Recent Research in Science and Technology 2013, 5(2): 68-72
ISSN: 2076-5061
Available Online: http://recent-science.com/

Studies on antibiotic resistance profiles of thermotolerant *Escherichia coli* and multiple antibiotic resistance index of different water sources

Mabel Varghese and M G. Roymon

Department of Microbiology.St. Thomas College, Ruabandha, Bhilai (C.G.), India.

Abstract

The rationale behind this study was that no clear data from Durg District is available on the antibiotic resistance profiles of *E. coli*, the most common pathogen of both humans and animals. Water being one of the most prevalent routes of transmission of *E. coli*, the present study aimed at isolation and characterization of the same from widely used water sources of Durg-Bhilai region. Thermotolerant *E. coli* (n=50) were isolated from municipal water, ground water, lake (Talpuri Talab), and river Shivnath. Multiple antibiotic resistance (MAR) indexing was performed using antibiotics viz. amoxicillin, chloramphenicol, co-trimoxazole, ceftriaxone, ciprofloxacin, cephotaxime, nalidixic acid, gentamycin and tetracycline. 85.4% of isolates showed resistance to 2 or more antibiotics. Resistance to three or more antibiotics (multidrug resistance) was shown by 24.4% of isolates. Plasmid bands ranging in size from 0.5 kb – 35 kb were present in 35 out of 50 isolates. Multiple plasmids were shown in 6 isolates. MAR index of isolates ranged from 0.11 to 0.55. 61% of isolates in this study had an index of 0.22, 14.63% had 0.44, 7.23% had 0.33 and 2.44% had 0.55. A high index of 0.79 was shown by River Shivnath followed by 0.2 by Talpuri Talab. The MAR index >0.2 is considered to originate from high risk source of contamination. High MAR indices of individual isolates and sampling sites in Durg-Bhilai region showed that water sources are contaminated with antibiotic resistant *E. coli* arising from high risk sources of contamination.

Keywords: Antibiotics, MAR indexing, membrane filtration, River Shivnath, thermotolerant *Escherichia coli*

INTRODUCTION

Antibiotic resistance among pathogens is emerging as a threat to human and veterinary medicine. This is due to the extensive and indiscriminate use of antibiotics for treatment, prophylaxis or growth promotion. Rise of antimicrobial resistance in *E. coli* to multiple antibiotics is a major concern both in developed and developing countries (1,2). Contamination of water sources with faecal bacteria like *E. coli* is a serious problem due to its ability to transmit diseases. The risk associated with these bacteria further increases if they are antibiotic resistant (3). Several waterborne outbreaks due to multidrug resistant *E. coli* have been reported world wide. It is difficult to accurately determine the sources of contamination of water bodies. Multiple antibiotic resistance (MAR) indexing is one such method used for determining the source of faecal contamination (4,5). Hence, the aim of this study was to isolate antibiotic resistant *E. coli* from water sources of Durg district and bring out the level of contamination of different sources with the same.

MATERIALS AND METHODS

Microbiological analysis of water samples

A total of 158 water samples were collected from surface water (54), municipal water (74) and ground water (30) of Durg-Bhilai region. Microbiological examination of samples was performed at 44.5°C by membrane filtration technique (MFT) using EMB agar (HiMedia Labs Pvt. Ltd., Mumbai) to isolate thermotolerant *E. coli* (6). Identification of *E. coli* was based on standard bacteriological tests such as cultural, morphological and biochemical tests.

Antibiotic susceptibility test

Nine antibiotics (HiMedia) amoxicillin (Amx 30μg), chloramphenicol (C 10μg), co-trimoxazole (Cot 25μg), ceftriaxone (Ctr 30μg), ciprofloxacin (Cip 5μg), cephotaxime (Cfx 30μg), nalidixic acid (Na 30μg), gentamycin (Gen 10μg) and tetracycline (Te 30μg) were used to determine the resistance pattern of isolates using disc diffusion method. The diameter of zone of inhibition was measured in millimetre, compared with standard interpretative chart and characterized as sensitive, intermediate and resistant (7). Isolates resistant to three or more antibiotics were considered multidrug resistant (MDR).

Multiple antibiotic resistance (MAR) indexing

MAR index of individual isolates and sampling sites were calculated using the equations given below (8):

MAR index of isolate = No. of antibiotics to which isolate was resistant / Total no. of antibiotics to which isolate was exposed.

