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Research Article

Antibiotic profiling of *Salmonella* species isolated from Bagmati River, Kathmandu, Nepal

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Abstract

With rapid population growth, unmanaged urbanization, and industrialization, the holy river Bagmati, has become one of the most contaminated water sources in Kathmandu, Nepal. As a result, the river has lost its capacity for self-purification and became ideal habitat for many pathogenic microorganisms such as *E. coli, Salmonella, Vibrio*, etc. The study aimed to perform the antibiogram profile of *Salmonella* species. According to the findings, *Salmonella* was identified in excessively polluted areas with sewage. Out of total 55 samples, 34.45% were *Salmonella* positive among which, 10.5% were found to be *S.* Typhi and *S.* Paratyphi, and the remaining 79% were found to be other *S. enterica* serovar. S. enterica serovar was detected in abundance from site B1. Site B2 yielded *Salmonella* Typhi and *Salmonella* Paratyphi isolates. Furthermore, *Salmonella* Paratyphi was isolated from Site B3. The water samples from Site B6 were proven to be *Salmonella*-free. Antibiotic Susceptibility Test was performed for the positive samples and all the serovars were found sensitive to Amikacin whereas least sensitive to Ampicillin i.e., 86.67%, 100%, and 50% resistant in case of other *S. enterica* serovar, *S.* Typhi and *S.* Paratyphi respectively. All the *Salmonella* species isolates were sensitive to Ciprofloxacin, Streptomycin, Ofloxacin, and Nitrofurantoin but 100 % resistant to Ampicillin. However, there is not a single data reported as Multi-Drug Resistant *Salmonella* species in this study. The study emphasized the use of effective treatments against the disease and addresses the health danger to people, animals, and many other living species living nearby the river.

Keywords: Antibiotics; Antibiotic susceptibility test; Bagmati river; *Salmonella* species; Water pollution.

Introduction

Water pollution has become today's one of the most serious global environmental issues along with its health impact as it is one of the modes of disease transmission (Wild, 2012). Bagmati River which is one of the highly polluted water sources originated from Baghdwar above the Southern edge of Shivapuri Hill about 15km North-East of Kathmandu, Nepal. It has a catchment area of 3710 square kilometers in Nepal and is fed by natural springs and monsoon rainfall (NTNC, 2009). As it passes the center of Kathmandu, this tributary becomes heavily polluted. Flowing generally the south although, with many curves, the Bagmati reaches the edge of the Kathmandu Valley and enters Chovar Gorge near the Dakshinkali temple complex. The gorge cuts through the Mahabharat Range or Lesser Himalaya (Davis, 2007).

Rapid population increment, unmanaged urbanization, and industrialization in Kathmandu valley have contributed to

the degradation of the quality of the Bagmati river and its tributaries (Thakur *et al.,* 2017). Religious activities like cremation and mismanagement of hospital wastes have led to water quality deterioration resulting in serious public health and environmental challenges (Kannel *et al.,* 2001). The river has now changed into the reservoirs of many pathogenic microbes such as *E. coli, Vibrio cholera, Salmonella, Shigella*, etc. (KC *et al.,* 2018).

Salmonella is a Gram-negative facultative intracellular anaerobe that has been reported as the pathogen for 1.3 billion cases of the disease annually worldwide (Ochman and Groisman, 1994). Most serotypes are responsible for gastroenteritis, while specific serotypes (*S.* Typhi, *S.* Paratyphi A and C, and *S. sendai*) cause enteric fever, and a few nontyphoid serovars, e.g., *S. cholerae-suis* and *S. dublin* are more likely to cause bacteremia than diarrhea (Fierer and Guiney, 2001). In Nepal, enteric fever has been established as an endemic and major public health problem (Acharya et al., 2013). Additionally, diarrhea is still recognized as a major problem for Nepalese children, being recorded as the second most prevalent diagnosis in out-patience services. Annually, a total of 27 million global populations are affected by Typhoid and also the reason for 300,000 deaths. Most of the population is from South Asia, especially in India, Pakistan, Bangladesh, Sri Lanka, and Nepal (Basnyat, 2010). Globally, two million ton of sewage and industrial and agricultural waste is discharged into waterways, and at least 1.8 million children under five-years-old die every year from water-related diseases, or one every 20 seconds (Corcoran et al., 2010).

