Journal of Applied & Environmental Microbiology, 2018, Vol. 6, No. 1, 25-29
Available online at http://pubs.sciepub.com/jaem/6/1/4

DOI:10.12691/jaem-6-1-4

Water Quality of River Narmada at Gwari Ghat Jabalpur (M.P., India) in Terms of Microbial Load, Drug Resistance and Potability

Poornima Sharma*

Shri Ram Institute of Pharmacy, Madhotal, Jabalpur, India
*Corresponding author: poornima.sharma.india@gmail.com

Abstract Regular assessment of river water quality is required to monitor the effectiveness of various schemes declared by municipal authorities in the direction of cleaning river Narmada. Though efforts have been made by the responsible authorities, the expected results are yet to be achieved. Water samples tested on selective and differential media indicate presence of potentially pathogenic microbes in high numbers such as *Pseudomonas*, *Salmonella* and *Shigella*, other than *E.coli*. Drug resistant forms of enteric bacteria were not detected in present research. Yeast and mould forms were also obtained in test samples where *Candida*, *Aspergillus*, *Penicillium* were at considerable levels, though dermatophytes were infrequently obtained.

Keywords: *Narmada river, microbial load, pathogen, drug resistance*

Cite This Article: POORNIMA SHARMA, "Water Quality of River Narmada at Gwari Ghat Jabalpur (M.P., India) in Terms of Microbial Load, Drug Resistance and Potability." *Journal of Applied & Environmental Microbiology*, vol. 6, no. 1 (2018): 25-29. doi: 10.12691/jaem-6-1-4.

1. Introduction

Fresh water pollution has direct implication on health of populations throughout the world. It is estimated that over 80% of the wastewater generated in the world is currently untreated or not reused and it flows into the environment contaminating fresh water-bodies and the soil where crops are grown [1].

It has been reported that half of India's interstate rivers are overwhelmed with pollution and poor water quality. Sewage Treatment Plants have been unable to manage the sewage that has increased from 26,200 million litre per day in 2009 to 38,000 million litre per day in 2015 [2]. Release of untreated sewage and industrial waste into rivers has appeared as main cause of pollution in 16 interstate rivers in the country [2].

Narmada is a revered river since ancient times and the banks of river involve extensive anthropologic activities and recurrent religious ceremonies throughout the year. The river originates from Amarkantak (Madhya Pradesh), and mingles with Arabian Sea at Gulf of Khambat (Gujrat). Major part of the river flows in Madhya Pradesh, and irrigates more than seventeen lakh hectare area in the state. The river provides drinking water to more than four crore people [3]. Several schemes of government over the years have not been successful enough to contain water pollution. Several small and large sewer drains still mingle with river with insufficient treatment or no treatment at all. Thus more synchronized efforts between various govt. authorities are required at state and district levels. The daily activities of bathing, washing clothes and releasing waste materials into the river leads to high microbial load at the banks of river. Regular assessment of water quality is necessary under such circumstances where the local residents also use the untreated river water for domestic purposes. The religious eminence of the river is such that the locals and pilgrims directly drink the river water and show full faith in the rituals at Narmada river. Although there is no official report of epidemics due to water borne diseases in the last five years, the possibility of such an occurrence cannot be ruled out.

2. Materials and Methods

Water samples were collected from near-shore region of proximal and distal banks of river Narmada at Gwari ghat from sites with high anthropologic activity and from sites close to sewage drain outlets to the river. Adopting heterotrophic plate count method, the freshly collected samples were brought to laboratory and spread on five different media, namely, MacConkey's agar, Eosin Methylene Blue (EMB) agar, *Salmonella-Shigella* (SS) agar, Sabouraud Dextrose (SD) agar, Dermatophyte Test Medium (DTM) agar with dermato-supplement, from Himedia. Assessment was conducted in three sets. In set I, 0.5 ml of undiluted sample was spread on 90 mm plate, whereas, in set II, the turbid sample obtained from near-shore region was diluted [1:4] with sterilized distilled water thereafter, 0.5 ml of the diluted sample was spread on 90 mm plate.

