

# Antibiotic Susceptibility Profile of Bacteria Isolated from Natural Sources of Water from Rural Areas of East Sikkim

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## ABSTRACT

**Background:** Contamination of water, food, and environment with antibiotic-resistant bacteria poses a serious public health issue. **Objective:** The objective was to study the bacterial pollution of the natural sources of water in east Sikkim and to determine the antimicrobial profile of the bacterial isolates. **Materials and Methods:** A total of 225 samples, 75 each during winter, summer, and monsoon season were collected from the same source in every season for bacteriological analysis by membrane filtration method. Antibiotic susceptibility test was performed using standard disc diffusion method. **Results:** A total of 19 bacterial species of the genera *Escherichia*, *Klebsiella*, *Proteus*, *Salmonella*, *Shigella*, *Enterobacter*, *Citrobacter*, *Morganella*, *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, and *Serratia* were isolated and their antimicrobial sensitivity tested. Generally, most bacterial isolates except *Salmonella* and *Shigella* species were found resistant to commonly used antibiotics such as ampicillin (57.5%), trimethoprim/sulfamethoxazole (39.1%), amoxicillin/clavulanic acid (37.4%), cefixime (34.5%), tetracycline (29.1%), ceftazidime (26.3%), ofloxacin (25.9%), amikacin (8.7%), and gentamicin (2.7%) but sensitive to imipenem and piperacillin/tazobactam. **Conclusion:** Natural sources of water in east Sikkim are grossly contaminated with bacteria including enteropathogens. The consumption of untreated water from these sources might pose health risk to consumers.

**Keywords:** Bacterial contamination, multi drug resistance, multiple antibiotic resistance index, water sources

## Introduction

Water pollution is a major health problem in the global context, there are nearly 1.7 billion cases of diarrheal disease every year.<sup>(1)</sup> Almost 90% of child deaths from diarrheal diseases are directly linked to contaminated water, and India alone accounts for 24% of all deaths under 5-years old.<sup>(2)</sup> Among the pathogens disseminated in water sources, enteric pathogens such as enterotoxigenic *Escherichia coli*, *Shigella* spp.,

*Salmonella* spp., and so forth are the ones most frequently encountered that are responsible for a variety of diseases like diarrhea, dysentery, and enteric fever. To further compound this problem, enteric bacterial pathogens have been widely reported to demonstrate resistance to several antibiotics.<sup>(3-5)</sup> Bacteria with highest level of resistance were isolated from environment, surface water is the main receptacle reservoir of antibiotics and antibiotic-resistant bacteria in the environment.<sup>(5)</sup> The rise in antibiotic resistance has been reported in the past two decades, and antibiotic resistance still remains a global problem.<sup>(6)</sup> The determination of the efficiency of antimicrobial agents against specific pathogens, either human or animal source, is essential for proper therapy.

In rural areas of Sikkim, surface water serves as major sources of water supply for drinking and domestic purposes. The use of untreated surface water sources

Access this article online	
Quick Response Code:	Website: www.ijcm.org.in
	DOI: 10.4103/0970-0218.137152

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Received: 30-10-13, Accepted: 27-01-14

for drinking and for domestic purposes remains a major threat to public health, as these could serve as reservoirs for transfer of antibiotic-resistant pathogens. The present study was conducted in order to determine the level of bacterial pollution of water in rural areas and antibiogram of the bacterial isolates from the natural sources of water.

### Materials and Methods

This study was conducted in rural habitations of east district of Sikkim, India [Figure 1], where sources of water are springs and streams. A total of 225 water samples, 75 each in winter, summer, and monsoon from the same source, were collected during the period from November 2011 to October 2012 from 75 villages. The samples were collected in sterile capped bottles using the standard technique described in Mackie and McCartney.<sup>(7)</sup> The bottle was labeled with full details of the source of the water and time and date of collection. The bottles were kept in an ice box and transported to microbiology laboratory within 6 h of collection.