Analyzing the health risk associated with the polluted water source, it is crucial to monitor both the physiochemical and microbiological aspects of the river water to evaluate its quality. Therefore, this study was carried out to evaluate the microbiological analysis through the isolation of pathogenic *Salmonella* species. The study also highlighted the distribution of the *Salmonella* species in the river site along with establishing the relation of the polluted area with its isolation. The isolated organisms have shown different anti-profiling patterns which assisted in finding effective antibiotics for the treatment of the disease caused by the *Salmonella* species.

Materials and Methods

Sampling site and Sample collection

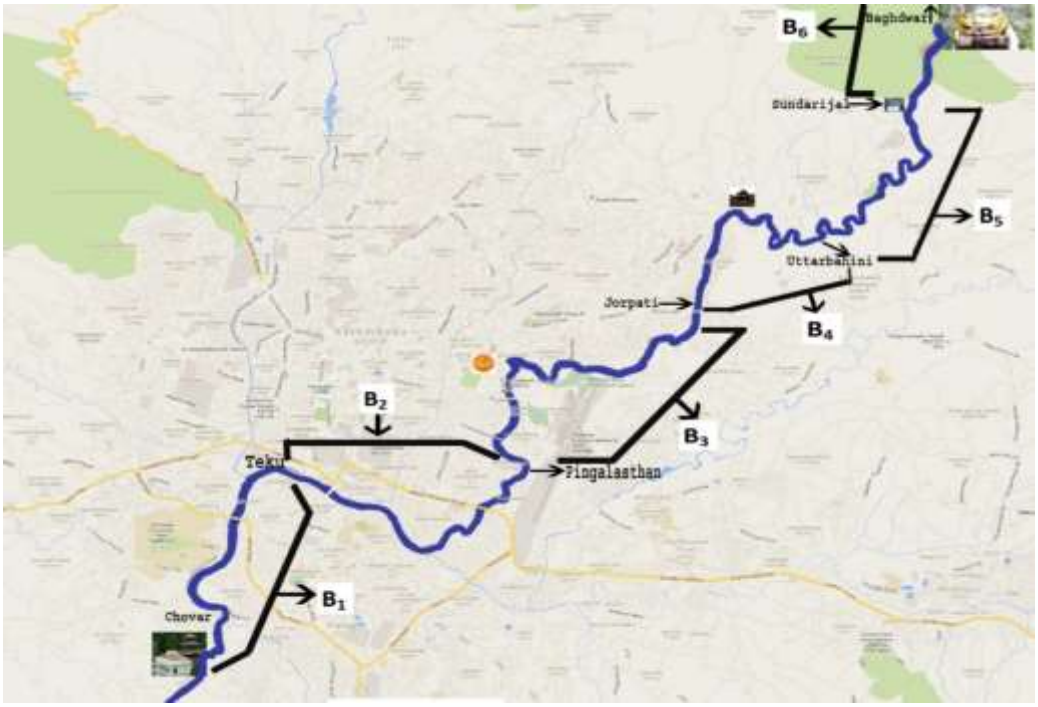

Fig. 1: Map of Bagmati River including sampling site B1 to B6.

To perform this study, the Bagmati river was divided into 6 different sites i.e., Brock B1-B6. Moreover, to represent each site, 10 samples were taken (approximately at a 600-meter distance) except for site B6 where a total of 5 samples were taken. Water Samples were collected in clean and sterilized glass stoppered bottles with tightly screw-capped. During sample collection, the bottle caps were opened aseptically, and they were lowered in water with their mouth directed against the water current. The water samples were transported to the laboratory and processed within 4 hours. Dilutions were also performed on those samples which were highly polluted and turbid (William, 2000). A total of 55 samples were collected from 6 different sites in Bagmati River from Chovar to Baghdwar.

Isolation and Identification of Salmonella species

Primarily, the enrichment was performed, 5ml of water sample was inoculated into 45ml of Selenite "F" broth and mixed thoroughly.

Table 1: Sampling sites of Bagmati River and the total number of sample collection.

