bacterial strains, most recently isolated from patients, indicated that erythromycin was most active against the Gram-positive cocci and quite active against strains of Neisseria, diphtheria bacilli and Hemophilus. However, it could be considered inactive against most coliform and enteric bacilli for practical purposes. Concentrations of erythromycin in plasma after single oral doses varied widely but generally proportional to the dose. Maximum concentrations were found 1 or 2 hours after a dose, and no activity was demonstrated at 6 hours except following doses of 1.0 g. Significant concentrations were maintained in the plasma with oral doses of 250 mg or more every 3 or 4 hours. The amounts of erythromycin activity recovered in the urine appeared to be small and erratic after ingestion of single doses or after repeated small doses, particularly early in the course of therapy; however, on continuous therapy with divided doses, up to 15% of the amount ingested daily could be demonstrated in active form in the urine. Results of studies on the mode of action of erythromycin and on the resistance of bacteria to that agent are presented in the succeeding papers. **(**Thomas H. Haight, Maxwell Finland)

Antimicrobial Activity.—Haight and Finland3 demonstrated a fourfold difference in the minimal inhibitory concentrations (0.2 to 0.02 µg/ml) of 102 - and 105 -fold diluted inocula of overnight broth cultures of a Streptococcus and a twofold difference in the minimal in Mayo Clin Proc 60:189-203, 1985 189 190 ERYTHROMYCIN Mayo Clin Proc, March 1985, Vol 60 inhibitory concentrations (0.04 to 0.02 µ^πιl) of similarly diluted inocula of Sarcina lutea. Hobson8 noted no differences in the minimal inhibitory concentrations when 40 strains of Staphylococcus aureus were tested at inocula ranging from 5 x 105 to 50 colony-forming units.(HaightTH, 1982)

Erythromycin continues to be a valuable and useful antimicrobial agent in children. Its low index of toxicity, freedom from sensitization, and reliable absorption, when

administered orally, make it an attractive agent in treating a variety of minor respiratory and skin infections, especially in situations with real or potential allergy to penicillin exists. Other major uses are in the eradication of the carrier state in whooping cough and in diphtheria, especially in those instances when oral therapy can be tolerated. Despite use over more than two decades, resistance developing in formerly susceptible organisms has not been a problem and thus seems unlikely to become so in the future. (M.D. Charles   M.Ginsburg M.D. Heinz F.Eichenwald 2006)

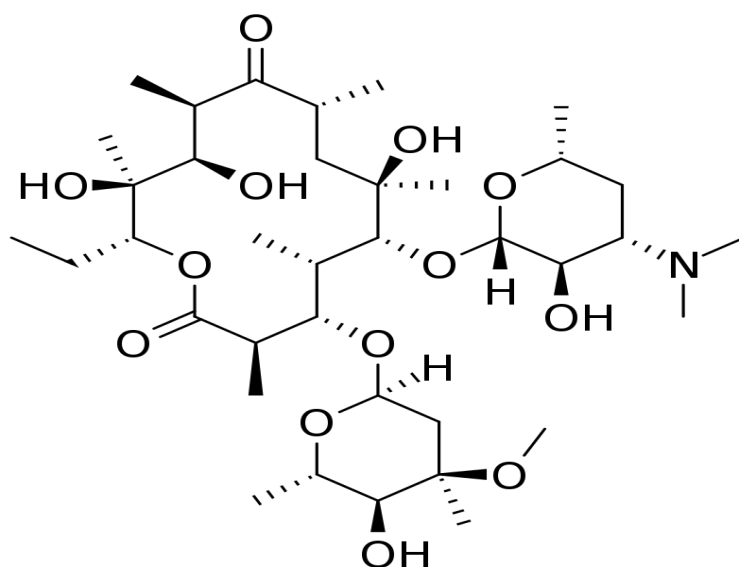Figure 1- Structure of Erythromycin

PHYSICAL PROPERTIES

Molecular formula   -   C37H67NO13

Molecular weight   -   733.937

Half-life, days and hours   -   11.5* (sediments)c

- 20* (soil)

- 5.8-365 (pond water system)

- 1.6-2.0 (humans)

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Physical Description –

Erythromycin stearate appears as fluffy colorless powder or fine white powder. (NTP, 1992)

Color/Form - Hydrated crystals from water. (NJ: Merck and Co., Inc., 2006)

Odor – odorless

Taste – Bitter

Boiling Point – 719.69

Melting Point - 212 to 219 °F (NTP, 1992)

Solubility - less than 1 mg/mL at 72° F (NTP, 1992)

pH Gradient —Weak bases accumulate in highly acidic intracellular compartments such as lysosomes and reach an equilibrium that is dependent on the pH gradient between the lysosomes and the intracellular fluid.

This material is assumed to be combustible. As with all dry powders, it is advisable to ground mechanical equipment in contact with dry material to dissipate the potential of static electricity.

