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A Study on Analysing Gram Negative Multidrug-Resistant Enteric Bacteria from the River Kshripa, Ujjain (MP), India

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ABSTRACT: The main aim of the study was to evaluate the antibiotic resistance among gram negative enteric bacteria that were isolated from different ghats of the river Khripra. For the investigation of gram negative enteric bacteria, a total of 8 sampling sites were selected, and a total of 6 gram negative bacteria were isolated. The members are *E. coli*, *Salmonella*, *Enterobacter*, *Kliebsella* and *Citrobacter* Along with this, *Pseudomonas* were also isolated, and the resistance mechanism was analysed by the antibiotic susceptibility test, also known as the disc diffusion method and the Kirby-bauer method. For the detection of multidrug resistance among the Enterobacteriaceae, the following types of beta-lactam antibiotics were used: aztreonam, ceftazidime, cefpodoxime, chloramphenicol, amikacin, ceftriaxone, ciprofloxacin, and ampicillin. Finally, after performing the disc diffusion test, we isolated a total of 21 strains of gram negative bacteria, out of which 8 strains were sensitive against these antibiotics, while 13 gram negative bacterial strains showed the mechanism of antibiotic resistance. Besides, the MAR index of all the isolates was calculated, which shows that the strains of gram negative bacteria in samples 1, 2, and 3 were sensitive except *Enterobacter*, which was present in sample no. 3. On analysis, we also detected the resistant strains that were present in samples 4, 5, 6, 7, and 8. After calculating the MAR index, it was found that the isolated strains from samples 4, 5, 6, 7, and 8 Together with one isolate from Sample No. 3, a MAR Index greater than 0.2 indicates a high risk source of contamination where antibiotics are commonly used.

Keywords: Multidrug-Resistant Bacteria, Antibiotics, Antibiotic Resistance (AR), Antibiotic Resistance Genes (ARG), Kshripra River, MAR Index.

INTRODUCTION

Coliforms are gram negative bacilli that are members of the family Enterobacteriaceae. Coliform bacteria are present in the normal flora inside the digestive tract of all warm-blooded and some cold-blooded animals. For the prevention of disease in animals, antibiotics are being used in livestock. The bacteria isolated from livestock show the mechanism of antibiotic resistance. Surface water also acts as a carrier of antibiotic-resistant bacteria. The River Missouri shows the presence of tetracycline-resistant bacteria bacteria (McDonnell *et al*., 2004; Gardea *et al*., 2016). The study done on the irrigation water system has also been polluted by multidrug-resistant bacteria, which have the possibility of entering our ecosystem directly. The strains of multidrug-resistant *Enterobacter* and enteric pathogens have been responsible for a main problem within a community. In various aquatic ecosystems, multiple antibiotic-resistant bacteria were observed. When this MDR bacteria causes infection inside the human being, it creates a problem during its treatment with a proper drug (Chatterjee *et al*., 2021). For the prevention and treatment of infection in different fields, including agriculture and poultry, antibiotic groups such as cephalosporins and fluoroquinolones are commonly used. In the last two decades, the increased and careless use of antimicrobial substances in animal husbandry, aquaculture, and food preservation has created an environment of antibiotic-resistant bacteria. Multidrug-resistant bacteria have been isolated from the main rivers of India, such as Ganga, Yamuna and Cauvery. The bacteria present in the environment receive the resistance gene by horizontal gene transfer. The mechanism of horizontal gene transfer occurs through a variety of mobile gene elements, such as plasmids, bacteriophages, genomic islands, integrative and conjugative elements, insertion sequences, transposons, integrons, and miniature inverted repeat transposable elements (Dhawde *et al*., 2021; Nain *et al*., 2021; Salikan *et al*., 2020; Ash *et al*., 2002; Purohit *et al*., 2020; Torkan *et al*., 2016; Schwartz *et al*., 2002; Mustafa *et al*., 2022; Tula *et al*., 2022; Besharati *et al*., 2018; Resende *et al*., 2009). It is estimated that antibiotic resistance might lead to ten million deaths

