ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 02, pp.12282-12288, February, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ASSESSMENT OF NATURAL WATER QUALITY USING MOST POTABLE NUMBER (MPN)

[1,*]Bhagwan N. Rekadwad, [2]Vikas B. Maske and [2]Anil E. Jogdand

[1]School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, India
[2]Yeshwant Mahavidyalaya, Nanded, India

ARTICLE INFO

Article History:
Received 10th November, 2014
Received in revised form
07th December, 2014
Accepted 25th January, 2015
Published online 26th February, 2015

Key words:
16S rRNA,
Coliform,
Escherichia coli,
LATEX TEST,
MPN,
Water.

ABSTRACT

Most potable number (MPN) method was used to reckon microorganisms per 100 mL in water samples collected from hot springs, ground water sources, domestic water sources and urban area. This method provided sufficient and accurate results to prove the presence of contamination in potable and recreational waters. Of the total 90 samples, six samples showed highest number of total coliform. Selected six samples found MPN total coliform greater than permissible value have detected the presence of *E. coli* ranging from 2 to 140 MPN per 100 mL. Total 15 isolates were isolated from these samples. Of 15 isolates, selected three isolates showed colourless to bright pink coloured colonies, highly motile small rods, positive for glucose, inositol, lactose, maltose, mannitol, salicin, sucrose, TSI test and negative for sorbitol. LATEX REAGENT TEST shows identified *Escherichia coli* isolate has serogroup O157:H7 antigen. Three biochemically identified *Escherichia coli* confirmed using16S rRNA gene sequence analysis. Obtained results indicate the presence of pathogenic microorganism in water samples. Hence, attributor to strict sanitation, high level of hygienic sanitary conditions, strict preventive practices should be adopted to reduce possibilities of infection.

INTRODUCTION

All living organisms need water for their survival. Of the abiotic component of environment, water is the major requisite of every living thing for better health. Among all living organisms, animals were very sensitive to changes in their surroundings and environment (**Srinivas, 2008**). Religious literature recorded that water have transformative power with respect to its purity. Water from sacred places is also used for various purposes by pilgrims and local peoples. Therefore, locally and globally water is used for various practical activities. The supply of clean, contaminant free water is utmost priority in maintaining better health because, water is one of the way for the transfer of pathogenic microorganisms (**Tambekar *et al.*, 2008**). Not only water used to for cleaning and keep contaminants away but also it also bring contaminants nearer to the living organisms. Natural water is contaminated by supplies of microorganisms from air, liquid and solids (**Hosseini *et al.*, 2014**). Source of contaminant include contaminated air, aerosols, sewage, washings of floors, water from hospitals, industries etc. Most common infectious agents transported by contaminated water are bacteria include *E.coli O157* (**Ratnam *et al.*, 1998**), *Bacillus etc.*, fungi and viruses. These heterotrophs cause serious damage to the personal health and social health.

Corresponding Author: Dr. Bhagwan N. Rekadwad,
School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, India.

Common water borne infections were bacterial associated diarrhea, typhoid, cholera, shigellosis, viral diarrhea and virus associated hepatitis. The extent of water pollution generally indicated by the presence of coliform and *Escherichia coli* (**Salem *et al.*, 2011**). The most-probable-number (MPN) is presently used and accepted test for counting microorganisms and in determining the potability of water (**Highsmith and Ashire, 1975; Gonzalez, 1995, 1996**). In present study, water samples were collected from various places in Maharashtra state include the water from hot springs, sacred places, natural water bodies, domestic drinking water and urban drinking water. The same water were supplied and used for recreational, sacred, domestic and urban purposes such as drinking, washing, cleaning, irrigation etc . The collected water samples were thoroughly analyzed for the presence of pathogenic microorganisms (total coliform) by the Most Potable Number (MPN) method followed by *Escherichia coli* MPN. Detected present in water was confirmed by 16S rRNA gene sequencing method. The purpose of this study to make hurriedly awareness of peoples for the presence of human entero-pathogenicbacteria (*Escherichia coli* and other pathovars).

MATERIAL AND METHODS

Collection of water samples

Water samples were collected by composite sampling method in sterilized Polystyrene bottles (100 mL) with tight fitting

screw cap. Mumbai (India). The composite samples were stored at 4 °C until use and analyzed (**Greenberg *et al*., 1992**).