Highly lipid-soluble antibiotics such as rifampin, chloramphenicol, and lincomycin accumulate within phagocytes.181 Formalinkilled or viable phagocytes concentrate intracellular rifampin or chloramphenicol similarly, suggesting that entry of these agents into phagocytes is not an energy-dependent process and is more likely related to lipid solubility, which allows the drug to penetrate through the cell-surface membrane. (Johnson JD, et al. 1980)

Erythromycin resistance determinants include Erm methylases, efflux pumps, and inactivating enzymes. To distinguish the different mechanisms of resistance in clinical isolates, PCR primers were designed so that amplification of the partial gene products could be detected in multiplex PCRs. This methodology enables the direct sequencing of amplified PCR products that can be used to compare resistance determinants in clinical strains. Further, this methodology could be useful in surveillance studies of erythromycin-resistant determinants.

Absorption

Orally administered erythromycin is readily absorbed. Food intake does not appear to exert effects on serum concentrations of erythromycin. Some interindividual variation exists in terms of erythromycin absorption, which may impact absorption to varying degrees. The Cmax of erythromycin is 1.8 mcg/L and the Tmax is 1.2 hours. In one pharmacokinetic study, the serum AUC of erythromycin after the administration of a 500mg oral dose was 7.3±3.9 mgh/l. Erythromycin is well-known for its variable bioavailability (18-45%) after oral administration and its susceptibility to breaking down under acidic conditions. Absorption of orally administered erythromycins occurs mainly in the duodenum. The bioavailability of the drugs is variable and depends on several factors including the particular erythromycin derivative, the formulation of the dosage form administered, acid stability of the derivative, presence of food in the GI tract, and gastric emptying time. In an in vitro model using human skin, erythromycin was absorbed into the stratum corneum following topical application of 10-20 mg of the drug in a vehicle containing dimethylacetamide and 95% alcohol. The drug does not appear to be absorbed systemically following twice daily application of a 2% solution of the drug in a vehicle containing 77% alcohol and polyethylene glycol and acetone. It is not known if erythromycin is absorbed from intact or denuded skin, wounds, or mucous membranes following topical application of an ointment containing the drug.

Route of Elimination

In patients who have normal liver function, erythromycin which specially concentrates in the liver and is then excrete in the bile. Under 5% of the orally dose of erythromycin is found excreted in the urine. A high percentage of absorbed erythromycin is not accounted for, but likely is metabolized.

Volume of Distribution

Erythromycin mostly found in body fluids and is present in leucocytes and inflammatory liquid. Spinal fluid concentrations of erythromycin are apparently low, however, the diffusion is through the blood-brain barrier increases in meningitis, likely

due to the presence of inflamed tissues which are easily penetrated. Erythromycin crosses the placenta.

Erythromycin is rather slowly absorbed after oral administration peak serum concentrations ranged from 0.1 to 4.8 ug/mL according to the erythromycin's form and coating. Oral absorption is less than 50%, and erythromycin is degraded by gastric acid. It is absorbed in the small intestine in duodenum of humans in the form of erythromycin base. Erythromycin diffuses mainly into intracellular fluids, achieving an antibacterial activity in essentially all sites but not affecting the brain and CSF. Erythromycin which penetrates into prostatic fluid, achieving concentrations approximately 40% of those in plasma. Concentrations in middle ear exudate reach only 50% of serum concentrations and thus may be inadequate for the treatment of otitis media caused by H. influenzae. Protein which is binded approximately to 70% to 80% for erythromycin base and also can be higher 96%, for the estolate. Erythromycin traverses the placenta, and drug concentrations in fetal plasma are about 5% to 20% of those in the maternal circulation. Concentrations in breast milk are 50% of those in serum. (Hardman, J.G. et.al 1184)

Resistance

Resistance to erythromycin is due to demethylation of a specific adenine residue in 23S ribosomal ribo-nucleic acid and can be expressed in an inducible or constitutive mode. Resistance due to mutation occurs at very low frequency and is usually unstable. Staphylococcal resistance is often "dissociated,' wherein populations of strains not previously exposed to erythromycin contain only a few. Is capable of growing in the presence of inhibitory concentrations of erythromycin and are inhibited by other macrolides, whereas strains previously exposed to subinhibitory concentrations of erythromycin become resistant not only to erythromycin but also to other macrolides. Constitutive resistance may be generalized and extend to all macrolide-, lincosamide-, and streptogramin B-type antibiotics or it may be partial and extend to several but not all such antibiotics. Resistance to macrolide-, lincosarnide, and streptogramin B-type antibiotics is commonly plasmid determined in Staphylococcus aureus and in streptococci and has been transferred from streptococci to staphylococci. Resistance determinants on the chromosome rarely occur in staphylococci and streptococci and may be transferred without evidence of a vector

intermediate such as a phage or plasmid. Plasmid-mediated, transferable resistance to clindamycin and erythromycin has also been reported in Bacteroides frag and Clostridium difficile The percentage of erythromycin-resistant staphylococci between 1953 and 1958 ranged from less than 1 % to 25% in a survey of the literature by Bauer and associates.'

