British Microbiology Research Journal
3(1): 84-95, 2013

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Bacteriological Screening of Water in Mangalore, India

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Authors' contributions

This work was carried out in collaboration between all authors. Author BD designed the study, wrote the protocol, helped in execution and successful completion of the study and reviewed the manuscript for submission. *Authors VCD and JA performed* laboratory experiments, *managed the literature searches, data compilation and wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

Research Article

Received 7th November 2012
Accepted 8th January 2013
Published 5th February 2013

ABSTRACT

Introduction: Diarrhoea caused by contaminated water is among the most prevalent waterborne diseases in the developing countries like India. In the interest of public health, water supplies should be tested regularly to confirm their freedom from contamination.
Objective: The objectives of the study were to screen different water sources for bacterial contamination, to know the antibiotic susceptibility of the common bacterial isolates and typing of the bacterial isolates by random amplification of polymorphic DNA (RAPD) technique.
Place and Duration of the Study: Kasturba Medical College Hospital, Microbiology Laboratory, Mangalore, Karnataka, India between August 2007 and August 2009.
Methodology: Water samples (n=324) were analyzed by standard microbiological techniques for bacterial contamination. Isolates were identified biochemically and antibiotic susceptibility testing was done by disc diffusion method. *Escherichia coli* isolates were typed by RAPD technique.
Results: Among the water samples tested, 246 were excellent and 78 were contaminated. Contaminated samples showed the growth of commensal bacteria belonging to the family

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Enterobacteriaceae along with pathogens like *Salmonella spp*. and *Vibrio spp*. Many of the isolates were found to be sensitive and a few were found to be resistant to the antibiotics tested. RAPD typing showed genetic similarity and differences among the *E. coli* isolates from different water sources.

Conclusion: Genetic similarity among isolates of *E. coli* indicates a common ancestral origin or a common source. Bacterial contamination of water samples with pathogens like, *Salmonella spp*. and *Vibrio spp*. as well as the faecal coliform is a concern, as water quality is an index of health and well - being of the society. Degree of contamination observed in this study suggests a need to be vigilant to monitor water quality, in order to prevent enteric diseases.

Keywords: Antibiogram; bacterial contamination; RAPD technique; water sample; faecal coliform; water quality.

1. INTRODUCTION

Water is a vital resource for humanity and a basic human right. Pollution of water bodies is one of the areas of major concern in the public health. It is well known that although water is essential to sustain life, it can be a hostile environment to humans when grossly polluted [1]. Diarrhoea caused by contaminated water is among the ten most prevalent waterborne diseases in the developing countries [2]. Microorganisms of concern in contaminated water include the bacterial agents of diarrhoea and gastroenteritis namely, *Salmonella spp., E. coli* and *Vibrio cholerae* [3]. An estimated 80% of all diseases and over one third of deaths in developing countries are caused by the consumption of contaminated water and on an average as much as one tenth of each person's production time is sacrificed to water-related diseases [4, 5]. In the interest of public health, water supplies should be tested regularly to confirm their freedom from contamination. Water supply / source is tested for indicator microorganisms which demonstrates whether faecal pollution has taken place and have not been killed or removed by purification process. Hence, the supply is liable to be contaminated with dangerous intestinal pathogens [6]. An international expert meeting concluded that the application of molecular methods has to be considered in a frame work of quality management for drinking water. The new methods will influence epidemiology and outbreak investigations more than the routine testing of finished drinking water [7]. In the present study, an attempt has been made to screen the water samples in Mangalore for microbial contamination, to study the antibiotic susceptibility pattern of the bacterial isolates and to type the common isolates by RAPD technique as no such study has so far been reported from this part of the country.

2. MATERIALS AND METHODS

2.1 Specimen Collection

Water samples (n=324) from different sources like wells, bore-wells, municipal supply, tankers and water purifiers received in sterile bottles at the Department of Microbiology, Kasturba Medical College, Mangalore for routine bacteriological screening were included in the study. All the culture media were procured from Hi-Media Laboratories (P). Ltd., Mumbai and prepared as per manufacturer's instructions. The bottle containing water was inverted several times to mix and distribute the deposit. Macroscopic appearance of the water was also noted down [8].