The Most Potable Number (MPN) Method for total coliforms

a. MPN for total coliforms

The most probable number (MPN) method was performed to detect the coliforms. All experiments were performed in triplicates. The three sets of test tubes containing 10 mL double strength MacConkey broth in which 10 mL water sample was added. Another six test tubes containing 10mL single strength MacConkey broth each, out of these six test tubes three test tubes were inoculated with one mL water sample and another three test tubes were inoculated with 0.1 mL water samples. Then all tubes were incubated at 37 °C for 24 h. The production of acid and gas in Durham's tube indicate positive results (**Gonzalez, 1996; Tambekar and Ahir, 2014**). MPN positive tubes showing the MPN beyond maximum acceptable and non objectionable concentration as per the drinking water quality standards were further preceded for detection *Escherichia coli* (**Gonzalez, 1996; Tambekar and Ahir, 2014**).

b. MPN for the presence of *Escherichia coli*

The most probable number (MPN) method was performed to detect *Escherichia coli*. All experiments were performed in triplicates. The five sets of test tubes containing 10mL double strength MacConkey broth in which 10 mL water sample was added. Another ten test tubes containing 10 mL single strength MacConkey broth each, out of these ten test tubes five test tubes were inoculated with one mL water sample and remaining five test tubes were inoculated with 0.1 mL water samples. Then all tubes were incubated at 37 °C for 24 h. The production of acid and gas in Durham's tube indicate positive results (**Oblinger and Koburger, 1975; Woomer, 1994; Gonzalez, 1996; Feng *et al*., 2002**).

Calculation of MPN, Low level confidence limit and Upper level confidence limit using MPN Calculator

MPN calculating software was used to calculate MPN (at 10 mL, 1.0 mL & 0.1 mL inoculum), % low level confidence limit and % upper level confidence limit (**USEPA, 2012**).

Isolation of culturable bacteria using selective medium

Culturable bacteria were isolated using MacConkey agar (pH 7.2) and Sorbitol-MacConkey agar (pH 7.2) (**Aneja, 2007**). 0.1 mL water sample were spread on agar and incubated at 37 °C for 24 h. After 24 h, isolated colonies were selected and colony characteristic were recorded. Selected colonies were sub-cultured on same medium. Pure cultures were stored on slants of respective medium at 4 °C (**Greenberg *et al*., 1992**).

Morphological and biochemical identification of isolates

Isolated strains were identified using Grams staining, micrometry, motility, carbohydrate utilization (glucose, inositol, lactose, maltose, mannitol, salicin, sorbitol and sucrose), IMViC (Indole production, MR test, VP test and citrate utilization), TSI test, catalase test, urease test and salt tolerance (0.5%, 2% and 5%) (**Aneja, 2007; Bergeys *et al*., 1984**).

***In vitro* diagnosis of isolates using latex test reagent kit**

LATEX REAGENT TEST KIT was purchased from PRO-LAB Diagnostics (Texas, U.S.A.) used for presumptive identification of *Escherichia coli* (sorbitol fermenting and non-sobritol fermenting) serogroup O157:H7 antigen (**Scotland *et al*., 1980; CDC, 1982; Ratnam *et al*., 1998**).

Confirmation of isolates using 16S rRNA gene sequencing method

Extraction of DNA from stable enrichment cultures in nutrient medium and the isolation was done by the phenol chloroform method. The method was modified as follows: cell pellet of 2 mL from each enrichment culture of isolate was suspended in extraction buffer (100 mM Tris-HCl (pH 8.0), 100 mM Na_2EDTA (pH 8.0) Proteinase K (Nitrogen, USA) was added at the final concentration of 100 mg/mL and set was incubated at 55 °C for 2 h with continuous shaking. Then 0.5 M NaCl was added and set was incubated at 72 °C for 30 min. Subsequently, DNA was extracted and washed twice with 70% ethanol and dissolved in Tris-EDTA buffer (pH 8.0). Set was analyzed by electrophoresis on a 0.8% agarose gel and visualized by ethidium bromide staining using UV trans-illuminator. The 16S rDNA of the enriched strains were amplified with a pair eubacteria specific of primers (forward primer 530 F: 5' GTGCCAGCAGCCGCGG 3' & reverse primer 1392 R: 5'ACGGGCGGTGTGTAC 3'. The PCR conditions used were an initial denaturation at 94 °C for two minutes, followed by 35 cycles of denaturation at 95 °C for one minute, annealing at 55 °C for one minute and extension at 72 °C for one minute then a final extension was given at 72 °C for 10 min. The amplified PCR product was a mixture of 16S rRNA genes from all the strains used for the amplification. The identity of the isolates was determined through a BLAST search (**Tamura *et al*., 2011; Pathak and Rekadwad, 2013**).

