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A Study On ESBL Production Inside The Members Of Enterobacteriaceae Present Inside The River Kshripra, Ujjain (M.P.), India.

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Abstract

The present study is designed to determine the occurrence of extended-spectrum beta-lactamase (ESBL)-producing producing gram negative bacteria inside the Kshripra river. Water samples were collected from different ghats of the river Kshripra. Isolation and identification of gram negative bacteria were done by using various biochemical tests. For the detection of multidrug-resistant bacteria, antibiotic susceptibility tests were performed, and to see the extended-spectrum beta-lactamase (ESBL) mechanism inside the gram negative bacteria, various types of ESBL detection methods were performed. During our study, we finally obtained a total of 21 strains of gram negative bacteria.Out of these 21 strains, 8 strains of gram negative bacteria were

sensitive, 9 strains of gram negative bacteria were non-conclusive, and 4 strains of gram negative bacteria were extended-spectrum beta-lactamase (ESBL) positive. All the isolates belonged to the family Enterobacteriace. The isolates were E.coli**,** Kliebsella**,** Enterobacter**,** Salmonella**,** Citrobacter, and Pseudomonas, all are gram negative bacteria.

Keywords: **Extended-Spectrum Beta-Lactamase (ESBL), Gram-Negative Bacteria, Enterobacteriaceae, Multidrug- Resistant Bacteria (MDR).**

1. INTRODUCTION

ESBL-producing Enterobacteriaceae have been investigated in river, effluent, and hospital sewage at the global level. **(Suzuki et al., 2020)**. At the global level, river water is mainly affected by human activity. The collection of toxic material in water habitats is responsible for affecting the aquatic organisms present inside the natural habitat as well as the health of humans. Antibiotic-resistant bacteria and antibiotic-resistant genes mainly develop in various habitats of surface water. Antibiotic-resistant bacteria and antibiotic-resistant genes can enter the water habitat or surrounding area through the release of unprocessed water from different sources, including agriculture and industry. Such unprocessed water is one of the main causes of contamination in the natural habitat. For treating patients who were infected by a multidrug-resistant strain of bacteria known as ESKAPE, mainly broad-spectrum antibiotics, including third-generation cephalosporin, are considered proper medicinal agents. In the European Union, the use of antibiotics is mainly favoured in the fields of human health and veterinary use. In Gram negative bacteria, due to the production of ESBL, bacteria become resistant to all beta-lactam antibiotics, and bacteria show the mechanism of resistance against third-generation cephalosporins. The ESBL enzyme was first investigated in 1983. Currently, these enzymes are spreading at the global level. CTX-M-type beta-lactamases are mostly present worldwide. The CTX-M gene is present on the plasmid. **(Tacao et al., 2022; Mechai et al., 2018; Sultana et al., 2016; Kurekci et al., 2017; Rawat et al., 2015; Yousef et al., 2016; Parvez et al., 2017; Shittu et al.,2022)**.WHO considers antibiotic-resistant bacteria to be one of the top 10 warnings all over the world. By 2050, at least 50 million people will die each year. **(Kimera et al., 2021; Akinola et al.,2022)**.In environments where bacteria are present in contact with antibiotic residues, such types of environments contribute to the selection of resistant bacteria. The residues of antibiotic and antibiotic-resistant bacteria pass into the natural ecosystem, including river water, through the waste of industrial sewage,hospital and municipal wastewater, and agriculture pollution. River water plays an important role in terms of carrying anitbitic-resistant bacteria. **(Abbas et al.,2015)**.E. coli acts as an indicator bacteria as well as an antibiotic-resistant bacteria in bacterial communities. **(Tissera et al., 2013; Akinola et al., 2022; Devraj et al., 2016; Tram et al., 2016; Ho et al., 2021; Graham et al., 2011; Hanna et al., 2020; Kittinger et al., 2016; Johnson et al., 2020; Ahsan et al., 2022; Rashid et al., 2015; Banu et al., 2021)**. The members of this family, including Enterobacteriaceae and Pseudomonadales, are antibiotic-resistant and gram negative rod-shaped bacteria. These above family members are mainly present in aquatic habitats, and they are

capable of exchanging their genetic material within the trans-species. At present, surface water acts as a reservoir for resistant genes, and sensitive strains can also carry new resistant mechanisms. According to a worldwide report document, resistance mechanisms inside the members of Enterobacteriaceae are mainly found in various water sources, including rivers, lakes, and the ocean. In the last decade, ESBL-producing bacteria have become ubiquitous. The ESBL-producing bacteria arise within hospitals, livestock, and veterinary medicine. **(Hassen et al., 2020; Siddiqui et al., 2018; Kittinger et al., 2016; Falodun et al., 2017; Kurekci et al., 2017; Tram et al., 2023; Banu et al., 2021)**. The misuse of antibiotics in the last few decades is responsible for antibiotic resistance inside the coliform bacteria. The use of antibiotics such as cephalosporins, mainly in fields including hospitals and animal husbandry, is responsible for ESBL production inside the enteric bacteria. The strains of gram negative bacteria produce the beta-lactamase enzyme, which breaks the beta-lactum ring of antibiotics. Due to this, antibiotics become inactive, so the bacterial strain becomes resistant to first, second, third, and fourth generation cephalosporin. Due to the ESBL enzyme production inside the gram-negative bacteria, it has become difficult to treat the patient with antibiotics. In such conditions, the treatment of patients becomes expensive, and limited medicinal options are available. ESBL production is mainly detected in the family Enterobacteriaceae **(Kebede et al., 2022; Kim et al., 2009; Falodun et al., 2017; Ahsan et al., 2022; Siddiqui et al., 2018; Tissera et al., 2013; Ebongue et al., 2018; Singh et al., 2017)**. Due to an antibiotic-resistant illness, 7,000 people die annually, and this number is calculated to reach 10 million by 2025. Antibiotic-resistant bacteria can transfer antibiotic-resistant genes between different bacteria with the help of genetic components, including plasmids, integrons, and transposons. Due to the transformation of genetic material, sensitive strains of bacteria become resistant. According to a study published in 2019, Vietnam consumes the most antibiotics, mainly in agriculture and industries, and the use of antibiotics has increased over the last 10 years. Such types of drugs have been used in the country without any control. **(Tram et al., 2023; Siddiqui et al., 2018)**. ESBL encoded by different families of bla genes, including CTX-M, TEM, and SHV, is either chromosomally localised or plasmid-based genetic material. Out of bla TEM, bla SHV, bla CTX-M, bla CTX-M was identified as the most present all over the world. It has been reported that such types of bacteria carry antibiotic resistance genes for different classes of antibiotics, including aminoglycosides, quinolone, and tetracycline **(Devraj et al., 2016; Siddiqui et al., 2018; Roopashree et al., 2021; Kebede et al., 2022; Anima et al., 2017; Chotinantakul et al., 2022; Parvez et al., 2017; Kurekci et al., 2017; Cornista et al., 2019)**. ESBL-producing organisms are resistant to important beta-lactam antibiotics, which are mainly used for treating infections. **(Singh et al., 2017; Banu et al., 2021)**. Carbapenems are antibiotics that are used for the treatment of patients who are affected by ESBL-producing bacteria. Carbapenems are broad-spectrum antibiotics that inhibit the enzyme traspeptidase, thus preventing peptide cross-linking during peptide synthesis. Carbapenemase enzymes are plasmid-mediated and readily transferred among bacteria. The most common type of carbapenemase that is found in India is NDM-1. **(Roopashree et al., 2021; Wadekar et al.,2021)**.ESBL-producing genes are found on large plasmids,which can carry the antibiotic-resistant gene. Due to these genes, bacteria show a resistance mechanism. **(Boron et**