MAR index of site = Average resistance index of all isolates from a site / (No. of antibiotics tested × no. of isolates from the site)

*Corresponding Author

Mabel Varghese
Department of Microbiology.St. Thomas College, Ruabandha, Bhilai (C.G.), India.

Email: varghesemmariam@gmail.com

Isolates with an MAR index of ≥0.2 are said to originate from high risk sources of contamination. An MAR index of 0.4 or higher is associated with human faecal source and less than 0.4 with non human faecal source of contamination (9).

Plasmid profiling

Plasmid DNA was extracted by the alkaline lysis method and resolved by electrophoresis in 0.8% agarose gel incorporated with ethidium bromide (10). Super mix DNA ladder (Genei Pvt. Ltd., Bangalore, India) was run along with samples as reference. The *E. coli* strain MTCC 723 was used as control. The gels were visualized on a trans-illuminator and the number of bands was noted with careful eye examination.

RESULTS AND DISCUSSION

The quality of water that is consumed is an important factor governing the health of an individual and the society as a whole. Thus there is a need to constantly maintain the quality of this resource by effective surveillance and control measures. Durg district occupies the south-western part of the Upper Shivnath Mahanadi valley of Chhattisgarh state and is abundantly drained by rivers Shivnath and Kharrun. There are several major and minor lakes also providing fresh water. Both the urban and rural communities are mostly dependant on the public utilities for supply of drinking water. Ground water is also developed in terms of dug cum tube wells. Thermotolerant *E. coli* (39 isolates) were isolated from all river and lake water sites giving a high rate of isolation of 71.43% and 75% respectively. Of the ten potable water sites studied, six sites showed the presence of *E. coli* (11 isolates) with a rate of isolation of 10.81% for municipal water and 10% for tube well water. Thermotolerant *E. coli* were isolated also by Tambekar *et al*. (5), Oyedeji *et al*. (11) from different water sources. The high rate of isolation of *E. coli* from surface water is a cause of concern as it indicated severe contamination which could be due to open defecation, surface run off during rains and percolation of waste water from surroundings.

In this study, varying levels of resistance was observed among the 50 biochemically identified *E. coli* isolates. Nine isolates viz. SA4, SC8, SD2, SD10, PH3, Pul1, LW4, Cha2 and Khu1 were sensitive to all the antibiotics tested. Among the other isolates, resistance was found to be highest for tetracycline (58.53%) followed by gentamycin (48.78%), amoxicillin (31.7%), ciprofloxacin (21.95%), chloramphenicol (17.07%), nalidixic acid and co-trimoxazole (14.63%) and ceftriaxone (12.19%). Least percent (9.75%) of isolates showed resistance to cefotaxime. In this study we observed moderate resistance to antibiotics and the results were consistent with previous studies on aquatic isolates of *E. coli* (12,13).

Of the 41 antibiotic resistant *E. coli* isolates obtained from different sampling sites, ten isolates (24.39%) were resistant to three or more antibiotics (MDR) (Table 1), which is typical of the results obtained by Sahoo *et al*. (14). Higher rates of occurrence of MDR *E. coli* in water sources have been reported from Hyderabad (15) and Saudi Arabia (16). Resistance to tetracycline was observed in all MDR isolates. The frequency of resistance to individual antibiotics among the MDR and non MDR isolates from the four sampling sites is shown in Fig. 1. Frequency of isolates resistant to all antibiotics was high for both river Shivnath and lake Talpuri. All the municipal water isolates were sensitive to cefotaxime and co-trimoxazole. Tube well water isolates were sensitive to amoxicillin, chloramphenicol, cefotaxime, co-trimoxazole, ciprofloxacin, ceftriaxone and nalidixic acid. Least resistance was observed for tube well (ground water) water site. The low prevalence of MDR bacteria in ground water was noted also by Soge *et al*. (17) who studied resistance in gram negative bacteria from different ground water sources in Uganda.