32 Rates of 5% at the Peter Bent Brigham Hospital in Boston and of 46% at the Hospital St. Joseph in Paris were reported for Staphylococcus aureus by O'Brien and associates133 in 1978. Resistance to erythromycin (minimal inhibitory concentrations of greater than 4 µg/ml) among isolates of Staphylococcus aureus from patients at the Mayo Clinic and affiliated hospitals was 5% in 1970 and 6% in 1983 (unpublished observations). Data regarding the erythromycin susceptibility of Staphylococcus aureus causing nosocomial infections in hospitals participating in the National Nosocomial Infections Study between 1975 and 1983. As many as 95 hospitals participated in this study. The hospitals ranged in size from 80 to more than 1,200 beds and were located in more than 28 states. Although the results were not a probability sample from the target population of all hospitals in the United States, the percentage of erythromycin-resistant Staphylococcus aureus isolates gradually increased in all the reporting hospitals from 1975 to 1982. Resistance was more than twice as high in large (more than 500 beds) medical-school-affiliated hospitals as in non-medical school-affiliated hospitals and small (500 or fewer beds) medical-school-affiliated hospitals (Weisblum B: Altered et al.)

Erythromycin continues to be a valuable and useful antimicrobial agent in children. Its low index of toxicity, freedom from sensitization, and reliable absorption when administered orally make it an attractive agent in treating a variety of minor respiratory and skin infections, especially in situations with real or potential allergy to penicillin exists. Additional major uses are in the eradication of the carrier state in whooping cough and in diphtheria, especially in those instances when oral therapy can be tolerated. Despite use over more than two decades, resistance developing in formerly susceptible organisms has not been a problem and thus seems unlikely to become so in the future. (M.D.Charles M.Ginsburg M.D.Heinz F.Eichenwald 2006) Erythromycin can be dangerous sometimes. Their safety health hazards and their symptoms are Symptoms of exposure to this compound include nausea, vomiting, diarrhea and abdominal cramps. Chronic overexposure may cause jaundice. This may be

accompanied by fever, leukocytosis, eosinophilia and elevated activities of transaminases in plasma. Allergic reactions to this compound may include fever, eosinophilia, skin eruptions, urticaria and anaphylaxis. Cholestatic hepatitis occurs rarely. Epigastric distress, possibly severe may also occur. Intramuscular injections of large quantities of this compound may cause extremely severe pain that persists for hours. Intravenous infusions of 1 gram doses have reportedly been followed by thrombophlebitis. Prolonged use may result in an overgrowth of non susceptible bacteria or fungi. There have been isolated reports of reversible hearing loss occurring after exposure to this chemical, chiefly in persons with renal insufficiency. ACUTE/CHRONIC HAZARDS: When heated to decomposition, this compound may emit toxic fumes of NOx. (NTP, 1992) Possible eye, skin, and/or respiratory tract irritation.

Toxicity

LD50 The oral LD50 of erythromycin in rats is 9272 mg/kg. Overdose information Symptoms of overdose may include diarrhea, nausea, stomach cramps, and vomiting. Erythromycin should immediately be discontinued in cases of overdose. Rapid elimination of unabsorbed drug should be attempted. Supportive measures should be initiated. Erythromycin is not adequately removed by peritoneal dialysis or hemodialysis.

Ecological Information

Erythromycin's production and use as an antibiotic may result in its release to the environment through various waste streams. Erythromycin is produced by a strain of Streptomyces erythreus which is found in soil. If released to air, an estimated vapor pressure of 2.1X10-25 mm Hg at 25 °C indicates erythromycin will exist solely in the particulate phase in the atmosphere. Particulate-phase erythromycin will be removed from the atmosphere by wet or dry deposition. Erythromycin contains chromophores that absorb at wavelengths >290 nm and, therefore may be susceptible to direct photolysis by sunlight. If released to soil, erythromycin is expected to have low mobility

based upon an estimated Koc of 570. The pKa of erythromycin is 8.9, indicating that this compound will exist almost entirely in the cation form in the environment and cations generally adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts. Volatilization from moist soil is not expected because the base exists as a cation, and cations do not volatilize. Erythromycin biodegradation in the soil is dependent on both temperature and addition of a readily biodegradable source of organic carbon as suggested by 0, 75, 100% biodegradation in 30 days at 4, 20, and 30 °C, respectively, in a sandy loam soil plus cattle feces. If released into water, erythromycin is expected to adsorb to suspended solids and sediment based upon the estimated Koc. Utilizing the Closed Bottle Test, -3% of the theoretical BOD was reported in 4 weeks, indicating that biodegradation is not an important environmental fate process in water. A pKa of 8.9 indicates erythromycin will exist almost entirely in the cation form at pH values of 5 to 9 and therefore volatilization from water surfaces is not expected to be an important fate process. An estimated BCF of 49 suggests the potential for bioconcentration in aquatic organisms is moderate. Hydrolysis is not expected to be an important environmental fate; however it may hydrolyze under basic conditions. Occupational exposure to erythromycin may occur through inhalation and dermal contact with this compound at workplaces where erythromycin is produced or used. Monitoring data indicate that the general population may be exposed to erythromycin via ingestion of contaminated drinking water, and dermal contact and ingestion of consumer products containing erythromycin.

