April 2009 Volume 3 Issue 2

Trade Science Inc.

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 3(2), 2009 [55-59]

Antibiotics resistance profiling for detection of microbial fecal pollution source in water

D.H.Tambekar*, R.R.Jane, R.K.Sethi, P.S.Talokar, G.S.Rajerkar, S.D.Tambekar
P.G. Department of Microbiology, S.G.B. University, Amravati-444602, (INDIA)
E-mail : dilipmicro@gmail.com

Received: 31st December, 2008 ; Accepted: 5th January, 2009

ABSTRACT

In present study, a total of 260 water samples from Akola (140) and Buldhana district (120) were analyzed for water quality and detection of thermotolerant coliform (*E.coli*) from salinity affected villages of Purna River basin of Vidarbha and 243 (95%) water samples were found contaminated by MTFT and 75 (29%) by MFT. The water from Akola, Akot, and Shegaon taluka showed 33% pollution due to human faecal matter followed by 26% in Telhara and 20% each in Nandura and Balapur taluka. The minimum human faecal pollution in water was observed in the J.Jamod taluka. Maximum fecal *E.coli* was recorded in surface water sources indicating entrance of fecal matter from open defecation or due to open drainage or sewage water from household. The deep ground water (hand pump and tube well) was not free from fecal contamination and it may be due to percolation of sewage and wastewater and construction of latrines near the tube wells. The public distribution system (Maharashtra Jeevan Pradhikaran) water was not free from fecal contamination regardless of degree to which the water is treated. Thus from the above data it is concluded that out of 75 contaminated water samples, 75% contamination was non-human faecal origin whereas 25% human faecal origin.

KEYWORDS

MAR index;
E.coli;
Faecal contamination;
Purna river;
Akola;
Buldhana;
Vidarbha.

INTRODUCTION

The Ground Water Survey and Development Agency (GSDA), Government of Maharashtra has identified 547 villages (136 in Amravati, 318 in Akola and 93 in Buldhana district) in the Purna River Valley of Vidarbha (Maharashtra State), which are severely affected by salinity and poor quality ground water[1]. According to Finance and Statistics, Govt. of Maharashtra[6], several deaths occurred by water borne diseases in 1998 in Akola. Every year around 2.2 million people die due to basic hygiene related diseases. In India 80% of infectious diseases are waterborne diseases such as typhoid, cholera, dysentery, and infectious hepatitis, which are due to contaminated water[21,18]. Faecal bacteria can be emitted from various sources including agricultural practices, wild and domestic animals and human beings. It is compounded by the fact that the faecal indicators may not be from one particular source, but rather from variety of sources[5].

Water gets contaminated by human and animal fecal matter and spreads the infections. Determination of source of fecal contamination in the water is difficult but with use of multiple antibiotic resistances (MAR) indexing it can be determined to some extent. Krumperman[11], reported that the MAR index of *Es-*

cherichia coli (*E.coli*) from wild animals was generally low, while human and poultry isolates had higher MAR indices. Kaspar and Burgess[10] showed higher MAR index of *E.coli* from urban areas than from rural area. Wiggins[19], reported discriminate analysis of MAR pattern in faecal Streptococci to differentiate man and animal sources of faecal pollution in natural water and 74% of the isolated were correctly classified into one of six possible sources (beef, chicken, dairy, human, turkey or wild). Parveen et al.[13], had reported association of multiple antibiotic resistance profiles with point and non-point sources of *E.coli in* Apalachicola Bay and isolates from point sources showed significantly greater resistance ($P<0.05$) to antibiotics and higher MAR indices than isolates from non-point sources. Wiggins et al.[20], determined the reliability and repeatability of antibiotic resistance analysis as a method of identifying the source of faecal pollution in surface and ground water. Tambekar and Charan[15] reported antibiotic sensitivity indexing of *Escherichia coli* to identify source of faecal contamination in drinking water in Purna Valley of Vidarbha. Shukui Guan, et al.[14], developed a procedure for discriminating among *Escherichia coli* isolated from animal and human sources and stated average MAR indices for human, livestock and wildlife isolate as 0.1339, 0.0966 and 0.027 respectively.