Sampling sites (Bagmati River)	Area covered	Number of samples
B1	Chovar to Teku	10 (S1-S10)
B2	Teku to Pingalasthan	10 (S11-S20)
B3	Pingalasthan to Jorpati	10 (S21-S30)
B4	Jorpati to Gokarna	10 (S31-S40)
B5	Gokarna to Sundarijal	10 (S41-S50)
B6	Sundarijal to Baghdwar	5 (S51-S55)

The information shown is the area of Bagmati River from where a total of 55 water samples were collected.

It was then incubated at 37ºC overnight. After enrichment, a loopful of the upper part of the broth was subcultured on *Salmonella-Shigella* agar. The plates were incubated at 37ºC for 24 hours. The black-centered colonies were selected and sub-cultured in Nutrient Broth and Nutrient Agar by pour plate method and then incubated for 4 hours at 37ºC and overnight at 37ºC respectively. The isolated colonies were subjected to Gram staining. The colonies showing Gram-negative rods were further selected for identification (Johnson *et al.,* 2003; Cappucino and Sherman, 2007; Haley *et al.,* 2008; Aryal M 2016).

Antibiotic Susceptibility Test of Salmonella species by Kirby-Bauer Method

Under the aseptic condition, each Muller Hinton Agar plate was labeled with the name of the sample organism to be inoculated. A sterile cotton swab was dipped into a well-mixed saline test culture (the density of half of the McFarland standard) and excess inoculum was removed by pressing the saturated swab against the inner wall of the culture tube. Using the swab, the entire agar surface was streaked horizontally and vertically to ensure a heavy growth over the entire surface.

Table 2: Biochemical test performed for the identification *Salmonella*

Sample	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S17	S18	S22	S26	S29	S37	S45
Tests																			
MR/VP	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
OF	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Citrate	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SIM	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M	-M
TSIA	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y	R/Y
Catalase	++	++	++	++	++	++	++	++	++	++	-+	++	-+	+-	++	++	+-	++	++
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Result	SO	SO	SO	SO	SO	SO	SO	SO	SO	SO	ST	SO	SO	SPt	SO	SO	SPt	SO	SO

Data shown is the biochemical reaction shown by *Salmonella*, Index: ST- *S.* Typhi, SPt- *S.* Paratyphi SO- Other *Salmonella enterica* serovar.

All culture plates were allowed to dry for about 5 minutes. Using the aseptic technique, the antibiotic discs were applied on the agar surface by using sterile forceps. Each disc was kept at least 15 mm from the edge of the plate. Gently each disc was pressed down with sterile forceps to ensure that the discs adhere to the surface of the agar. All the culture plates were incubated in an inverted position for 24 to 48 hours at 37ºC. The susceptibility of an organism to a drug is determined by the size of the zone of inhibition. A measurement of the diameter of the zone of inhibition in millimeters is made and its size is compared to that contained in a standardized chart (zone size interpretive chart). Based on this comparison, the test organism is determined to be resistant, intermediate, or susceptible to the antimicrobics (Bauer *et al.,* 1966; Reller *et al.,* 2009; CLSI, 2018).

Results and Discussion

Bagmati River, especially in the urban area is excessively polluted with sewage; therefore, isolation of *Salmonella* species was highly anticipated. Out of total samples, 34.45% were *Salmonella* positive and the rests were *Salmonella* negative. Among the positive samples, 10.5% were found to be *S.* Typhi and *S.* Paratyphi each, and the remaining 79% were found to be other *S. enterica* serovar. A similar study conducted on Bagmati River showed 45.83% (N= 11) samples exhibiting growth of *Salmonella* spp. of which 12.5% (n=3) isolates were found to be *S.* Typhi, 8.33% (n=2) to be *S.* Paratyphi and rest 25% (n=6)