Submission of nucleotide sequences

Nucleotide sequence of 16S rRNA genes from isolated strain was submitted to NCBI repository.

RESULTS AND DISCUSSION

Collected water samples from hot spring, ground water sources, domestic water sources and urban waters were used to detect MPN per 100 mL and recorded (Table 1-5; Figure 1). Of the tested 90 samples, six samples showed highest number of total coliform such as *Nagpur* (M.S.), *Mudkhed* (Nanded district, M.S.), *Bhokar* (Nanded district, M.S.), *Mahur* (Nanded city, M.S.), *Itwara Chowk* (Nanded city, M.S.), *Degloor Naka* (Nanded city, M.S.) and *Doctors lane* (Nanded city, M.S.) such as 150, 150, 210, 210, 290, 460 and 1100 MPN per 100 mL respectively (Table 6; Figure 2).

Table 1. Total coliform- MPN index and 95% confidence limits for MPN of five hot springs water in Maharashtra (India) indicating various combinations of positive tubes in a 3 tube dilution series using inoculum quantities of 10 mL, 1 mL and 0.1 mL

Place	Number of tubes per dilution	Number of positive tubes per dilutions for inoculum volume			Most Potable Number (MPN) Results			MPN per 100 mL
		10 mL	1.0 mL	0.1 mL	MPN per mL	Confidence Limit Lower 95%	Upper 95%	
Akoli hot spring (M.S.)	3	1	0	0	0.036	0.0051	0.25	3.6
Rajapur hot spring (M.S.)	3	1	0	0	0.036	0.0051	0.25	3.6
Unkeshwar (M.S.)	3	2	0	0	<0.03	0.0051	0.25	3.0
Unapdev hot spring (M.S.)	3	1	0	0	0.036	0.0051	0.25	3.6
Vajreshwari hot spring (M.S.)	3	0	1	0	<0.03	0.0051	0.25	3.0

Table 2. Total coliform- MPN index and 95% confidence limits for water at Bus Stands (Maharashtra, India) indicating various combinations of positive tubes in a 3 tube dilution series using inoculum quantities of 10 mL, 1 mL and 0.1 mL

Place	Number of tubes per dilution	Number of positive tubes per dilutions for inoculum volume			Most Potable Number (MPN) Results			MPN per 100 mL
		10 mL	1.0 mL	0.1 mL	MPN per mL	Confidence Limit Lower 95%	Upper 95%	
Ahemadnagar	3	1	0	1	0.072	0.016	0.32	7.2
Akola	3	2	1	1	0.2	0.059	0.71	20
Aurangabad	3	3	1	0	0.43	0.1	1.8	43
Buldhana	3	2	1	1	0.2	0.059	0.71	20
Hingoli	3	3	1	1	0.75	0.18	3.2	75
Jalgaon	3	2	1	1	0.2	0.059	0.71	20
Kolhapur	3	3	1	0	0.43	0.1	1.8	43
Latur	3	2	1	1	0.2	0.059	0.71	20
Mumbai (Dadar)	3	3	2	0	0.93	0.23	3.8	93
Nagpur	3	3	2	1	1.5	0.42	5.4	150.0
Nasik	3	2	2	0	0.21	0.061	0.73	21
Navi Mumbai (Washi)	3	2	1	1	0.28	0.077	0.99	28
Osmanabad	3	2	1	1	0.2	0.059	0.71	20
Pune	3	2	2	1	0.28	0.077	0.99	28
Sangli	3	2	1	1	0.2	0.059	0.71	20
Solapur	3	2	1	1	0.2	0.059	0.71	20
Thane	3	2	2	0	0.21	0.061	0.73	21
Wardha	3	1	0	0	0.036	0.0051	0.25	3.6
Yavatmal	3	2	1	0	0.15	0.041	0.52	1.5

Table 3. Total coliform- MPN index and 95% confidence limits for water at Railway stations (Maharashtra, India) indicating various combinations of positive tubes in a 3 tube dilution series using inoculum quantities of 10 mL, 1 mL and 0.1 mL

