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Paper

Isolation and Characterization of Campylobacter and Salmonella Species from Water Sources in Uttarakhand, India: Assessing Colistin Resistance in the Isolates

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Abstract

Waterborne diseases can affect a large number of individuals in a short span of time; hence the possibility of them turning into an outbreak is high. Zoonotic pathogens represent an important fraction as causative organisms of waterborne illnesses. WHO has ranked *Campylobacter spp*. and *Salmonella spp*. as the two leading zoonotic pathogens in the world. The aim of this study was to isolate and identify *Salmonella* and *Campylobacter* species from the surface water bodies in two districts of Uttarakhand, India- Udham Singh Nagar and Nainital. Water samples from various locations were analyzed for the presence of these bacteria, with the latter coming out to be the predominant one. Thereafter, they were tested for resistance against Colistin, an antibiotic considered to be the last resort antibiotic against gram-negative bacteria and for the presence of *mcr* genes as the cause behind the resistance. The number of isolates showing the presence of these genes was significantly higher as compared to a previous study where an attempt was made to test their presence.

Keywords

Campylobacter, Salmonella, Drinking water, colistin resistance, mcr genes

Introduction

A large portion of world's population still experiences some sort of water scarcity even though water is a basic and essential necessity for every living being. The water bodies are becoming increasing polluted and contaminated with a wide variety of pathogens, reasons for which can be rise in human population, increasing industrialization and urbanization (Bhumbla *et al*., 2020). This will eventually lead to an increase in consumption of water, which in turn will worsen the scarcity. Rural households in India usually draw water from surface water sources like rivers, dams, streams etc. for their daily utilities including drinking, which is most often untreated. These surface water sources are usually unprotected and are prone to contamination from multiple sources like agricultural run-off, rainfall, sewage effluents etc. (Mulamattathil *et al*., 2014). Water borne illnesses are not only a cause of concern for developing countries but developed nations as well (Sirisha *et al*., 2017). A huge burden of water borne pathogens is shared by the zoonotic pathogens. Two most important leading bacterial pathogens, *Salmonella* (non -typhoidal) spp. and *Campylobacter* spp. are very well known zoonotic pathogens whose prevalence in water is based on the nature of the source and the water supply, excreta and other waste disposal processes along with environmental and climatic factors (Tracogna *et al*., 2013). According to a report by European Union One Health on Zoonosis (2022), *Campylobacter* and *Salmonella* remained the most commonly reported zoonosis in 2022 (EFSA and ECDC, 2022). Though outbreaks of human campylobacteriosis through contaminated water has been observed sparsely (Taylor*et al*., 2013; CDC, 2016), the severity of illness caused by the organisms is a concern. As minimum as 500 organisms are enough to cause a disease (Same and Tamma, 2018) with complications as severe as Guillain-Barre syndrome, Miller Fisher syndrome, Reactive Arthritis and Infectious Bowel Syndrome. Foodborne illnesses caused by *Salmonella* organisms also occur worldwide. According to Majowicz *et al.* (2010) approximately 93.8 million gastroenteritis cases

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occur each year because of *Salmonella* globally, and these are responsible for 155,000 deaths. In 2016, the European Union reported around 65,208 confirmed cases of human salmonellosis and 137,107 cases of campylobacteriosis in 2022. Both *Campylobacter* and *Salmonella* are listed in the 4 major diarrhoeal causes in the world (WHO, 2020). India is still a developing nation that faces many challenges, scarcity of food and water, one amongst many.

While working on Non Typhoidal *Salmonella* (NTS), Kumar *et al .,* (2022) reported that in 2016 20.22% samples analysed were positive for NTS, and this percentage was 59.92 in 2017 and 19.83 in 2018. Rajendran *et al.,* 2012 while working with *Campylobacter* spp. in faeces of children below 5 years, reported that 4.5% of the samples tested positive for the bacteria. Vaishnavi *et al.,*(2015) found a very low prevalence (2.6%) of *Campylobacter* spp. in their research centred in North of India but the isolates showed resistance against antibiotics like ciprofloxacin, tetracyclin, Nalidixic acid.