annually by 2050. Thousands of deaths occur annually due to the antibiotic resistance mechanism that occurs inside the bacteria. The sewage treatment plant contains a huge population of antibiotic-resistant bacteria with antibiotic-resistant genes, which further discharge into aquatic habitats. Due to antibiotic-resistant bacteria with antibiotic-resistant genes, when they infect a human population, such types of pathogens create a negative effect on health, including treatment failure, long periods of treatment, and in chronic cases, death may occurs (Singh *et al*., 2020; Graham *et al*., 2011). The most common resistance mechanism against beta-lactam antibiotics is the production of an extended-spectrum beta-lactamase enzyme by gram-negative bacteria (Djenadi *et al*., 2017). The species of *Pseudomonas* carries antibiotic-resistant genes and also exchanges such genes with the members of the Enterobacteriaceae (Kittinger *et al*., 2016). The various types of water sources, such as rivers, lakes, sea, groundwater and drinking water, inside this bacteria show resistance mechanisms (Hanna *et al*., 2023; Ghabalo *et al*., 2022; Bartley *et al*., 2019). Antibiotics have played an important role in controlling various types of diseases that occur in animals and humans (Tadesse *et al*., 2012). According to the World Health Organisation, antibiotic resistance is a major threat to public health (Ogura *et al*., 2020; Wengenroth *et al*., 2021; Mustafa *et al*., 2022). Waste water treatment plants act as a source of antibiotic-resistant bacteria with antibiotic-resistant genes (Teshome *et al*., 2020). Hospital wastewater can be dangerous to people and ecosystems since it is contaminated with various types of pollutants, including radioactive, chemical, and pharmaceutical waste, as well as harmful microbes and antibiotic-resistant bacteria, along with antibiotic residue at concentrations that inhibit sensitive bacteria (Mogs *et al*., 2014). Using too many antibiotics and misuse of antibiotics in human and veterinary medicine are the main causes of the evolution and dissemination of antibiotic-resistant bacteria all over the world. Excretory material from animals and humans is responsible for spreading resistant enteric bacteria in aquatic habitats (Abo-State *et al*., 2012). According to the World Health Organisation, 80% of illnesses occur due to unsafe water (Odonkor *et al*., 2018; Ulfat *et al*., 2021). In recent years, strains of multidrug-resistant bacteria have increased fourfold (Basak *et al*., 2015). Per year in the United States alone, approximately 2 million infections occur due to antibiotic-resistant bacteria. Multidrug resistance among gram negative bacteria shows harmful effects (Riedel *et al*., 2019). Metropolitan wastewater treatment plants show the presence of antibiotic-resistant bacteria with antibiotic-resistant genes (Silva *et al*., 2007). There is a possibility of antibiotic-resistant bacteria being consumed by pilgrims during the mass bathing or drinking of river water. In Indian rivers, different antibiotic-resistant bacteria have been reported (Purohit *et al*., 2020). Bacteria can produce extracellular enzymes that can inactivate antibiotics such as penicillin, monobactam, carbapenems, and cephalosporins. These antibiotics are known as beta-lactum antibiotics. The enzyme beta-lactamase, which is normally produced by gram negative bacteria, breaks the beta-lactum ring of antibiotics, which makes the antibiotic inactive (Alam *et al*., 2018; Aujoulat *et al*., 2021; Azevedo *et al*., 2014). The dissemination of antibiotic resistance among bacteria is regarded as a universal hazard to human, animal, and environmental health (Moreira *et al*., 2014). *Peudomonas aeruginosa* and Enterobacteriaceae members are resistant to carbapenems and third-generation cephalosporins (Mustafa *et al*., 2022; Muller *et al*., 2018). Municipal wastewater treatment plants are antimicrobial-resistant hotspots (Mustafa *et al*., 2022). Bacteria genera isolated from water, including *Enterobacter*, and *E. coli*, show the mechanism of multidrug resistance (Babalola *et al*., 2021). The main cause of pollution inside the Halda River is the unplanned activities of various industries, including textile mills, powerplants, papermills, tanneries, etc. (Rahman *et al*., 2022). Due to urbanisation, pathogenic bacteria discharge into water bodies (Afzal *et al*., 2021). In the past few years, the increased use of antibiotics has caused antibiotic resistance in bacteria, including coliform bacteria. Multidrug-resistant bacteria have been isolated from hospital effluent and sewage water. The presence of multiple antibiotic resistances in different water habitats has been reported in Malaysia (Lihan *et al*., 2017). *E. coli* is an important indicator organism with respect to faecal pollution in environmental water; therefore, *E. coli* has also been used in analysing antibiotic resistance in gram-negative bacteria (Wambugu *et al*., 2015). There is no proper treatment of effluents that belong to hospitals and municipalities due to this infectious agent, and antibiotic-resistant microbes are passed into rivers, which further reach into communities (Belachew *et al*., 2018). The rivers of Metropolitan are very prone and serve as a reservoir of antibiotic-resistant bacteria with antibiotic resistance genes. According to a report by the WHO (2018), there should be serious awareness with respect to the control of antibiotics by different international organisations in terms of pollution inside the surface water by antibiotics (Ravi *et al*., 2022).

