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Prevalence of Antibiotic Resistant Coliforms in Penganga River Residing Hadgoan.

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ABSTRACT:

The goal of the present investigation was to identify multiple antibiotic resistance in coliform bacteria that were collected from water samples of Penganga River residing Hadgaon town. Penganga River Hadgaon site PRHS-1, PRHS -2, PRHS -3 & PRHS -4 were the four sites selected and studied Seasonal variations in total coliforms and faecal coliforms count. Monsoon season revealed the highest levels of total coliforms and faecal coliforms during the seasonal examination of the water samples, with PRHS-4 having 1800 CFU/100 ml and PRHS-2 having 220 CFU/100 ml, respectively. Using a culture-dependent methodology, the 39 isolated bacterial cultures were identified. The most common isolates from the river water samples were *Citrobacter sp., Enterobacter sp., K. pneumoniae, and E. coli.* Multiple antibiotic Resistance (MAR) profile of isolates studied by the antibiotic disc diffusion method and zone of inhibition (mm) was measured. Out of total 39 isolates studied, maximum isolates i.e. 80% showed resistance against penicillin whereas highest sensitivity was found against streptomycin i.e. 70% of the isolates.

Keywords: Antibiotics, Resistance Profile, Disc diffusion method, coliforms.

Introduction:

The Penganga River, a pivotal water resource for Hadgaon City, plays a crucial role in sustaining and developing the local ecosystem. Concerns regarding the emergence of antibiotic resistance in coliform bacteria within this aquatic environment triggered targeted exploration of specific locations along the river. This study focuses on high-prevalence sites of antibiotic-resistant coliforms to inform strategy of water management to counter the antibiotic resistance.

Coliforms are commonly found in the environment, including water sources, and can serve as reservoirs for antibiotic resistant genes. Understanding the prevalence of antibiotic-resistant coliforms in water is crucial for assessing the risk of transmission of disease threat to public health and to animals (Stange et al; 2016).

Escherichia coli (E.coli) is a faecal indicator organism linked with faecal discharge in water resources, is a member of faecal coliforms that contaminate the drinking water sources through human and animal faecal waste discharge. In the recent years, in *E. coli* multiple antibiotic resistance against many broad spectrum antibiotics and antimicrobial drugs has been reported. It's a crucial way to protect humans from getting sick, monitoring of water with these microbial indicators is very important for keeping our communities healthy (Krista *et al*; 2022).

The increased resistance in bacterial populations against commonly used antibiotics has become a critical issue in treating infections in both humans and animals (WHO 2014). The World Health Organization reports that annually, water-related illnesses like cholera, diarrhoea, and dysentery kill the lives of 3.4 million peoples and majorly children's. (Pandey *et al*; 2014).

Increase in antibiotic resistance level of the bacteria leads to the majority of nosocomial, neonatal infections leading to bacteraemia, wound infection, urinary tract infection which is often fatal in newborns and immune-compromised patients.

The increasing antibiotic resistance in bacteria towards some regular antibiotics (concentration in mcg) like, Ceftazidime(30), Penicillin(10), Polymyxin B(10), Tigecycline(15), Streptomycin(10), Gentamycin (10), Tobramycin (10), Linezolid (30), Ciprofloxacin (5), Levofloxacine (5), Azithromycin (15), Clarithromycin(15) leading to global issues. Analysis of the density of coliforms in water resources is crucial parameter in order to protect public health.

Materials & Methods:

Sampling of water: Water Sampling Sites along the Penganga River were chosen based on their proximity to Hadgaon town. The Water Samples were collected in sterile plastic container over a period of March 2019 to December 2019. As per the guidelines given by American Public Health Association (APHA), water samples were collected and further processed from Penganga river at four sites designated as PRHS-1, PRHS-2, PRHS-3 and PRHS-4 (APHA, 1995).

Enumeration of total coliforms and faecal Coliform: Membrane filtration technique was performed to isolate and enumerate coliform from the water samples. The Samples were collected from river and 10-folds diluted, and filtered using membrane filter of 0.45µm porosity (Britton et al. 1988). M-FC agar and M-Endo Agar were used as selective and differential media for enumeration of faecal coliform and total coliform. Membranes filters after filtration of water samples, were transferred on The M-FC agar plates and incubated for 24 hrs at 44.5°C and M- Endo Agar plates and incubated at 37°C for 24 hrs. The colony count was recorded from M-FC agar plates as faecal coliforms and M- Endo Agar plates as total coliforms (Buckalew *et al;* 2006). Total coliform and Faecal coliform count was represented as the colony forming units (CFU/100ml) (Table 01).