Place	Number of tubes per dilution	Number of positive tubes per dilutions for inoculum volume			Most Potable Number (MPN) Results			MPN per 100 mL
		10 mL	1.0 mL	0.1 mL	MPN per mL	Confidence Limit Lower 95%	Upper 95%	
Akola	3	2	1	0	0.15	0.041	0.52	15.0
Aurangabad	3	1	1	0	0.074	0.017	0.33	7.4
Jalgaon	3	1	1	0	0.074	0.017	0.33	7.4
Latur	3	1	0	0	0.036	0.0051	0.25	3.6
Mumbai (Dadar)	3	1	0	0	0.036	0.0051	0.25	3.6
Nagpur	3	2	1	0	0.15	0.041	0.52	15.0
Nasik	3	2	1	0	0.15	0.041	0.52	15.0
Pune	3	0	0	0	<0.03	0.0051	0.25	3.0
Thane	3	1	1	0	0.074	0.017	0.33	7.4

Table 4. Total coliform-MPN index and 95% confidence limits for water near the public places in Nanded district indicating various combinations of positive tubes in a 3 tube dilution series using inoculum quantities of 10 mL, 1 mL and 0.1 mL

Place	Number of tubes per dilution	Number of positive tubes per dilutions for inoculum volume			Most Potable Number (MPN) Results			MPN per 100 mL
		10 mL	1.0 mL	0.1 mL	MPN per mL	Confidence Limit Lower 95%	Upper 95%	
Ardhapur	3	2	1	0	0.15	0.041	0.52	15.0
Bhokar	3	3	2	2	2.1	0.61	7.6	210
Biloli	3	1	0	0	0.036	0.0051	0.25	3.6
Dharmabad	3	1	0	0	0.036	0.0051	0.25	3.6
Hadgaon	3	1	0	0	0.036	0.0051	0.25	3.6
Himayatnagar	3	1	0	0	0.036	0.0051	0.25	3.6
Islapur	3	1	0	0	0.036	0.0051	0.25	3.6
Kandhar	3	1	0	0	0.036	0.0051	0.25	3.6
Kinwat	3	1	0	0	0.036	0.0051	0.25	3.6
Loha	3	2	0	0	0.092	0.023	0.37	9.2
Mahur	3	3	2	2	2.1	0.61	7.6	210.0
Mudkhed	3	3	2	1	1.5	0.42	5.4	150
Mukhed	3	1	0	0	0.036	0.0051	0.25	3.6
Nanded (North)	3	2	1	0	0.15	0.041	0.52	15.0
Nanded (South)	3	1	0	0	0.036	0.0051	0.25	3.6
Umari	3	1	0	0	0.036	0.0051	0.25	3.6

Table 5. Total coliform- MPN index and 95% confidence limits for water near the public places in Nanded city indicating various combinations of positive tubes in a 3 tube dilution series using inoculum quantities of 10 mL, 1 mL and 0.1 mL

Place	Number of tubes per dilution	Number of positive tubes per dilutions for inoculum volume			Most Potable Number (MPN) Results			MPN per 100 mL
		10 mL	1.0 mL	0.1 mL	MPN per mL	Confidence Limit Lower 95%	Upper 95%	
Ambedkar Chowk	3	2	0	0	0.092	0.023	0.37	9.2
Annabhau Sathe Chowk	3	1	0	0	0.036	0.0051	0.25	3.6
Bafna	3	3	2	0	0.93	0.93	3.8	9.3
Bhagat Singh Chowk	3	0	0	0	<0.03	0.0051	0.25	3.0
Bhagya Nagar	3	1	0	0	0.036	0.0051	0.25	3.6
Bust stand	3	2	1	0	0.15	0.041	0.52	150.0
Chaitanya Nagar	3	1	0	0	0.036	0.0051	0.25	3.6
Chanda Singh M.I.D.C.	3	2	1	0	0.15	0.041	0.52	150.0
Chikhalwadi	3	1	0	0	0.036	0.0051	0.25	3.6
CIDCO	3	1	0	0	0.036	0.0051	0.25	3.6
Degloor Naka	3	3	3	1	4.6	1.0	21	460.0
Doctors lane	3	3	3	2	11	2.6	47	1100.0
Hingoli Naka	3	2	1	1	0.2	0.059	0.71	20.0
Holi	3	2	1	1	0.2	0.059	0.71	20.0
HUDCO	3	0	0	0	<0.03	0.0051	0.25	3.0
Itwara Chowk	3	3	2	3	2.9	0.78	11	290.0
Labour colony	0.2	0.059	0.71	20	0.2	0.059	0.71	20.0
Latur phata	3	1	0	0	0.036	0.0051	0.25	3.6
M.I.D.C. Nanded	3	1	0	0	0.036	0.0051	0.25	3.6
Mahavir Chowk	3	0	0	0	<0.03	0.0051	0.25	3.0
Namaskar Chowk	3	0	0	0	<0.03	0.0051	0.25	3.0
New Kautha	3	1	0	0	0.036	0.0051	0.25	3.6
New Mondha	3	1	0	0	0.036	0.0051	0.25	3.6
Old Kautha	3	1	0	0	0.036	0.0051	0.25	3.6
Old Mondha	3	1	0	0	0.036	0.0051	0.25	3.6
Railway station	3	2	1	1	0.2	0.059	0.71	20.0
Shivaji Nagar	3	2	1	1	0.2	0.059	0.71	20.0
Sri Nagar	3	2	1	1	0.2	0.059	0.71	20.0
SRTM University	3	1	0	0	0.036	0.0051	0.25	3.6
Taroda (Budrukh)	3	0	0	0	<0.03	0.0051	0.25	3.0
Taroda (Khurd)	3	1	0	0	0.036	0.0051	0.25	3.6
Vajirabad	3	2	1	0	0.15	0.041	0.52	15.0
Vishnupuri	3	2	1	1	0.2	0.059	0.71	20
Waman Nagar	3	1	1	0	0.036	0.0051	0.25	3.6
Workshop	3	1	0	0	0.036	0.0051	0.25	3.6