2.2 Presumptive Coliform Count

In this study, municipal water, sample from domestic water purifier and tanker water samples were presumed to be of “good quality” as they are treated water. Water samples that were “clear” and from untreated sources like wells and bore-wells were presumed to be of “doubtful quality”. “Turbid” Water samples from wells and bore-wells were presumed to be “polluted”. Multiple tube technique was used to determine the presumptive coliform count in water samples [6]. For water of good quality, one 50ml volume and five 10ml volumes of water was added aseptically into bottles containing corresponding volumes of double strength MacConkey’s lactose purple (MLP) broth with inverted Durham’s tube. For water of doubtful quality, one 50ml volume and five 10ml volumes of water was added aseptically into bottles containing corresponding volumes of double strength MLP broth. In addition, five 1ml volumes of water were added to bottles containing 5ml of double strength medium. For water known to be polluted, five 10ml volumes of the water was added aseptically into bottles containing 10ml double strength MLP broth and five 1ml volume into bottles containing the corresponding volumes of single strength medium. All the bottles were incubated aerobically at 37ºC for up to 48 h. After 24 h and 48 h of incubation, the media were checked and the number of tubes of each volume of water that showed the production of acid and gas was noted. From the number and distribution of positive and negative reactions, the most probable number (MPN) of indicator organisms in the sample was estimated by referring to McCardy’s statistical tables [9]. These acid and gas producing cultures were considered as presumptive positives for the growth of coliform bacilli. Using McCardy’s statistical tables, the presumptive coliform count of 0, 1-3, 4-10 and >10 MPN/100ml was interpreted as excellent, satisfactory, suspicious and unsatisfactory, respectively [6].

2.3 Differential Coliform Count (Eijkman Test) [4]

Tubes containing 5-10ml of single strength MLP broth with an inverted Durham’s tube were taken and before inoculation, the media were incubated in a thermostatically controlled water bath at 44ºC and at 37ºC. When the media reached the respective incubation temperature, a loopful of each presumptive positive culture was inoculated into tubes of MPL medium. The tubes were further incubated at 37ºC and at 44ºC. After 48 h, tubes were examined for acid and gas production and the results were recorded. From the combination of positive and negative results at 37ºC, the most probable number of coliform bacilli per 100ml of water was read to get the confirmed coliform count. From the combination of positive and negative results at 44ºC, the MPN of *E. coli* per 100ml of water was read to get the confirmed *E. coli* count [6]. The tubes showing positive results for coliform count were subcultured onto MacConkey’s agar and Deoxycholate-citrate agar and the growth was identified by standard biochemical reactions [10].

2.4 Isolation of Enteric Pathogens

Selenite F broth was used as enrichment culture medium for the isolation of *Salmonella spp*. and *Shigella spp*. from water samples. 10ml of the water sample was aseptically transferred into bottles containing 10ml Selenite F broth and incubated at 37ºC for 6h, then subcultured onto Deoxycholate-citrate agar and MacConkey’s agar. The plates were incubated at 37ºC for 18-24 h and the colonies were identified by standard biochemical tests and confirmed by agglutination with specific antisera [10]. For the isolation of *Vibrio spp*., 10ml water sample was aseptically transferred into bottles containing 10ml alkaline peptone water and incubated at 37ºC for 4h and subcultured onto thiosulphate citrate bile-salt sucrose agar and

MacConkey's agar. Plates were incubated at 37ºC for 18-24h and the colonies were identified by standard biochemical tests and confirmed by agglutination with specific antisera [11].