MATERIALS AND METHODS

Sample Site

Water is very essential for all forms of life. Most of the time our civilizations were generated on the water bank. The Gomti river is located in south of the Himalayan foot hills near Madhogani Tanda village in Pilibhit district in northern Uttar Pradesh. It flows south eastward for almost 940 km through nine districts of Uttar Pradesh. Large amounts of human waste, agricultural and industrial pollutants are discharged in this river as it flows through the highly populated regions of Uttar Pradesh. The cities located near the river are the main reason of municipal and domestic waste and sewage water causing pollution in this river. But in recent times , the conditions of water quality are very badly affected. The reasons for this due to increase in population growth, rapid industrialization and agriculture methods resulting deterioration of water quality. The water pollution has many negative consequences such as destruction of marine habitat, development of various fatal human diseases such as cholera, malaria, tuberculosis, etc. Therefore, water pollution is indeed a major and serious global topic of concern.
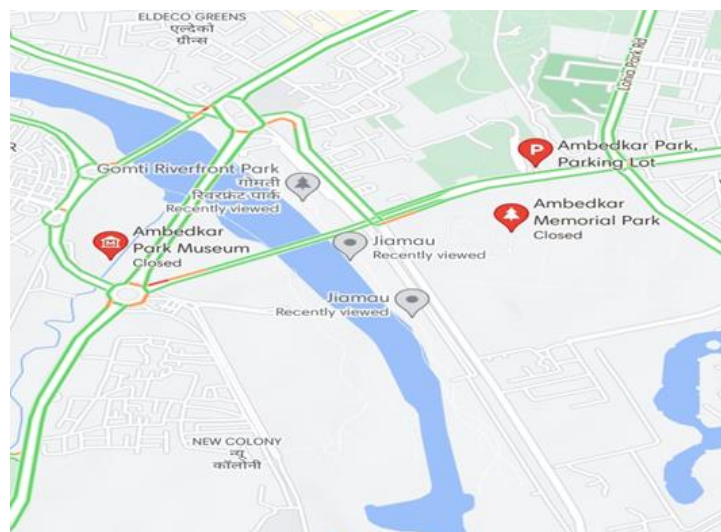

Figure 2:Map showing Gomti river showing sampling location

1. **Collection of water sample:** We have collected contaminated waste water sample in a sterilized plastic/centrifuge tubes from different sources and then transferred into the laboratory immediately for initial processing. The city of Lucknow has come up on the banks of the river Gomti. The river's significance has been time and again stated in various historical annals, which essentially point out the manner in which the city in its early days was dependent on the Gomti. To further prove that pollution is changing the river's biodiversity, scientists have tested water samples from the tributaries of Gomti.

Figure 3: Figure showing the actual site for collection of water sample

Culture media used for enumerationof normal and erythromycin 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 erythromycin in the media. The Nutrient agar was amended with erythromycin to get final concentration of 100 µg/ml to enumerate the erythromycin resistant microbial population. Serial dilutions of river water (10 mL) were media is 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 were counted and the population was evaluated using the formula

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

Isolation of erythromycin resistant bacteria from water

20

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 erythromycin and incubated at 35°C for 24 h. Ten different fast growing bacterial isolates with distinct colony morphology were picked and purified by repeated streaking on nutrient agar.

Sub culturing for pure culture preparation

Under 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 it cooled . Gone back to 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 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 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 got cooled, the bacteria were inoculated by the loop and the slant test tubes were incubated at 37°C for overnight. This process was done aseptically in laminar air flow. 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 by means of disc diffusion method (Bauer et al.1966). The following antibiotics (all from Hi-media, Mumbai, India) were used. Concentration of the antibiotics used is given in µg∕disc. The abbreviations and concentrations of the respective antibiotics are given in parentheses: Ofloxacin (C5mcg), Streptomycin (C 10mcg), Sulfadiazine(C 100mcg), Amoxicillin (C 30mcg), ,nalidixic acid (c 30mcg).

RESULT AND DISSCUSION

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 erythromycin in the medium.