Bacteriological analysis was done using the standard membrane filtration technique as described in Mackie and McCartney.<sup>(7)</sup> Each sample (100 ml) was filtered through a 47-mm diameter and 0.45-µm pore size cellulose acetate membrane filter grid (Millipore) held in a filtration unit. Then the membrane, with upper side upwards, with the help of sterile flat-ended forcep was transferred to the surface of MacConkey agar. Plates were incubated at 37°C. The primary identification of the bacterial isolates was done by the conventional methods of morphological and the cultural characteristics. Further confirmation was done on the basis of biochemical characteristics of the isolates.

Antimicrobial susceptibility was performed by the Kirby – Bauer disk diffusion method on Muller – Hinton agar.<sup>(8)</sup> Antibiotic discs were procured from Himedia, Mumbai, India. The antibiotics tested were ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), cefixime (5 µg), tetracycline (30 µg), ceftazidime (30 µg), ofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin/tazobactam (100/10 µg), imipenem (10 µg), chloramphenicol (30 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg). The antimicrobials were chosen on the basis of their common use in treatment in the area. The control strain used was *E. coli* ATCC 25922.

Multiple antibiotic resistance (MAR) index is a useful tool for bacterial source tracking. This method helps in differentiating between human and nonhuman fecal sources.<sup>(9)</sup> MAR indices of the present isolates, belonging to *Enterobacteriaceae*, against the tested antibiotics were calculated based on the formula as follows.<sup>(10-12)</sup>

$$\text{MAR index for isolates} = a/b$$

a = Number of antibiotics to which the isolate is resistant  
 b = Number of antibiotics tested

$$y = \text{Total number of resistance scored}$$

$$n = \text{Total number of isolates}$$

$$x = \text{Total number of antibiotics tested}$$

### Results

In the present study, most of the water sources were found to be contaminated in all three seasons. Out of total 541 isolates, 102 in winter, 241 in summer, and 198 in monsoon were isolated. During winter few organisms were detected as compared with that in summer and monsoon.

All bacterial isolates were found sensitive to imipenem and piperacillin/tazobactam but maximum resistance to ampicillin (57.5%), trimethoprim/sulfamethoxazole (39.1%), and amoxicillin/clavulanic acid (37.4%)

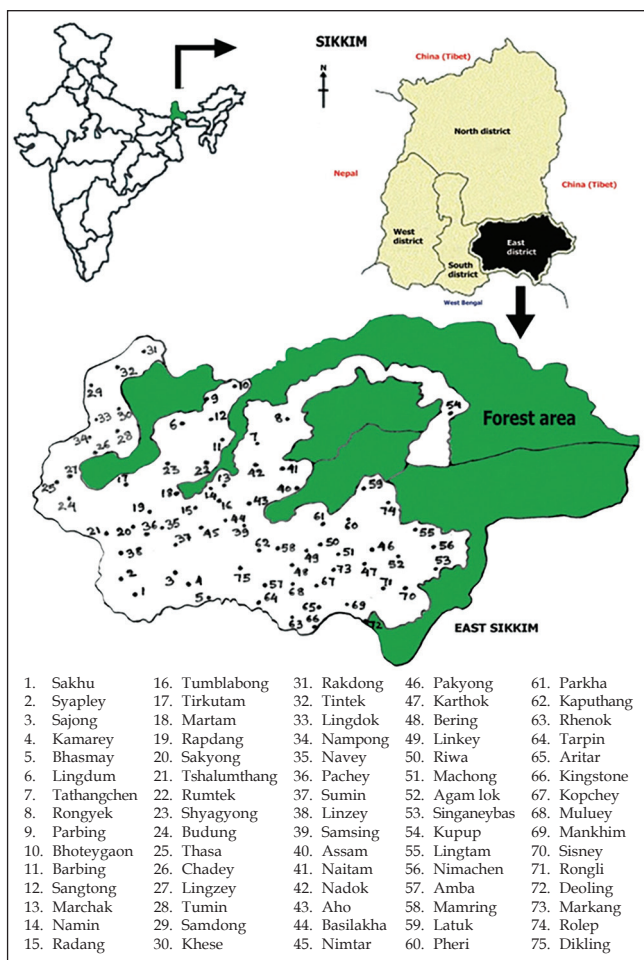
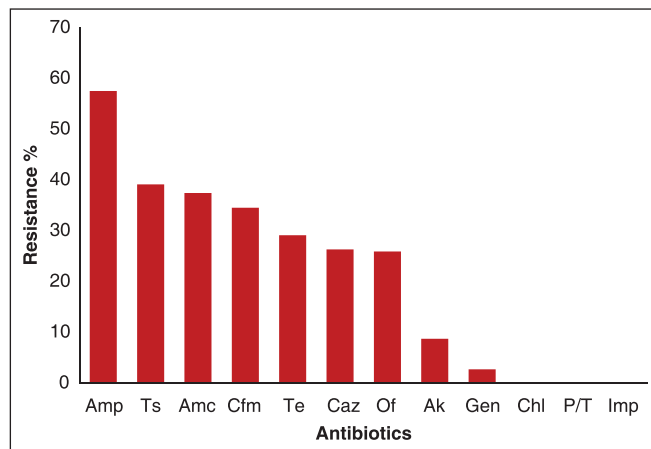