2.5 Antimicrobial Susceptibility Testing

Disk diffusion method was used as per Clinical and Laboratory Standards Institute [CLSI] guidelines for performing the antimicrobial susceptibility testing [12]. Two to three colonies of the biochemically confirmed isolates were inoculated into Muller Hinton broth and incubated at 37ºC for 4h. The turbidity of the inoculum was adjusted to 0.5 McFarland standard. With the help of a sterile cotton swab, the broth culture was swabbed over the entire surface of the Muller Hinton agar plates so as to have an even distribution of the inoculum. Antimicrobial disks, as listed in Table 2, were placed on the inoculated plate with sterile forceps and incubated at 37ºC for 16-18 h. After incubation, the diameter of the zones of growth inhibition was measured and interpreted as either susceptible, intermediate or resistant [13].

2.6 Molecular Typing by RAPD

For the extraction of DNA, bacterial isolates were grown in 2ml of Luria Bertani broth for 18 h at 37ºC. The broth culture was centrifuged at 10000 rpm for 10 minutes. The pellet was washed twice in distilled water and resuspended in 100µl of distilled water and lysed by boiling for 10 minutes in a dry bath. The lysate was centrifuged briefly, DNA in the supernatant was spectrophotometrycally quantified (Bio Spec-1601, DNA/protein/ enzyme analyzer, Shimadzu Japan) and 2µl of supernatant was used as the source of DNA. Two custom synthesized decamer random primers R1 (5'GCGATCCCCA3') and R2 (5'CAGCACCCAC 3') procured from Bangalore Genei, India were used in RAPD reaction [14]. Amplification were performed in 25µl reaction mixture consisting of genomic DNA (2µl); 1X reaction buffer; 100 mM each of dATP, dCTP, dGTP, and dTTP; 0.2 mM random primer; 2.5 mM MgCl2 and 1U of Taq polymerase. A single primer was used in each reaction. The PCR tubes containing master mix, primer and DNA were amplified in a thermocycler (BioRad Inc., USA). PCR reaction was carried out up to 35 cycles. The reaction conditions were: initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 1 minute, annealing at 36°C for 1 minute and extension at 72°C for 2 minutes and the final extension at 72°C for 10 minutes. The amplified product was resolved by agarose gel electrophoresis using 2% agarose in 1X Tris acetate EDTA (TAE) buffer containing 0.5 mg/ml ethidium bromide [15]. Gels were visualized under UV trans-illuminator and photographed using a gel documentation system (Alpha View 1.3.0, Alpha Innotech Corporation Multi Image Light Cabinet). Bands were arbitrarily numbered and visually analyzed.

3. RESULTS AND DISCUSSION

Out of 324 water samples tested from five different water sources, 246 were excellent and 78 were contaminated. Among the contaminated water samples, 40 were satisfactory (1-2 MPN/ml). A coliform count of >10 MPN/ml was detected in 38 samples and these were unsatisfactory for consumption. The MPN test revealed that 45.79%, 42.85% and 5.59% of water samples collected from wells, bore-wells and purified sources, respectively, found contaminated and thus unsuitable for drinking. Source-wise results of water samples tested are depicted in Table 1. The bacterial species identified from the contaminated water samples (n=78) were mainly members of the family *Enterobacteriaceae* (n=135). Of these,