The use of antibiotics resistance profiles to identify sources of fecal contamination is promising and emerging procedure and the patterns of MAR in different animal populations vary according to the types and quantities of agent used[16]. Hence, attempt was undertaken to determine the source of fecal pollution by determining the antibiotics resistance profile and MAR indexing of *Escherichia coli* isolated from drinking water in salinity-affected villages of Akola and Buldhana district of Vidarbha.

MATERIAL AND METHODS

A total of 260 drinking water samples were collected from surface water (13) shallow ground water (42), deep ground water (129) and public water supply (76) to study water quality and contamination of thermotolerant coliform (*E.coli*) from different salinity affected villages of Akola and Buldhana district of Vidarbha from June 2007 to December 2007. The bacteriological examination was performed within the 24 h of collection using standard Multiple Tube Fermentation Technique (MTFT) for determination of Most Probable number (MPN) index, nine multiple tube dilution technique using double and single strength Bromo-Cresol Purple MacConkey medium and Membrane filter techniques (MFT) by using M-EC test agar (Hi-media Lab. Mumbai), for detection of *E.coli* (Thermotolerant coliform, TTC) with production of yellow colour colonies on membrane filter at 44.5^0C. The MPN Index was calculated from MPN table and index more than 10 coliforms/dl is designated as polluted or non-potable water[3].

The isolation and identification of *E.coli* was made based on MFT plates incubated at 44.5^0C and standard bacteriological tests such as morphological, cultural, biochemical and special tests by subculturing the MFT positive (yellow color colonies on membrane filter in M-EC test agar) colonies in respective medium. Antibiotic resistance pattern (by disc diffusion technique) of these isolates were carried with 15 different antibiotic supplied by Hi-media Pvt Ltd, Mumbai. The multiple antibiotic resistance indices (MARI) were calculated for these *E.coli* isolates[11].

RESULTS AND DISCUSSION

In present study, a total of 260 water samples from Akola (140) and Buldhana district (120) were analyzed for water quality; 13 from surface water (river and lakes), 42 from shallow ground water (open wells), 129 from deep ground water (tube wells and hand pumps) and 76 samples from public water supply scheme, and isolation and detection of thermotolerant coliform (*E.coli*) from salinity affected villages of Purna River basin of Vidarbha. All water samples were analysed by MTFT, and MFT and 243 (95%) water samples were found contaminated by MTFT, and 75 (29%) by MFT (TABLE 1). Out of these, 39 (93%) in shallow ground water, 124 (96%) in deep ground water and 71 (93%) treated water and all 13 (100%) in surface water were found polluted by MTFT; whereas 10(24%) in shallow ground water, 37(29%) in deep ground water and 21 (28%) treated water and 7(54%) in surface water by MFT (TABLE 2). Out of 10 taluka's, the water in five were 100% polluted, while in four in the range of 81-96%, except in Balapur where it was 33% by MTFT (TABLE 1). The detection of thermotolerant *E.coli*

TABLE 1: Quality of drinking water in Akola and Buldhana district of Vidharbha (in %)

Test	Result	Akola district				Buldhana district						
		Akola	Akot	Balapur	Murtijapur	J.Jamod	Malkapur	Nandura	Sangrampur	Shegaon	Telhara	Total
MTFT	Safe	4	5	67	0	0	9	0	0	13	0	5
	Polluted	96	95	33	100	100	91	100	100	87	100	95
E.coli by MFT	Absent	69	69	67	100	69	36	14	100	16	25	71
	Present	31	31	33	0	31	64	86	0	84	75	29

TABLE 2: Source wise water quality

Test	Source	Public water supply	Deep ground water	Shallow ground water	Surface water	Total
MTFT	Safe	7%	4%	7%	0%	5%
	Polluted	93%	96%	93%	100%	95%
E.coli by MFT	Absent	72%	71%	76%	46%	71%
	Present	28%	29%	24%	54%	29%