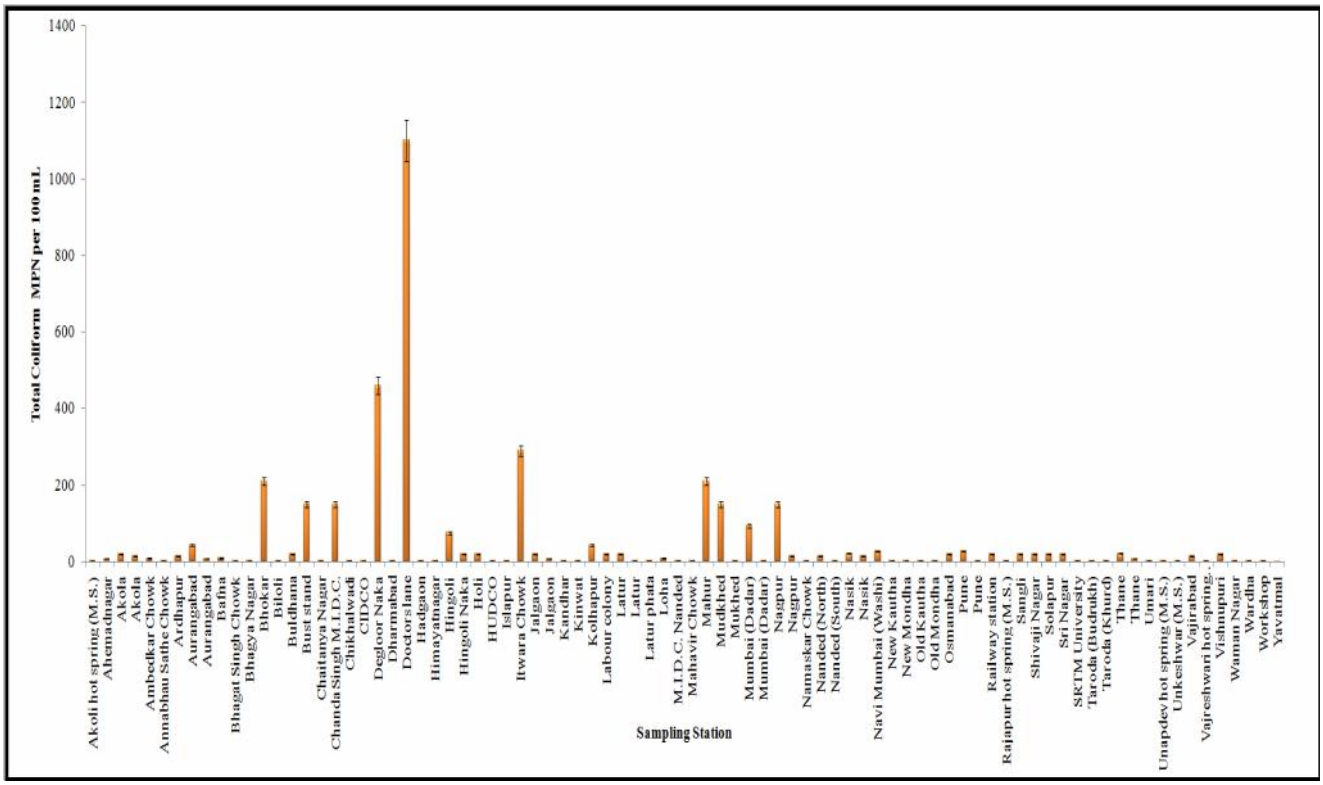

Figure 1. Relationship of the geometric mean of total coliforms count between sample stations in Maharashtra (India)