al., 2021). Carbapenem-resistant Enterobacteriaceae were investigated in aquatic environments all over the world. Classical and molecular microbiological methods are the best techniques available for the microbiological community and monitoring antibiotic-resistant bacteria. **(Ho et al., 2021)**. Aquatic surroundings create a hotspot for transferring antibiotic-resistant genes among bacterial species with the help of horizontal gene transfer, including integrons, transposons, and plasmids. **(Zhang et al., 2017; Wiegand et al., 2007; Ali et al., 2021; Stadler et al., 2018; Kurekci et al., 2017; Tissera et al., 2013; Adenike et al., 2022; Ahmed et al., 2017)**. E. coli and Kliebsella pneumoniae are the major ESBL-producing organisms all over the world **(Kebede et al., 2022; Sultana et al., 2016; Andy et al., 2019; Cormican et al., 1996; Pillai et al., 2022; Cornista et al., 2019; Teklu et al., 2019; Yousef et al., 2016; Medegar et al., 2019)**. In enterobacteria, the main cause of resistance mechanisms is ESBL production. The ESBL-producing bacteria show a resistance mechanism against oxyimino-cephalosporins. **(Sivaramn et al., 2020)**. In developing countries, hospital waste water is a source of environmental pollution. Water contains a huge population of coliform bacteria as well as viruses and helminths that infect the human population. The effluents of hospitals consist of antibiotic residues,which further stop the growth of sensitive bacterial strains. In the presence of such residues, bacteria act as antibiotic-resistant. **(Falodun et al., 2019; Kebede et al., 2022)**. ESBLs are a group of plasmid-mediated enzymes. The presence of the ESBL enzyme inside gram negative bacteria makes them resistant to penecillin, first, second, and third generation cephalosporins, and aztreonam. But not against cephamycins (cefoxitin and cefotetan). Bacteria producing the ESBL enzyme were killed by a beta-lactamase inhibitor known as clavulanic acid. In the last few years, the production of the ESBL enzyme has been observed in members of the Enterobacteriaceae. Enterobacteriaceae commonly produce ESBL enzymes, which are known as SHV and TEM types. This enyme causes a nosococal infection. **(Chetna et al., 2017; Andy et al., 2019; Pillai et al., 2022)**. Since 1990, ESBL-forming E.coli has originated globally. **(Andy et al. 2019)**. Several resistance mechanisms have been detected, including penicillin-binding protein (PBP) modification, the efflux system, and ESBL enzyme production **(Pillai et al., 2022)**. ESBL genes have now been identified in wastewater, sewage, and sediment samples. ESBL-producing bacteria were first noted in Germany in 1980; presently, these ESBL-producing bacteria are present in underdeveloped nations **(Parvez et al., 2017)**. Waterborne diseases, including dysentery,cholera, diarrhoea, and typhoid, are caused by polluted water. 80% of illnesses are caused by waterborne pathogens that are present in polluted water. Some of the faecal enteric bacteria include E.coli, Kliebsella,Citrobacter,Enterobacter, Morganella, and Hafnia. Carbapenems, including imipenem, meropenem, and ertapenem, are beta-lactam antibiotics that are mainly used against serious infections caused by multidrug-resistant bacteria that belong to the Enterobacteriaceae. **(Devraj et al., 2016; Akinola et al., 2022)**. ESBL-producing bacteria show resistance mechanisms against different types of antibiotics, including flurouinolones,trimethoprim, sulfuramethoxazole, aminoglycosides, and metronidazole. The first ESBL-producing bacteria were identified in Germany in 1983; later, they were identified in France in 1985 in the bacteria Kliebsella pneumoniae. Later, ESBL production was reported globally in members of the Enterobacteriaceae and Pseudomonas species. The rate of ESBL production is found to be high, mostly in Asia

and Europe. **(Ahmed et al.,2017)**.Beta-lactamase enzymes are present in many species of gram-positive and gram-negative bacteria. Some beta-lactamase enzymes are plasmid-mediated. In the case of Staphylococcus.aureus, the ESBL enzyme is plsmid-mediated. While others are chromosomally mediated in the case of gram positive bacteria. **(Abdelmoktader et al., 2017)**. ESBL enzymes are usually plasmid-mediated beta-lactamases, mostly found in Kliebsella pneumoniae but also reported in E.coli, Proteus mirabilis, and other Gram-negative bacteria. This bacteria is responsible for the failure of therapy, including cephalosporin and other classes of antibiotics, at the global level. **(Goyal et al.,2008)**.In 2008, a new beta-lactamse enzyme was identified in Kliebsella pneumonia.This enzyme breaks down beta-lactams except aztreonam antibiotics. Later, these NDM-1-producing enterobacteria were isolated from different parts of the world. India and other nations have investigated the presence of blaNDM-harbouring bacteria in the environment. **(Singh et al., 2017)**. ESBL production is most common in members of the gram-negative bacteria family Enterobacteriaceae. **(Butt et al., 2017)**. Various labs have used the guidelines of the Clinical Laboratory Standard Institute (CLSI) in terms of ESBL detection inside gram-negative bacteria, including E.coli, Kliebsella pneumoniae, K. oxytoca, and Proteus mirabilis. ESBL genes can be communicated from one strain of bacteria to another strain of bacteria **(Anima et al., 2017)**. The water environment is the main source of resistant bacteria in which resistance genes are present. **(Olatunji et al., 2016)**. MDR bacteria is present in different sources, including rivers, lakes, groundwater, and drinking water.