Figure 1: Location of study sites in the map of east district of Sikkim, India

[Figure 2]. Table 1 shows the antibiotic susceptibility pattern of the all bacterial isolates obtained in this study. *E. coli* showed more than 50% resistance to ampicillin and cefixime. Among *Klebsiella spp.* more than 50% resistance was seen to tetracycline, trimethoprim/sulfamethoxazole, ofloxacin, and cefixime. Interestingly, enteropathogens, namely, *Salmonella typhi* (88.8%), *Salmonella paratyphi A* (90%), and *Shigella sonnei* (100%) were found to be fairly sensitive to the antibiotics tested.

Most of the isolates showed resistance to multiple antibiotics. Of all the bacterial isolates ( $n = 541$ ), 213 (39.4%) isolates were sensitive to all the antibiotics tested,



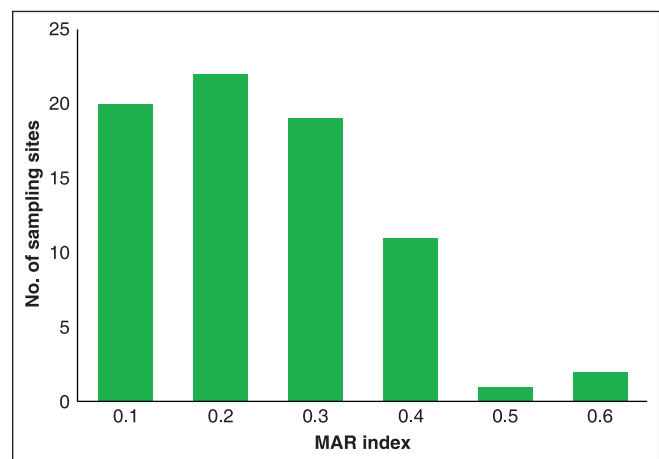
**Figure 2:** Antibiotic susceptibility test results for the isolated bacteria ( $n = 541$ ) Amp: Ampicillin, Ts: Trimethoprim/sulfamethoxazole, Amc: Amoxicillin/clavulanic acid, Cfm: Cefixime, Te: Tetracycline, Caz: Ceftazidime, Of: Ofloxacin, Ak: Amikacin, Gen: Gentamicin, Chl: Chloramphenicol, P/T: Piperacillin/tazobactam, Imp: Imipenem

88 (16.3%) were resistant to at least one antibiotic, and 240 (44.4%) of these strains were multiresistant [Table 2].

The overall resistance of isolates to the antibiotics is represented as MAR index. MAR index of the isolates ranged from 0 to 0.7 [Table 3], whereas MAR index of the sampling sites ranged from 0 to 0.5 [Figure 3].