Development of resistance among the pathogens is another matter that has garnered the attention of public health experts around the world. This owes to the hazardous increase in antibiotic usage, both for therapy and agriculture (Vaseeharan *et al.*, 2005; Martinez, 2009). Multi drug resistance exhibited by the bacteria against drugs of choice is a cause of concern, not only for humans but for animals and environment too because nothing in nature woks in solitude. The antibiotics used in animals or humans find their way into nature and then back into the humans and animals. Resistance against fluoroquinolones and first, second, third and even fourth generation cephalosporins have been reported by many researchers (Tack *et al.*, 2020). Colistin is a polymyxin antibiotic (Polymyxin E), and was discovered in Japan in 1949. It was first used clinically in the 1959 but its use was stopped after its toxic properties were discovered, though in veterinary medicine its use continued. It began to be used again in mid 1990s (Ansari *et al.,* 2021). To treat serious or life threatening infections, colistin is being dubbed as 'last-resort' antibiotic (Oliatan *et al.*, 2016). Unfortunately, even colistin is no exception to bacterial resistance and several colistin resistance genes are reported (Aghapour *et al.*, 2019, Elbediwi *et al*., 2019), indicating that resistance is rapidly developing even against this antibiotic. The mechanism for such resistance can be many, such as- intrinsic, seen in *Neisseria* spp, *Moraxella* spp etc. or it can be because of presence of capsule which is seen to render colistin useless as seen in *K. pneumonia.* Alterations in the phoQ or pmrAB operons can also give rise to resistance but the most terrifying mechanism is via *mcr* genes because it is a type of horizontal transfer of resistance and hence can rapidly give rise to resistant mutants in the bacterial population (Ahmed *et al.*, 2020).

The *mcr* (mobile colistin resistance gene) has been identified to be responsible for the fast developing colistin resistance in bacteria and was first reported from China (Liu *et al.*, 2016). These genes are located on the plasmid and till date nine genes or variants have been reported across the world (*mcr*-1, -2, -3, -4, -5, -6, -7, -8, and -9) with vast distribution in the animal species. Their distribution has been shown to be widely varied, from poultry, pigs to cows and rabbits (Gharaibeh *et al*., 2019).

Uttarakhand is region in North of India populated by approximately 10.1 million people but around 2 million in the state do not get quality water for drinking and double that number do not have access to sanitation in the hilly parts of the region. More than 2 million people do not have consistent access to clean water and more than twice that number lack access to sanitation in hills as well as plane areas of the state. Sources of surface drinking water are gradually drying up and the level of groundwater is continuously declining (Singh *et al*., 2012) in most of the cities and towns, like Haldwani, Nainital and adjoining areas etc. This study was designed to determine the prevalence of non-typhoidal *Salmonella* spp. and thermophilic *Campylobacter* spp. in the study area and to determine colisitin resistance in the isolates.

Figure 1. Maps showing the area of sample collection with orange star marks.

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Materials and Methods

Sampling

Between April 2022 and March 2023, a total of 1600 water samples were collected from various sources supplying water to residents in different areas of Uttarakhand. The sample collection included Pantnagar (n=350), Rudrapur (n=180), Nainital (n=470), Bhimtal (n=280), Sattal (n=150), Sariyatal (n=70), Kathgodam (n=60), and Lalkuan (n=40). The samples were obtained from lakes (n=870), rivers (n=530), barrage (n=60), industries (n=40), and irrigation pipes (n=100) (Table I).

District	Location	Number of Samples
Nainital (n=107)	Bhimtal	280
	Nainital	470
	Sariyatal	70
	Sattal	150
	Kathgodam	60
	Lalkuan	40
U.S. Nagar (n=53)	Pantnagar	350
	Rudrapur	180
Total		1600

Table I. Syntax of sample collection

Isolation, Purification, and Characterization of the Bacteria

Isolation

The isolation of both the bacteria was done by pre-enriching the samples, followed by culture on specific media (enrichment in case of *Salmonella* was also done). For *Campylobacter* spp., 1 ml of the sample was pre- enriched in 9 ml of Bolton Broth with incubation at 42^0C for 24-48 hours with 5% CO_2. A loopful of the pre-enriched culture was then inoculated on Blood Free Campylobacter Selectivity Agar and incubated at 42^0C for 48 hours with 5% CO_2.