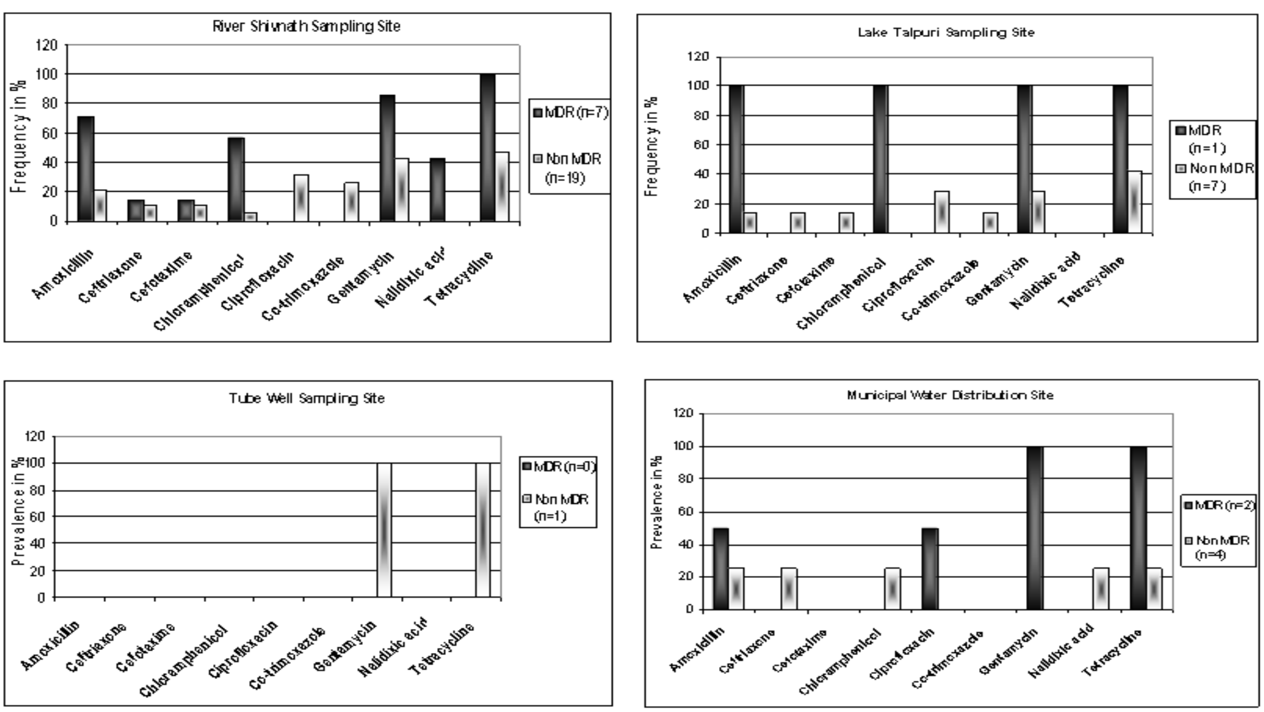

Fig 1. Frequency of antibiotic resistance of MDR and non MDR *E. coli* in water samples from river, lake, tube well and municipal distribution sites

Plasmid profiling of both MDR and non MDR isolates was performed and 36 isolates showed the presence of plasmid. The molecular size of plasmids ranged from 35 kb to 0.5 kb. High molecular size resistance plasmids from antibiotic resistant *E. coli* obtained here was in conformity with results of Nazir *et al*. (18), Akturk *et al*. (19). Table 1 illustrates the number of plasmid in both MDR and non MDR isolates. Multiple plasmids were found more significantly in MDR than non MDR *E. coli*. A strong positive correlation (0.717) was observed between number of antibiotics to which an isolate was resistant and its number of plasmid bands. The isolates with more plasmids were more likely to be antibiotic resistant ($p<0.01$, Fisher's Exact test, SPSS Inc. Version 16, USA).

Table 1a. Resistance pattern, plasmid profile and MAR index of *E. coli* isolates

MDR *E. coli*			
Isolate No.	**Resistance Pattern**	**Plasmid Bands**	**MAR Index**
SC1	AmxCNaTe	3	0.44
SC5	AmxCGenNaTe	2	0.55
SD4	AmxCGenTe	2	0.44
SB2	AmxCtrGenTe	1	0.44
SA3	CGenNaTe	1	0.44
LW7	AmxCGenTe	1	0.44
PH2	CipGenTe	1	0.33
Pul2	AmxGenTe	1	0.33
SD9	AmxGenTe	1	0.33
SC4	CfxGenTe	0	0.33

Table 1b. Resistance pattern, plasmid profile and MAR index of *E. coli* isolates