25.9% were *E. coli* (n=35), 40.7% *Klebsiella spp*. (n=55) and 25.9% were *Citrobacter spp*. (n=35). The tested samples also showed the presence of *Salmonella spp*., out of which one was identified as *Salmonella enterica paratyphi B* and two others agglutinated with polyvalent O antisera. *Vibrio spp*. was isolated from four samples of well water and one sample of tanker water. These *Vibrio spp.* agglutinated with polyvalent antisera but did not agglutinate with Ogawa or Inaba antisera. However, 75.92% (n=246) of the water samples did not show the growth of enteric pathogens and other bacteria. These included 152 from water purifier, 58 from well, 12 from bore-well, two from tanker water and 22 from municipal water sources. Other bacterial species isolated from water samples are shown in Table 1. All the isolates were tested for their susceptibility to different antibiotics. 100% of *Salmonella*, 85.7% of *Klebsiella spp,* 25.71% of *E. coli* and 55.8% of *Citrobacter spp*. showed resistance to ampicillin. 2.85%of *E. coli* and 1.78% of *Klebsiella* isolates were moderately sensitive to amikacin. All the isolates tested were sensitive to cephotaxime, gentamicin and ciprofloxacin. One isolate of *Pseudomonas spp*. was resistant, one isolate of *E. coli* was moderately sensitive and remaining bacterial isolates were sensitive to netilmicin. *Pseudomonas spp.* (2.9%) and *E. coli* (2.3%) showed resistance to tobramicin. Whereas, rest of the isolates tested were sensitive. Antibiotic susceptibility pattern of the isolates is shown in Table 2. As *E. coli* is the most reliable indicator of faecal pollution, they were subjected to molecular typing by RAPD technique to know the genetic similarity and difference among the isolates. Among the 35 isolates of *E. coli* tested by RAPD, major clearly visible bands were considered for comparison*. E. coli* isolates from well water (n=20), tested by using R1 primer, showed 14 different profiles. Isolates 2, 4 and 6 showed similarity in their banding pattern. Isolates 8 and 11, 12 and 14, 15 and 16, 18 and 19 showed similarity and formed four distinct clusters. Among the *E. coli* isolated from bore-well water (n=5), four isolates (22, 23, 24 and 25) showed similarity in their banding pattern with R1 primer. Whereas, one isolate was different from the rest. Among the four *E.coli* isolates from water purifier, two (27 and 28) were similar and other two (29 and 30) were similar in their banding pattern among themselves. Three *E. coli* isolates from tanker water (n=5) had similar banding pattern and three had non identical banding pattern when tested with R1 primer. RAPD profiles obtained with R1 primer for different *E. coli* isolates are shown in Fig. 1. With R2 primer, *E. coli* isolates from well water showed 11 profiles. Eight isolates were without any bands. Isolates 5 and 8, 15 and 19 showed similarity and formed two distinct clusters. Isolates of bore-well water and tanker water did not show any similarity. However, one isolate, each from bore-well water (lane 24) and tanker water (lane 32) did not show any band. Among the *E. coli* isolates from water purifier, two had similar banding pattern and others had no similarity when tested with R2 primer. In Fig. 2 similarity in the banding pattern between lane 21 and 26 as well as lane 19 and 25 could be noticed in case of *E. coli* isolated from well and bore-well waters, respectively. Similar observations could be noticed for lane 12, 27 and 29, and lane 9 and 30 (Fig. 2). However with R2 primer lanes 29 and 30 did not show any similarities. One isolate from municipal water resembled one of the isolate from well water [Fig. 2: lane-1 and 4].

In the present study, microbial quality of water from different sources was evaluated using coliform count. Of all the water sources tested, well water was found to be highly contaminated. Coliform counts have been used extensively as a basis for regulating the microbial quality of drinking water [1]. In this study, 75.92% of samples were above the WHO guideline [16] value (0 MPN/100ml) for coliform count. Water quality and isolation of different bacterial species indicates that pollution of ground water is common in this part of the country and may create a serious threat to human health and the environment. Bacteriological pollution of drinking water may be either due to failure of disinfection of raw water at the treatment plant or due to the infiltration of contaminated water (sewage) through