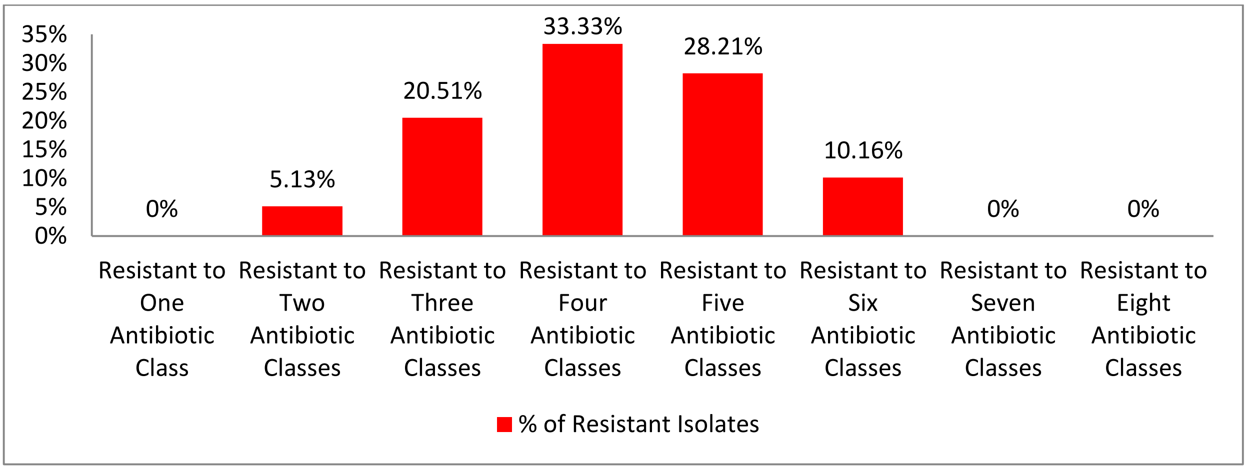
Seasons	Months	Total Coliform (MPN/100)								Total Faecal Coliform (CFU/100ml)							
		*PRHS1	Mean	PRHS2	MEAN	PRHS3	MEAN	PRHS4	MEAN	PRHS1	Mean	PRHS2	MEAN	PRHS3	MEAN	PRHS4	MEAN
Summer	Mar-19	1000	950	1000	1100	1400	1300	1000	950	100	145	150	125	140	120	130	125
	May-19	900		1200		1200		900		90		100		100		120	
Monsoon	Jul-19	1200	1300	1300	1400	1700	1700	2000	**1800**	220	200	200	**220**	160	175	210	205
	Sep-19	1400		1500		1700		1600		180		240		190		200	
Post Monsoon	Nov-19	800	750	900	800	1200	1100	900	850	80	75	120	120	100	95	100	110
	Dec-19	700		700		1000		800		70		120		90		120	

***Penganga River Hadgaon Site (PRHS)**

Table 1: Seasonal variation of microbial characteristics of Penganga River at study site.

Isolation and identification of coliforms: The selected 39 colonies were sub-cultured on EMB agar and incubated for 24 hours at 37 °C. The characteristic coliform colonies were selected and confirmed using biochemical tests using Bergy's manual of systemic bacteriology. The colonies with a green metallic sheen were identified as faecal coliforms, and the other colonies with a pink color and no metallic sheen were identified as non faecal coliforms. Further identification and confirmation of the selected faecal and non faecal coliforms was done using 16S rRNA analysis (Data not given here) (Edward and Ewing, 1972).