Non MDR *E. coli*							
Isolate No.	**Resistance Pattern**	**Plasmid Bands**	**MAR Index**	**Isolate No.**	**Resistance Pattern**	**Plasmid Bands**	**MAR Index**
Cha1	GenTe	2	0.22	SD8	CtrGen	1	0.22
SA1	AmxTe	1	0.22	LW1	CotTe	1	0.22
SA2	GenTe	1	0.22	LW5	AmxNa	1	0.22
SB1	AmxCip	1	0.22	LW8	CipTe	1	0.22
SB3	CotGen	1	0.22	LW9	CtrGen	1	0.22
SB4	CCip	1	0.22	Sup1	CtrTe	1	0.22
SB5	CfxCip	1	0.22	SD6	Te	1	0.11
SB6	CotTe	1	0.22	LW3	Te	1	0.11
SB7	AmxCot	1	0.22	Sup2	Amx	1	0.11
SC2	CipTe	1	0.22	PH1	C	1	0.11
SC7	GenTe	1	0.22	Ru1	Na	1	0.11
SC9	CipTe	1	0.22	SC3	CotGen	0	0.22
SD1	GenTe	1	0.22	SC6	AmxCip	0	0.22
SD3	CfxGen	1	0.22	LW2	CipGen	0	0.22
SD5	CtrTe	1	0.22	LW6	Cfx	0	0.11
SD7	CotGen	1	0.22				

Amx- Amoxicillin, Cfx- Cefotaxime, C- Chloramphenicol, Cot- Cotrimoxazole, Cip- Ciprofloxacin, Ctr- Ceftriaxone, Gen- Gentamycin, Na- Nalidixic acid, Te- Tetracycline

Multiple antibiotic resistance indexing of the *E. coli* isolates in this study showed that 70% of isolates were form high risk sources of contamination, with an MAR index ranging from 0.22 – 0.55 (Table 1). The MAR index of isolates from different water samples was comparable with those of previous studies (20,2,19). Six isolates had human faecal origin (MAR index of 0.44 and 0.55). River Shivnath and lake Talpuri were found to have a high MAR index suggesting that both were contaminated with *E. coli* arising from high risk sources (Table 2). Similar observation was made by Tambekar *et al*. (5) who reported high MAR index due to human and non human faecal contamination, of surface, ground and public supply water sites in Akola and Buldhana of Vidarbha district. Chatterjee *et al*. (21) noted that drinking water sources of Uttarakhand region were contaminated with high MAR index *E. coli* originating from potential risk sources.

Table 2. Multiple antibiotic resistance index of sampling sites

Site	No. of *E. coli* isolates	% of MDR isolates	MAR index of sites
River Shivnath	30	23.33	0.79
Lake Talpuri	9	11.11	0.20
Municipal distribution lines	8	25.00	0.13
Tube wells	3	0	0.02

CONCLUSION

Thermotolerant *E. coli* isolated from the surface water, ground water and municipal distribution sites were found to be highly resistant to tetracycline, gentamycin and amoxicillin which are commonly used in human and animal medicine. High multiple antibiotic resistance index of isolates as well as sampling sites suggested high risk sources such as human and non human faeces as the origin of contamination. Prevalence of plasmid mediated resistance among isolates is a cause of concern due to the possibility of dissemination of resistance gene to other species and other genera. The results of this study highlighted the need for controlled use of antibiotics and strict pollution monitoring programmes in Durg district of Chhattisgarh.

REFERENCES

[1] Ajamaluddin M., Khan M.A. and Khan A.U. 2000. Prevalence of multiple antibiotic resistance and R-plasmid in *Escherichia coli* isolates of hospital sewage of Aligarh city in India. *Indian J Clin. Biochem*., 15:104-109.

[2] Chandran A., Hatha A.A.M., Varghese S. and Sheeja K.M. 2008. Prevalence of multiple drug resistant *Escherichia coli* serotypes in a tropical estuary, India. *Microbes Environ*., 23:153-158.

[3] Da Silva G.J. and Mendonca N. 2012. Association between antimicrobial resistance and virulence in *Escherichia coli*. *Virulence*, 3:18–28.

[4] Wiggins B.A., Andrews R.W., Conway R.A., Corr C.L., Dobratz E.J., Dougherty D.P., Eppard J.R., Knupp S.R., Limjoco M.C., Mettenburg J.M., Reinehardt J.M., Sonsino J., Torrijos R.L. and Zimmerman M.E. 1999. Use of antibiotic resistance analysis to identify nonpoint sources of fecal pollution. *Appl. Environ. Microbiol*., 65:3483-3486.