MATERIAL AND METHODS

A. Sampling Method

Between March 2021 and April 2022, water samples were collected. We selected eight locations on the Kshipra River to serve as sample sites. Plastic bottles with sterile screw caps were used to collect samples. After being kept at a constant 4°C in an ice box to prevent the growth of microbes, each bottle was placed in a thermal stabilising box. Within two hours, the bottles were transferred to a microbiology research laboratory for research. With the use of a pH strip and a thermometer, the temperature and pH were determined at each sample location (Wambugu *et al*., 2015; Teshome *et al*., 2020; Babalola *et al*., 2021; Nain *et al*., 2015).

B. Study Area

The Kshripra river in Ujjain is regarded as a holy river. Many pilgrims visit Ujjain in order to partake in sacred baths. It has been observed that as the water quality declines, a number of anthropogenic activities take

place, including mass bathing, clothes washing, and the disposal of trash like coconuts, ashes, photos, and garland. A lot of funeral-related activities were also going on. The river rises in the northern Dhar district and flows north across the Malwa Plateau to meet the Chambal River in the Mandsaur district, which is where the MP and Rajasthan borders meet. It is a sacred river in Hinduism. The holy city of Ujjain is situated on its east bank. The Kumbh Mela (Sinhastha fair), which is held after every 12 years of period.

C. Isolation and Identification of Gram-negative Enteric Bacteria

For the isolation of gram-negative enteric bacteria streak plate method were emplyoed, one loopful of enriched media was inoculated on different culture media like Mac-Conkey Agar, Eosine Methylene Blue Agar, Nutrient Agar, Hi-chrome MM Agar, and Salmonella Shiegella Agar for the isolation of *E. coli*, *Kliebsella*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Pseudomonas*. For further confirmation of Gram negative bacteria, biochemical tests IMViC were employed. Besides this, triple sugar tests, oxidase tests, catalase, gas production tests, motility tests, and gram staining techniques were also used for the identification of gram negative bacteria. After identification, the pure culture of bacteria was preserved in a nutrient agar slant for the antibiotic susceptibility test (Singh *et al*., 2020).

(i) Antibiotic Susceptibility Test. For the identification of multidrug-resistant bacteria, an antibiotic susceptibility test, also called the disc diffusion method, was performed on all 8 isolates. For confirmation of multidrug-resistant bacteria, Mueller-Hinton agar plates were prepared. To confirm the multidrug-resistant bacteria, a pure culture of the test bacteria was prepared, and the bacterial turbidity was set to 0.5 McFarland standards. By taking the pure culture of bacteria with the help of sterilised cotton swapped, we spread the pure culture of bacteria over the plates of Mueller Hinton agar plates. After spreading the pure culture of bacteria, we were allowed to settle down for 5–10 minutes. After this, we placed the beta lactam antibiotics at a distance of 2.5 cm with the help of a sterilised forcep. After placing the antibiotics, the plates were kept inside the incubator at 37 °C for 24 to 48 hours of incubation. After the incubation period, observe the zone of inhibition. These zones of inhibition were further compared with the CLSI standards (Clinical and Laboratory Standards Institute). The following types of beta-lactam antibiotics were used for confirmation of multidrug-resistant bacteria: aztreonam, ceftazidime, cefpodoxime, chloramphenicol, amikacin, ceftriaxone, ciprofloxacin, and ampicillin. The bacterial isolates that show resistance to three or more classes of antibiotics are termed multidrug-resistant bacteria (MDR). (Singh *et al*., 2020).

(ii) Calculation of the Multiple Antibiotic Resistance (MAR) Index. The multiple antibiotic resistance (MAR) index was calculated using the formula

MAR = Number of antibiotics to which an isolate showed resistance

Total Number of Antibiotics (Teshome *et al*., 2020)