Antibiotic Susceptibility Test: Selected 39 isolates were analyzed for antibiotic sensitivity against selected 8 classes of antibiotics, and the antibiotic sensitivity of the isolates was determined by the Kirby-Bauer Disk Diffusion Method on Muller Hinton Agar. E. coli ATCC 25922 was used as a control for testing sensitivity to selected antibiotics (NCCLS 2000). The homogenous suspension in sterile saline of isolated test culture was spread on Muller Hinton Agar; the plates were rested at room temperature for 2 hours for drying, and the antibiotic susceptibility disc was placed on the pre-inoculated Muller Hinton Agar plates. The plates were then incubated at 37°C for 24 hours, and the diameter of the zone of inhibition was recorded. A total of 13 antibiotics belonging to the selected 8 classes were studied, and results were recorded and compared with standards (Graph 01).



Graph 01: Percentage resistance of the coliform isolates against eight antibiotic classes.

Results: In the present study, during the seasonal examination of the water samples, it was observed that from site PRHS-4, the highest total coliform count was 1800 MPN/100 ml, and PRHS-2 had the highest total faecal coliform count of 220 CFU/100 ml during the monsoon season and lowest MPN count was found during summer season (Table 01). The MPN count was compared to standards given by the Central Pollution Control Board (CPCB), which suggest that the water should be used for drinking purposes only after conventional treatment and disinfection. (Source: hppt://cpcb.nic.in/water_Quality_Criteria.php).

Using a culture-dependent methodology, the coliform bacteria were isolated, and the 39 isolated bacterial cultures were identified using biochemical analysis as per Bergy's Manual of Systemic Bacteriology (Bergy et al., 1984). The most common genera identified (number of isolates) from the Penganga river water samples were Citrobacter sp. (06), Enterobacter sp. (09), K. pneumoniae (08), and E. coli (16) (Fig.01).

