Detection of Antibiotic-Resistant *E. coli* from Ponds of Janakpurdham, Nepal

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ABSTRACT

Objectives: The purpose of the study was to determine the extent of antibiotic-resistant *E. coli* in various sacred ponds of Janakpurdham.

Methods: A cross-sectional study was conducted and 16 samples were processed from 16 different revered ponds around Janakpurdham. Isolation of *E. coli* was done through cultural and biochemical analysis. Kirby-Bauer disc diffusion test was employed to test the susceptibility of isolates to antibiotics.

Results: A total of 16 *E. coli* isolates, one from each sampling site, were analysed for their antibiotic-resistant pattern against the eight most commonly used antibiotics. From the total samples, 100% (16) were sensitive to tetracycline, Ciprofloxacin, Amikacin, and Gentamicin while 88% (14), 25% (4), 62% (10), and 44% (7) were resistant to Ampicillin, Amoxycillin/Clavulanate, Cefotaxime and Ceftazidime respectively. Two isolates had the lowest estimated multiple antibiotic resistance index (0.125). In addition, 10 isolates showed indices ranging from 0.25 to 0.375. Two isolates showed the highest indices i.e., 0.5.

Conclusion: The holy ponds of Janakpurdham were found to be highly contaminated with pathogenic bacteria. The results may indicate the origin of *E. coli* isolates from an area of high antibiotic use.

Keywords: *Escherichia coli*, antibiotic resistance, pond water, Janakpurdham, water quality

INTRODUCTION

Water-borne pathogen pollution and related diseases are major concerns for water quality around the world (Sahoo et al 2012). Bacteria are well-known water contaminants, with several contributing to waterborne diseases. The most common human pathogens responsible for water contamination and water-borne diseases are *Salmonella, Campylobacter, Staphylococcus, Clostridium, Pseudomonas,* and *Escherichia coli* (Parveen et al 1997; He et al 2007).

The majority of *E. coli* strains are not harmful, but some of them can cause diseases that can be fatal (Cho et al. 2018). In addition, it is generally accepted that the presence of *E. coli* in water bodies constitutes faecal contamination. Additionally, according to contamination of water bodies by faecal coliform bacteria carrying drug-resistance genes has grown to be a problem of significant national importance (Panneerselvam and Arumugam 2012), *E. coli* is also thought to play a significant role in the spread of genes for antibiotic resistance (AR) in various bacteria, including human pathogens, in natural environments (Mohanta and Goel 2014; Bisht et al 2019). In Janakpurdham, a number of ponds are considered sacred and mass bathing in these ponds is an age-old ritual. Due to the religious importance mass bathing of large or small scales occurs throughout the year at its bank. People take a 'holy dip' and even drink a handful of holy water as a part of religious rituals in the form of mass bathing. So, there may be a high possibility of

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ingestion and thus dissemination of antibiotic-resistant bacteria when they bathe and/or drink pond water. Therefore, our aim was to gain knowledge about the presence of antibiotic-resistant *E. coli* in such sacred ponds of Janakpurdham.

MATERIALS AND METHODS

Study site and duration: The laboratory investigation of this project was carried out at the microbiology laboratory of Ramswaroop Ramsagar Multiple Campus, Janakpurdham, Nepal. The study was conducted from May-June 2023.

Selection of Ponds: A total of 16 sacred ponds were identified based on local knowledge. Most of these ponds were located at the core of the city.

Collection of samples

Wide-mouth bottles were dipped into the water at least three times with a sterile string of the desired length to collect samples of pond water in 100-mL sterile bottles. Sample bottles were kept in containers containing ice packs; and were transported to the laboratory within an hour for further processes.

Isolation and Identification of *E. coli*

Two samples from each pond were mixed to get the maximum number of organisms possible. The MPN technique (WHO 2017) was then used to estimate the viable number of organisms in the sample. The diluted samples were incubated for 24 hours at room temperature. The tubes in which the colour of the media was changed and produced gases in Durham's tubes were recorded and streaked onto EMB Agar and incubated at 37°C for 24 hours. After incubation, the characteristics of colonies on EMB agar plates were recorded. For identification, the pure colonies that were cultured on the EMB plates were sub-cultured on nutrient agar plates for the final test.