2. MATERIAL AND METHODS:

2.1. Water Sample Collection:

A total of 8 ghats of Kshripra were selected for sample collection. The study was conducted from March 2021 to April 2022, and water samples were placed on a 4°C ice box to inhibit the growth of microorganisms and immediately sent (within 2 hours) to the microbiology research laboratory for analysis. Distilled water will be used as a control during the analysis **(Mahato et al., 2019)**.

2.2. Antibiotic Susceptibility Test:

This test was performed for the phenotypic conformation of multidrug-resistant bacteria. To perform this test, test organism growth was compared with the 0.5 McFarland standards, and the culture of the test organism was swabbed over Mueller Hinton agar media, and an antibiotic disc was placed at a distance of 2.5 cm. Besides this, all the necessary precautions were taken while performing. One plate of Mueller-Hinton agar was taken as a control. **(Falodun et al., 2007)**

2.3. Method for ESBL detection

2.3.1. Screening Method

The screening method was performed by the disc diffusion technique using 3rd-generation cephalosporins, ceftazidime (CAZ 30 mcg), ceftriaxone (CTR 30 mcg), and cefpodoxime (CPD 10 mcg). Isolates resistant to more than one of these agents were identified as possible ESBL producers. **(Chaudhary et al., 2021)**

2.3.2. Confirmatory method

For confirmation, a combined disc test was performed by using ceftazidime (CAZ 30µg) alone, ceftazidime with clavulanic acid (CAC 30µg/10µg), cefotaxime (CTX 30µg), and cefotaxime with clavulanic acid (CT/CTL 30µg/10µg) at a distance of 2 cm. A difference in zone of inhibition of ≥5mm between ceftazidime clavulanic acid with ceftazidime alone and cefotaxime clavulanic acid with cefotaxime alone was interpreted as confirmed ESBL. **(Chaudhary et al., 2021)**

2.3.3. Double disk Synergy Test (DDST)

This is another method for ESBL detection. In this method, the discs of third-generation cephalosporin Cefpodoxime (CPD 10 mcg) were placed at a distance of 1.5 cm from amoxicillin-clavulanic acid (AMC 30 mcg (20/10 mcg). Enhanced inhibition indicates ESBL. **(Dashti et al., 2006).**

2.3.4. E-Test (MIC ESBL Strip)

In this method, MIC strips were used in such a way that a two-sided strip contained CAZ (ceftazidime) on one side and CAZ-CA (ceftazidime/ceftazidime + clavulanic acid) on the other. The ratio of the MIC of the combination to that of CAZ alone >8 or the presence of a phantom zone (or both) indicates ESBL. **(Dashti et al., 2006)**.

3. RESULTS AND DISCUSSION

In the study of **Ho, J.Y.** et al. **(2020)** with respect to multidrug-resistant bacteria and microbial communities inside a river estuary, it was observed that there was a huge population of ESBL-producing E.coli in the upstream area of the river. In Malaysia, ESBL-producing bacteria have been identified in surface water and hospitals. The presence of ESBL-producing gram negative bacteria and multidrug-resistant E.coli was observed in Matang mangrove estuaries. This contamination is due to man-made activities. A large population of E.coli isolates shows resistance against ciprofloxacin, which belongs to the fluoroquinolone class. Pseudomonas sp. (other than Pseudomonas aeruginosa) is resistant or intermediate to meropenem. In our studies on the Kshripra river with respect to the isolation of multidrug-resistant bacteria and ESBL-positive strains of gram negative bacteria.We isolated resistant strains of E. coli, and on ESBL detection, it was found