RESULT AND DISCUSSION

In the study of Wambugu *et al*. (2015) on the Athi river water in Machakos, Kenya, they isolated a high-resistant strain of *E. coli*. which shows a resistant mechanism against ampicillin, cefoxitin, amoxicillin, clavulanic acid, and sulfamethoxazole, whereas the *E. coli* strain shows the least resistance mechanism against gentamicin, cefepime, and ceftazidime. Similar types of resistant strains of *E. coli* were also isolated from the Shripra River from samples 4 and 7. Besides the *E. coli* strain, we also studied the resistant strains of *Enterobacter*, *Kliebsella*, *Salmonella*, *Citrobacter*, and *Pseudomonas*, which are clearly mentioned in Table 1, 3. In the study of Belachew *et al*. (2018), they isolated a high level of drug-resistant strains of gram-negative bacteria from an urban river in Addid Ababa, Ethiopia. Their studies show that the strains of *E. coli* show a high level of resistance to ampicillin, cefalotin, cefuroxime, ceftriaxone, and cefepime. In addition to this, they also studied the resistance mechanism inside the *Kliebsella* bacteria, which shows resistance against ampicillin. Another strain of gram negative bacteria, *Citrobacter*, also shows a resistance mechanism against cefazolin and amoxicillin-clavulanic acid. However, all isolates, including *E. coli*, *Kliebsella*, and *Citrobacter*, were sensitive against Ceftriaxone, tetracycline, nitrofurantoin, and trimethoprim-sulfamethoxazole. Similar types of resistance strains of gram negative bacteria were also studied from the Kshripra River. The strains of gram negative bacteria were *E. coli*, *Kliebsella*, *Enterobacter*, *Citobacter*, *Salmonella*, and *Pseudomonas*, which were isolated from samples 1–8, as mentioned in Table 1, 3. In the study of Ravi *et al*. (2022) on the Ghaghara River, India, it was shown that the strain of *Kliebsella* isolated from the Ghaghara River showed resistance mechanisms against penicillin G, cefuroxime, amoxicillin, and ampicillin. In the study of Ravi *et al*. (2022) on the Ghaghara River, India, it was shown that there were multiple antibiotic-resistant bacteria and provided a route to spread the multidrug-resistant pathogen in the human and animal populations through the aquatic environment. In our study on the Kshripra River with respect to analysing multidrug-resistant bacteria, we found similar findings to the study done by Ravi *et al*. (2022) on the Ghaghara River. In our study, the resistant strain of *Kliebsella* was isolated from sample no. 5, which is mentioned in Table 1. In the study of Purohit *et al*. (2022) on the Kshripra River, an isolated resistant strain of *E. coli* from river water and river sediment shows the resistance mechanism against different types of antibiotics such as ceftazidime, cefotaxime, cefapime, ampicillin, tetracycline, and co-trimoxazole. In our study on the Kshripra River, similar types of resistant strains of *E. coli* were also isolated from samples no -4, 7. The isolates of *E. coli* from sample no. 4 show a resistance mechanism with aztreonam and ceftazidime, while sample no. 7 shows a resistance mechanism with similar antibiotics as aztreonam and ceftazidime in addition to ampicillin. In the study of Besharati *et al*. (2018) on the Karoon River, enteric bacteria were isolated, including *E. coli*, *Salmonella*, *Kliebsella*,

Enterobacter, and *Pseudomonas aeruginosa*. Multidrug resistance has been found in many bacterial isolates. The highest number of bacterial isolates were resistant to cephalexin, and the least amount of resistance was found against ciprofloxacin. Similar isolates were also isolated from the Kshripra River. The isolates were *E. coli*, *Salmonella*, *Kliebsella*, *Enterobacter*, and *Pseudomonas*. Out of these isolates in our study, the multidrug resistance mechanism was shown by *E. coli*, *Kliebsella*, *Enterobacter*, and *Salmonella*, as mentioned in Table 1, 3. In the study of Afzal *et al*. (2021) on the swat river, they isolated a *Pseudomonas* resistant strain from the swat river, which shows the resistance mechanism against ampicillin. A similar result was also obtained with respect to the multidrug-resistant strain of *Pseudomonas*, which shows resistance against ampicillin. We obtained the resistant strain of *Pseudomonas* from sample no. 8, as clearly mentioned in Table 1, 3. In the same field, another study done by Lihan *et al*. (2017) on recreational river water of a community resort in Baram, Sarawak, Malaysian Borneo found that *Enterobacter* spp. shows resistance mechanisms with nitrofurantoin, ampicillin, and piperacillin. *Kliebsella* spp. shows resistance mechanisms to ampicillin, piperacillin, and tobramycin. Similar types of resistance strains of *Enterobacter* and *Kliebsella* were also isolated from the Kshripra River, which is clearly mentioned in Table 1, 3. In the study of Kittinger *et al*. (2016) on the Danube, they isolated a resistant strain of *Pseudomonas* spp. The bacterial strain *Pseudomonas* spp. shows resistance mechanisms against meropenem, piperacillin/tazobactam, and ceftazidime. In our studies on the Kshripra River, a similar resistant strain of gram negative bacteria, *Pseudomonas* spp., was isolated from sample no. 8 and shows the resistance mechanism against ceftazidime, chloramphenicol, ceftriaxone and ampicillin. In the same field, another study done by Abo-State (2012) on the Rosetta branch of the Nile, Egypt, studied the Enterobacteriacae members, including *Salmonella typi*, *E. coli*, and *Citrobacter freundii*, which show 100% resistance against ampicillin, methicillin, vancomycin, erythromycin, clindamycin, trimethoprim/ sulfamethoxazole and tetracycline. Also, they fail to show resistance mechanisms against norfloxacin and ofloxacin. MAR index value of *Salmonella typhi* shows (0.69). *Citrobacter freundii* shows (0.60, 0.55) and *E. coli* shows (0.46). Similarly, in our study, we also isolated a resistant strain of *Salmonella* from sample no. 6. which show resistance mechanisms against cefpodoxime, ceftazidime, ceftriaxone, ciprofloxacin, and ampicillin, and sample no. 7 *Salmonella* shows resistance mechanisms against aztreonam, ceftazidime, and ampicillin. Similarly, *E. coli* isolated from sample no. 4 shows a resistance mechanism against aztreonam, ceftazidime, and ampicillin, while *E. coli* isolated from sample no. 7 shows a resistance mechanism against aztreonam and ceftazidime. In our study on the Kshripra River, the resistant strain of *Salmonella* was isolated from sample 6, which showed a MAR index of 0.62, and *Salmonella* was isolated from sample 7, which showed a MAR index of 0.37. The *Citrobacter* strain we isolated from sample no. 6 shows a MAR index of 0.37; the *Citrobacter* of sample no. 7 shows a MAR index of 0.25; and the *Citrobacter* of sample no. 8 shows a MAR index of 0.75. Similar to *E. coli*, we were isolated from sample no. 4, which shows a MAR index of 0.37, and *E. coli*, isolated from sample no. 7, shows a MAR index of 0.25.