To obtain Colony forming units (CFU)/100 ml of sample [for Set I and Set II] following formula was applied –

CFU on plate per 0.5 ml of sample= X
CFU / ml = X*2=Y,
CFU / 100 ml = Y*100

When turbid sample was diluted with sterilized distilled water then the dilution factor was multiplied with the CFU per ml

In set III, 100ml water sample from off-shore region was filtered through membrane filter (0.2 µm) and the filter was inverted on medium; and removed after three hours. Plates were incubated at 37°C Observation was taken after 24 h and 48 h. For SD agar and DTM agar plates, observations were also taken after 72 h, 96 h and 120 h. Results were analyzed and average colony counts were determined from the three sets. CFU/100ml was calculated.

Besides routine Gram staining, Urease test was performed on Urea Agar Slant (Himedia) for bacterial isolates giving black centered colonies on SS agar medium and bacterial isolates giving purple colonies with metallic sheen on EMB agar medium [4]. Methyl Red test in Glucose Phosphate broth was also performed for bacterial isolates giving purple colonies with metallic sheen on EMB agar medium [4,5]; with intention to agreeably identify *Salmonella* sp. and *E.coli* bacteria from culture plates.

Antimicrobial susceptibility assay was performed on isolated gram negative rod forms of enteric bacteria identified as *Salmonella* sp. and *E.coli* through above mentioned procedures. Disc diffusion method on Mueller-Hinton agar (Himedia) was adopted for the assay. With reference to McFarland standard 0.5, inoculum density of test bacteria was adjusted to ca. $1x10^8$ CFU/ml (O.D. at 600nm = 0.12) and concentration of test antibiotic was taken as mentioned in table no.1. Antimicrobial discs (9mm diameter, whatman filter paper) were prepared in laboratory. The concentrations of drugs were taken with reference to Antimicrobial susceptibility test discs supplied by Fluka (Sigma-Aldrich) [6].

Antimicrobial susceptibility assay discs were placed on inoculated medium and incubated at 37°C for 18 hours and then diameter of the inhibition zone surrounding the disc on each plate was measured in millimeters [7].

Table 1. Antimicrobial disc content

Antibiotic	Concentration [Microgram per 10 microlitre]
Norfloxacin	5
Ofloxacin	5
Ceftriaxone	30
Amoxacillin	25
Azithromycin	15
Chloramphenicol	30
Nalidixic acid	30
Tetracycline	30
Amikacin	30
Gentamycin	30

3. Results and Discussion

The water samples were taken from banks of river Narmada where anthropologic activity was high and organic waste/ debris was apparent in the littoral region as seen in the photograph (Figure 1). The density of enteric bacteria obtained on EMB agar and SS agar medium was high enough to affect quality of littoral water at Gwari Ghat proximal bank of river as seen in photographs (Figure 2 and Figure 3), indicating risk of enteric diseases in the local population. The observations for bacteria and fungi obtained from water samples are summarized in Table 2 and Table 3. In present study, the quality of water sampled from Narmada river bank was not at par with required standards of potability, domestic use or swimming. The fecal coliforms crossed 200 colonies per 100 ml of sample as evident from the tabulated data. If fecal coliform count is over 200 colonies/ 100 ml of water in the river, there is a probability that pathogenic organisms may also be present, swimming in such contaminated water poses a risk of skin or ear diseases; drinking such water may lead to gastro-enteric diseases [8]. Water used for drinking must have no *E.coli* or thermo-tolerant coliforms in any 100ml water sample; as suggested by World Health Organisation [9].