cross connection, leakage points and back siphonage [2]. In the present study, infiltration of the sewage could be the probable source of contamination of bore-well and well water. In this study, 24% of the water samples tested was contaminated. Diseases causing bacterial pathogens like *Salmonella spp*. and *Vibrio spp*. as well as antibiotic resistant coliforms were isolated from the water samples. Our results are similar to the earlier studies conducted in tsunami hit coastal areas of Kanyakumari, Tamilnadu [17], which revealed that 37% (56) of water samples tested (151), showed the presence of coliforms, majority of which were from wells and bore-well. The same study also reported the isolation of *Salmonella paratyphi B* and NAG *Vibrio* from well water sample. But no cases of acute diarrhoea or typhoid illness were reported from that area. Even in the present study, gastrointestinal tract infection outbreaks from the city were not reported during the study period. However, the health condition of the consumers of these water sources may throw some light on the impact of consumption of such polluted water on public health. A study from Nepal reports contamination of urban water supply by different *Salmonella* serotypes [18]. However, in the present study only *S. paratyphi B* was isolated and characterized. Enteric pathogens are usually present in low doses in water. However, they may pose danger to people whose defence mechanisms are impaired as infectious doses for these people are significantly low. Also, water polluted by bacteria when used in raw food preparations would lead to multiplication of the pathogen to very large doses and can lead to food spoilage [19]. *Pseudomonas spp*. detected in the present study is an important opportunistic pathogen and can cause food spoilage [6]. The high level of *E. coli* can be attributed to poor sanitation, low level of hygiene education, poor supervision, maintenance and or irregular disinfection. Contamination of water from domestic water purifier could be due to poor maintenance of this equipment or non-regulation of water flow at prescribed rate. The isolation of drug resistant *Enterobacteriaceae* members, along with pathogens like *Salmonella spp*. and *Vibrio spp*. as seen in this study, is a potential threat to humans if such water is consumed. Moreover, few of the isolates from well water, tanker water, water purifiers and bore-well water showed genetic similarity with R1 and R2 primers. It is interesting to note that, *E. coli* isolate from well water showed similarity to bore-well water and also with municipal water when tested with R2 primer. Genetic similarity was also observed among *E. coli* isolated from water purifier and well water with R2 primer. These *E. coli* isolates also showed similar antibiograms. This finding probably indicates a common source of origin of isolates or common source of contamination of these water bodies. However, highly discriminating typing methods like pulse field gel electrophoresis, if performed and interpreted using a good quality dendrogram would help to understand and trace the source of contamination.

Table 1. Source-wise results of water sample tested

Source (n)	Number tested		Organisms isolated (n)	Water quality (n)		
	Clear	Turbid		Unsatisfactory	Satisfactory	Excellent
Well water (107)	58	49	*Citrobacter spp*. (22) *Klebsiella spp*. (35) *Escherichia coli* (20) *Acinetobacter spp*. (3) *Pseudomonas spp*. (23) *Enterobacter spp*. (2) *Salmonella spp*. (1) *Vibrio spp*. (4) *Proteus spp*. (2)	28	21	58
Borewell water (21)	10	11	*Citrobacter spp*. (7) *Klebsiella spp*. (7) *Escherichia coli* (5) *Pseudomonas spp*. (4) *Enterobacter spp*. (1) *Salmonella paratyphi B* (1)	5	4	12
Municipal water (29)	22	7	*Citrobacter spp*. (1) *Klebsiella spp*. (4) *Escherichia coli* (1) *Pseudomonas spp*. (3) *Enterobacter spp*. (1)	1	6	22
Tanker water (6)	2	4	*Citrobacter spp*. (1) *Klebsiella spp*. (4) *Escherichia coli* (5) *Pseudomonas spp*. (3)	2	2	2

			Enterobacter spp. (1) *Salmonella spp*. (1) *Vibrio spp*. (1)			
Domestic water purifier (161)	152	9	*Citrobacter spp*. (4) *Klebsiella spp*. (5) *Escherichia coli* (4) *Acinetobacter spp*. (1) *Pseudomonas spp*. (4)	2	7	152
Total 324	244	80	*Citrobacter spp*. (35) *Klebsiella spp*. (55) *Escherichia coli* (35) *Acinetobacter spp*. (4) *Pseudomonas spp*. (37) *Enterobacter spp*. (5) *Salmonella spp*. (3) *Vibrio spp*. (5) *Proteus spp*. (2)	38	40	246

Table 2. Antibiotic susceptibility pattern of bacterial isolates from water samples