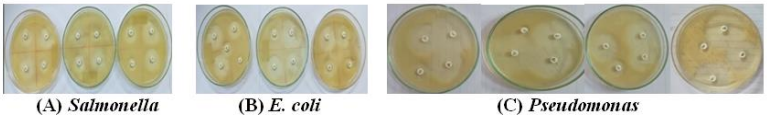


Fig. 1. Antibiotic Susceptibility Test for Sample-1 Isolates.

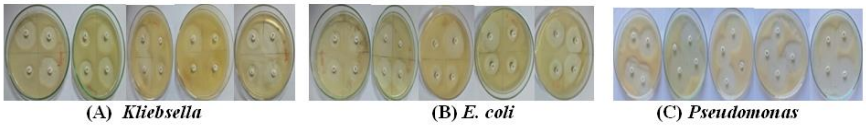


Fig. 2. Antibiotic Susceptibility Test for Sample 2 Isolates.

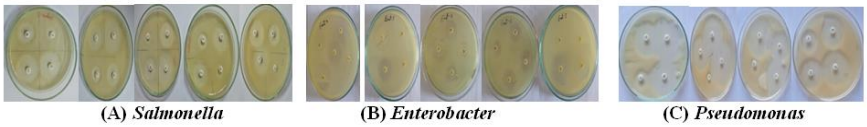


Fig. 3. Antibiotic Susceptibility Test for Sample 3 Isolates.

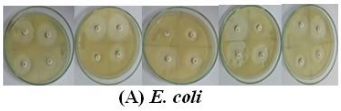


Fig. 4. Antibiotic Susceptibility Test for Sample 4 Isolates.

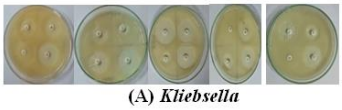


Fig. 5. Antibiotic Susceptibility Test for Sample 5 Isolates.

Fig. 6. Antibiotic Susceptibility Test for Sample 6 Isolates.

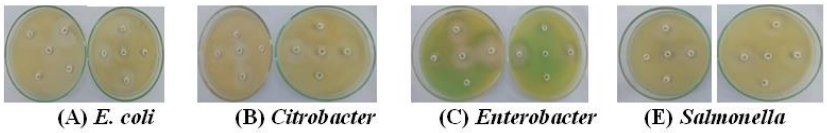


Fig. 7. Antibiotic Susceptibility Test for Sample 7 Isolates.

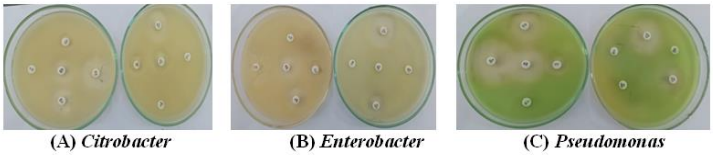


Fig. 8. Antibiotic Susceptibility Test for Sample 8 Isolates.

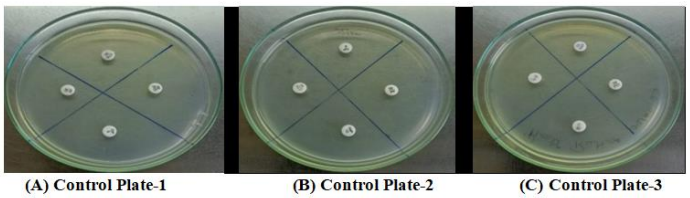


Fig. 9. Control for Antibiotic Susceptibility Test.