Figure 1. Bank of river Narmada with high anthropologic activity

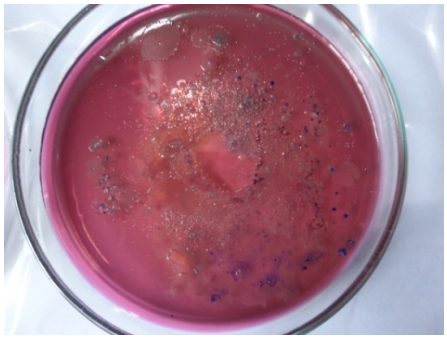

Figure 2. EMB agar medium showing presence of Enteric bacteria such as *E.coli* in the test sample

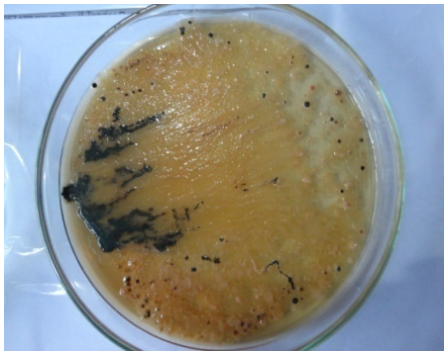

Figure 3. SS Agar medium showing presence of *Salmonella* species and *Shigella* species in test sample

Fungi, such as, *Aspergillus*, *Penicillium*, *Mucor* were found in moderate density while dermatophytes were in low density. Yeast forms were present in high density on SD agar medium; and in moderate density on DTM agar. Presence of yeast forms of *Candida* sp. and *Malassezia* sp. was noted indicating possibility of skin infections in people who regularly bathe in this water. Actinomycetes were also obtained on SD agar. It has been reported that actinomycetes inhibit or reduce biofilming of potentially pathogenic bacteria [10]. This property of actinomycetes may be exploited in cleaning river water if appropriately applied in water treatment processes. In the present research, the proximal bank of river at Gwari Ghat with high anthropologic activity showed higher load of microbes than the distal bank. Possibility of pathogenic forms of bacteria cannot be ruled out when anthropologic activity is high during festivals and also when human and animal wastes merges with river water at the banks regularly.

Over a period of several years anthropologic activities at the river banks, untreated sewage disposal, sand mining, deforestation in the catchment area of river, permanent constructions by human society influencing river bed and river bank have affected physico-chemical and microbiological nature of water; making it unfit for drinking without purification methods [11,12]. Even in 2017 researchers have reemphasized that improper management of domestic wastes, municipal sewage, industrial effluent and agricultural run-off are continuing to disturb physical and chemical nature of river water; this has adversely affected the ecosystem and human society [13].

Further, the enteric bacteria (*Salmonella* sp. and *E.coli*) obtained on selective and differential media were subjected to antimicrobial susceptibility test. Black centered colonies picked from SS Agar and established negative on Urease test were identified as *Salmonella* sp. Purple colonies with metallic sheen picked from EMB Agar, demonstrating Urease test as negative and Methyl Red test as positive were identified as *Escherichia coli*. These isolates were taken up for antimicrobial susceptibility test on ten allopathic drugs listed in Table 1.

While *E.coli* isolates from river Narmada bank were clearly sensitive to all the ten antibiotics tested, *Salmonella* isolates showed slightly decreased sensitivity towards Amoxicillin and Nalidixic acid. *Salmonella* sp. showed intermediate response to Tetracycline and Gentamycin as depicted in Figure 4. No drug resistance was found against any of the tested antibiotics for the considered bacterial isolates from Narmada water in the present study. Fluka (Sigma-Aldrich) Inhibition Zone Diameter Interpretation chart [6] has been referred for interpretation of results depicted in Figure 4.

But a repeated sampling and testing is required for further assessments as the possibility of drug resistant pathogenic microbes in river water cannot be ruled out in small scale sampling.

It has already been stated by researchers in 2016 [14] that Narmada river water quality was poor enough to pose health hazards in people directly using the river water for drinking and other domestic purposes. The researchers also stated that deteriorated water quality is already affecting health of people in rural areas. Similar results were found by Katakwar [15] where chemical as well as microbiological values obtained in water samples were much beyond tolerable limits leading to risk of gastro-intestinal, respiratory diseases and vector borne diseases like malaria.