### Discussion

In the present study, the bacteria were isolated from natural sources of water and has indicated high levels of antibiotic resistance to ampicillin, whereas less resistance to amikacin and gentamicin. Similar to our study, Manisha *et al.*, isolated multidrug-resistant *E. coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Citrobacter*



**Figure 3:** MAR index of the sampling sites, MAR: Multiple antibiotic resistance

**Table 1: Antibiotic resistance pattern of the bacterial isolates**

Antibiotics→Organisms↓	Gen	Amc	Amp	Te	Of	Caz	Cfm	Ts	Ak	Chl
<i>E. coli</i> ( $n = 122$ )	0	34 (27.8)	84 (68.8)	56 (45.9)	48 (39.3)	—	61 (50)	54 (44.3)	2 (1.6)	—
<i>K. pneumoniae</i> ( $n = 18$ )	1 (5.5)	8 (44.4)	—	12 (66.6)	5 (27.8)	—	13 (72.2)	11 (61.1)	8 (44.4)	—
<i>K. oxytoca</i> ( $n = 50$ )	0	23 (46)	—	0	0	—	26 (52)	12 (24)	0	—
<i>K. ornitholytica</i> ( $n = 38$ )	0	15 (39.5)	—	12 (31.6)	24 (63.1)	—	5 (13.2)	24 (63.1)	6 (15.8)	—
<i>S. typhi</i> ( $n = 18$ )	—	—	1 (5.5)	1 (5.5)	1 (5.5)	—	0	0	—	0
<i>S. paratyphi A</i> ( $n = 10$ )	—	—	1 (10)	0	0	—	0	0	—	0
<i>P. mirabilis</i> ( $n = 47$ )	0	20 (42.6)	29 (61.7)	—	0	—	0	23 (48.9)	3 (6.4)	—
<i>P. vulgaris</i> ( $n = 34$ )	7 (20.7)	—	—	—	7 (20.5)	—	8 (23.5)	16 (47.1)	0	—
<i>E. cloacae</i> ( $n = 13$ )	0	—	—	7 (53.8)	3 (23.1)	—	4 (30.7)	4 (30.7)	0	—
<i>C. freundii</i> ( $n = 39$ )	0	—	—	7 (17.9)	9 (23.1)	—	14 (35.8)	13 (33.3)	0	—
<i>C. koseri</i> ( $n = 9$ )	0	5 (55.5)	—	4 (44.4)	0	—	0	3 (33.3)	0	—
<i>M. morgani</i> ( $n = 18$ )	4 (22.2)	—	—	—	0	—	—	—	9 (50)	—
<i>P. aeruginosa</i> ( $n = 41$ )	4 (9.7)	—	—	—	21 (51.2)	14 (34.2)	—	—	13 (31.7)	—
<i>P. alkaligenes</i> ( $n = 29$ )	0	—	—	—	20 (68.9)	19 (65.5)	—	—	18 (62.1)	—
<i>A. lwoffii</i> ( $n = 17$ )	2 (11.7)	—	—	2 (11.7)	—	1 (5.8)	—	3 (17.6)	1 (5.8)	—
<i>F. odoratum</i> ( $n = 2$ )	0	—	—	6 (27.3)	8 (36.4)	6 (27.3)	—	9 (40.9)	5 (22.7)	—
<i>S. marcescens</i> ( $n = 10$ )	4 (40)	—	—	0	0	—	2 (20)	6 (60)	0	—
<i>P. stutzeri</i> ( $n = 5$ )	1 (20)	—	—	—	2 (40)	2 (40)	—	—	2 (40)	—
<i>S. sonnei</i> ( $n = 1$ )	—	—	0	0	0	—	0	0	—	0

Gen: Gentamicin, Amc: Amoxicillin/clavulanic acid, Amp: Ampicillin, Te: Tetracycline, Of: Ofloxacin, Caz: Ceftazidime, Cfm: Cefixime, Ts: Trimethoprim/sulfamethoxazole, A: Amikacin, Chl: Chloramphenicol, Values in parentheses represent percentages

*freundii*, *Morganella morganii*, *Pseudomonas aeruginosa*, and *Serratia marcescens*, and the isolates showed high resistance to ampicillin and less resistance to gentamicin and amikacin.<sup>(13)</sup>