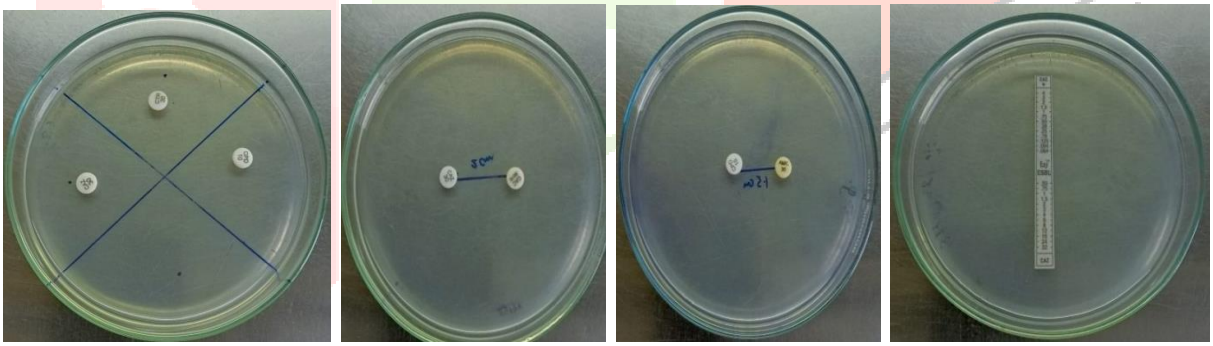
to be a non-conclusive strain, a mechanism of resistance other than ESBL by E.coli. In a similar manner, we also isolated strains of Pseudomonas**.** On the ESBL detection test, it was found to be a non-conclusive strain. Here is also the resistant mechanism inside Pseudomonas that occurs other than the ESBL. In the study of **Mechai** et al. **(2018)** on river water in terms of ESBL production inside the members of Enterobacteriaceae, the authors isolated strains of gram negative bacteria, including Enterobacter, Kliebsella, Citrobacter, Serretia, Salmonella, Shiegella, Yersinia, Providencia and Morganella.The following bacteria show a high level of resistance against amoxicillin/clavulanic acid, amoxicillin, ticarcillin, cefoxitin, cefalotin, ertapenem, and fosfomycin. According to the authors, the main cause of antibiotic resistance inside the river water is a sewage treatment plant that disseminated antibiotic-resistant genes and antibiotic-resistant bacteria inside the river. In our studies on the Ksripra River, we isolated the following types of bacteria: Salmonella, E.coli, Kliebsella, Enterobacter, Citrobacter, and Pseudomonas. In our studies in the Kshripra River, the species of Salmonella showed resistance mechanisms against ceftazidime, cefpodoxime, ceftriaxone, aztreonam, and ampicillin. E.coli shows resistance mechanisms to aztreonam, ceftazidime, ampicillin, and cefpodoxime. Kliebsella sp. shows resistance mechanisms to aztreonam, ceftazidime, chloramphenicol, and ampicillin. Enterobacter shows resistance mechanisms with aztreonam, ceftazidime, chloramphenicol, ampicillin, cefpodoxime, ciprofloxacin, and ampicillin. Citrobacter shows resistance mechanisms against chloramphenicol, ampicillin, aztreonam, ceftazidime, cefpodoxime, cefpodoxime, ciprofloxacin, and ampicillin. Pseudomonas species shows resistance mechanisms against ciprofloxacin, ceftazidime, chloramphenicol, ceftriaxone, and ampicillin. Out of the following strains of gram negative bacteria, Kliebsella from sample no. 5, Enterobacter from sample no. 7, and Citrobacter and Enterobacter from sample no. 8, ESBL production is shown. Another study was conducted by **Boron** et al**. (2017)** with respect to antibiotic resistance and the presence of the ESBL gene in E.coli from a river in Podhale. They were isolated E.coli strains, which show a resistance phenomenon against penicillin, ampicillin, amoxicillin, clavulanic acid, and ticarcillin. Ciprofloxacin (fluoroquinolones) was the most effective antibiotic for E.coli strains isolated from Zakopianka. Besides this, the strains were ESBL-positive. In our findings on the Kshripra River, the strains isolated from sample no. 4 show a resistance mechanism, and on ESBL detection, it shows non-conclusive results. In a similar manner, E.coli strains isolated from sample no. 7 also show the resistance mechanism, and on ESBL detection, E.coli shows a non-conclusive result. Therefore, in both E.coli strains. Therefore, both E.coli strains have resistance mechanisms other than ESBL. Another study was performed by **Falodun** et al**. (2017)** with respect to ESBL detection in gram-negative bacteria inside the rivers Alaro,Kudati,Yemetu.In their studies, Kliebsella showed a higher rate of resistance. These gram negative resistant bacteria show resistance mechanisms, and the strains were ESBL positive. These bacterial strains were Pseudomonas, Enterobacter, Kliebsella**,** E.coli**,** and Proteus**.** In the study of **Falodun** et al**. (2017)**, Kliebella pneumoniae and Proteus mirabilis show resistance against cefuroxime and cefepime. Enterobacter.aerogens and Pseudomonas.putida**.** also showed the resistance mechanism against seven different antibiotics, including cefuroxime, cefepime, amoxicillin, azithromycin, ciprofloxacin, florfenicol, and gentamicin. In our findings on the Kshripra River, the gram negative bacterial strains, including Kliebsella,

Enterobacter, and Pseudomonas**,** also show resistance mechanisms. Kliebsella strains isolated from sample no. 5 show a resistance mechanism against aztreonam, ceftazidime, chloramphenicol, and ampicillin. On ESBL detection, the Kliebsella species shows ESBL production. The Enterobacter species isolated from samples no. 3, 6, 7, and 8 also show the resistance mechanism against aztreonam, ceftazidime, chloramphenicol, ceftriaxone, ampicillin, cefpodoxime, and ciprofloxacin. In our studies, samples 3 or 6 on ESBL detection inside Enterobacter show a non-conclusive result, meaning that resistance is other than ESBL production. While in our findings on Kshripra River sample no. 7, 8, Enterobacter shows ESBL detection as positive. In our studies, Pseudomonas strains showed resistance mechanisms against ceftazidime, chloramphenicol, ceftriaxone, and ampicillin. On ESBL detection, Pseudomonas strains show a non-conclusive result, meaning the resistance mechanism is other than ESBL. Another study was conducted by **Tram et al**. **(2023)** on the Perfume River with respect to antibiotic resistance inside the E.coli. E.coli strains were identified in the river water, and these isolated E.coli strains show a resistance mechanism against seven antibiotics: amoxicillin, nalidixic acid, ciprofloxacin, cefotaxime, and ceftazidime. But E.coli strains were sensitive to meropenem. Antibiotic resistance inside the E.coli was high against amoxicillin. Besides this, in the Perfume River, multidrug-resistant strains of E.coli show the phenomenon of ESBL production. In our finding on the Kshripra River, the E.coli strains isolated from samples 4–7 also show the phenomenon of resistance against aztreonam, ceftazidime, and amoxicillin. Besides this, ESBL detection in E.coli isolates from samples 4–7 shows a non-conclusive result, which means the resistance mechanism is other than ESBL production. In the study of **Ahsan** et al**. (2022)** on different places of the Korang River and its tributaries with regard to antibiotic resistance patterns inside the E.coli . The water samples belong to the wet market, and the waste water treatment plant is highly polluted with E.coli. The E.coli isolated was an ESBL producer. All the E.coli isolates that produce ESBL show 100% resistance against streptomycin (aminoglycosides), neomycin (aminoglycosides), enrofloxacin (fluoroquinolones), lincomycin (lincosamides), ampicillin, penicillin G (penicillin), and oxytetracycline (tetracycline)**.** The results of the antibiotic susceptibility test show that E.coli that produces ESBL shows a weaker resistance mechanism against second-generation cephalosporins such as cefoxitin, fourth-generation cephalosporins such as cefepime, and first-generation cephalosporins such as cephradine. Third-generation cephalosporins such as cefixime. Whereas this ESBL-producing E.coli was sensitive against carbapenems, including imipenem and amoxicillin with clavulanic acid. In our findings on the Kshripra River with respect to gram-negative bacteria, we also isolated the resistant strains of E.coli from samples 7. On ESBL detection, the strains of E.coli were non-conclusive. The strains of E.coli show resistance mechanisms against aztreonam, ceftazidime, and ampicillin**.** The strains of E.coli were sensitive to cefpodoxime, chloramphenicol, ceftriaxone, and ciprofloxacin. Another study was conducted by **Abbas** et al**. (2015)** on the Hilla River with respect to the identification of multidrug-resistant and ESBL-producing coliform bacteria. **Abbas et al**. **(2015)** isolated resistant strains and ESBL-producing strains of E.coli**,** Kliebsella pneumoniae, and Enterobacter species. The bacterial strains show a high rate of resistance against ampicillin, piperacillin, amoxiclav, cefoxitin, ceftazidime, cefpodoxime, ceftriaxone, aztreonam, nalidixic acid,