Enteric fever and other gastro-enteric ailments are one of the major health issues in developing and under-developed nations due to poor sanitation. The problem increases further with prevalence of multidrug resistant strains of pathogens such as *Salmonella* and *Shigella*. This problem of drug resistance persists across the continents. Over a period of two decades drug resistant pathogenic enteric bacteria have been reported by various researchers from Indian subcontinent, South East Asia and Africa. Strains of *Salmonella* and *Shigella* resistant to antibiotics, namely, Ampicillin, Chloramphenicol, Trimethoprim [16]; Nalidixic acid, Ciprofloxacin [17]; Co-trimoxazole [18]; Tetracyclin [19,20] have been reported. Also, there are reports of multidrug resistant *E.coli* and other bacteria in the Indian rivers for several new generation antibiotics [21] which is a matter of concern for villages and tribes at river banks directly using river water for drinking and other domestic purposes. Drug resistance posed by *E.coli* has been reported in the recent period for antibiotics such as Amoxicillin, Ceftriaxone, Chloramphenicol, Tetracyclin, Ampicillin [22,23]; Ciprofloxacin and Co-trimoxazole [24].

Table 2. Densities of most frequently isolated micro-organisms from littoral water of river at Gwari Ghat proximal bank

Proximal bank -water sample on selective & differential medium	DENSITY ; colonies per 100 ml [provisional identification]		
	Category I- [more than 300 colonies]	Category II- [150-300 colonies]	Category III- [less than 150 colonies]
MacConkey's agar	*E.coli*,	----------	*Pseudomonas* sp.
EMB agar	*E.coli*, *Enterobacter* sp.,	*Klebsiella* sp. *Pseudomonas* sp. *Proteus* sp, *Salmonella* sp.	---------
SS agar	*Salmonella* sp., *Shigella* sp.	----------	*Enterobacter* sp. *E.coli*
SD agar	yeasts	*Aspergillus* sp., *Penicillium* sp. *Mucor* sp.	Actinomycetes
DTM agar	----------	yeasts	Dermatophytes

Table 3. Densities of most frequently isolated micro-organisms from littoral water of river at Gwari Ghat distal bank

Distal bank -water sample on selective & differential medium	DENSITY; colonies per 100 ml [provisional identification]		
	Category I- [more than 300 colonies]	Category II- [150-300 colonies]	Category III- [less than 150 colonies]
MacConkey's agar	*E.coli*	*Pseudomonas* sp.	---------
EMB agar	*E.coli*	---------	*Salmonella* sp., *Shigella* sp., *Proteus* sp.
SS agar	--------	---------	*Salmonella* sp. *Shigella* sp.
SD agar	--------	Yeasts, Actinomycetes	*Aspergillus* sp.
DTM agar	--------	Yeasts	Dermatophytes