to be other *S. enterica* serovar (KC *et al.*, 2018). The water sample from site B1 (Chovar to Teku) was found to be rich with *S. enterica* serovar as this area is highly polluted because of the direct discharge of domestic sewage and untreated industrial effluents. One of the most polluted tributary Bishnumati is also merged with Bagmati River in this site at Balkhu making 50% well source and 100% surface water source microbially contaminated (Wolfee, 2000; Shrestha *et al.*, 2005). *Salmonella* typhi and *Salmonella* Paratyphi were isolated from site B2 (Teku to Pingalasthan). This area is surrounded by a huge population of slum dwellers who are settled nearby Pingalasthan due to the generation, storage, collection, and transportation of municipal solid waste (Alam *et al.*, 2006). The bank of this river is also inhabited by many residents, and hospitals, and therefore, direct discharge of effluents was found. Additionally, *Salmonella* Paratyphi was isolated from site B3 (Pingalasthan to Jorpati) which is a highly polluted area for religious activities such as a funeral, as there is a UNESCO listed world heritage site, one of the famous Hindu temples, Shree Pashupatinath. This study was supported by the study conducted at the Guheswori sewage treatment plant with 85% *Salmonella* Typhi with other pathogens isolated from the treatment plant (Gautam *et al.*, 2018). Site B6 was found to be free from *Salmonella* species as is located at the Shivapuri hill and is the origin of the river which is also supported by the study conducted in pre-monsoon, monsoon, and post-monsoon seasons (Bhandari *et al.*, 2017). Therefore, no human activities are found in that area. Bagmati River, especially in an urban area is excessively polluted with sewage so, isolation of *Salmonella* spp was highly anticipated. Some of the important human pathogens such as *Escherichia* coli, *Salmonella* spp, *Shigella* spp, *Proteus* spp, and *Klebsiella* spp, were isolated from similar studies (Paudyal, 2001) during his study which supports our findings. *E.* coli, *Salmonella* spp, *Pseudomonas* spp, *Micrococcus* spp, *Vibrio* spp and *Bacillus* spp were also isolated from the study done in Bagmati River (Baral, 1998).

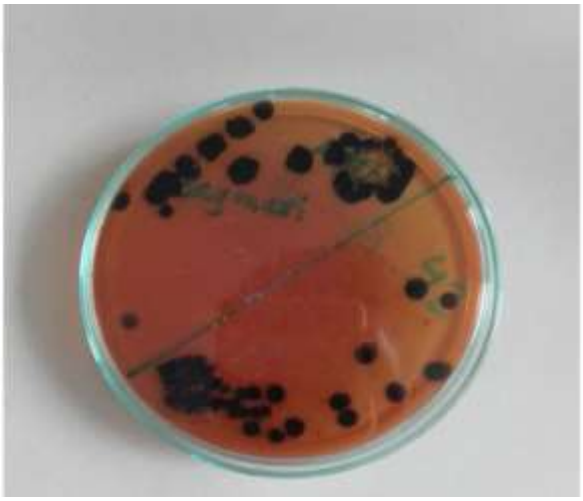

Fig. 2: Isolated colonies of *Salmonella* species on SSA media

Antibiotic Susceptibility Pattern of different Salmonella enterica serovar

In this study, Antibiotic Susceptibility Test (AST) was performed for the positive samples and all of the serovars were found to be sensitive to Amikacin i.e.,100% whereas least sensitive to ampicillin i.e., 86.67%, 100% and 50% resistant in case of other *S.* enterica serovar, *S.* Typhi and *S.* Paratyphi respectively. Similar results were obtained in other studies where none of the *Salmonella* isolates were resistant to amikacin and the least effective antibiotic found was ampicillin i.e., 41% (Murugkar et al., 2005; Al-Bahry *et al.*, 2007). All the *Salmonella* species isolates were sensitive to Ciprofloxacin, Streptomycin, Ofloxacin, and Nitrofurantoin but 100% were resistant to Ampicillin and *Salmonella* strains resistant to ampicillin were also observed in the study conducted in a freshwater environment (Carvalho *et al.*, 2013).