and erythromycin. Lower rates of resistance were observed for imipenem, meropenem, and levofloxacin. In our studies on the Kshripra River, we also isolated resistant strains of E.coli from samples 4–7, which show resistance mechanisms against aztreonam, ceftazidime, and ampicillin**.** Besides this, E.coli. isolated on ESBL detection shows a non-conclusive result, meaning the resistance mechanism is other than ESBL. In a similar manner, we also isolated resistance strains of Kliebsella from sample no. 5, which show resistance against aztreonam, ceftazidime, chloramphenicol, and ampicillin. We also isolated the resistance strains of Enterobacter from samples 3, 6, 7, and 8. The Enterobacter strains isolated from sample no. 3 show a resistance mechanism against aztreonam,ceftazidime, chloramphenicol, and ampicillin. On ESBL detection, the Enterobacter strains were non-conclusive, meaning their resistance mechanism was other than ESBL. The strains of Enterobacter isolated from sample no. 6 also show a resistance mechanism against aztreonam, chloramphenicol, ceftriaxone, and ampicillin. On ESBL detection, the bacteria show a non-conclusive result, which means the resistance mechanism is other than ESBL. The strains of Enterobacter isolated from sample 7 also show a resistance mechanism against ceftazidime, chloramphenicol, and ampicillin. On ESBL detection, the bacteria show ESBL production. The strains of Enterobacter isolated from sample no. 8 show resistance against aztreonam, ceftazidime, cefpodoxime, chloramphenicol, ciprofloxacin, and ampicillin. On ESBL detection, the Enterobacter shows ESBL production.

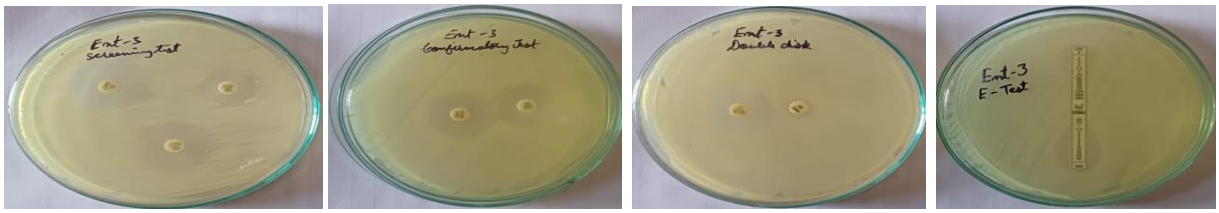
ESBL detection in those bacterial isolates shows a resistance mechanism against three or more antibiotics.

Figure 1: Control Plates for ESBL Detection



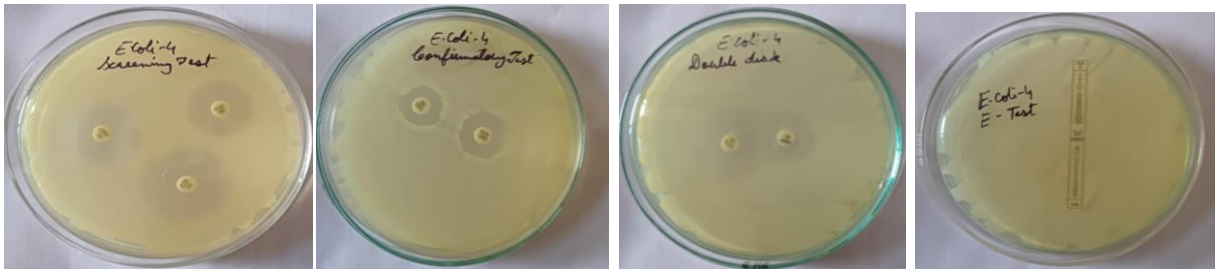
(A) Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 2: **ESBL Detection in Sample 3: Enterobacter**



(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 3: **ESBL Detection in Sample 4: E. coli**

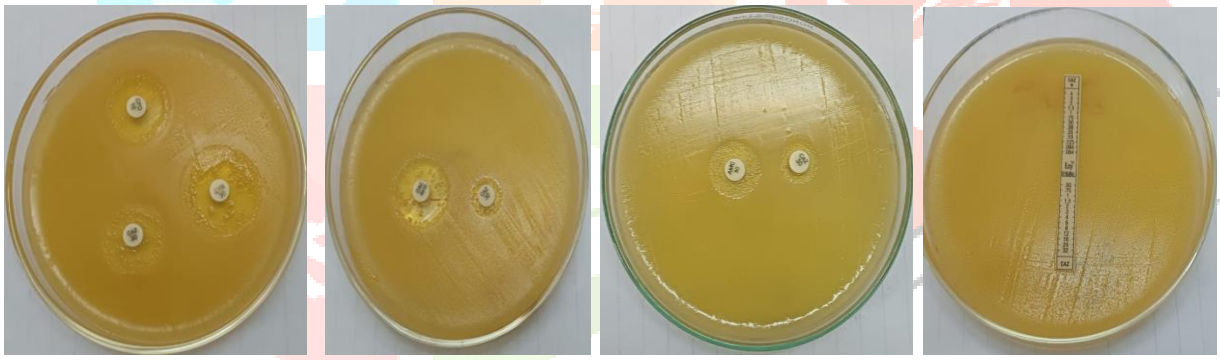


(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 4: ESBL Detection in Sample 5: Kliebsella