For isolating *Salmonella* spp., firstly, pre-enrichment was done with 1 ml of the sample being added to 9 ml of Buffered Peptone water and incubated for 24 hours at 37°C. Thereafter, enrichment of the samples was done by adding 0.1 ml of the culture from BPW to 9.9 ml of RV-10 media and incubating at 42°C for 24 hours. Then a loopful of culture was drawn from RV and streaked onto XLT4 agar plates, supplemented with XLT4 supplement and kept in the incubator at 37°C for 24 hours.

Purification of Colonies

Colonies were subcultured multiple times using the streak plate method. Fresh cultures were Gram stained and all isolates were identified as Gram-negative (rods in case of *Salmonella spp*. and 'S' shaped or curved in case of *Campylobacter spp*.). All the primary isolates were then subjected to biochemical identification tests.

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Morphological identification

The isolates were tested for their colony characteristics on Blood Free Campylobacter Selectivity Agar (BFCSA). The appearance of greyish, slightly raised, sticky colonies were indicative of *Campylobacter* spp. The isolates were also tested for their colony characteristics on Xylose lysine tergitol 4 Agar (XLT4), on which the appearance of red colonies with black centre was indicative of *Salmonella* spp.

Growth in aerobic conditions for Campylobacter spp.

The isolates were streaked on the BFCSA plates and incubated at 36^0C for 48 hours aerobically. Absence of growth on the plates is indicative of the *Campylobacter* genus.

Biochemical Identification of Campylobacter spp.

A series of biochemical tests were performed.

A. Hippurate Hydrolysis Test

This test was performed to differentiate between *C. jejuni* from other species of *Campylobacter*.

B. Oxidase Test

A loopful of culture was rubbed over a filter paper soaked with Oxidase solution.

C. Catalase Test

A loopful of culture was inoculated onto a clean slide containing 3% H_2O_2 and mixed properly.

D. Urease Test

The presumptive culture was inoculated in Christensen's Urease Agar Slant and the tubes were incubated at 42^0C for 48 hours with 5% CO_2.

E. Triple Sugar Iron Test

On incubation of a TSI agar slant, inoculated with *Campylobacter* culture, at 42°C for 48 hours.

F. Latex Agglutination Test

This test was carried out by using HiCampylobacter™ Latex Test Kit. Overnight incubated colony of *Campylobacter* were mixed with 50 µl of sample diluent on the inoculation strips provided by the manufacturer (HiMedia) and mixed properly. Then Campylobacter Latex Reagent was added to it and mixed using a sterile stick provided with the kit and spread evenly. The mixture was rocked gently, appearance of clumps was noted.

Biochemical Identification of Salmonella spp.

The following biochemical tests were performed for identification of *Salmonella* spp.

A. Urease Test

The presumptive culture was inoculated in Christensen's Urease Agar Slant and the tubes were incubated at 37^0C for 18 hours.

B. Triple Sugar Iron Test

On incubation of a TSI agar slant, inoculated with *Salmonella* spp. culture, at 37°C for 12-18 hours, give red slant with yellow butt along with blackening of the agar (H_2S production) indicative of *Salmonella* spp.

Extraction of Genomic DNA and PCR for the Identification of Culture Species

DNA extraction of the *Campylobacter* and *Salmonella* organisms was done by using the heat lysis method (Reichl*et al.*, 2000) with some modifications.

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Confirmation of the Isolates by PCR Assays

The identities of the isolates were confirmed by targeting the gene- *cad* F for *Campylobacter* genus. The amplification was carried out according Konkel with some modifications (Konkel *et al.,* 1999). For confirming the species, gene *hip* O was targeted for C. *jejuni*, while *asp* for C. *coli*. The amplification consisted of Early Denaturation- 95º C for 5 minutes (1 cycle), Denaturation- 95º C for 45 sec, Annealing- 48º C for 30 sec, Extension- 72º C for 30 sec (30 cycles for the three steps) and a Final Extension at 72º C for 5 min (1 cycle).

The gene *omp* C was the target gene for *Salmonella* genus identification and *typh* for Typhimurium serotype. The amplification was carried out as follows: Early Denaturation- 95º C 2 min, 30 cycles of- Denaturation at 95 º C 1 min, Annealing at 57º C for 1 min, Extension at 72º C 2 min and finally 1 cycle of Final Extension at 72º C for 5 min.