Sample	Heterotrophic bacteria without antibiotic (A) (CFU/ml)	Heterotrophic bacteria with erythromycin added in the medium (B) (CFU/ml)
GRW4	$9.25X10^8 \pm 1.77X10^8$	$5.31X10^4 \pm 8.55X10^4$

The water samples collected from the Gomti River, Lucknow, show a high population of Erythromycin-resistant bacteria. The average heterotrophic bacterial count in the Gomti water was found to be $9.25X10^8 \pm 1.77X10^8$ CFU/ ml when no antibiotic was added to the growth media (Table 1). However, the bacterial population reduced to $5.31X10^4 \pm 8.55X10^4$ CFU/ ml when Erythromycin 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 significant population of Erythromycin-resistant bacteria. The analysis of the results revealed that the Gomti water contains about 26% of Erythromycin-resistant bacteria (Figure 2).

Similar to our results, reported erythromycin-resistant bacteria in Larut River and Sangga Besar River receiving different wastewater. They found a high population of erythromycin-resistant bacteria in river water. They further reported that the hospital wastewater contains the highest erythromycin-resistant bacteria (10^7 CFU/ml). The presence of erythromycin in the water inhibits bacterial growth and changes the water's microbial diversity. Water contamination by environmentally realistic concentrations of erythromycin affected both the heterotrophic and autotrophic communities with various effects according to the sulfonamide and the exposure level. Thus, exposure to erythromycin modified bacterial structure and impaired microbial enzyme functions. Moreover, sulfonamide exposure also had adverse effects on the autotroph component of the periphytic biofilm.

These results suggest first a direct effect of the sulfonamides on the exposed communities, resulting in impairment of the bacterial functions as already observed in soil microbial communities exposed to erythromycin or in the biofilm which is exposed to river water contaminated by antibiotic. Further, sulfonamide exposure acted as a selection pressure on the microbial communities, selecting the most tolerant species able to maintain the reference level of extracellular enzyme activities on exposure to sulfonamides. Many studies observed the impact of antibiotics, including sulfonamides, on the bacterial structure in soil sediments.
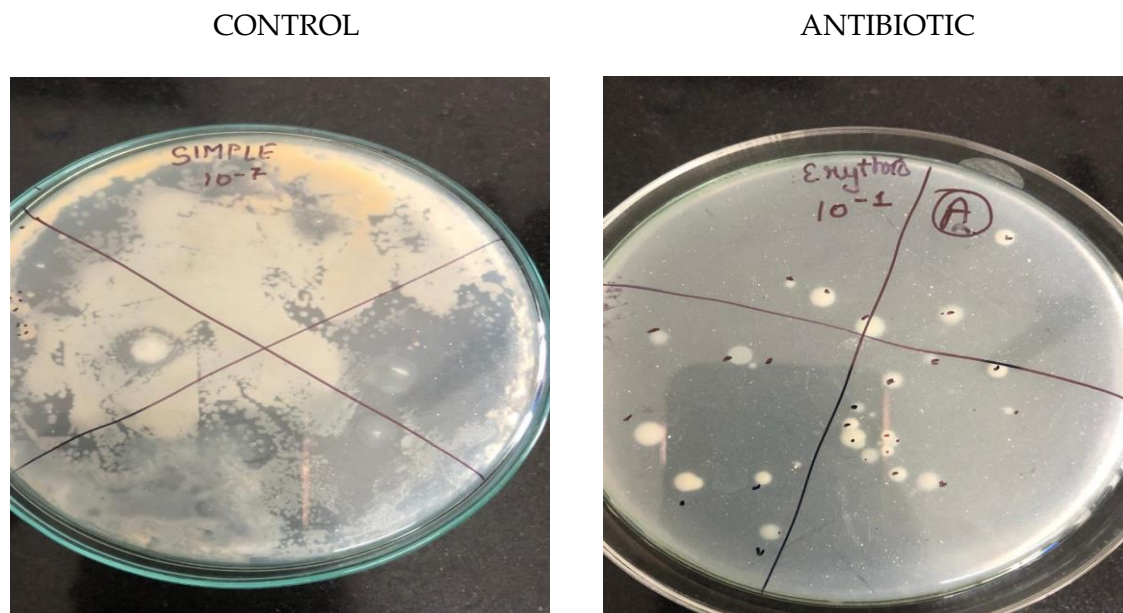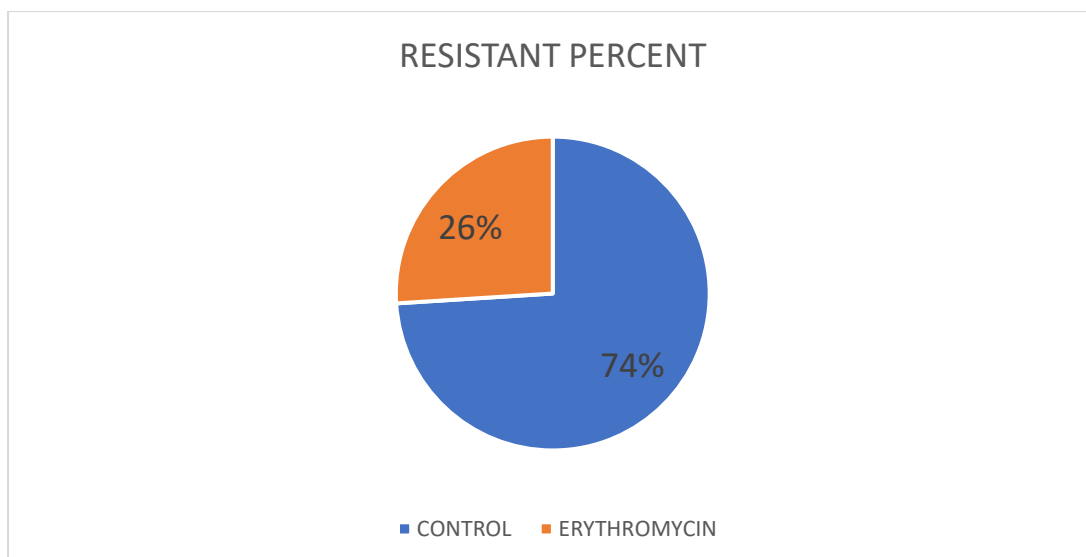
CONTROL   ANTIBIOTIC