Antibiotic tested		*Acinetobacter* N=4	*Citrobacter* N=35	*Enterobacter* N=5	*E. coli* N=35	*Klebsiella* N=56	*Proteus* N=2	*Pseudomonas* N=34	*Salmonella* N=3	*Vibrio* N=5
Ampicillin	**S**	2 (50)	13 (37.30)	1 (20.0)	24 (69)	5 (8.60)	1 (50)	9 (26.5)	2 (66.66)	2 (40)
	I	0 (0)	2 (6.90)	0 (0)	3 (7.2)	3 (5.20)	0 (0)	0 (0)	0(0)	1(20)
	R	2 (50)	20 (55.80)	4 (80.0)	8 (23.8)	48 (86.20)	1 (50)	25 (73.5)	1 (33.34)	2 (40)
Amikacin	**S**	4 (100)	35 (100)	5 (100)	34(97.15)	55 (98.30)	2 (100)	34 (100)	3 (100)	5 (100)
	I	0 (0)	0 (0)	0 (0)	1 (2.85)	1 (1.70)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Carbenicillin	**S**	-	-	-	-	-	-	34 (100)	-	-
	I	-	-	-	-	-	-	0 (0)	-	-
	R	-	-	-	-	-	-	0 (0)	-	-
Cephatoxime	**S**	4 (100)	35 (100)	5 (100)	35 (100)	56 (100)	2 (100)	34 (100)	3 (100)	5 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ceftazidime	**S**	-	-	-	-	-	-	34 (100)	-	-
	I	-	-	-	-	-	-	0 (0)	-	-
	R	-	-	-	-	-	-	0 (0)	-	-
Chloramphenicol	**S**	3 (75)	35 (100)	5 (100)	35 (100)	56 (100)	2 (100)	34 (100)	3 (100)	5 (100)
	I	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Co-trimoxazole	**S**	4 (100)	35 (100)	5 (100)	35 (100)	56 (100)	2 (100)	19 (55.8)	3 (100)	5 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	11 (32.3)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (11.7)	0 (0)	0 (0)
Ciprofloxacin	**S**	4 (100)	35 (100)	5 (100)	35 (100)	56 (100)	2 (100)	34 (100)	3 (100)	5 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gentamicin	**S**	4 (100)	35 (100)	5 (100)	35 (100)	56 (100)	2 (100)	34 (100)	3 (100)	5 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Netilmicin	**S**	4 (100)	35 (100)	5 (100)	34 (97.6)	56 (100)	2 (100)	33 (97.1)	3 (100)	5 (100)
	I	0 (0)	0 (0)	0 (0)	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.9)	0 (0)	0 (0)
Piperacillin	**S**	-	-	-	-	-	-	34 (100)	-	-
	I	-	-	-	-	-	-	0 (0)	-	-
	R	-	-	-	-	-	-	0 (0)	-	-
Tobramicin	**S**	4 (100)	35 (100)	5 (100)	34 (97.6)	56 (100)	2 (100)	32 (94.20)	3 (100)	5 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.90)	0 (0)	0 (0)
	R	0 (0)	0 (0)	0 (0)	1 (2.4)	0 (0)	0 (0)	1 (2.90)	0 (0)	0 (0)