The primer sequence used and the reaction conditions employed are given in Tables II-IV. The volume of reaction mixture was 50 μ L in all the reactions. The PCR products were stored at 4 °C until further use.

Name	Gene Targeted	Sequence	bp	Reference
*cad*F F	*cad*F	5'-TGGAGGGTAATTTAGATATG-3'	400	Konkel *et al*., 1999
*cad*F R		5-CTAATACCTAAAGTTGAAAC -3'		

Table II. Primers used for detection of Campylobacter genus.

Name	Gene Targeted	Sequence	bp	Reference
*hip*O F	*hip*O	5'- GAAGAGGGTTTGGGTGGTG-3'	735	Shams *et al.,* 2017
*hip*O R		5'-AGCTAGCTTCGCATAATAACTG-3'		
asp F	*asp*	5'- GGTATGATTTCTACAAAGCGAG-3'	500	Shams *et al.,* 2017
asp R		5'- ATAAAAGACTATCGTCGCGTG-3'		

Table III. Primers used for detection of C. jejuni and C. coli.

Name	Gene Targeted	Sequence	bp	Reference
*omp*C F	*omp*C R	5'-ATCGCTGACTTATGCAATCG-3'	204	Alvarez *et al.,* 2004
*omp*C R		5'-CGGGTTGCGTTATAGGTCTG-3'		
typh F	*typh*	5'-TTGTTCACTTTTTACCCCTGAA-3'	401	Alvarez *et al.,* 2004
typh R		5'- CCCTGACAGCCGTTAGATATT-3'		

Table IV. Primers used for duplex PCR for Salmonella Typhimurium detection.

Electrophoresis of PCR Products

The PCR products were loaded onto 1.5% agarose gel, stained with 0.5 µg/ml Ethidium Bromide and electrophoresed for 1 hour at 85 V. Then visualization of the gel was done to observe for the bands at the characteristic base pair length. Along with the PCR product, a positive control (commercially acquired) and a 100 bp DNA ladder was also electrophoresed and visualized.

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Resistance against Colistin

The obtained *Salmonella* and *Campylobacter* isolateswere screened for resistance against colistin using E-strips (HiMedia India) (Reller *et al.* 2009) and by Broth Micro-dilution method NCDC (2020).

The isolates showing a Minimum Inhibitory Concentration of >2 mcg/ml was considered to be resistant to colistin and the ones showing MIC <2 mcg/ml as sensitive as per the recommendation of EUCAST (2020) and by the standard protocol followed for broth microdilution assay using colistin sulphate (NCDC, 2020). The presence of mobile colistin resistance genes in the obtained isolates (*mcr* 1-9) was evaluated by multiplex PCR. The primers and conditions were as described by Rebelo *et al.* (2018) for *mcr* 1-5 and for *mcr* 6-9 Borowiak *et al.* (2020) (Table V).

Primer	Sequence	bp	Target Gene	Reference
mcr_1 F	AGT CCG TTTGTT CTT GTG GC	320	*mcr* 1	Rebelo *et al*., 2018
mcr_1 R	AGA TCC TTG GTC TCG GCT TG	320	*mcr* 1	
mcr_2 F	CAA GTG TGT TGG TCG CAG TT	715	*mcr* 2	
mcr_2 R	TCTAGCCCGACAAGCATACC	715	*mcr* 2	
mcr_3 F	AAATAAAAATTGTTCCGCTTATG	929	*mcr* 3	
mcr_3 R	AATGGAGATCCCCGTTTTT	929	*mcr* 3	
mcr_4 F	TCACTTTCATCACTGCGTTG	1116	*mcr* 4	
mcr_4R	TTGGTCCATGACTACCAATG	1116	*mcr* 4	
mcr_5 F	ATGCGGTTGTCTGCATTTATC	1644	*mcr* 5	
mcr_5 R	TCATTGTGGTTGTCCTTTTCTG	1644	*mcr* 5	
mcr_6 F	AGCTATGTCAATCCCGTGAT	252	*mcr* 6	Borowiak *et al*., 2020
mcr_6 R	ATTGGCTAGGTTGTCAATC	252	*mcr* 6	
mcr_7 F	GCCCTTCTTTTCGTTGTT	551	*mcr* 7	
mcr_7 R	GGTTGGTCTCTTTCTCGT	551	*mcr* 7	
mcr_8 F	TCAACAATTCTACAAAGCGTG	856	*mcr* 8	
mcr_8 R	AATGCTGCGCCGAATGAAG	856	*mcr* 8	
mcr_9 F	TTCCCTTTGTTCTGGTTG	1011	*mcr* 9	
mcr_9 R	GCAGGTAATAAGTCGGTC	1011	*mcr 9*	

Table V. Primers used for multiplex PCR for detecting mcr genes.