Table 1: Showing Sensitive and Resistance Mechanism as well as MAR Index by Gram Negative Bacteria.

Kshripra River Water Sample	Coliform Bacteria Isolated	Interpretive Criteria Analyzed as Per CLSI Standards (Clinical and Laboratory Standards Institute)		
			MAR Index	
1	*Salmonella*	Sensitive	0	The isolated bacterial strains did not show any resistance mechanisms.
	E. coli	Sensitive	0	The isolated bacterial strains did not show any resistance mechanisms.
	Pseudomonas	Sensitive	0	The isolated bacterial strains did not show any resistance mechanisms.
2	*Kliebsella*	Sensitive	0	The isolated bacterial strains did not show any resistance mechanisms.
	E. coli	Sensitive	0	The isolated bacterial strains did not show any resistance mechanisms.
	Pseudomonas	Sensitive	0	The isolated bacterial strains did not show any resistance mechanisms.
3	*Salmonella*	Sensitive	0	The isolated bacterial strains did not show any resistance mechanisms.
	Enterobacter	Resistant	0.62	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
	Pseudomonas	Sensitive	0	The isolated bacterial strains did not show any resistance mechanisms.
4	*E. coli*	Resistant	0.37	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
5	*Kliebsella*	Resistant	0.5	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
6	*Salmonella*	Resistant	0.62	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
	Citobacter	Resistant	0.37	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
	Enterobacter	Resistant	0.5	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
7	*E. coli*	Resistant	0.25	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
	Citobacter	Resistant	0.25	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
	Enterobacter	Resistant	0.37	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
	Salmonella	Resistant	0.37	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
8	*Citobacter*	Resistant	0.75	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
	Enterobacter	Resistant	0.72	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.
	Pseudomonas	Resistant	0.5	A MAR greater than 0.2 means that the high-risk source of contamination is where antibiotics are frequently used.

Table 2: Total Coliform Bacterial Strains Isolated from River Kshripra Water Samples.

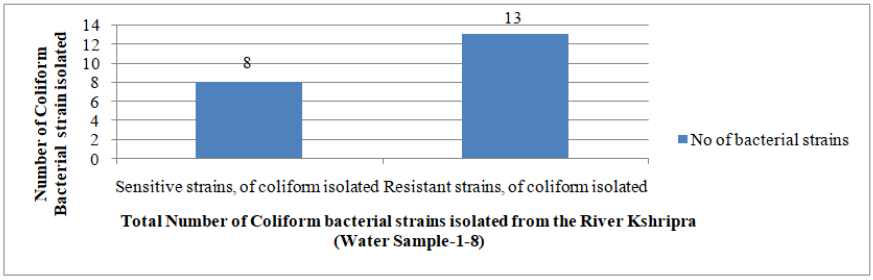
Total water samples of the River Kshripra	Interpretive Criteria	Number of bacterial strains	Interpretation
1-8	Sensitive strains of coliform were isolated	08	Bacterial strains were sensitive
	Resistant strains of coliform were isolated	13	Bacterial strains were multidrug-resistant

Table 3: Antibiotic Sensitivity Test for Detection of Multidrug Resistant Bacteria.