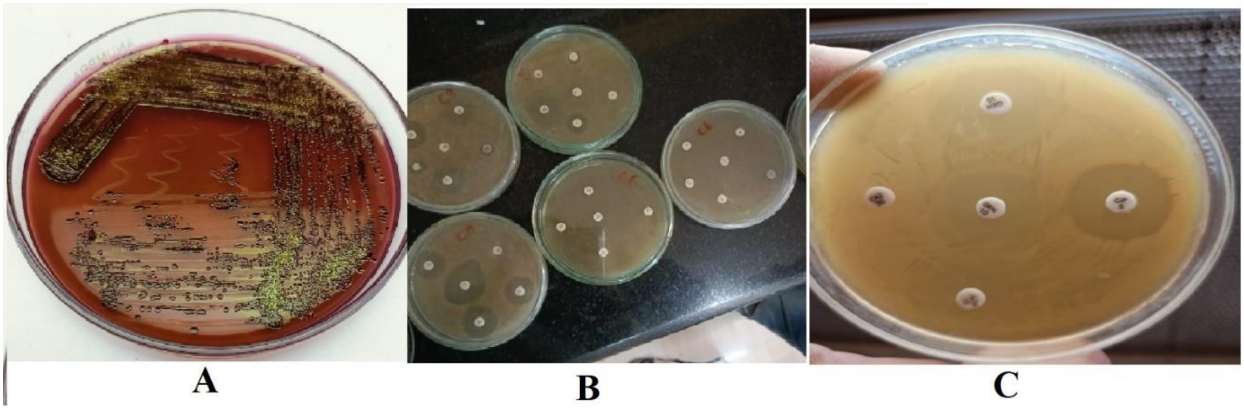
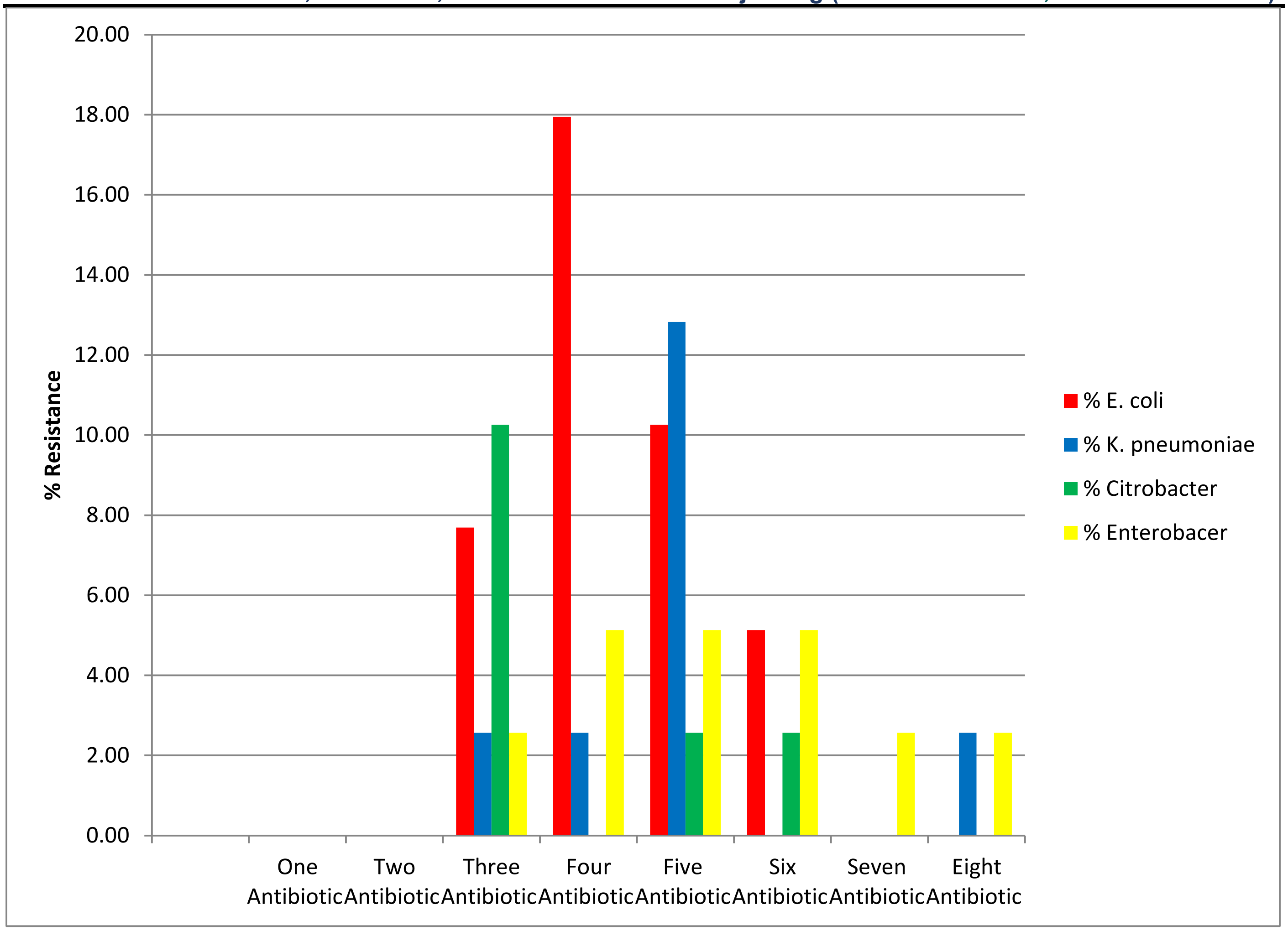