Results

Salmonella spp. and *Campylobacter* spp. isolated from Water

Out of the 1600 samples, 370 (23.12%) colonies grown were identified with having pink-coloured gram-negative bacteria with an S or curve shape on Gram's staining were observed, indicating that they could be *Campylobacter* spp. A total of 271 (73.24%) out of these 370 isolates showed positive reaction on addition of Campylobacter Latex Reagent, gave purple coloration on oxidase test, had bubble with 3% H2O2 on catalase test, exhibited negative reaction on urease test and also had negative results on TSI slant confirming as *Campylobacter spp.* Additionally, on subjection to hippurate hydrolysis test, 166 (61.25%) out of the suspected 271 colonies showed appearance of deep blue color and were suspected to be *C. jejuni* .

In our study the prevalence of *Campylobacter* spp. was highest in rivers (20.75%), followed by 'Other' sources (15%) and then lakes (14.94%). The *C. jejuni* was more prominent in rivers (90.91%) while *C. coli* in lakes (38.46%).

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For selective isolation of *Salmonella*, 223 (13.93%) out of the 1600 samples on XLT4 agar yielded characteristic growth with pink-coloured colonies and rod-shaped appearance. Moreover, on urease test the colours of the slant did not change in 197 (88.34%) of the isolates and those same isolates showed alkaline red slant, acid yellow butt with blackening of the agar showing H_2S production with air space at the bottom of the slant indicating that only 197 isolates are *Salmonella spp.* In the present study, among different source 'Others' (25%) were found to harbour more *Salmonella* than rivers (11.32%) and lakes (9.19%).

The isolates giving the characteristic reactions in the biochemical tests were then confirmed by PCR reactions.

Molecular Identification

Out of the 197 presumptive *Salmonella* isolates, 124 (62.95%) were confirmed to be serotype Typhimurium based on the presence of the *typh* gene.

Genus and species specific PCRs were run to confirm the identity of the presumptive isolates by amplification with *cad* F for *Campylobacter* genus and *hip* O (C. *jejuni*), *aps* (C. *coli*) and *omp* C for *Salmonella* genus and *typh* for serotype identification with PCR reaction at characteristic bp of 400, 750, 350, 401, 204 for *Campylobacter* genus, C. *jejuni*, C. *coli*, *Salmonella* genus and Typhimuriumserotype respectively. A total of 271 (16.94%) samples out of the 1600 were positivefor *Campylobacter spp* and 197 (12.31%) for *Salmonella spp*.

All 271 presumptive *Campylobacter* isolates were confirmed to be *Campylobacter species* as they possessed the *cad* F gene. Among them, 166 isolates exhibited the *hip* O gene, identifying them as *C. jejuni*, while the remaining 105 isolates carried the *aps* gene, classifying them as *C. coli*.

Resistance against Colistin

Campylobacter spp.

Among the 271 confirmed *Campylobacter* isolates, 111 exhibited phenotypic resistance to colistin, representing a prevalence rate of 40.96% (111/271). Notably, the highest prevalence of colistin resistance in *Campylobacter spp*. was observed in water samples collected from Nainital, with a rate of 53.15% (59/111) in terms of location, specifically from rivers, which accounted for 65.77% (73/111) of the resistant isolates.

When using the broth microdilution method, all *Campylobacter* isolates (100%) displayed resistance to colistin, as defined by an MIC exceeding 2 mcg/ml.

Salmonella spp.