Kshripra River Water Samples	Coliform Bacteria Isolated from the River Kshripra	Beta-Lactum Antibiotic Used	Symbol of Antibiotic	Con. (µg)	Zone of Inhibition millimetre (mm)	Interpretive Criteria
1.	*Salmonella*	Aztreonam	AT	10	27	Sensitive
		Ceftazidime	CAZ	30	20	Intermediate
		Cefpodoxime	CPD	10	23	Sensitive
		Chloramphenicol	C	30	24	Sensitive
		Amikacin	AMK	30	19	Sensitive
		Ceftriaxone	CTR	30	27	Sensitive
		Ciprofloxacin	CIP	5	26	Sensitive
		Ampicillin	AMP	30	16	Sensitive
	E. coli	Aztreonam	AT	10	28	Sensitive
		Ceftazidime	CAZ	30	20	Intermediate
		Cefpodoxime	CPD	10	32	Sensitive
		Chloramphenicol	C	30	27	Sensitive
		Amikacin	AMK	30	19	Sensitive
		Ceftriaxone	CTR	30	28	Sensitive
		Ciprofloxacin	CIP	5	97	Sensitive
		Ampicillin	AMP	30	18	Sensitive
	Pseudomonas	Aztreonam	AT	10	20	Intermediate
		Ceftazidime	CAZ	30	34	Sensitive
		Ceftriaxone	CTR	30	45	Sensitive
		Amikacin	AK	30	33	Sensitive
		Ciprofloxacin	CIP	5	35	Sensitive
	Kliebsella	Aztreonam	AT	10	28	Sensitive
		Ceftazidime	CAZ	30	23	Sensitive
		Cefpodoxime	CPD	10	30	Sensitive
		Chloramphenicol	C	30	27	Sensitive
		Amikacin	AMK	30	19	Sensitive
		Ceftriaxone	CTR	30	32	Sensitive
		Ciprofloxacin	CIP	5	38	Sensitive
		Ampicillin	AMP	30	21	Sensitive
	Pseudomonas	Aztreonam	AT	10	20	Intermediate
		Ceftazidime	CAZ	30	35	Sensitive
		Ceftriaxone	CTR	30	45	Sensitive
		Amikacin	AK	30	32	Sensitive
		Ciprofloxacin	CIP	5	40	Sensitive
	E. coli.	Aztreonam	AT	10	31	Sensitive
		Ceftazidime	CAZ	30	23	Sensitive
		Cefpodoxime	CPD	10	35	Sensitive
		Chloramphenicol	C	30	26	Sensitive
		Amikacin	AK	30	22	Sensitive
		Ceftriaxone	CTR	30	30	Sensitive
		Ciprofloxacin	CIP	5	41	Sensitive
		Ampicillin	AMP	30	19	Sensitive
3.	*Salmonella*	Aztreonam	AT	10	29	Sensitive
		Ceftazidime	CAZ	30	21	Sensitive
		Cefpodoxime	CPD	10	24	Sensitive
		Chloramphenicol	C	30	25	Sensitive
		Amikacin	AK	30	21	Sensitive
		Ceftriaxone	CTR	30	28	Sensitive
		Ciprofloxacin	CIP	5	29	Sensitive
		Ampicillin	AMP	30	0	Resistant
	Enterobacter	Aztreonam	AT	10	0	Resistant
		Ceftazidime	CAZ	30	0	Resistant
		Cefpodoxime	CPD	10	22	Sensitive
		Chloramphenicol	C	30	0	Resistant
		Amikacin	AK	30	18	Sensitive
		Ceftriaxone	CTR	30	0	Resistant
		Ciprofloxacin	CIP	5	25	Intermediate

		Ampicillin	AMP	30	0	Resistant
	Pseudomonas	Aztreonam	AT	10	20	Intermediate
		Ceftazidime	CAZ	30	32	Sensitive
		Ceftriaxone	CTR	30	45	Sensitive
		Amikacin	AK	30	30	Sensitive
		Ciprofloxacin	CIP	5	34	Sensitive
4.	*E. coli*	Aztreonam	AT	10	0	Resistance
		Ceftazidime	CAZ	30	15	Resistance
		Cefpodoxime	CPD	10	26	Sensitive
		Chloramphenicol	C	30	26	Sensitive
		Amikacin	AK	30	21	Sensitive
		Ceftriaxone	CTR	30	30	Sensitive
		Ciprofloxacin	CIP	5	31	Sensitive
		Ampicillin	AMP	30	0	Resistance
5.	*Kliebsella*	Aztreonam	AT	10	0	Resistant
		Ceftazidime	CAZ	30	0	Resistant
		Cefpodoxime	CPD	10	23	Sensitive
		Chloramphenicol	C	30	0	Resistant
		Amikacin	AK	30	20	Sensitive
		Ceftriaxone	CTR	30	25	Sensitive
		Ciprofloxacin	CIP	5	28	Sensitive
		Ampicillin	AMP	30	0	Resistant
6.	*Salmonella*	Aztreonam	AT	10	25	Sensitive
		Ceftazidime	CAZ	30	0	Resistant
		Cefpodoxime	CPD	10	16	Resistant
		Chloramphenicol	C	30	22	Sensitive
		Amikacin	AMK	30	18	Sensitive
		Ceftriaxone	CTR	30	0	Resistant
		Ciprofloxacin	CIP	5	0	Resistant
		Ampicillin	AMP	30	0	Resistant
	Citrobacter	Aztreonam	AT	10	20	Sensitive
		Ceftazidime	CAZ	30	20	Intermediate
		Cefpodoxime	CPD	10	27	Sensitive
		Chloramphenicol	C	30	0	Resistant
		Amikacin	AK	30	24	Sensitive
		Ceftriaxone	CTR	30	18	Resistant
		Ciprofloxacin	CIP	5	37	Sensitive
		Ampicillin	AMP	30	0	Resistant
	Enterobacter	Aztreonam	AT	10	0	Resistant
		Ceftazidime	CAZ	30	18	Intermediate
		Cefpodoxime	CPD	10	21	Sensitive
		Chloramphenicol	C	30	0	Resistant
		Amikacin	AMK	30	18	Sensitive
		Ceftriaxone	CTR	30	12	Resistant
		Ciprofloxacin	CIP	5	26	Sensitive
		Ampicillin	AMP	30	0	Resistant
7.	*E. coli*	Aztreonam	AT	10	0	Resistant
		Ceftazidime	CAZ	30	0	Resistant
		Cefpodoxime	CPD	10	26	Sensitive
		Chloramphenicol	C	30	25	Sensitive
		Amikacin	AMK	30	18	Sensitive
		Ceftriaxone	CTR	30	29	Sensitive
		Ciprofloxacin	CIP	5	28	Sensitive
		Ampicillin	AMP	30	17	Sensitive
	Citrobacter	Aztreonam	AT	10	0	Resistant
		Ceftazidime	CAZ	30	11	Resistant
		Cefpodoxime	CPD	10	27	Sensitive
		Chloramphenicol	C	30	23	Sensitive
		Amikacin	AK	30	20	Sensitive
		Ceftriaxone	CTR	30	28	Sensitive
		Ciprofloxacin	CIP	5	30	Sensitive
		Ampicillin	AMP	30	18	Sensitive
	Enterobacter	Aztreonam	AT	10	25	Sensitive
		Ceftazidime	CAZ	30	0	Resistant
		Cefpodoxime	CPD	10	24	Sensitive
		Chloramphenicol	C	30	0	Resistant
		Amikacin	AK	30	25	Sensitive
		Ceftriaxone	CTR	30	20	Intermediate
		Ciprofloxacin	CIP	5	33	Sensitive
		Ampicillin	AMP	30	0	Resistant
	Salmonella	Aztreonam	AT	10	0	Resistant
		Ceftazidime	CAZ	30	0	Resistant
		Cefpodoxime	CPD	10	22	Sensitive
		Chloramphenicol	C	30	21	Sensitive
		Amikacin	AK	30	16	Intermediate
		Ceftriaxone	CTR	30	23	Sensitive