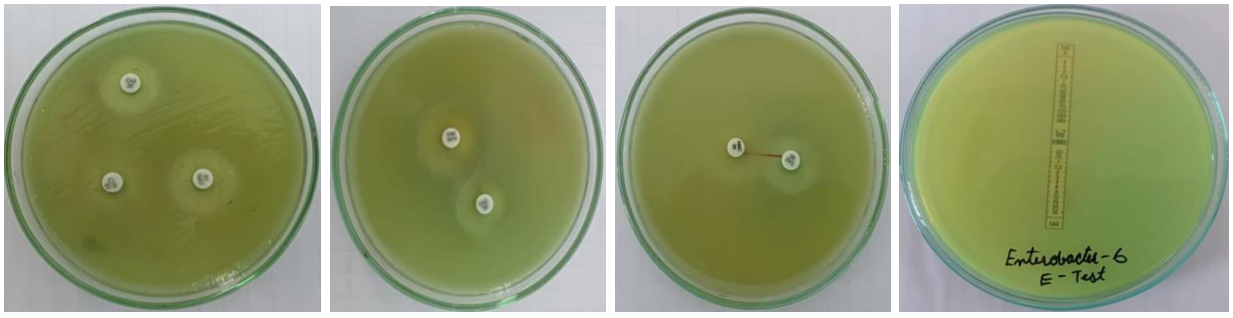
(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 5: ESBL Detection in Sample 6: Salmonella



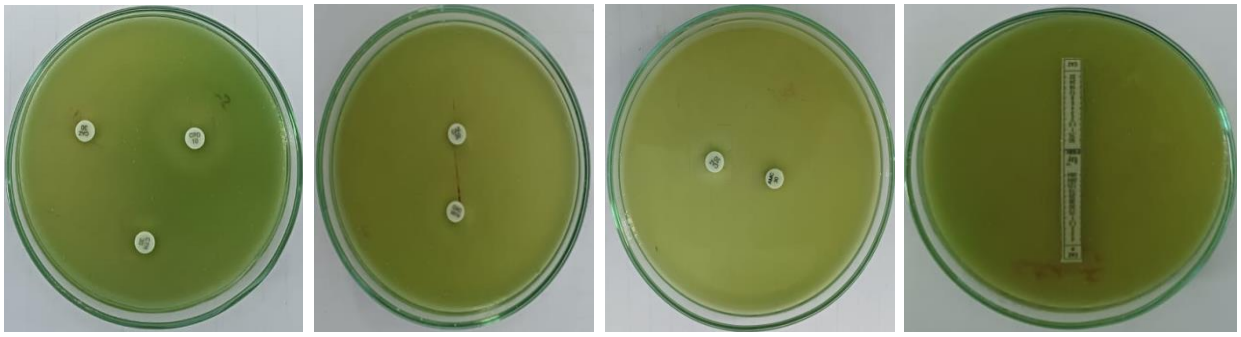
(A)Screening Test Contro (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 6: ESBL Detection in Sample 6: Enterobacter



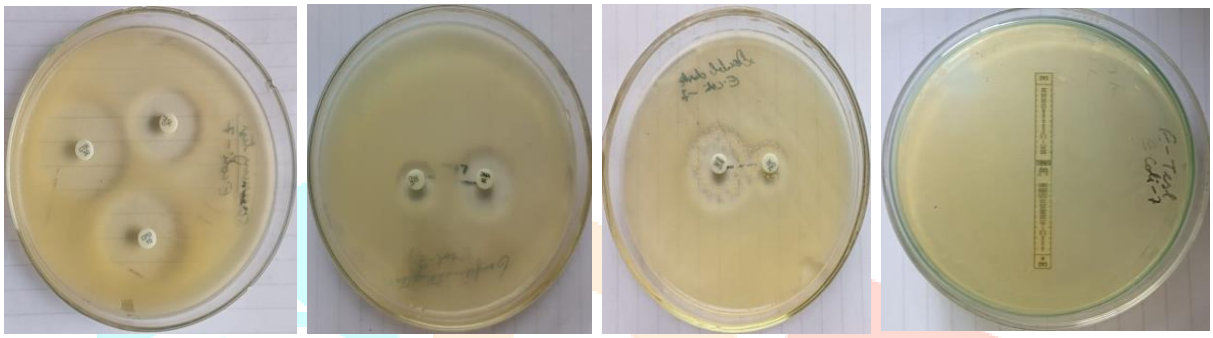
(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 7: ESBL Detection in Sample 6:Citrobacter



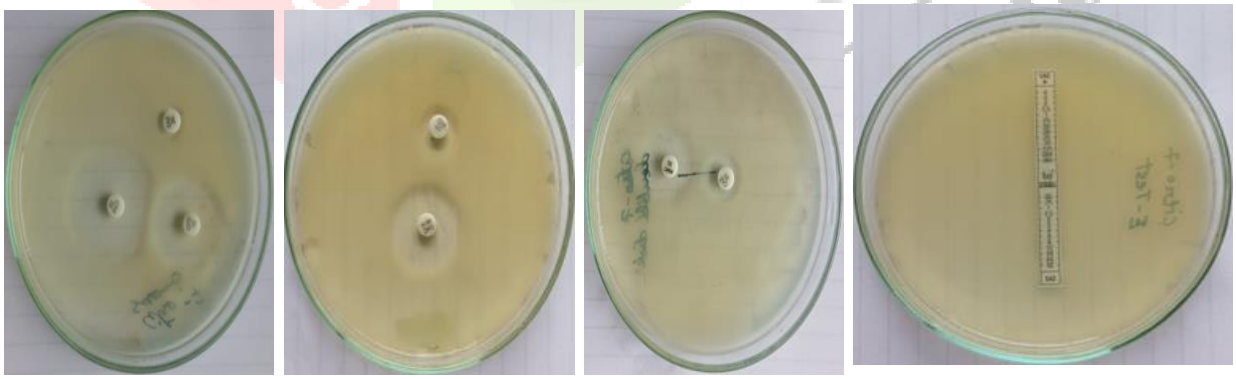
(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 8: ESBL Detection in Sample 7: E. coli