MIC determination of 197 isolates revealed that 126 isolates demonstrated phenotypic resistance to colistin using E-strips, resulting in a resistance rate of 63.96% (126/197). Among these resistant isolates, 86 (68.25%) exhibited high resistance levels exceeding >256 mcg/ml. Regarding *Salmonella*, out of the 126 resistant isolates, 57.14% (72/126) were obtained from the Nainital district, while 42.86% (54/126) were from the U.S. Nagar district. Furthermore, in the current study, 68.02% (134/197) of the isolates demonstrated resistance to colistin when assessed using the broth micro-dilution assay with colistin sulphate powder.

Genotypic colistin resistance (*mcr* genes)

All the 197 isolates irrespective of showing phenotypic resistance to colistin were screened for possessing the mobile resistance genes (*mcr* 1 to 9) using two separate multiplex PCRs, one for *mcr* 1-5 and another for *mcr* 6-9. The *Salmonella* isolates were found to possess genes *mcr*-1 (71/197; 36.04%), *mcr*-2 (13/197; 6.59%) and *mcr*-3 (14/197; 7.11%). None of the isolates tested positive for the other *mcr* genes. All the *Campylobacter* isolates tested negative and did not have any *mcr* genes.

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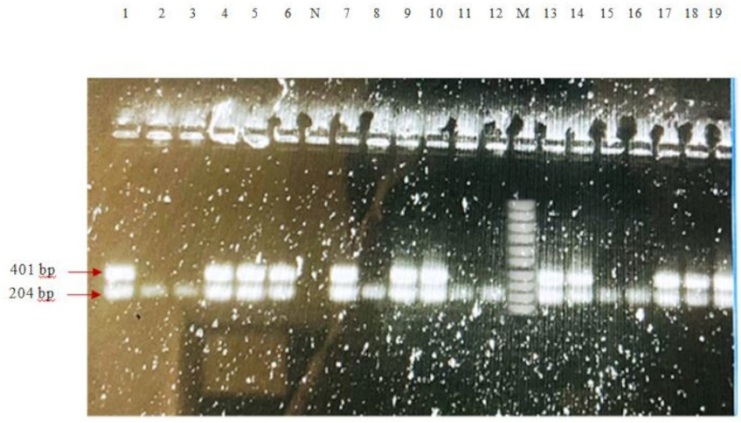

Figure 2. PCR detection of Salmonella genus and serovar Typhimurium; Lane M- Ladder; 1- Positive Control; N-Negative Control; 2-19- Suspected Salmonella genus and serovar Typhimurium.

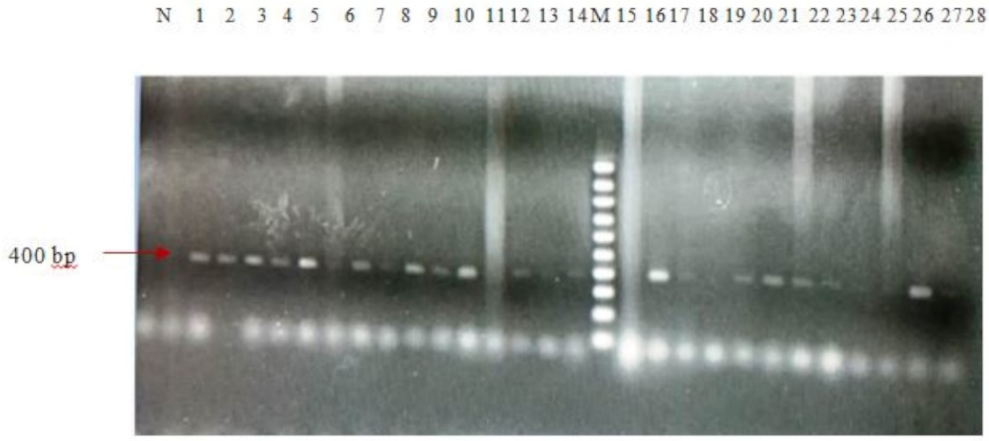

Figure 3. PCR detection of Campylobacter genus; Lane M- Ladder; N- Negative Control Postive control; 2-28- suspected Campylobacter spp.

