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

Narayan Kunwar¹*, Manoj KC¹*, Rashika Shrestha¹*, Sushil Chaudhary¹*, Anima Shrestha¹*, Bibek Rana Chhetri¹*

¹Department of Microbiology, St. Xavier's College, Tribhuvan University, Kathmandu, Nepal

**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 become ideal habitat for many pathogenic microorganisms such as *E. coli*, *Salmonella*, *Vibrio*, etc. The study aimed to perform the antibiotic 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 Ciprofloxacain, Streptomycin, Ofloxacain, 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 river has lost its capacity for self-purification and become ideal habitat for many pathogenic microorganisms such as *E. coli*, *Salmonella*, *Vibrio*, etc. The study aimed to perform the antibiotic 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 Ciprofloxacain, Streptomycin, Ofloxacain, 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.

This paper can be downloaded online at http://ijasbt.org & http://nepjol.info/index.php/IJASBT
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 outpatient 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**

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.

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

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

Table 2: Biochemical test performed for the identification Salmonella

<table>
<thead>
<tr>
<th>Sample</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
<th>S9</th>
<th>S10</th>
<th>S11</th>
<th>S12</th>
<th>S13</th>
<th>S17</th>
<th>S18</th>
<th>S22</th>
<th>S26</th>
<th>S29</th>
<th>S37</th>
<th>S45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR/VP</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SIM</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>TSIA</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
<td>R/Y</td>
</tr>
<tr>
<td>Catalase</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Result</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
<td>SO</td>
</tr>
</tbody>
</table>

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 37°C. 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 microbiologically 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 Pushpatinath. 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).

![Graph showing AST pattern of Other S. enterica serovar](image)
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.
Acknowledgment
Sincere thanks to the Department of Microbiology, St. Xavier’s College, Kathmandu, Nepal for providing us with the Microbiology laboratory to complete this study. We would also like to express our gratitude to the supervisor, principal, head of the department, laboratory staff, and other faculty members for their help, support, and kind cooperation during the research period.

References


Society of Tropical Medicine and Hygiene 91-192. DOI: https://doi.org/10.1016/j.trstmh.2007.10.003