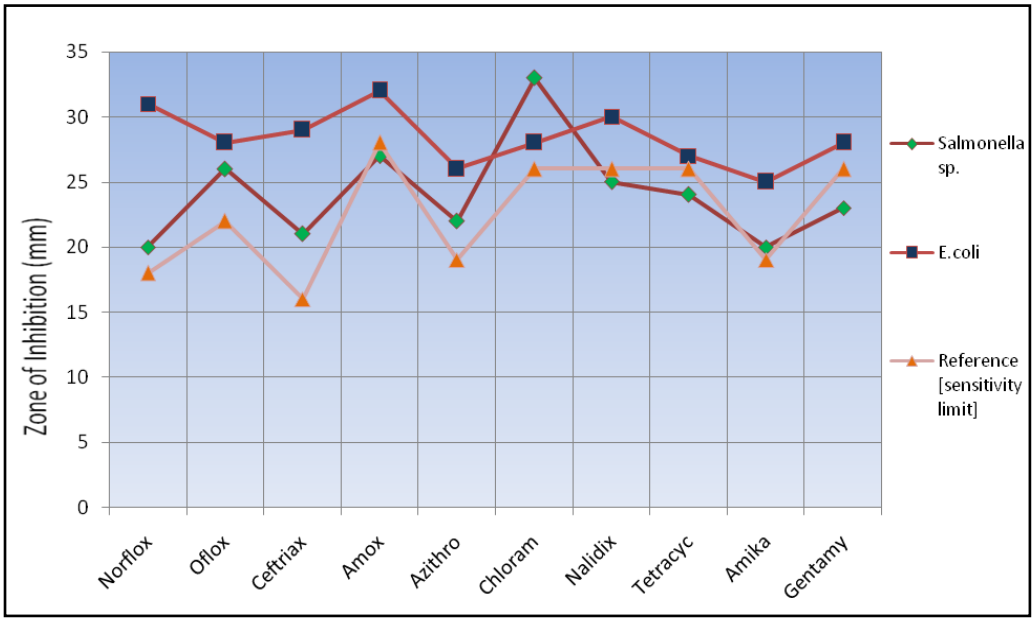

Figure 4. Antimicrobial susceptibility assay evaluating ten antimicrobial drugs on bacterial isolates from Gwari Ghat banks of river Narmada

4. Conclusion

The quality of river water has not improved enough to be used for domestic purposes even after efforts from local activists and government; thus, fool-proof rules and proper implementations are required in order to raise the quality of river water. Effective sewage treatment is necessary before mingling city drainage into the river. Only a concerted effort in use of water purification technology and social awareness can lessen water pollution. Heavy load of enteric bacteria in water may further become a challenge if drug resistant microbial forms increase in number; although in present research drug resistant forms were not detected. Repeated sampling and drug sensitivity tests on large sample size from different sites of river is required for routine assessment of drug resistance in microbial load of river.

Acknowledgements

I extend my gratitude to Dr.V. Tiwari, Principal, ShriRam Instt. of Pharmacy, Jabalpur for providing laboratory facility under SERB Project - YSS/2015/000800; I thank Mr. Rajesh Patel, Resident, Gwari Ghat, Jabalpur for his help in water sample collection. Dr.A. Ayachi, Professor, Science College, Jabalpur, for his suggestion on Narmada water study.

References

[1] Singh, P.K., "Why Waste Water", Message from the Regional Director, WHO South-East Asia Region, on World Water Day 2017 World Health Organisation., 2017.

[2] Kelkar-Khambete, A., "Half of India's interstate rivers plagued by pollution and poor water quality, India Water Portal", http://www.indiawaterportal.org/; https://yourstory.com/2017/03/interstate-rivers-india/, 02 March 2017.

[3] Vyawahare, M., "PM Modi launches river conservation project", *Hindustan Times*, http://www.hindustantimes.com/india-news, May 23, 2017.

[4] Identification of *Salmonella* species. UK Standards for Microbiology Investigations Issued by the Standards Unit, Public Health England, ID 24, Issue no: 3, 25.06.2015. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-qualityand-consistency-in-clinical-laboratories, 2017.

[5] https://corn.org/wp-content/uploads/2009/12/IV-B.pdf; Microbiological Methods, IV-B, 2009.

[6] Antimicrobial Susceptibility Discs Leaflet, Fluka, https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/General_Information/antimicrobial_suscept_discs_leaflet.pdf, 2017.

[7] Balouiri, M., Sadiki, M. and Ibnsouda, S.K., "Methods for in vitro evaluating antimicrobial activity: A review", *Journal of Pharmaceutical Analysis* 6, 71-79, 2016.

[8] Flint River Green Notebook, www.FlintRiver.org, April, 2011.

[9] Guidelines for Drinking Water Quality, Fourth Edition, World Health Organisation, 2017.