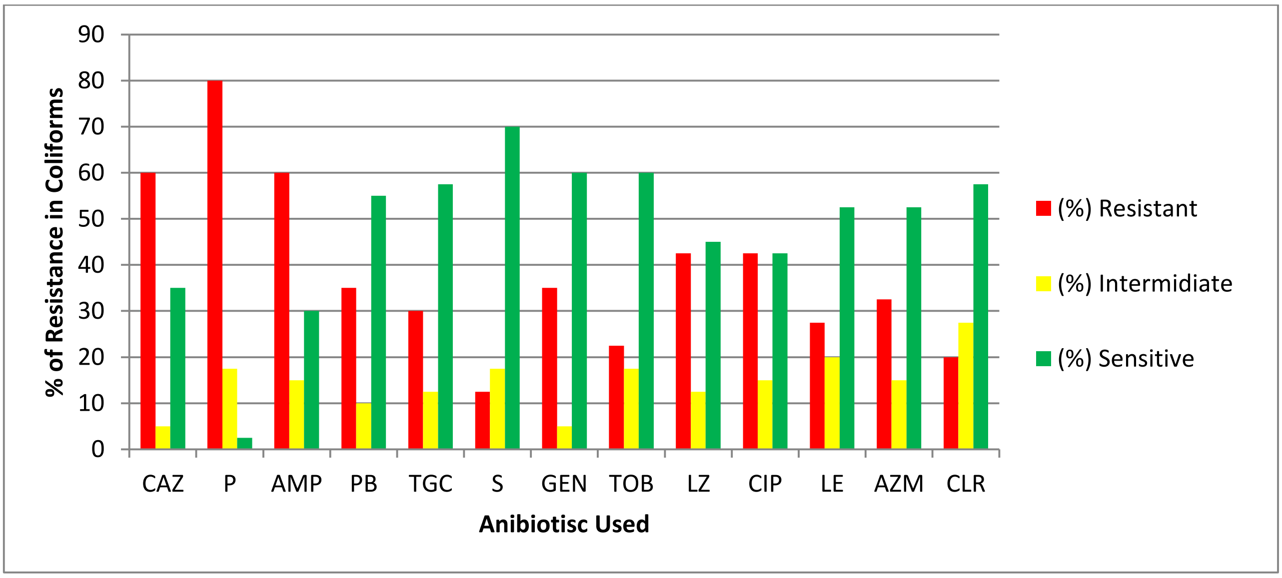