[5] Tambekar D.H., Wankahde S.J., Yadav S.D. and Tambekar S.D. 2008. Correlation of antibiotics resistance profiling of *E. coli* and source of faecal pollution in water. *Poll. Res*., 27:507-510.

[6] APHA 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th ed. American Public Health Association, Washington D.C.

[7] CLSI 2007. *Performance Standards for Antimicrobial Susceptibility Testing*: Seventeenth informational supplement, M100-S17. Clinical and Laboratory Standards Institute. Pennsylvania: USA. pp. 19-37.

[8] Krumperman P.H. 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of foods. *Appl. Environ. Microbiol.*, 46:165-170.

[9] Kaneene B.J., Miller R., Sayah R., Jihnson Y.J., Gilliland D. and Gardiner J.C. 2007. Considerations when using discrimination function analysis of antimicrobial resistance profiles to identify sources of fecal contamination of surface water in Michigan. *Appl. Environ. Microbiol.*, 73:2878-2890.

[10] Birnboim H.C. and Doly J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res*., 7:1513-1523.

[11] Oyedeji O., Olutiola P.O., Owolabi K.D. and Adeojo K.A. 2011. Multiresistant faecal indicator bacteria in stream and well waters of Ile-Ife City, Southwestern Nigeria: Public health implications. *J Public Health Epidemiol.*, 3:371-381.

[12] Watkinson A.J., Micalizzi G.B., Graham G.M., Bates J.B. and Costanzo S.D. 2007. Antibiotic-resistant *Escherichia coli* in wastewaters, surface waters, and oysters from an urban riverine system. *Appl. Environ. Microbiol*., 73:5667–5670.

[13] Sharma B.C. and Rai B. 2012. Incidence of multi-drug resistance in *Escherichia coli* strains isolated from three lakes of tourist attraction (mirik lake, jorepokhari lake and Nakhapani lake) of darjeeling hills, India. *Indian J Fundam. Appl. Life Sci.*, 2:108-114.

[14] Sahoo K.C., Tamhankar A.J., Sahoo S., Sahu P.S., Klintz S.R. and Lundborg C.S. 2012. Geographical variation in antibiotic-resistant *Escherichia coli* isolates from stool, cow-dung and drinking water. *Int. J Environ. Res. Public Health*, 9:746-759.

[15] Patoli A.A., Patoli B.B. and Mehraj V. 2010. High Prevalence of multi-drug resistant *Escherichia coli* in drinking water samples from Hyderabad. *Gomal J Med. Sci.*, 8:23-26.

[16] Alzahrani A.M. and Gherbawy Y.A. 2011. Antibiotic resistance in *Escherichia coli* strains isolated from water springs in Al-Ahsa Region. *African J Microbiol. Res.*, 5:123-130.

[17] Soge O.O., Giardino M.A., Ivanova I.C., Pearson A.L., Meschke J.S. and Roberts M.C. 2009. Low prevalence of antibiotic-resistant gram-negative bacteria isolated from rural south-western Ugandan groundwater. *Water SA.*, 35:343-347.

[18] Nazir K.H.M.N.H., Rahman M.B., Nasiruddin K.M., Akhtar F., Khan M.F.R. and Islam M.S. 2005. Antibiotic sensitivity of *Escherichia coli* isolated from water and its relation with plasmid profile analysis. *Pakisthan J Biol. Sci.*, 8:1610-1613.

[19] Akturk S., Dincer S. and Toroglu S. 2012. Determination of microbial quality and plasmid mediated multidrug resistant bacteria in fountain drinking water sources in Turkey. *J Environ.*

Sci., 33:1127-1136.

[20] Parveen S., Murphee R.L., Edmiton L., Kaspar C.W., Portier K.M. and Tamplin M.L. 1997. Association of multiple-antibiotic-resistance profiles with point and nonpoint sources of *Escherichia coli* in Apalachicola Bay. *Appl. Environ. Micorbiol.*, 63:2607-2612.

[21] Chatterjee R., Sinha S., Aggarwal S., Dimri A.G., Singh D., Goyal P., Chauhan A., Aggarwal M.L. and Chacko K.M. 2012. Studies on susceptibility and resistance patterns of various *E. coli* isolated from different water samples against clinically significant antibiotics. *Int. J Bioassays*, 1:156-161.