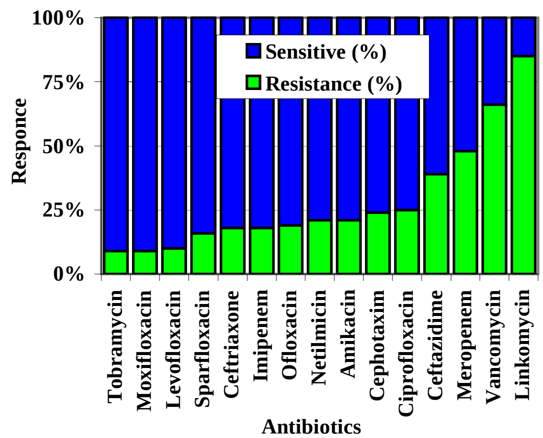

Figure 1: Antibiotic resistance pattern of *E.coli*

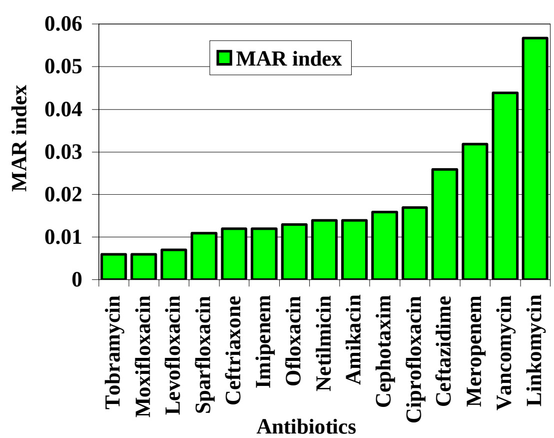

Figure 2: MAR index of antibiotics against *E.coli*

analysis of water gives the proper status of drinking water sources and results showed that 67% *E.coli* in Balapur taluka, 31% each in Akola and Akot taluka and no E.coli in Murtizapur taluka water in Akola district while 75% in Telhara, 64% in Malkapur, 31% in J.Jamod, 16% in Shegaon, 14% in Nandura and 0% in Sangrampur taluka's of Buldhana districts (TABLE 1). Analysis of drinking water from different sources demonstrated, 54% of thermotolerant *E.coli* pollution in surface water while 24%, 29%, and 28% in shallow ground water, deep ground water, and public water supply respectively (TABLE 2). Bacteriological analysis of different water indicated that surface water was highly polluted by fecal *E.coli* as compare to other water sources. It may be due to contamination in water by fecal matter or due to the percolation of contaminated water in these resources. The quality of drinking water also affected by seasonal variation and high coliforms count detected in rainy season. Lomate and Samant[12], Tambekar et al.[17], and Bahador et al.[4], showed the seasonal variation of microbial pollution in surface water of Pune and Amravati and reported maximum coliform count in monsoon than the winter and summer.

Antibiotic resistance profile was used to identify the sources of faecal and non-faecal contamination in drinking water. In the present study, *E.coli* isolated from various sources showed highly resistant to lincomycin (85%) and vancomycin (66%) and sensitive to tobramycin, moxifloxacin (91%), levofloxacin (90%), sparfloxacin (84%), ceftriaxone, imipenem (82%), ofloxacin (81%) and amikacin (71%) (Figure 1). Begum et al., (2004) studied isolates obtained from different sources of drinking water (river, well, supply water and tube well) and showed highest susceptibility to ciprofloxacin (92.96%), and least with cephotaxime (3.29%).

The MAR indices for the antibiotics tobramycin (0.0059), Moxifloxacin (0.0059), levofloxacin (0.0069), sparfloxacin (0.0109), ceftriaxone (0.0119), imipenem (0.0119), ofloxacin (0.0129), Netilmicin (0.0139), amikacin (0.0139), cephotaxime (0.0159), ciprofloxacin (0.0169), and ceftazidime (0.0258) representing isolates from non human faecal origin while antibiotics meropenem (0.0318), vancomycin (0.0437) and lincomycin (0.0567 represents isolates from human faecal origin (Figure 2). The study showed that surface water was highly contaminated with fecal *E.coli*