**S = Sensitive, I = Intermediate and R = Resistance; Numbers in parenthesis indicate percentage*

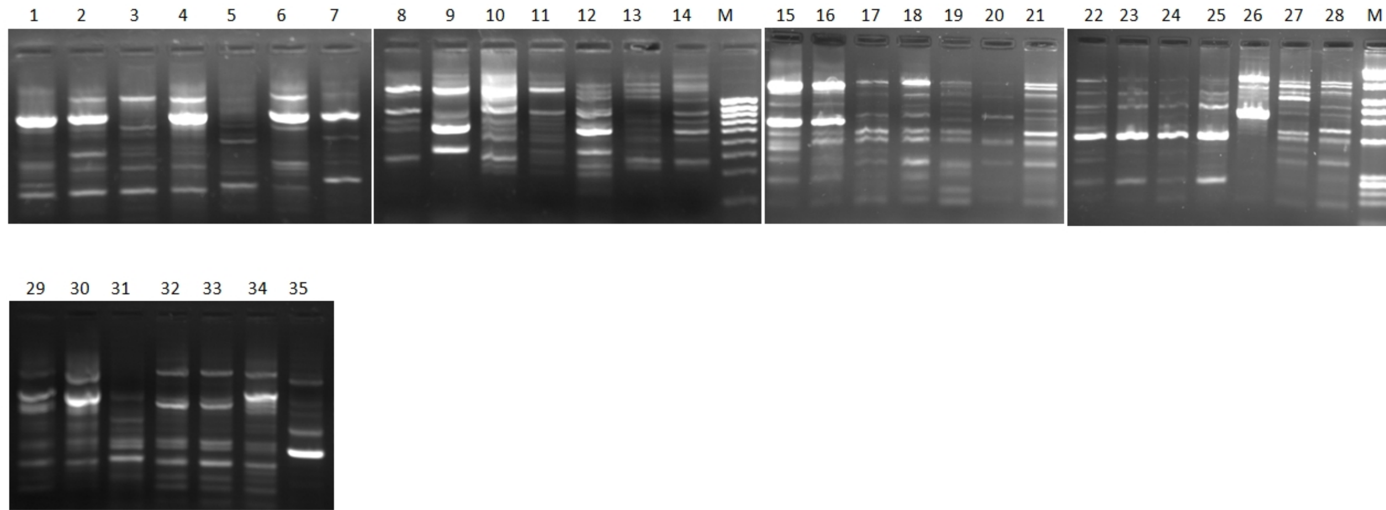

Fig. 1. RAPD typing of *E. coli* isolates using R1 primer. Lanes 1, isolate from municipal water; 2-21, isolates from well water; 22-26, isolates from borewell water; 27-30, isolates from domestic water purifier; 31-35, isolates from tanker water; M, molecular weight marker.

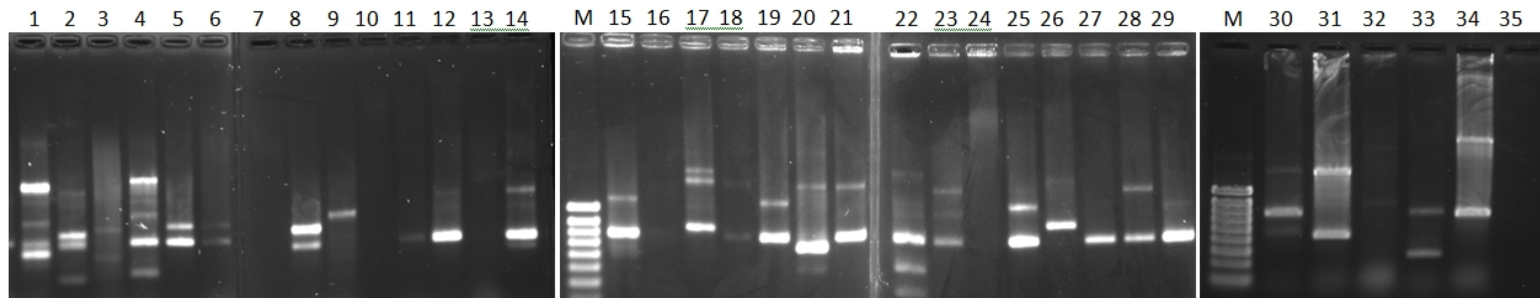

Fig. 2. RAPD typing of *E. coli* isolates using R2 primer. Lanes 1, isolate from municipal water; 2-21, isolates from well water; 22-26, isolates from borewell water; 27-30, isolates from domestic water purifier; 31-35, isolates from tanker water; M, molecular weight marker.

4. CONCLUSION

The degree and rate of contamination in this study suggests a need to be cautious and vigilant to avert the possibility of waterborne diseases from water sources. Regular monitoring of water quality serves to prevent the enteric diseases and also checks the water resources from going further polluted. The conservation of water sources is also very important to provide safe water as there can be no state of positive health and well-being without safe water.

ACKNOWLEDGEMENTS

The authors are grateful to the Head of the Microbiology Department, Kasturba Medical College, Manipal University, Mangalore, Karnataka, for giving permission to conduct the study. They thank all staff of Microbiology department for helping to conduct laboratory tests used in the study.

FINANCIAL DISCLOSURE

A self - financed study and all expenses were borne by authors.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Institutional ethics committee and have therefore been performed in accordance with the ethical standards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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