[10] Waturangi, D.E., Rahayu, B.S., Lalu, K.Y., Michael and Mulyono. N., "Characterization of bioactive compound from actinomycetes for antibiofilm activity against Gram-negative and Gram-positive bacteria", Malaysian Journal of Microbiology, 12(4), 291-299, 2016.

[11] Sharma S., Vishwakarma R., Dixit S. and Jain P., "Evaluation of Water Quality of Narmada River with reference to Physcochemical Parameters at Hoshangabad city, MP, India", *Research Journal of Chemical Sciences*, 1(3) June 2011.

[12] Saxena, D.; "Madhya Pradesh's lifeline- Narmada, battles for survival", *Times of India*, https://timesofindia.indiatimes.com; Jun 5, 2013.

[13] Jain, D., Rahi, D.C. and Verma, S., "Assessment of Water Quality Status by Using Water Quality Index (WQI) Method in Narmada River in Jabalpur Madhya Pradesh", *International Journal for Scientific Research & Development*, 4 (12), 122- 124, 2017.

[14] Upadhyay, A. and Chandrakala, M., "Water quality index of Narmada river water Jabalpur,MP, India", *International Journal of Science and Nature*, 7 (4), 809-812, 2016.

[15] Katakwar, M., "Narmada river water: Pollution and its impact on the human health", *International Journal of Chemical Studies*, 4(2): 66-70, 2016.

[16] Rowe, B., Ward, L.R. and Threlfall, E.J., "Multidrug-resistant *Salmonella* typhi: a worldwide epidemic", *Clinical Infectious Diseases*, 24 Suppl 1: S106-109, Jan, 1997.

[17] Hirose, K., Tamura, K., Sagara, H. and Watanabe, H., "Antibiotic Susceptibilities of *Salmonella enterica* Serovar Typhi and *S. enterica* Serovar Paratyphi A Isolated from Patients in Japan", *Antimicrobial Agents and Chemotherapy*, 45 (3), 956-958, Mar. 2001.

[18] Raveendran, R., Datta, S. and Wattal, C., "Drug resistance in *Salmonella enterica* serotype typhi and paratyphi A", *JIMSA Journal of International Medical Sciences Academy*, 23(1); January - March 2010.

[19] Lamboro, T., Ketema, T., and Bacha, K., "Prevalence and Antimicrobial Resistance in *Salmonella* and *Shigella* Species Isolated from Outpatients, Jimma University Specialized Hospital, Southwest Ethiopia", *Canadian Journal of Infectious Diseases and Medical Microbiology*, Vol 2016, Article ID 4210760, 8 pages, 2016.

[20] Chiou, C., Lauderdale, T., Phung, D., Watanabe, H., Kuo, J., Wang, P., Liu, Y., Liang, S. and Chen, P., "Antimicrobial Resistance in *Salmonella enterica* Serovar Typhi Isolates from Bangladesh, Indonesia, Taiwan, and Vietnam", *Antimicrobial Agents and Chemotherapy*, 58 (11), 6501-6507, November 2014.

[21] Verma, S. and Rawat, A., "Multi-Drug Resistance in Indian Rivers: Review", *International Journal of Multidisciplinary and Current Research*, 2, Sept/Oct 2014.

[22] Me, I., Ne, B., and Me, H., "Increased multi-drug resistant Escherichia coli from hospitals in Khartoum state, Sudan", *African Health Sciences*, 12(3), 368-375, 2012.

[23] Wambugu, P., Habtu, M., Impwi, P., Matiru, V. and Kiiru, J., "Antimicrobial Susceptibility Profiles among *Escherichia coli* Strains Isolated from Athi River Water in Machakos County, Kenya", Advances in Microbiology, 5, 711-719, 2015.

[24] Chen, Z., Yu, D., He, S., Ye, H., Zhang, L., Wen, Y., Zhang, W., Shu, L. and Chen, S., "Prevalence of Antibiotic-Resistant *Escherichia coli* in Drinking Water Sources in Hangzhou City", *Frontiers in Microbiology*, 8, Article 1133, June 2017.