FULL PAPER

TABLE 3: MAR index, type of pollution, collection site and source of water

Type of pollution	MAR index of *E.coli*	District	Taluka	Source: Surface water	Source: Deep ground water	Source: Public water supply	Source: Shallow ground water	Total
Non-human fecal contamination	0.06	Buldhana	J.Jamod	0	1	1	0	2
			Shegaon	0	1	0	0	1
		Total		0	2	1	0	3
	0.13	Akola	Akola	0	1	2	0	3
			Akot	0	0	2	0	2
			Balapur	0	1	0	0	1
		Buldhana	Malkapur	0	1	0	0	1
			Telhara	0	0	0	1	1
			J.Jamod	0	2	0	0	2
		Total		0	5	4	1	10
	0.2	Akola	Akola	0	3	0	0	3
			Akot	0	2	2	0	4
		Buldhana	Telhara	0	1	1	0	2
			J.Jamod	0	1	0	0	1
			Malkapur	0	1	0	0	1
			Nandura	0	1	0	2	3
			Shegaon	0	1	0	0	1
		Total		0	10	3	2	15
	0.27	Akola	Akola	0	3	0	0	3
			Akot	0	0	2	0	2
			Balapur	0	1	0	0	1
		Buldhana	Telhara	0	3	0	0	3
			J.Jamod	0	0	0	2	2
			Malkapur	0	1	0	1	2
			Nandura	0	1	0	0	1
			Shegaon	0	1	1	0	2
		Total		0	10	3	3	16
Human fecal contamination	0.33	Akola	Akola	1	1	5	0	7
			Akot	1	0	1	0	2
		Buldhana	J.Jamod	0	1	1	0	2
			Shegaon	0	1	0	0	1
		Total		2	3	7	0	12
	0.4	Akola	Akola	0	1	0	0	1
			Akot	1	1	2	0	4
		Buldhana	J.Jamod	0	0	0	2	2
			Malkapur	0	1	0	0	1
			Nandura	0	1	0	0	1
		Total		1	4	2	2	9
	0.47	Akola	Akola	0	1	0	0	1
			Akot	1	0	0	0	1
		Buldhana	Malkapur	0	0	0	1	1
			Balapur	1	0	0	0	1
		Total		2	1	0	1	4
	0.53	Akola	Akola	0	0	0	1	1
		Total		0	0	0	1	1
	0.6	Akola	Akola	0	1	0	0	1
		Buldhana	Malkapur	1	1	0	0	1
		Total		1	2	0	0	3
	0.66	Akola	Akola	0	0	1	0	1
			Akot	1	0	0	0	1
		Total		1	0	1	0	2
		Total *E.coli*		7(54%)	37 (29%)	21 (24%)	10 (24%)	75(29%)

(54%) and showed maximum MAR index 0.055 for ceftazidime, 0.050 for lincomycin, meropenem, followed by 0.044 cephotaxime. Maximum fecal *E.coli* was recorded in surface water sources indicating entrance of fecal matter from soil (open defecation) or due to open drainage or sewage water from household. The deep ground water (hand pump and tube well) was not free from fecal contamination and it may be due to percolation of sewage and wastewater and construction of latrines near the tube wells. The public distribution system (Maharashtra Jeevan Pradhikaran) water was not free from fecal contamination regardless of degree to which the water is treated.

The study showed 75-76% isolates susceptibility to the ciprofloxacin, cephotaxime, where as Alhaj et al.[2], recorded it in the tune of 44-24% for the same. Tambekar et al.[18] in their studies showed maximum resistance to ofloxacin (92%) followed by ciprofloxacin (79%). The antibiotic ceftriaxone (58%) was moderately sensitive against isolates. In the present study, ceftriaxone and amikacin were highly sensitivity (82%) to the isolated fecal *E.coli*. Kasper et al.[10], observed low MAR to *E.coli* isolated from rural water whereas Parveen et al.[13], showed high MAR for isolates from municipal waste and river and estuarine water. Alhaj et al[2], (recorded low antibiotic resistance to coliform isolates from ground water. Graves et al.[7], studied the MAR of isolates recovered from the stream samples and showed various contaminations as human (10%), wild life (40%) and livestock (50%). Hagedon et al.[8], and Kaneene et al.[9] observed that the livestock contribute more contamination than the humans in surface water. The water from Akola,

Akot, and Shegaon taluka showed 33% pollution due to human faecal matter followed by 26% in Telhara and 20% each in Nandura and Balapur taluka. The minimum human faecal pollution in water was observed in the J.Jamod taluka. Thus from the above data it is concluded that out of 75 contaminated water samples, 75% contamination was non-human faecal origin whereas 25% human faecal origin (TABLE 3).