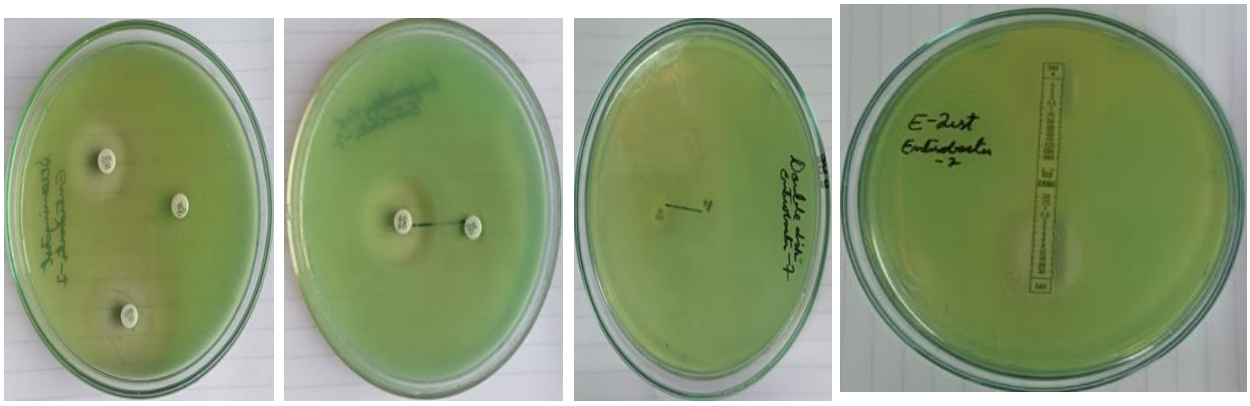
(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure9: ESBL Detection in Sample7: Citrobacter



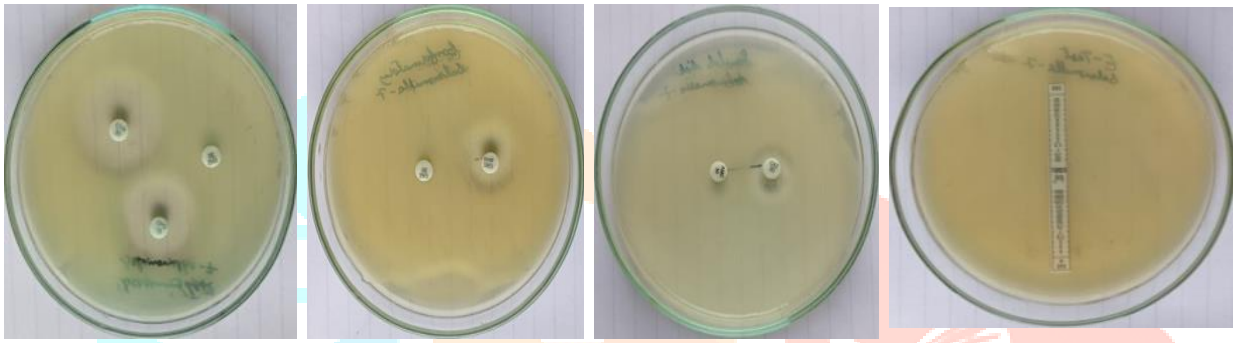
(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 10: ESBL Detection in Sample 7:Enterobacter



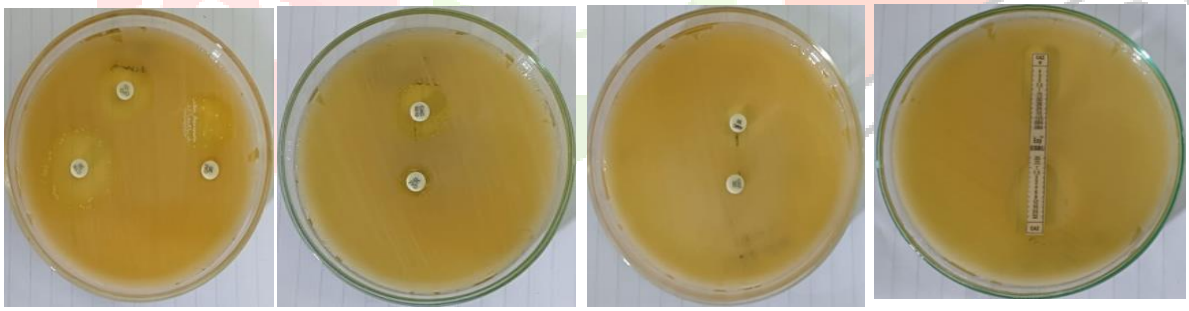
(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 11: ESBL Detection in Sample 7: Salmonella



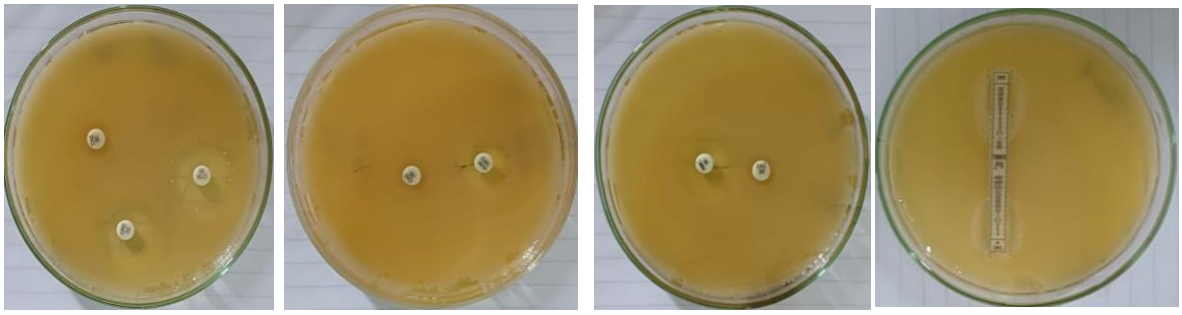
(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 12: ESBL Detection in Sample 8: Enterobacter



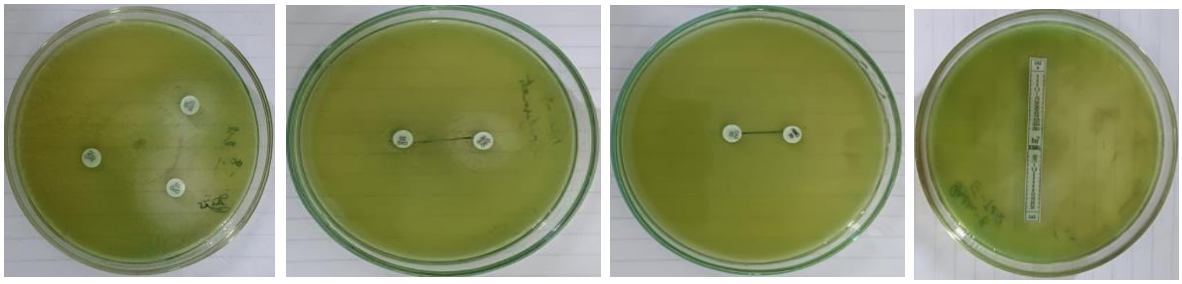
(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 13: ESBL Detection in Sample 8: Citrobacter