Biochemical test

For identification of isolated bacteria, Gram stain and biochemical tests such as Indole, Methyl red, Voges-Proskauer, Citrate, Motility, catalase, oxidase, Triple Sugar Iron Agar test, and urease test were done (Osek 1999; Cheesbrough 2006; IB et al 2018).

Antibiotics susceptibility test of isolated *E. coli*

The antibiotic's bacterial susceptibility tests were conducted in vitro on a Muller Hinton agar plate using the Kirby Bauer disc diffusion method, following CLSI-2021 guidelines. Most common antibiotic discs like Ciprofloxacin, Tetracycline, Gentamicin, Ampicillin, Cefotaxime, Amikacin, Ceftazidime, and Amoxycillin/Clavulanate were employed.

Calculation of the MAR index

MAR indices were determined for each isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (i.e., a/b, where a is the number of antibiotics to which the isolate was resistant and b is the total number of antibiotics to which the isolate was exposed; i.e., b = 8).

RESULTS

All of the 16 water samples collected showed growth of *E. coli*. The most probable number (MPN) index of faecal contamination of each pond is shown in Table 1.

Table 1: Number of Pond/MPN index per 100 ml

S. N	Name of the ponds	MPN index per 100ml
1.A	Dhanush Sagar	43
2.B	Ganga Sagar	210
3.C	Dasharatha Talau	43
4.D	Aargaza	29
5.E	Visahara	460
6.F	Kamal pond	43
7.G	Bidal Sagar	29
8.H	Rukmani	460
9.I	Sitakund	43
10.J	Biharkund	1100
11.K	Ratan Sagar	75
12.L	Sita maiya	93
13.M	Matkhorba	75
14.N	Bhuthi	150
15.O	Manimandap	43
16.P	Paap mochan	210

The antibiotic susceptibility-resistance (AMR) patterns have been presented in Table 2. Out of 16 isolates screened 100% (16 samples) were sensitive to tetracycline, ciprofloxacin, amikacin, and gentamicin, while 88% (14), 25% (4), 62% (10), and 44%(7) were resistant to ampicillin, amoxycillin/clavulanate, cefotaxime, and ceftazidime respectively. Results indicated that most isolates were resistant to Ampicillin while being sensitive to tetracycline, ciprofloxacin, and amikacin.

Table 2: Antibiotic resistance patterns of *E. coli* isolated from ponds of Janakpurdham

Antibacterial Agent with Concentration of Antibiotic	Number of Isolates (n = 16); Percentages in Parenthesis		
	Sensitive	Intermediate	Resistance
Ampicillin (AMP) (10 mcg)	0%	12% (2)	88% (14)
Amoxycillin/clavulanate (AMC) (20/10 mcg)	50% (8)	25% (4)	25% (4)
Cefotaxime (CTX) (30 mcg)	19% (3)	19% (3)	62% (10)
Ceftazidime (CAZ) (30 mcg)	50% (8)	6% (1)	44% (7)
Gentamicin (GEN) (30 mcg)	100% (16)	0%	0%
Amikacin (AK) (30 mcg)	100% (16)	0%	0%
Tetracycline (TE) (30 mcg)	100% (16)	0%	0%
Ciprofloxacin (CIP) (5 mcg)	81% (13)	19% (3)	0%

Multiple Antibiotic Resistance Index (MAR index)

The MAR indices for each isolate were calculated in the present study by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (Krumperman 1983; Odjadjare et al 2012). The MAR indices in the range of 0 to 0.375 were identified in this study indicating many isolates were resistant to multiple number of antibiotics (Table 3.).

Table: 3 MAR index of *E. coli* isolates

S.N.	Name of the ponds	Resistance Percentage (%)	MAR INDEX
1.A	Dhanush Sagar	37.5%	0.375
2.B	Ganga Sagar	37.5%	0.375
3. C	Dasharatha Talau	37.5%	0.375
4.D	Aargaza	12.5%	0.125
5.E	Visahara	50%	0.5
6.F	Kamal pond	37.5%	0.375
7.G	Bidal Sagar	37.5%	0.375
8.H	Rukmani	37.5%	0.375
9.I	Sitakund	12.5%	0.125
10.J	Biharkund	50%	0.5
11.K	Ratan Sagar	25%	0.25
12.L	Sita maiya	25%	0.25
13.M	Matkhorba	0%	0
14. N	Bhuthi	0%	0
15.O	Manimandap	25%	0.25
16.P	Paap mochan	25%	0.25

DISCUSSION

In the present study, water samples were collected from 16 ponds in Janakpurdham, Nepal. All samples showed the growth of *E. coli*. The MPN index of all ponds is presented in Table 1. All sample stations had total faecal coliforms (*E. coli*) levels that were considerably higher than the allowable limit, indicating that the water at these locations is contaminated and should not be used for bathing or drinking. The most probable sources of antibiotic-resistant bacteria in the pond are the mixing of environmental water contaminated with sewage, food, and water (Witte 2000).