Faecal pollution contributed maximum (54%) contamination in surface water followed by deep ground water, shallow ground water and in public water supply. The main sources of fecal contamination in surface water were open defecation, domestic wastewater, and sewage discharge in the surface water. The study indicated that the animals were the major source; however man was also significant contributor of water contamination.

REFERENCES

[1] P.G.Adyalkar; Proc.Nat.Inst.Sci.Ind., **29(1)**, 25-45 **(1963)**.

[2] N.Alhaj, N.S.Mariana, A.R.Raha, Z.Ishak; Int.J.Poll.Sci., **6(4)**, 293-297 **(2007)**.

[3] APHA, AWWA, WPCP; 'Standard Method for the Estimation of Water and Wastewater', 20th Edn., American Public Health Association, New York, USA, **(1998)**.

[4] N.M.Bahador, Baseri Salehi, D.N.Patil, B.P. Kapadnis; Nature Envir.Poll.Tech., **4(1)**, 53-56 **(2005)**.

[5] S.Carroll, A.Goonetilleke, M.Hargreaves, R.A.Patterson, M.J.Johnes; 'Sourcing Faecal Contamination Through Antibiotic Resistance Pattern Classification', Proceeding of on site 05 Conference, Performance Assessment for on Site Systems: Regulations, Operation and Monitoring, **(2005)**.

[6] Finance and Statistics, Government of Maharashtra, Mumbai, 133 **(1998)**.

[7] A.K.Graves, C.Hagedon, A.Teeter, M.Mahal, A.M.Booth, R.B.Reneau; J.Environ.Quality, **31**, 1300-1308 **(2002)**.

[8] C.Hagedon, S.L.Roberson, J.R.Filtz, S.M.Grubbs, T.A.Angier, R.B.Reneau, (Jr.); Appl.Environ. Microbiol., **65**, 5522-5531 **(1999)**.

[9] B.J.Kaneene, R.Miller, R.Sayah, Y.J.Johnson, D.Gilliland, J.C.Gardiner; Michigan. Appl.Environ. Microbial., **73(9)**, 2878-2890 **(2007)**.

[10] C.W.Kasper, J.L.Burgess; Can.J.Microbiol., **36**, 891-894 **(1990)**.

[11] P.H.Krumperman; Applied Environ.Microbial., **46**, 165-170 **(1983)**.

[12] V.Lomate, J.Samant; Poll.Res., **24(3)**, 689-694 **(2005)**.

[13] S.Parveen, L.Rendi, M.L.Edmiston, C.W.Kaspar, K.M.Portier, M.L.Tamplin; Appl.Environ. Microbiol., **63**, 2607-2612 **(1997)**.

[14] Shukui Guan, Xu Renlin, She Chen, Odumeru Joseph, Gyles Carlton; Appl.Environ .Microbiol., **68**, 2690-2698 **(2002)**.

[15] D.H.Tambekar, A.B.Charan; Nat.Environ.Poll. Tech., **3(3)**, 413-418 **(2004)**.

[16] D.H.Tambekar, N.B.Hirulkar, A.S.Waghmare; Dat.Environ.Poll.Tech., **4(4)**, 525-528 **(2005)**.

[17] D.H.Tambekar, V.G.Bochare, B.B.Gole, Y.S. Banginwar; Poll.Res., **26(3)**, 473-475 **(2007)**.

[18] D.H.Tambekar, N.B.Hirulkar, M.V.Kalikar, Y.S.Patil, S.R.Gulhane; Res.J.Microbiol., **1(5)**, 458-462 **(2006)**.

[19] B.A.Wiggins; Appl.Environ.Microbiol., **62(11)**, 3997-4002 **(1996)**.

[20] B.A.Wiggins, R.W.Andrews; Conway; Applied and Environmental Microbiology, **65(5)**, 3483-3486 **(1999)**.

[21] World Health Organization (WHO); Global water Supply and Sanitation Assessment 2001 Report, Geneva, **(2001)**.