(A)Screening Test Control (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

Figure 14: ESBL Detection in Sample 8: Pseudomonas



(A)Screening Test Contro (B) Confirmatory Test Control (C) Double Disc Test Control (D) E- Test Control

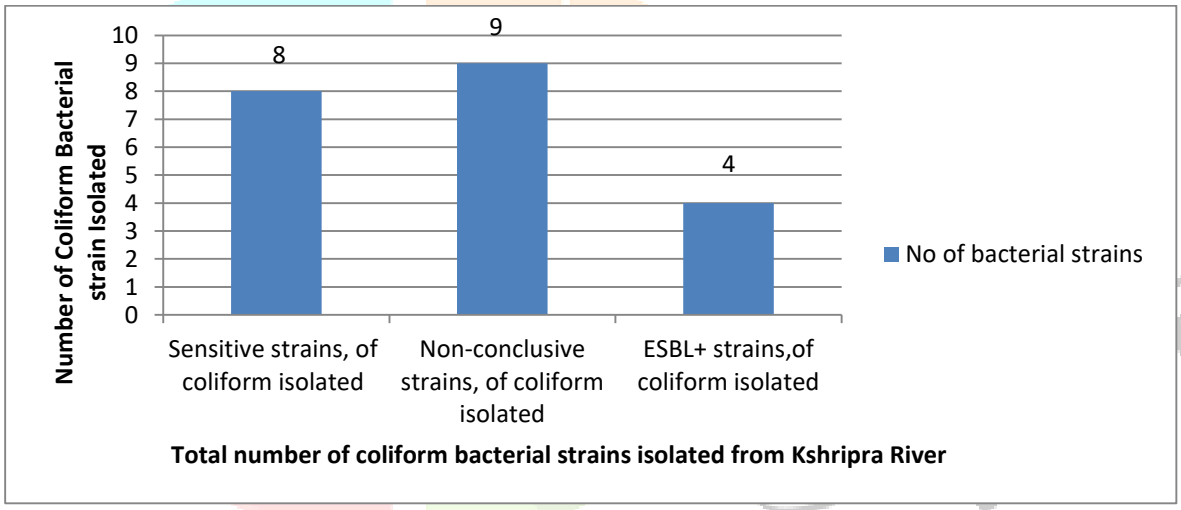
Table: 1 Identification of Sensitive,Non-conclusive and ESBL positive strain of Gram Negative Coliform Bacteria

Kshripra River Water Sample	**Coliform Bacteria Isolated**	**Interpretive Criteria Analysed as Per CLSI Standards (Clinical and Laboratory Standards Institute)**	**Interpretation of a bacterial strain of gram negative bacteria with respect to ESBL detection.**
1	Salmonella	Sensitive	Sensitive
	E.coli	Sensitive	Sensitive
	Pseudomonas	Sensitive	Sensitive
2	Kliebsella	Sensitive	Sensitive
	E.coli	Sensitive	Sensitive
	Pseudomonas	Sensitive	Sensitive
3	Salmonella	Sensitive	Sensitive
	Enterobacter	Resistant	Non-conclusive
	Pseudomonas	Sensitive	Sensitive
4	E.coli	Resistant	Non-conclusive
5	Kliebsella	Resistant	ESBL positive
6	Salmonella	Resistant	Non-conclusive
	Citobacter	Resistant	Non-conclusive
	Enterobacter	Resistant	Non-conclusive
7	E.coli	Resistant	Non-conclusive
	Citobacter	Resistant	Non-conclusive
	Enterobacter	Resistant	ESBL positive
	Salmonella	Resistant	Non-conclusive
8	Citobacter	Resistant	ESBL positive
	Enterobacter	Resistant	ESBL positive
	Pseudomonas	Resistant	Non-conclusive

Table: 2 Total Colifom Bacterial strains isolated from River Kshripra water Samples

Total River Kshripra Water samples	Interpretive Criteria	No of bacterial strains	Interpretation
08	Sensitive strains of coliform were isolated.	08	Bacterial strains were sensitive.
	Non-conclusive strains of coliform were isolated.	09	Bacterial strains were multidrug-resistant.
	ESBL-positive strains of coliform were isolated.	04	Bacterial strains were multidrug-resistant.

Graph: 1: Representation of Sensitive, Non-Conclusive, and ESBL+ Strains of Gram Negative Bacteria



4. CONCLUSIONS

Findings of the current work have shown that Kshripra river water is polluted with extended-spectrum beta-lactamase-producing gram negative bacteria belonging to the family Enterobacteriaceae. The presence of pathogenic bacteria, including Salmonella, E.coli, Enterobacter, Kliebsella, and Pseudomonas, provides an indicator of waterborne diseases including diarrhoea, typhoid, cholera, and shigellosis. In our study, resistant strains were identified, including E.coli, Kliebsella, Salmonella, Enterobacter, and Pseudomonas. The presence of antibiotic-resistant bacteria inside the Kshripra River is creating problems for the human population. The Kshripra River is utilised for irrigation, livestocks and other household purposes. Therefore, before using such types of water for human consumption. Water should be purified inside the water treatment system with respect to the removal of harmful pathogenic and antibiotic-resistant bacteria from the river water. Antibiotics should only be used when they are required. To overcome the problem of the spread of such types of antibiotic-resistant bacteria, the Kshripra River should be properly monitored in terms of various microbial and

chemical pollutants. All types of parameters should be within the permissible limit. Then only we will control the spreading of such types of pathogenic bacteria inside rivers. In this way, we will control the waterborne diseases that occur due to the presence of such types of gram negative bacteria. In this way, we can save the lives of various human populations because 80% of diseases in humans occur due to the consumption of unsafe drinking water.

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