		Ciprofloxacin	CIP	5	25	Sensitive
		Ampicillin	AMP	30	0	Resistant
8.	*Enterobacter*	Aztreonam	AT	10	0	Resistant
		Ceftazidime	CAZ	30	0	Resistant
		Cefpodoxime	CPD	10	16	Resistant
		Chloramphenicol	C	30	0	Resistant
		Amikacin	AK	30	17	Sensitive
		Ceftriaxone	CTR	30	24	Sensitive
		Ciprofloxacin	CIP	5	19	Resistant
		Ampicillin	AMP	30	0	Resistant
	Citrobacter	Aztreonam	AT	10	0	Resistant
		Ceftazidime	CAZ	30	13	Resistant
		Cefpodoxime	CPD	10	15	Resistant
		Chloramphenicol	C	30	0	Resistant
		Amikacin	AMK	30	16	Intermediate
		Ceftriaxone	CTR	30	23	Sensitive
		Ciprofloxacin	CIP	5	18	Resistant
		Ampicillin	AMP	30	0	Resistant
	Pseudomonas	Aztreonam	AT	10	24	Sensitive
		Ceftazidime	CAZ	30	0	Resistant
		Cefpodoxime	CPD	10	30	Sensitive
		Chloramphenicol	C	30	0	Resistant
		Amikacin	AK	30	25	Sensitive
		Ceftriaxone	CTR	30	16	Resistant
		Ciprofloxacin	CIP	5	35	Intermediate
		Ampicillin	AMP	30	0	Resistant



Graph 1: Showing the sensitive strains as well as the resistance mechanisms of Gram Negative Coliform Bacteria.

CONCLUSIONS

Enterobacteriaceae, which shows the resistance mechanisms against different types of test antibiotics. According to the obtained data with respect to multidrug-resistant bacteria, which shows the resistance mechanism against different test antibiotics, it can be concluded that the Kshripra River is facing a water quality change problem and there is a threat of antibiotic resistance occurrence among Enterobacteriaceae members of this aquatic water ecosystem. So, some necessary measures must be taken by higher authorities to prevent unprocessed wastewater discharge to this river and to execute different types of biological filtration processes for the degradation of antibiotics before their discharge into the river water. In this way, we can prevent the spreading of multidrug-resistant bacteria and save mankind against the occurrence of different types of water-borne diseases that occur with multidrug-resistant bacteria.

FUTURE SCOPE

The research will help in the prevention of waterborne diseases that may occur due to contamination by multidrug-resistant bacteria. Besides, this research also gives us the idea that during the time of infection in the human population with these pathogenic Gram-negative bacteria, which types of antibiotics are more effective in treatment of multidrug-resistant bacteria. In this way, we can easily save the lives of various mankinds and make people aware that the unnecessary use of antibiotics shall be restricted.

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