Fig. 01: A: Isolated E. coli with green metallic sheen B: Antibiotic resistance study by disc diffusion method C: Zone of inhibition (mm) of MAR Resistant and Sensitive isolates on Muller Hinton agar plate.

In the present study, the coliforms isolated were showing a variable resistance to different class of antibiotics used in the study. Out of the 39 isolates confirmed as coliforms every organism exhibited antibiotic resistance to the commonly used antibiotics, resistance profile of isolates studied by the Kirby Bauer disc diffusion method as a zone of inhibition (mm). The resistance profile of all the isolated coliforms was (Resistance to Number of antibiotics) like E. coli(04), K. pneumoniae(05), Citrobacter sp.(03), and Enterobacter sp. (04) (Graph 02), Out of the 39 isolates confirmed as coliforms, penicillin resistance was present in 80% of the isolates, but streptomycin sensitivity was observed in 70% of the isolates (Graph 03).

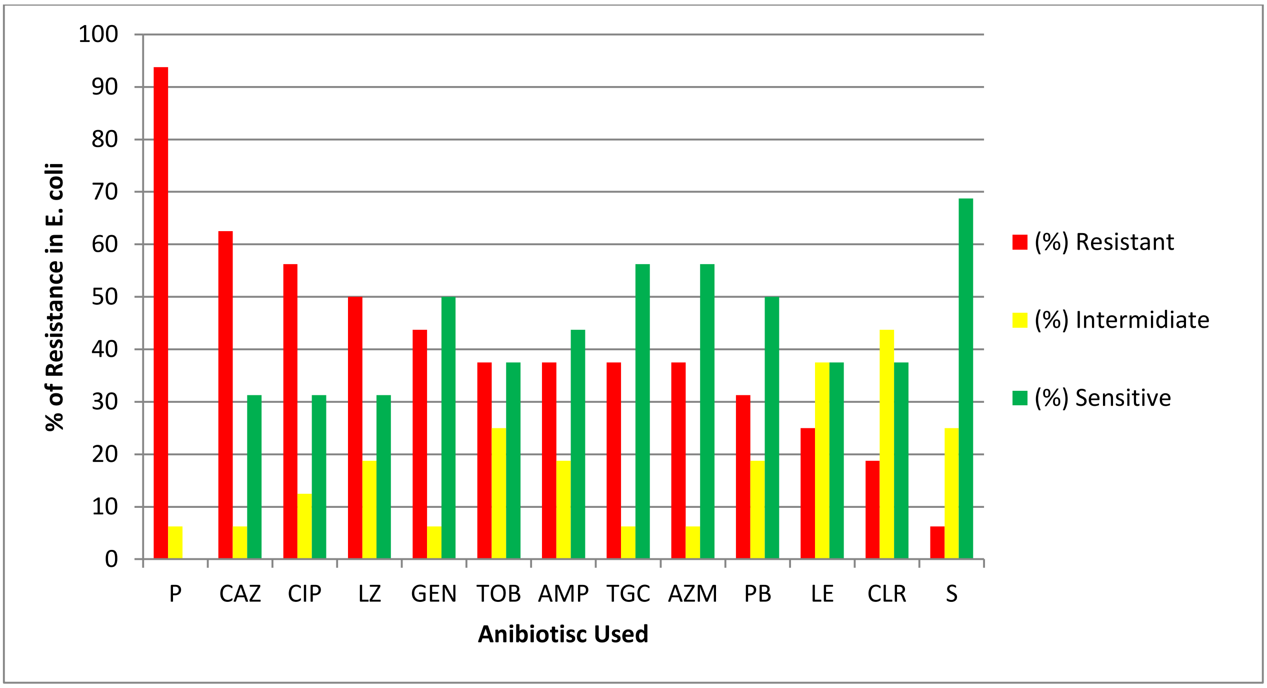


Graph 02: Multiple Drug Resistance profile of Coliform Isolates



Graph 03: Antibiotic resistance and sensitivity profile of total coliforms coliforms

In the present study, the isolated E. coli showed a gradient Resistance against the group of antibiotics used in the study. The isolated E. coli showed resistance against Penicillin(93.75%), followed by Ceftazidime (62.50%), Ciprofloxacin (56.25%), Linezolid (50.00%), Gentamycin (43.75%), Azithromycin, Tobramycin, Tigecycline and Amphicilin (37.50%) and Polymyxin B (31.25%), Levofloxacine (25.00%), Clarithromycin (18.75%), Streptomycin (6.25%); amongs all E. coli isolates there is highest sensitivity towards Streptomycin (68.75%), followed by Tigecycline (56.25%), Azithromycin (56.25%), Amphicilin (43.75%); this shows that some E. coli isolates showed similar resistance and sensitivity pattern towards four major classes of antibiotics (Graph 04).



Graph 04: Percentage of Resistance and Sensitivity against different antibiotics in E. coli

Discussion:

Antibiotics are one of the most important emerging pollutants in water bodies; they are used in the feed for poultry and live stock industries for illness prevention, growth promotion, and the treatment of bacterial infections in animals (Redwan Haque, Ahmed, et al., 2023). For over thirty years, tetracycline and penicillin have been supplemented in feed on swine farms to promote growth in live stocks (Burbee, C. R., et al., 1985). Total colifom and Faecal Coliform population were studied to determine the quality of water for domestic purpose as per the guideline of Central Pollution Control Board (CPCB), Total coliform organism MPN/100ml shall be 50 or less for Drinking Water Source without conventional treatment but after disinfection (Class A) and Total coliform organism MPN/100ml shall be 5000 or less for Drinking water source after conventional treatment and disinfection (Class C) and in the present study the water sample is having total coliforms count within the limit for Drinking water source after conventional treatment and disinfection i.e. the quality of water comes under class C (Source: hppt://cpcb.nic.in/water_Quality_Criteria.php).

The pattern of total coliforms shows seasonal variation in the CFU/100 ml; the maximum count was observed in the monsoon due to surface run-off, followed by the summer and winter. The lower CFU of coliforms in the winter are attributed to the lowering of temperature in the season as compared to the summer and monsoon. (Anilda Kokali and Sulejman Sulce, 2016; N. V. Biradar, 2014)

This study represents the imperative of mapping high-prevalence sites of antibiotic-resistant coliforms in the Penganga River near Hadgaon City. This study shows that the selected sites for sampling are moderately resistant to antibiotics among the coliforms, which helps in understanding the localized factors contributing to resistance. This study shows that some of the coliforms are resistant to major classes of antibiotics (B-Lactum and Penicillin), and specifically, E. coli showed a similar resistance and sensitivity pattern towards four major classes of antibiotics (Tetracycline and Tigecycline).

Conclusion:

The present study focuses on the antibiotic resistance of coliforms isolated from the Penganga River, which shows variable and moderate antibiotic resistance among the bacteria. This study gives an insight into the fecal waste that is disposed of from the sewers of the city in the river, causing an increase in the most probable number of coliforms in the river, leading to a high total coliform and fecal coliform count, which is an indication of poor sanitation and hygiene. Thus, it is indicative of the risk of a number of fatal gastrointestinal-related diseases due to direct consumption of untreated water. The fecal coloniform count of CFU/100 mL did not fall within the permissible limit of BIS standards.

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