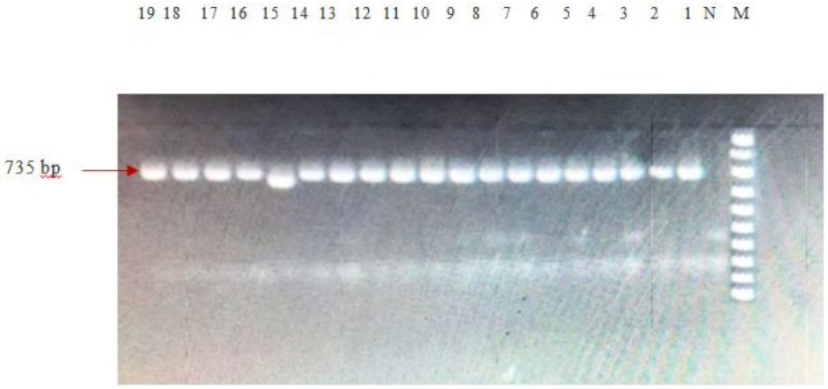

Figure 4. PCR detection of Campylobacter jejuni; Lane M- Ladder; N- Negative Control; 1- Postive control; 2-19- suspected C. jejuni.

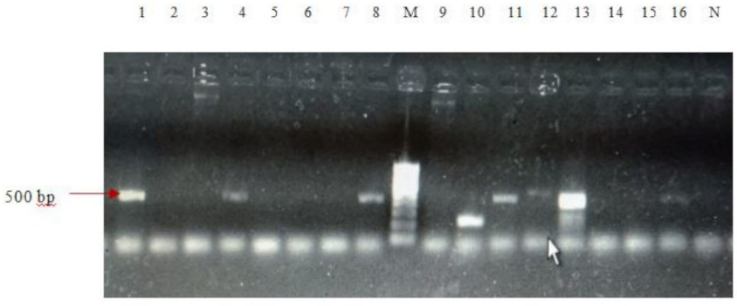

Figure 5. PCR detection of Campylobacter coli; Lane M- Ladder; N- Negative Control; 1-17- suspected C. coli.

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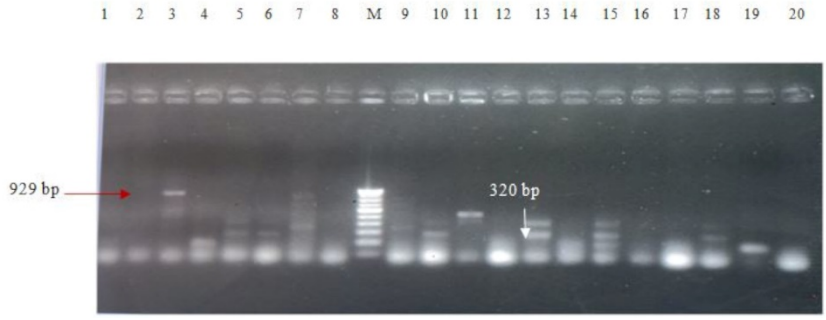

Figure 6. Multiplex PCR for detection of mcr 1-5 giving bands at 320 bp for mcr-1 and 929 bp for mcr -3.

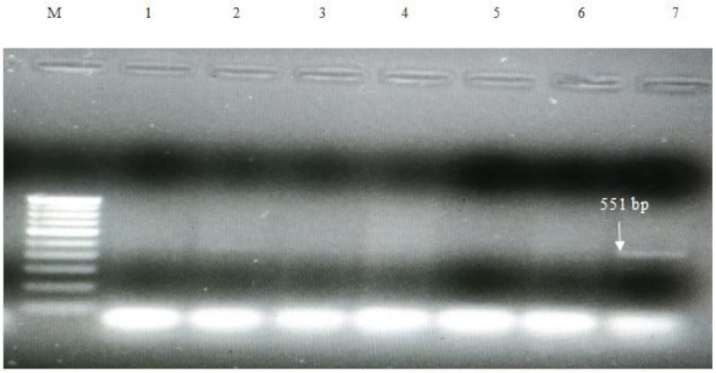

Figure 7. Multiplex PCR for detection of mcr 6-9 giving bands at 551 bp for mcr-7.