Table 6. *E. coli*- MPN index and 95% confidence limits for water near the public places in Nanded district indicating various combinations of positive tubes in a 5 tube dilution series using inoculum quantities of 10 mL, 1 mL and 0.1 mL

Place	Number of tubes per dilution	Number of positive tubes per dilutions for inoculum volume			Most Potable Number (MPN) Results			MPN per 100 mL
		10 mL	1.0 mL	0.1 mL	MPN per mL	Confidence Limit Lower 95%	Upper 95%	
Doctors lane	5	3	1	1	0.14	0.051	0.37	140.0
Degloor Naka	5	2	1	1	0.092	0.031	0.27	9.2
Itwara Chowk	5	1	1	1	0.061	0.018	0.21	6.1
Bhokar	5	1	1	0	0.04	0.0095	0.17	4.0
Mahur	5	1	1	0	0.04	0.0095	0.17	4.0
Mudkhed	5	1	0	0	0.02	0.0028	0.14	2.0

Table 7. Morphological and biochemical characteristics of *Escherichia coli* isolates

Character	Isolate NW1	Isolate NW2	Isolate NW3
Accession number	KM998072	KM998073	KM998074
Shape	Rod	Rod	Rod
Size (µm)	3.0x1.0	2x1.0	2.5x1.0
Gram staining	-	-	-
Arrangement	Single	Single	Single
Endospore	Absent	Absent	Absent
Motility	Motile	Motile	Motile
Colour of colony	Colourless	Colourless	Bright pink
Colony size (mm)	2	2	2
Form of colony	Circular	Circular	Circular
Margin of colony	Entire	Entire	Entire
Elevation of colony	Raised	Raised	Elevated
Density of colony	Opaque	Opaque	Opaque
Optimum temperature	37±0.2 °C	37±0.2 °C	37±0.2 °C
Optimum pH	7±0.2	7.4±0.2	7.4±0.2
Glucose	+	+	+
Inositol	+	-	-
Lactose	+	+	+
Maltose	+	+	+
Mannitol	+	+	+
Salicin	+	+	+
Sorbitol	-	-	+
Sucrose	+	+	+
TSI Test	+	+	+
Catalase test	+	+	+
Urease	-	-	-
Salt tolerance			
0.5%	+	+	-
02%	+	+	+
5%	-	-	-
IMViC			
Indole production	+	+	+
Methyl red	+	+	+
Voges-Proskauer	-	-	-
Citrate utilization	-	-	-
LATEX TEST	+	+	+

Selected six samples further analyzed and have detected the presence of *E. coli* maximum in *Doctors lane* (MPN 140 per 100 mL) followed by *Degloor Naka* (MPN 9.2 per 100 mL) followed by *Itwara Chowk* (MPN 6.1 per 100 mL) followed by *Bhokar* (MPN 4 per 100 mL) followed by *Mahur* (MPN 4 per 100 mL) and followed by *Mudkhed* (MPN 2 per 100 mL). From these samples, one composite sample was prepared aseptically. From composite sample, total 15 isolates were isolated. Of these three (NW1, NW2 and NW3) isolates showing luxuriant growth were identified using morphological and biochemical method. Colonies of isolates were up to 2 mm in size, bright pink, circular, entire, elevated and opaque having optimum growth at temperature 37 °C and pH 7.0-7.4. All isolates were aerobic, 1.0x3.0 (µm) small rods, Gram negative, highly motile.

Of the three isolates, all were positive for glucose, lactose, maltose, mannitol, salicin, sucrose, TSI test. NW2 was inositol positive. NW1 and NW2 was sorbitol negative. All isolated showed NaCl tolerance ranging from 0.5% to 5% (Table 7). LATEX REAGENT TEST confirmed the serogtype of *E.coli* strains. Both isolates NW1 & NW2 non-sorbitol fermenting colonies (NSFC) and NW3 sorbitol fermenting colonies (SFC) of *Escherichia coli* have serotype O157:H7.

The 16S rRNA gene sequence analysis confirms identification of *Escherichia coli* made in former step. Sequences NW1, NW2 and NW3 were deposited in NCBI repository under the accession number KM998072, KM998073 and KM998074 respectively.