With an increase in the antibiotic load, the prevalence of acquiring resistance increases within a bacterial community. The MAR index is an excellent tool that permits us to analyze the relative prevalence of resistant bacteria in the environment. For all the sites, MAR indices were between 0.1 and 0.5. The water sources having MAR index more than 0.4 are usually from human fecal origin and MAR indices less than 0.4 are from nonhuman fecal contamination.<sup>(11,14)</sup> On the basis of this assumption, 17.3% of the samples were human fecal contaminated. When isolates are exposed to high risk sources of contamination where antibiotics are frequently used, an MAR index value >0.2 is observed. When antibiotics are seldom or never used, an MAR

index value less than or equal to ≤0.2 is observed.<sup>(9,15)</sup> In this study, 36.2% of the bacterial isolates showed MAR value more than 0.2, which is a possible indication that the bacterial isolates have been exposed to several antibiotics. The high resistance seen may be due to the selective pressure exerted by the use of antibiotics in the management of bacterial infections in animals or humans in the area.

In this study, finding enteropathogens *S. typhi*, *S. paratyphi A* and *S. sonnei* sensitive to the commonly used antibiotics is fortunate, but the existence of other Multiple Drug Resistance (MDR) bacteria with the susceptible ones increases the chances of transfer of antibiotic resistance to the sensitive ones. This maintains a pool of resistant bacteria with a pool of resistance genes in the population that further contributes to the general increase and dissemination of bacterial resistance and can be a source of resistance genes for pathogens.<sup>(16)</sup> High resistance of *E. coli* to antimicrobial agents tested was observed in this study. Similar to our study, *E. coli* strains were isolated from water that showed high resistance for ampicillin and were resistant to more than one antibiotic.<sup>(3,17,18)</sup> In another study conducted in Agra, multidrug-resistant *Klebsiella spp.* was isolated from water and showed maximum resistant to gentamicin, whereas in our study *Klebsiella spp.* showed high sensitivity to gentamicin.<sup>(4)</sup> There can be many ways of transmission of resistant bacteria such as municipal, agricultural sewage, and human and animal excrement on the open ground surface and, these are the sources of spreading antibiotic-resistant bacteria in the aquatic environment.<sup>(19)</sup> Mode of transmission through water exposes the whole flock of population to the antibiotics. Therefore, knowledge of proper waste disposal in the environment should be acknowledged to avoid the indiscriminate spread of antibiotic-resistant bacterial strains.

The effectiveness of antibiotic treatment of diseases will decline due to the development of antibiotic resistance in the environment. It is evident from the present results that multiple antibiotic-resistant bacteria can thrive in

**Table 2: Showing percentage of MAR by individual bacterial species**

Number of antibiotics → Organisms↓	No antibiotic (%)	One (%)	Two (%)	Three or more
<i>E. coli</i>	38 (31.1)	16 (13.1)	8 (6.5)	60 (49.2)
<i>K. pneumoniae</i>	4 (22.2)	1 (5.5)	2 (11.1)	11 (61.1)
<i>K. oxytoca</i>	19 (38)	7 (14)	18 (36)	6 (12)
<i>K. ornitholytica</i>	13 (34.2)	1 (2.6)	3 (7.8)	21 (55.3)
<i>S. typhi</i>	16 (88)	1 (5.5)	0 (0)	1 (5.5)
<i>S. paratyphi A</i>	9 (90)	1 (10)	—	—
<i>P. mirabilis</i>	14 (29.7)	7 (14.8)	9 (19.1)	17 (36.2)
<i>P. vulgaris</i>	16 (47)	4 (11.7)	11 (32.3)	3 (8.2)
<i>E. cloacae</i>	6 (46.1)	0 (0)	3 (23.1)	4 (30.7)
<i>C. freundii</i>	19 (48.7)	6 (15.4)	7 (17.9)	7 (17.9)
<i>C. koseri</i>	4 (44.4)	1 (11.1)	1 (11.1)	3 (33.3)
<i>M. morganii</i>	3 (16.6)	5 (27.7)	3 (16.6)	7 (38.8)
<i>P. aeruginosa</i>	13 (31.7)	14 (34.2)	8 (19.5)	6 (14.6)
<i>P. alkaligenes</i>	13 (44.8)	11 (37.9)	5 (17.2)	—
<i>A. lwoffii</i>	8 (47)	9 (52.9)	—	—
<i>F. odoratum</i>	10 (45.4)	2 (9.1)	3 (13.6)	5 (22.7)
<i>S. marcescens</i>	4 (40)	2 (20)	2 (20)	—
<i>P. stutzeri</i>	3 (60)	0 (0)	1 (20)	—
<i>S. sonnei</i>	1 (100)	—	—	—