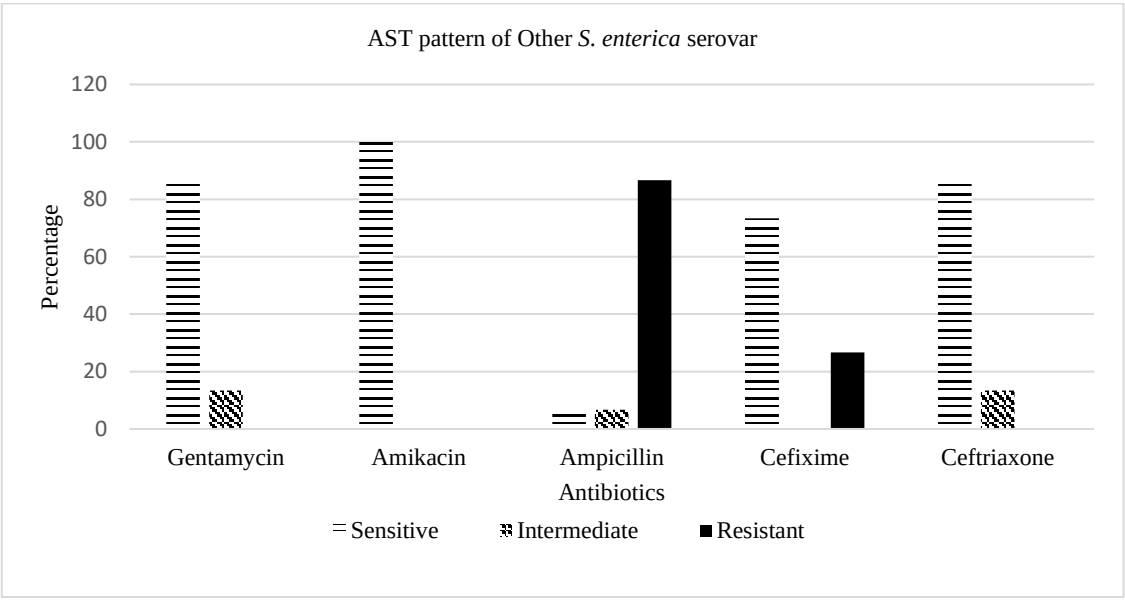

Fig. 3: Graph showing AST pattern of Other *S. enterica* serovar

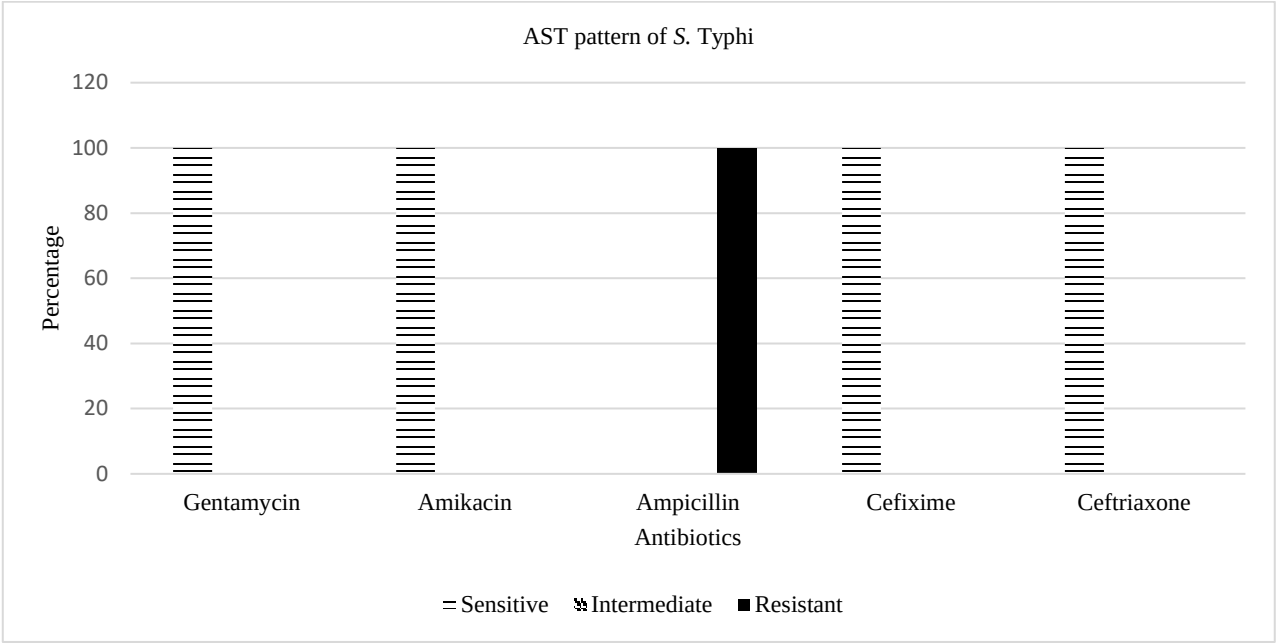

Fig. 4: Graph showing AST pattern of *S.* Typhi

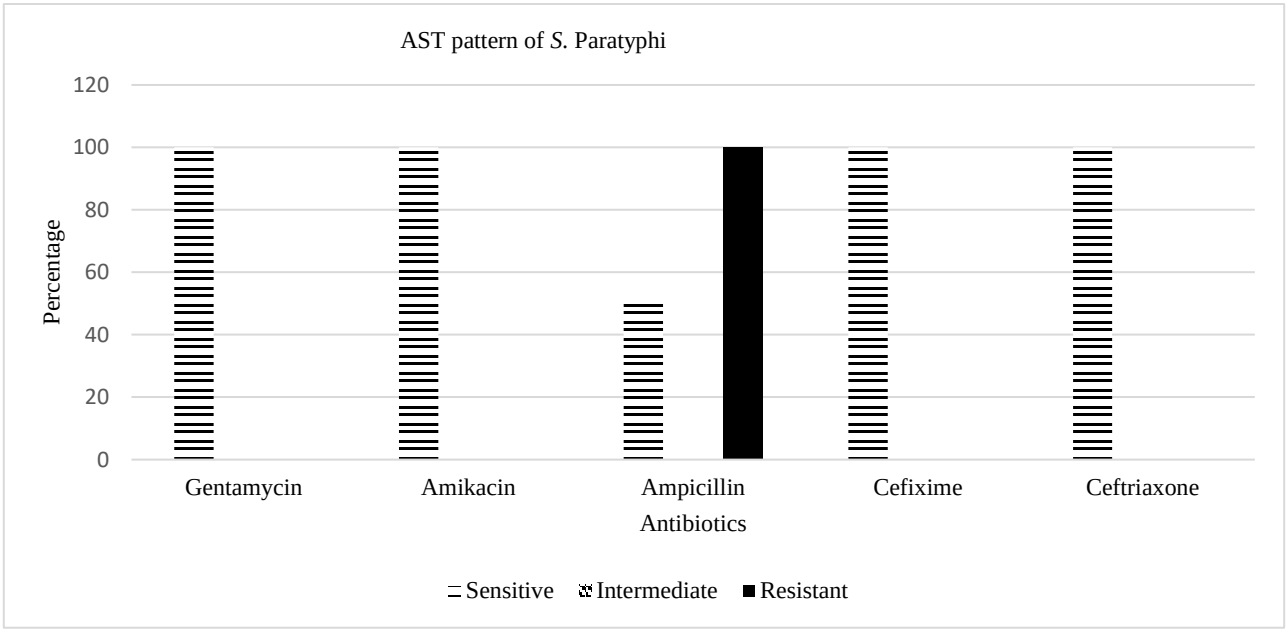

Fig. 5: Graph showing AST pattern of *S.* Paratyphi

Nepal has faced a series of enteric fever epidemics over the last decade (Maskey *et al.*, 2008; Lewis *et al.*, 2005) with changing resistance patterns (Lakshmi *et al.*, 2006; Malla and Dumre, 2008). The first report on Multi-Drug Resistant (MDR) *S.* Typhi in Nepal was published in 1991 (Watson and Pettibone, 1991). However, there is not a single data reported as Multi-Drug Resistant *Salmonella* species in this study. This study highlighted the effective drugs against *S.* Typhi and *S.* Paratyphi which were Gentamycin, Amikacin, Cefixime, and Ceftriaxone as they were all 100% sensitive to these drugs. In the case of other *Salmonella enterica* serovar variations in sensitivity against these drugs were observed. Therefore, concluding with the reference of this study, it can be stated that there's a high risk to the health of the people living nearby the Bagmati River and the risk is at its peak among the slum dwellers, as this microorganism is transmitted easily through contaminated food and water.

Author's Contribution

All of the authors contributed equally in the manuscript. Final form of the manuscript was approved by all of the authors.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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