Figure 4 : Percent of Erythromycin resistant bacteria in Gomti River Water

RESISTANT PERCENT

26%

74%

■ CONTROL   ■ ERYTHROMYCIN

Erythromycin resistant bacteria were isolated on the LB agar containing Erythromycin at a concentration of 100 µg/ml. From which 10 bacterial isolates were selected on the basis of morphology and colour. The well separated colonies were selected and purified by repeatedly re-streaking (Table 2). The bacterial isolates were named as GSM-1, GSM-2, GSM-3, GSM-4, GSM-5, GSM-6, GSM-7, GSM-8, GSM-9, and GSM-10

2. Objective – Isolation of antibiotic resistance Bacteria

Table 3 - Morphological and physical appearance of isolated and purified isolates

ISOLATES	COLONY MORPHOLOGY		
	COLOUR	SHAPE	SIZE
GEM -1	White	Round	Small
GEM -2	White	Round	Small
GEM -3	Yellow	Round	Large
GEM -4	White	Irregular	Small
GEM -5	Yellow	Irregular	Large
GEM -6	Yellow	Round	Small
GEM -7	White	Irregular	Small
GEM -8	Cloudy	Round	Large
GEM -9	Yellow	Round	Large
GEM -10	White	Irregular	Small

STREAKED PLATES
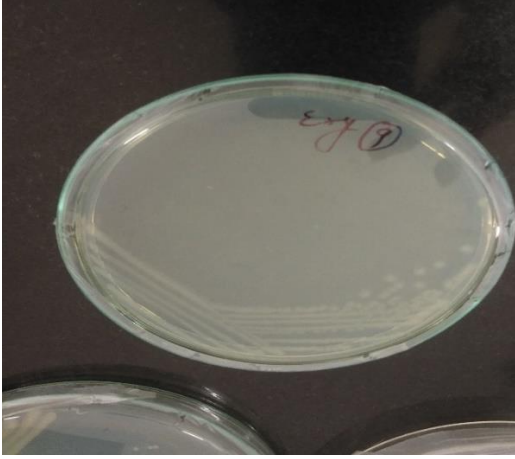
SLANT

3.OBJECTIVE – (a) Isolation of antibiotic resistance pattern.

BACTERIA	ANTIBIOTIC				
	OFLOXACIN	SULFADIAZINE	AMOXICILLIN	STREPTOMYCIN	NALIDIXIC ACID
GEM-1	R	R	R	16(S)	R
GEM-2	R	R	R	R	R
GEM-3	22 (S)	R	R	17(S)	R
GEM-4	R	R	R	19(S)	R
GEM-5	22 (S)	30(S)	R	R	25 (S)
GEM-6	R	R	R	16(S)	18(S)
GEM-7	R	R	R	16(S)	R
GEM-8	R	R	R	16(S)	R
GEM-9	22 (S)	20(S)	R	17(S)	21(S)
GEM-10	R	21 (S)	R	18 (S)	R

Five antibiotic discs i.e , Ofloxacin(5 mcg) , Sulfadiazine (100 mcg), Amoxicillin (30 mcg), Streptomycin(10 mcg), Nalidixic acid (30 mcg) were used to check the antibiotic resistance pattern.