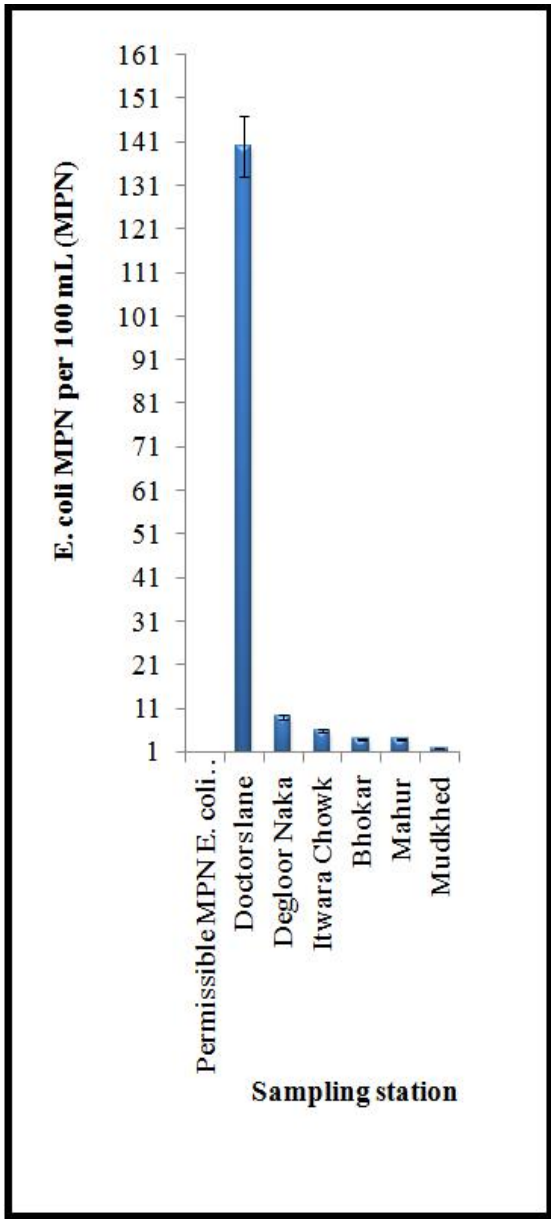

Figure 2. Relationship of the geometric mean of *E. coli* between sample stations in Nanded district (Maharashtra, India)

Water quality index of devotee place water and natural water assessed by research groups worldwide (**Rekadwad and Pathak, 2011; Pathak and Rekadwad, 2011; Pathak and Rekadwad, 2012, 2013; Tambekar and Ahir, 2014**). Various research groups reported number of religious place and drinking water source contaminated by coliform and indicate the presence of bacteria such as *Escherichia coli*, *Bacillus* sp. and other pathogenic microorganisms (**Gonzalez, 1996; Tambekar *et al*., 2008; Sutton, 2010**), (**Rizwan and Gupta, 2011; Pathak and Rekadwad, 2011; Mangalekar *et al*., 2014**). They have provided sufficient and reported accurate results to prove the presence of contamination in potable and recreational water.

Conclusion

The result indicates the presence of enteric gut coliforms (*E. Coli*) having serogroup O157:H7. Hence, attributor to strict sanitation, high level of hygienic sanitary conditions, strict preventive practices should be adopted to minimize possibilities of infection.

Acknowledgments

BNR is thankful to Dr. Juan M. Gonzalez, Senior Scientist, IRNAS-CSIC, Sevilla (Spain) and Professor C. N. Khobragade, Director, School of Life Sciences, S. R. T. M. University for ideal support. BNR is also grateful to Vikas and Anil for their efforts during sampling and for outsourcing.

REFERENCES

Aneja, K.R. 2007. Experiments in microbiology, plant pathology and biotechnology, Edn 4, New Age International (India). ISBN 812241494X.

Bergeys, D.H., Krieg, N.R., and Holt, JG. 1984. Bergey's Manual of Systematic Bacteriology. Williams & Wilkins, Baltimore. ISBN 978-0-387-95041-9. http://dx.doi.org/10.1007/978-0-387-68489-5

Centers for Disease Control (CDC). 1982. Isolation of *Escherichia coli* O157:H7 from sporadic cases of hemorrhagic colitis. United States MMRW, 31: 580-585.

Feng, P., Weagant, S.D., Grant, M.A., and Burkhardt, W. 2002. Chapter 4. Enumeration of *Escherichia coli* and the Coliform Bacteria," In: Food and Drug Administration (FDA), Bacteriological Analytical Manual Online, 8th Edition, Silver Spring, Berlin.