MAR: Multiple antibiotic resistance

**Table 3: Frequency of MAR index of the bacteria**

MAR index	Frequency of MAR index													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
0	38 (31.1)	4 (23.5)	19 (38)	13 (34.2)	17 (94.4)	9 (90)	1 (100)	14 (29.7)	19 (55.8)	19 (48.7)	4 (44.4)	6 (46.2)	3 (16.6)	4 (40)
0.1	16 (13.1)	1 (5.8)	7 (28)	1 (2.6)	—	1 (10)	—	7 (14.8)	3 (8.8)	4 (10.2)	1 (11.1)	—	5 (27.7)	2 (20)
0.2	8 (6.5)	2 (11.7)	18 (36)	3 (7.8)	—	—	—	9 (19.1)	—	9 (23.1)	1 (11.1)	3 (6.9)	—	2 (20)
0.3	10 (8.1)	1 (5.8)	6 (12)	4 (10.5)	—	—	—	14 (29.7)	12 (35.3)	—	3 (33.3)	—	3 (16.6)	—
0.4	5 (4.1)	4 (23.5)	—	12 (31.5)	1 (5.5)	—	—	3 (6.3)	—	5 (12.8)	—	4 (44.4)	3 (16.6)	2 (20)
0.5	16 (13.1)	3 (17.6)	—	5 (13.2)	—	—	—	—	—	2 (5.1)	—	—	—	—
0.6	29 (23.7)	2 (11.7)	—	5 (13.2)	—	—	—	—	—	—	—	—	4 (22.2)	—
0.7	0	1 (5.8)	—	—	—	—	—	—	—	—	—	—	—	—

A: *E. coli*, B: *K. pneumoniae*, C: *K. oxytoca*, D: *K. ornitholytica*, E: *S. typhi*, F: *S. paratyphi A*, G: *S. sonnei*, H: *P. mirabilis*, I: *P. vulgaris*, J: *C. freundii*, K: *C. koseri*, L: *E. cloacae*, M: *M. morganii*, N: *S. marcescens*, Values in parentheses represent percentages



water that can serve as an environmental reservoir and can therefore provide a route to multidrug-resistant pathogens to enter human population. The study of resistance in the environmental bacteria can help in guiding the development of strategies to counteract this resistance. From the study, it can be concluded that to detect any changing patterns, there is a need to monitor antibiotic sensitivity at regular intervals and knowledge of local presence of bacterial organisms and antibiotic sensitivities and not universal guidelines should provide the direction of antibiotic selection. Treatment of water is essential not only to kill the pathogenic bacteria but also to stop the transfer of antibiotic resistance from the resistant bacteria to the sensitive ones.

Observation of incidence of multidrug-resistant bacterial isolates in water sources of rural areas of east Sikkim in our study may serve as database for formulation of immediate and future sanitation and health-related programs in this area.

## Acknowledgement

The authors are grateful to the Vice Chancellor, Sikkim Manipal University and Dean, Sikkim Manipal Institute of Medical Sciences, Tadong, Gangtok for providing facilities to conduct the valuable research and allowing us to publish this paper. The authors also thank the Departments of Forest, Rural Management and Development, Government of Sikkim for their kind support in providing various information and statistics of the study areas which led to the fruitful completion of this paper.

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**How to cite this article:** Poonia S, Singh TS, Tsering DC. Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim. *Indian J Community Med* 2014;39:156-60.

**Source of Support:** Nil, **Conflict of Interest:** None declared.