The antimicrobial-resistance (AMR) pattern of all *E. coli* isolates was tested against 8 different antibiotics and the results are presented in Table 2. All isolates (16) were sensitive to gentamicin, amikacin, and tetracycline while being not sensitive to ampicillin. Half of the isolates (50%) were resistant to amoxicillin/ clavunate and ceftazidime. The majority of *E. coli* isolates (81%) were sensitive to ciprofloxacin. The pattern of resistance to these antibiotics shows how differentiable and relevant the widely prescribed medications are against water-borne pathogens in the digestive system. Consequently, it's advised to routinely assess the antibiotic sensitivity of frequently identified water-borne enteric bacteria. A previous study (Sayah et al 2005) reported that Tetracycline or ampicillin resistance in environments might increase the risk of becoming resistant to additional drugs. Although resistance genes were not investigated in this study, it is clear that the isolates have resistance genes. According to Roberts'(2005) report, 42 genes have been found so far in several bacterial species, and these genes are responsible for drug resistance in bacteria.

Concerns about antibiotic-resistant isolates in water bodies are significant in developing nations like Nepal. The resistance among the isolates might be related to contamination of sources with antibiotic-resistant *Enterobacteriaceae* from the hospital/industrial/ domestic wastewater that does not undergo effective treatment. The prolonged exposure creates the perfect environment for the spread of antibiotic resistance (Diwan et al 2010).

The *E. coli* isolates were resistant to not only to a single but multiple antibiotics. Commonly speaking, these organisms are called MAR bacteria. The highest MAR was 0.5 and the lowest was 0. The high MAR index of isolates could be the result of sewage MAR bacteria getting into the pond through subterranean sewage and domestic wastewater discharge. In human, animal, and aquaculture therapy, a wider spectrum of medications is used, and there is a chance that resistance will exchange during the course of treatment, which accounts for the isolates' higher MAR levels. The high MAR index of such ponds also suggests the possibility of finding multi-resistant strains of enterobacteria (WHO 2008).

The study done by Giri et al (2021) showed that *E.coli* isolates were highly sensitive to gentamicin as found in our study. Another study done by Indira et al (2021) showed that the *E.coli* isolates were sensitive to ampicillin; However, our study showed that the *E.coli* isolates were resistant to ampicillin.

The discovery of antibiotic resistance in *Enterobacteriaceae* is highly noteworthy since it indicates the existence of antibiotic-resistant microbes in sources of recreational water. According to recent observations, drug-resistant bacteria known as "superbugs" are a source of concern for many countries across the world (Walsh et al 2011). The difficulty in treating these newly resistant pathogens with traditional antibiotics drives up the expense of healthcare for those living in developing nations. In order to secure a better and healthier world in the future, such studies acquire growing significance and demand continuous monitoring of water sources.

Our work is special since it is the first to investigate the antibiotic-resistant bacteria found in Janakpurdham ponds. There are certain restrictions to this study. We do not compare the resistance burden of pre- and post-religious events. Further, the use of Polymerase Chain Reaction (PCR) for the detection of resistant genes could be employed to get better results.

CONCLUSIONS

In Janakpur Dham, where anthropogenic activity is remarkably high, water samples were taken from 16 specifically chosen ponds. Several biochemical analyses revealed *E. coli* in the pond samples, which could indicate that the water is of poor quality. All of the tested water samples contained *E. coli* that was susceptible to antibiotic treatment, with maximum sensitivity to Tetracycline, Gentamicin, Ciprofloxacin, and Amikacin when compared to other antibiotics and the highest resistance to Ampicillin, which is concerning because it is a widely used antibiotic. This research, which compares and characterizes the antibiotic sensitivity of *E. coli* isolated and characterized from these 16 ponds, is the first of its kind.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest of interest.

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