Gonzalez, J.M. 1996. A general purpose program for obtaining most probable number tables. J. Microbiological Meth., 26: 215-218. http://dx.doi.org/10.1016/0167-7012(96)00818-4

Greenberg, A., Clesceri, L., and Eaton, A. 1992. Standard methods for the examination of water and wastewater. American Public Health Association, Washington.

Hosseini, M., Jamsidi, A., and Khanzadi, S. 2014. Quantification of *Listeria monocytogenes* in milk by MPN-PCR and MPN-culture methods. *J. Microbiol. Biotechnol. Food Sci.*, 4 (2): 149-151. http://dx.doi.org/10.15414/jmbfs.2014.4.2.149-151

Mangalekar, S.B., Jadhav, A.S., and Raut, P.D. 2014. Assessment of water quality of Nullahs from Kolhapur City-Maharashtra (India). *PARIPEX - Indian J. Res.*, 3 (5): 117-120.

Oblinger, J.L., and Koburger, J.A. 1975. Understanding and teaching most potable number technique. International Association of Milk., Food and Environ Sanitarians., *J. Milk Food Technol.*, 38(9): 540-545.

Pathak, A.P., and Rekadwad, B.N. 2011. First report on physicochemical analysis of Unkeshwar hot water spring located in Maharashtra. India. Int. J. Chem. Sci. Appli., 2 (3): 169-171.

Pathak, A.P., and Rekadwad, B.N. 2012. High dissolved oxygen content of Indian hot water spring a novel report. *Int. J. Curr. Res.*, 4 (2), 001-001.

Pathak, A.P., and Rekadwad, B.N. 2013. Isolation of thermophilic *Bacillus* sp. strain EF_TYK1-5 and production of industrially important thermostable α-amylase using suspended solids for fermentation. *J Scientific Ind Res.*, 72: 685-689.

Ratnam, S., March, S.B., Ahemad, R., Bezanson, G.S., and Kasatiya, S. 1998. Characterization of *Escherichia coli* serotype O157:H7. *J. Clin. Microbiol.,* 26: 20006-20012.

Rekadwad, B.N., and Pathak, A.P. 2011. Characterization, antibiotic sensitivity of a thermostable amylase producing *Haemophilus haemolyticus* isolated from Unkeshwar hot spring and prediction of origin using antibiotic target site. *Int. J. Adv. Biotechnol. Res.,* 2(1), 224-229.

Rizwan, A.S., and Gupta, S.G. 2011. Bacterial contamination of surface water in and around Beed district, Maharashtra, India. *J. Microbial Biochem. Technol.,* 3 (5): 088-091. http://dx.doi.org/10.4172/1948-5948.1000057

Salem, I.B., Ouardani, I., Hassine, M., and Aouni, M. 2011. Bacteriological and physico-chemical assessment of wastewater in different region of Tunisia: Impact on human health. *BMC Res. Notes.,* 4: 144, http://dx.doi.org/10.1186/1756-0500-4-144

Scotland, S.M., Day, N.P., and Rowe, B. 1980. Production of a cytotoxin affecting vero cells by strains *Escherichia coli* belonging to traditional enteropathogenic serogroups. FEMS Microbiol. Lett., 7: 15-17.

Srinivas, T. 2008. Environment Biotecnhonology. New Age International (P) Ltd., Publishers, New Delhi (India). ISBN (13): 978-81-224-2544-4.

Sutton, S. 2010. The most probable number method and its uses in enumeration, qualification, and validation. *J. Valid. Technol.,* 16 (3): 35-38.

Tambekar, D.H., and Ahir, M.R. 2014. Water quality index assessment of devotee places in Amaravati region. *Indian J. Pharma. Sci. Res.,* 4 (1): 4-8.

Tambekar, D.H., Wankhede, S.J., Yadav, S.D., and Tambekar, S.D. 2008. Correlation of antibiotic resistance profiling of *E. coli* and source of fecal pollution in wateri. *Pollu. Res.,* 27 (3): 507-508.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony method. *Mol. Biol. Evol.,* 28: 2731-2739.

Usepa, Most Probable Number (mpn) calculator. 2012. Microbiological and Chemical Exposure Assessment, Version 3.0, U. S. Environmental Protection Agency, USA.

Woomer, P.L. 1994. Most probable number counts. SSSA Book Series. 5: 59-79.

****