(b) Antibiotic resistance isolates

ANTIBIOTICS	CONCENTRATION	NO. OF ISOLATES	RESISTANCE ISOLATES (%)
Ofloxacin	5 mcg	7	70
Sulfadiazine	100 mcg	7	70
Amoxicillin	30mcg	10	100
Streptomycin	10 mcg	2	20
Nalidixic acid	30 mcg	7	70

(c) Antibiotic resistance pattern of 10 isolates from water of Gomti River

NO. OF ANTIBIOTICS	NO. OF ISOLATES	RESISTANCE PATTERN
1	1	Amx
2	1	Amx , S
3	1	Sz, Amx, Na
	1	Of, Sz, Amx
	1	Of , Amx , Na
4	4	Of , Sz , Amx , Na
5	1	Of, Sz , Amx ,S, Na

we can conclude that 100% of bacterial isolates were resistant to Amoxicillin; 70% were resistant to Nalidixic acid, Ofloxacin, sulfadiazine and 20% were against streptomycin. shows that from the ten bacterial isolates, one isolates were resistant to 1 antibiotics (Amx). One isolates were resistance to 2 antibiotic (Amx , S). one isolates 3 antibiotic in three different pattern (Sz, Amx,Na), (Of, Sz, Amx), (Of , Amx , Na). four isolates were resistant to 4 antibiotic (Of , Sz , Amx , Na), one isolate were resistant to 5 antibiotic (Of, Sz , Amx ,S, Na). 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.

REFERENCES

American Society of Health-System Pharmacists, 2009. AHFS Drug Information 2009 Bethesda.

Brittain, D.C., Scully, B.E., McElrath, M.J., Steinman, R., Labthavikul, P. and Neu, H.C., 1985. The pharmacokinetics and serum and urine bactericidal activity of ciprofloxacin. *The Journal of Clinical Pharmacology*, *25*(2), pp.82-88.

Birnbaum, L.S., Thayer, K.A., Bucher, J.R. and Wolfe, M.S., 2013. Implementing systematic review at the National Toxicology Program: status and next steps. *Environmental health perspectives*, *121*(4), pp.a108-a109.

Brunton, L.L., Lazo, J.S. and Parker, K.L., 2006. The pharmacological basis of therapeutics. *Goodman, Gilmans, editors*, *11*

Bauer, A.W., Perry, D.M. and Kirby, W.M., 1960. Drug usage and antibiotic susceptibility of staphylococci. *Journal of the American Medical Association*, *173*(5), pp.475-480

Bundschuh, M., Hahn, T., Gessner, M.O. and Schulz, R., 2009. Antibiotics as a chemical stressor affecting an aquatic decomposer–detritivore system. *Environmental Toxicology and Chemistry: An International Journal*, *28*(1), pp.197-203.

Chabbert, Y., 1956. Antagonism in vitro between Erythromycin and Spiramycin. *Ann. Inst. Pasteur*, *90*(6), pp.787-90.

Clewell, D.B., 1981. Plasmids, drug resistance, and gene transfer in the genus Streptococcus. *Microbiological Reviews*, *45*(3), pp.409-436.

Davies, J.H. and Davies, D.R., 2010. Earth's surface heat flux. *Solid Earth*, *1*(1), pp.5-24.

Engel, H.W., Soedirman, N., Rost, J.A., Van Leeuwen, W.J. and van Embden, J.D.,

1980. Transferability of macrolide, lincomycin, and streptogramin resistances between group A, B, and D streptococci, Streptococcus pneumoniae, and Staphylococcus aureus. *Journal of bacteriology*, *142*(2), pp.407-413.

Finland, M. and Haight, T.H., 1953. Antibiotic resistance of pathogenic staphylococci: study of five hundred strains isolated at Boston City Hospital from October, 1951, to February, 1952. *AMA archives of internal medicine*, *91*(2), pp.143-158.

Galvin, S., Boyle, F., Hickey, P., Vellinga, A., Morris, D. and Cormican, M., 2010. Enumeration and characterization of antimicrobial-resistant Escherichia coli bacteria in effluent from municipal, hospital, and secondary treatment facility sources. *Applied and environmental microbiology*, *76*(14), pp.4772-4779.

Gribble, M.J. and Chow, A.W., 1982. Erythromycin. *Medical Clinics of North America*, *66*(1), pp.79-89.

Griffith, R.S. and Black, H.R., 1970. Erythromycin. *Medical Clinics of North America*, *54*(5), pp.1199-1215

Garrod, L.P., 1957. The erythromycin group of antibiotics. *British Medical Journal*, *2*(5036), p.57

Haight, T.H. and Finland, M., 1952. The antibacterial action of erythromycin. *Proceedings of the Society for Experimental Biology and Medicine*, *81*(1), pp.175-183.

Iwane, T., Urase, T. and Yamamoto, K., 2001. Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. *Water Science and technology*, *43*(2), pp.91-99.

Johnson, J.D., Hand, W.L., Francis, J.B., King-Thompson, N. and Corwin, R.W., 1980. Antibiotic uptake by alveolar macrophages. *The Journal of laboratory and clinical medicine*, *95*(3), pp.429-439.

Judson, R., Richard, A., Dix, D.J., Houck, K., Martin, M., Kavlock, R., Dellarco, V., Henry, T., Holderman, T., Sayre, P. and Tan, S., 2009. The toxicity data landscape for environmental chemicals. *Environmental health perspectives*, *117*(5), pp.685-695.