Discussion

Food borne illnesses of bacterial origin are mainly associated with contaminated products of animal origin. Approximately 93.8 million illnesses and 155,000 deaths in the world occur because of bacteria such as *E. coli*, *Salmonella* spp., *Campylobacter* spp., *S. aureus* etc. Non-typhoidal *Salmonella* and *Campylobacter* are important pathogens having zoonotic potential. Their presence is ubiquitous ranging from animals to associated environment. The occurrence of these organisms in water bodies utilized for human use is of great concern. An estimate by WHO states that such illnesses cause around 2 million deaths in developing countries and leave at least 30% population affected as far as developed nations are concerned. For a developing country like India, the huge burden of such diseases can prove to be a major set-back, not only in terms of development but basic needs also.

The present study demonstrated that the water sources were contaminated with the target bacteria as both *Campylobacter* and *Salmonella* were isolated from the samples, indicating the risk of health hazard if the water was consumed. Results from this study shows that drinking water produced from the sampled sources should be further analysed and that general microbiological tests that only include faecal coliforms and *E. coli* may be insufficient.

C. jejuni was more prevalent in rivers (90.91%), while *C. coli* was more common in lakes (38.46%). This aligns with previous findings (Kemp *et al.,* 2005) reporting *Campylobacter* prevalence at 56.7% in running water, such as rivers, and 45.9% in stagnant water, such as lakes. Specifically, *C. jejuni* prevalence was 36.7% and *C. coli* 10% in running water, whereas in stagnant water, C. jejuni prevalence was 6.6% and C. coli 31.1%.

The highest prevalence (25%) compare to rivers (11.32%) and lakes (9.19%) in the present study is due to easy accessibility of both human and animal population to this source (Parveen, 2021). The high prevalence of serotype Typhimurium correlates with the most commonly isolated serotype from the animal population of this region (Nagpal 2017).

The high prevalence of colistin resistance in the isolates is an indicator that even this antibiotic will lose its effectiveness in the coming years. Combined with the fact that the R&D in antibiotics had reached a standstill, the healthcare sector will be left with eve fewer option to treat bacterial infections. The presence of *mcr* genes in Salmonella isolates also point to the fact that the horizontal transmission of resistance against colistin is increasing at an alarming rate.

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None of the *Campylobacter* isolates were found to have the *mcr* genes in the present study (Hassen *et al.,* 2022; El-Sayed Ahmed *et al.,* 2020). Hence their resistance to colistin was due to some other phenomenon and not due to *mcr* genes. Cullen and Trent (2010) stated that the resistance against colistin in *Campylobacte* r spp. is due to attachment of phosphoethanolamine to lipid A in bacterial cell wall. In another study, Garcia-Fernandez *et al.,* (2024) found that plasmids play a crucial role in conferring AMR in *Campylobacter spp*. and also the horizontal spread of resistance in the bacteria. So it is ruled out as a possibility of *Campylobacter* showing resistance against colistin. But we need to understand the complexities of the different and varied mechanisms of the resistance development and transfer before reaching a solid conclusion.

National Centre for Disease Control reported that majority of foodborne illnesses go unnoticed in India, as they do in majority of developing and underdeveloped countries because they are seldom reported and hence the burden of foodborne pathogens is unknow in India, much less that of waterborne pathogens. These illnesses are more often than not, self-limiting and resolve within days, except in cases where children, elderly or immuno-compromised people are affected. People don't usually seek medical care or don't have the access to it and the surveillance system in India is still lacking so these pathogens go underreported.

The increasing and unchecked use of antibiotics is a major reason why AMR has become such a huge concern in the present times. Since the illnesses caused by *Salmonella* and *Campylobacter* resolve on their own, antibiotics should be administered in severe cases like gastroenteritis, extraintestinal infections, or immunocompromised patients.

We need to make sure that the water and food we consume has undergone proper treatment, especially when it's the vulnerable group of people (children, pregnant women etc.) who are consuming it. Hygienic practises need to become everyday habits and everyone's responsibility and awareness among the people is of utmost importance. The laws related to food and water safety, environmental pollution, policies and guidelines regarding use of antibiotics need to be strictly enacted. The healthcare system in underdeveloped and developing countries is still fragile and efforts are needed to strengthen it with better surveillance and laboratory facilities. The innovation in antibiotics discovery and development has reached a standstill and needs a push. But until that happens, we need to be careful in their usage, whether it be in animals sector or human, because ultimately everything is interconnected. The leading organizations in the world need to come together and guide the regional bodies and governments on how to tackle the issues that threaten the public health.

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The authors report there are no competing interests to declare.

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