Klempner, M.S. and Styrt, B., 1981. Clindamycin uptake by human neutrophils. *Journal of Infectious Diseases*, *144*(5), pp.472-479

Liu, Y., Davies, J.A., Luhmann, J.G., Vourlidas, A., Bale, S.D. and Lin, R.P., 2010. Geometric triangulation of imaging observations to track coronal mass ejections continuously out to 1 AU. *The Astrophysical Journal Letters*, *710*(1), p.L82.

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S. and Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the total environment*, *473*, pp.619-641.

Lacey, R.W., 1977. Lack of evidence for mutation to erythromycin resistance in clinical strains of Staphylococcus aureus. *Journal of Clinical Pathology*, *30*(7), pp.602-605.

Lacey, R.W., 1975. Antibiotic resistance plasmids of Staphylococcus aureus and their clinical importance. *Bacteriological Reviews*, *39*(1), pp.1-32

Privitera, G.A.E.T.A.N.O., Fayolle, F.R.A.N.Q.O.I.S.E. and Sebald, M., 1981. Resistance to tetracycline, erythromycin, and clindamycin in the Bacteroides fragilis group: inducible versus constitutive tetracycline resistance. *Antimicrobial Agents and Chemotherapy*, *20*(3), pp.314-320.

McEvoy, G.K. ed., 2000. *AHFS drug information, 2000.* American society of health-system pharmacists

O'Neill, M.J., 2006. The Merck Index. An Encyclopedia of Chemicals, Drugs and Biologicals. Whitehouse Station, New Jersey: Merck Research Laboratories, Division of Merck and Co.

O'Brien, T.F., Acar, J.F., Medeiros, A.A., Norton, R.A., Goldstein, F. and Kent, R.L., 1978. International comparison of prevalence of resistance to antibiotics. *Jama*, *239*(15), pp.1518-1523.

Prokesch, R.C. and Hand, W.L., 1982. Antibiotic entry into human polymorphonuclear leukocytes. *Antimicrobial agents and chemotherapy*, *21*(3), pp.373-380.

Privitera, G.A.E.T.A.N.O., Fayolle, F.R.A.N.Q.O.I.S.E. and Sebald, M., 1981. Resistance to tetracycline, erythromycin, and clindamycin in the Bacteroides fragilis group: inducible versus constitutive tetracycline resistance. *Antimicrobial Agents and Chemotherapy*, *20*(3), pp.314-320.

Pikkemaat, M.G., Yassin, H., Fels-Klerx, H.J. and Berendsen, B.J.A., 2016. *Antibiotic residues and resistance in the environment* (No. 2016.009). RIKILT Wageningen UR.

Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M.C., Michael, I. and Fatta-Kassinos, D., 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of the total environment*, *447*, pp.345-360.

Semblante, G.U., Hai, F.I., Ngo, H.H., Guo, W., You, S.J., Price, W.E. and Nghiem, L.D., 2014. Sludge cycling between aerobic, anoxic and anaerobic regimes to reduce sludge production during wastewater treatment: performance, mechanisms, and implications. *Bioresource Technology*, *155*, pp.395-409.

Schaberg, D.R., Clewell, D.B. and Glatzer, L., 1982. Conjugative transfer of R-plasmids from Streptococcus faecalis to Staphylococcus aureus. *Antimicrobial agents and chemotherapy*, *22*(2), pp.204-207

Tally, F.P., Snydman, D.R., Gorbach, S.L. and Malamy, M.H., 1979. Plasmid-mediated, transferable resistance to clindamycin and erythromycin in Bacteroides fragilis. *Journal of Infectious Diseases*, *139*(1), pp.83-88.

Vanghel, M., 2012. Effects of the antibiotic Tetracycline: sublethal nematode toxicity tests

WASHINGTON II, J.A. and WILSON, W.R., 1985, March. Erythromycin: a microbial and clinical perspective after 30 years of clinical use (first of two parts). In *Mayo Clinic Proceedings* (Vol. 60, No. 3, pp. 189-203). Elsevier.

Weisblum, B., 1975. Altered methylation of ribosomal RNA in erythromycin-resistant Staphylococcus aureus. In *Drug Receptor Interactions in Antimicrobial Chemotherapy* (pp. 145-155). Springer, Vienna.

Welch, R.A., Jones, K.R. and Macrina, F.L., 1979. Transferable lincosamide-macrolide resistance in Bacteroides. *Plasmid*, *2*(2), pp.261-268.

Wüst, J. and Hardegger, U., 1983. Transferable resistance to clindamycin, erythromycin, and tetracycline in Clostridium difficile. *Antimicrobial agents and chemotherapy*, *23*(5), pp.784-786

Yan, X., Zhu, Z. and Li, T., 2019. Pollution source localization in an urban water supply network based on dynamic water demand. *Environmental Science and Pollution Research*, *26*(18), pp.17901-17910.

Yuan, Q.B., Guo, M.T. and Yang, J., 2015. Fate of antibiotic resistant bacteria and genes during wastewater chlorination: implication for antibiotic resistance control. *PloS one*, *10*(3), p.e0119403