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Determination of Microbiological quality and detection of Thermotolerant fecal *E. coli* in ready to use water from Navsari city of South Gujarat

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ABSTRACT

Fecal bacterial indicator analyses have been widely used for monitoring the water quality. The present study was designed to determine the density of Coliform and Thermotolerant fecal E. coli from ready to use water samples collected for a five month(from november-2016 to March-2017) period of monitoring from in and around Navsari city. A total of 73 samples were collected from different places and processed under standard bacteriological techniques. The EMB Agar was used as selective medium for isolation. The presumptive isolates of thermotolerant fecal E. coli were identified by Indole and Mac Conkey broth test (Acid & Gas) at 44^{0}C incubation and coliform were enumerated by MPN technique and E. coli were identified by metallic seen on EMB agar plate and biochemical test. Analysis of result revealed 11 isolates (15.06 %) (3 samples from Abrama village, 3 samples from fish market, 1 sample from PG hostel, 1 samples from Krishna hotel, 1 sample from Jay Ambe tea stall, 1 sample from Mahadev mandir and 1 sample from panipuri centre) of Thermotolerant fecal E. coli as well as E. coli from 73 samples. All 11 isolates having presumptive number of coliform (MPN/100ml) ranging from 13-242 which may higher than the standard number which is below 10 according to BIS. The sensitivity pattern of Thermotolerant fecal E. coli with different antimicrobial agents was evaluated by D-test and showed cent percent resistant towards Amoxycilline followed in reducing levels by 18.18% each, of Cephalothine, Trimethoprime and Gatifloxacin and 9.09% each, of Tetracycline and Chloramphenicol. The pattern clearly indicated overall intermediate resistance to Cephalothine (36.36%), Gatifloxacin (36.36%). The isolates showed sensitivity towards 90.90% each, of Tetracycline and Chloramphenicol followed in reducing levels by Trimethoprime (81.81%) and 45.45% each, of Cephalothine and Gatifloxacin. The multiple antibiotic resistance MAR index was determined for each isolate which may raging from 0.16-0.83. The use of E. coli as the main bacterial indicator instead of other coliform bacteria has been proposed in water quality monitoring programs which tailors the microbiological quality of water.

Keywords: Antibiogram pattern, Thermotolerant, fecal, E. coli, MPN, MAR, Water, Navsari.

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INTRODUCTION

Earth consist of approximately 70% surface area covered with water and remaining is land which have only 2% water which is drinkable (Lim *et al*., 1999). Water is considered a vehicle for the propagation and dissemination of human associated bacteria (Faria *et al*., 2009). Safe drinking water is a fundamental human right and if contaminated with opportunistic pathogenic environmental bacteria, it may have health implications for consumers (WHO, 2004; Fawell and Nieuwenhuijsen, 2003). Human health should therefore be protected by preventing microbial contamination of water that is intended for consumption (Völker *et al*., 2010).

Coliform bacteria are the commonly used bacterial indicator for sanitary quality of water (Rompré *et al*., 2002; Tallon *et al*., 2005). They are defined as members of genera or species within the family Enterobacteriace capable of growth at 37º C (total coliforms) or 44º - 45º C (thermotolerant coliforms) that possess β-galactosidase (Edberg *et al*., 2000) that are aerobic/facultatively anaerobic, Gram-negative, non-spore forming rods fermenting lactose with acid and gas production in 24-48 hours at 35°C. Amongst these, *Escherichia coli* has been frequently used as a fecal pollution indicator organism (Toranzos *et al*., 2002). *E. coli* is a thermotolerant coliform which, among other things, produces indole from tryptophane at a temperature of 44 ± 0.5°C, gives a positive methyl red test result, is unable to produce acetyl–methyl carbinol and does not use citrate as its sole carbon source.

Coliform bacteria are abundant in the feces of warm-blooded animals but can also be found in soil, aquatic environments and vegetation. Unlike other coliform bacteria, *Escherichia coli* are almost exclusively of fecal origin and can be detected in elevated densities in human and animal feces, sewage and water subjected to recent fecal pollution. It is therefore considered the best fecal indicator microorganism (Edberg *et al*., 2000; WHO, 2004; Kumar *et al*., 2017).

Monitoring for fecal pollution of waters that are used for drinking, recreation and/or industry is important for public health and economic reasons. Pathogens introduced from fecal contamination can lead to disease in humans and livestock and economic losses to industries that depend on high water quality (Bernhard and Field, 2000). Contamination of water with human waste can introduce microorganisms causing a variety of infectious diseases such as typhoid, salmonellosis, and cholera (Scott *et al*., 2002; Kumar *et al.,* 2014).

Alarming increases in the consumption of antibiotics through human therapy and agricultural processes have been reported (Vaseeharan *et al*., 2005; J. L. Martinez, 2009) and this extensive usage in both human and animal medicine has resulted in the development of antibiotic-resistant bacteria which affect the treatment of infections (Akram *et al*., 2007; Łuczkiewicz *et al*., 2010). Antibiotic resistance has therefore become a major public health issue (Moore *et al*., 2010) and its presence in waste water, surface water, and drinking water is well documented (Łuczkiewicz *et al*., 2010; Moore *et al*., 2010; Lobova *et al*., 2008). The hazard associated with the pathogenicity of microbes is aggravated by its ability to resist destruction by antibiotics. Biological treatment processes in the waste water treatment plants may result in a selective increase of antibiotic-resistant bacteria and therefore increase the occurrence of multidrug-resistant organisms (Zhang *et al*., 2009). Moreover, the presence of antibiotic resistance in microorganisms has been previously reported (Mulamattathil *et al*., 2000; J. Lin and P. T. Biyela, 2005 and C. W. Kinge and M. Mbewe, 2010). Considering the fact that the public health of a community may be related to the quality of treated waste water supplied and that public health can be protected by reducing the pathogenic microorganisms in drinking water. Therefore, the use of *E. coli* as the main

bacterial indicator instead of other coliform bacteria has been proposed in water quality monitoring programs which tailors the microbiological quality of water.

MATERIAL AND METHOD

Sampling sites

Current study was carried out to examine the quality of ready to use water of Navsari city, South Gujarat. Knowing the public health risk from unsafe drinking water in Navsari city including most of the public places were chosen to study the quality water.

Sample collection

A total of 73 ready to use water samples (approximately 100 ml from each) were collected from different places of Navsari city of south Gujarat. 100 ml water sample was collected and transferred it into disposable sterilized plastic tubes. After collection of sample tubes were tightly closed to avoid any contamination and protection to make it protected from environmental pathogen contamination. The samples were collected from November 2016 to March 2017 and investigation was carried out of following after collection were accomplished in the laboratories.

Isolation and enumeration of bacteria

The selective medium used for isolation of Thermotolerant fecal *E. coli* and *E. coli* was Double strength MacConkey's and Single strength MacConkey's broth (HiMedia). A10 ml of water sample was inoculated in first set of three test tube containing 10 ml of Double strength MacConkey's broth with Durham's tubes and 1 ml of water sample was inoculated in second set of 5 ml Single strength MacConkey's broth with Durham's tubes and 0.1 ml water sample was inoculated in third set of 5 ml Single strength MacConkey's broth with Durham's tubes then incubated at 37 °C for 24 – 48 hours. Continued for another 24-48 hours for those tube showing negative reactions. Mark the positive test tube showing both acid and gas production after 24 and 48 hrs incubation as presumptive positive for coliforms. Compute the results using MaCrady's probability table as Most ProbableNumber(MPN) of coliforms in 100 ml of the water sample. According to Bureau of Indian Standards No water sample should contain more than 10 coliforms/100 ml of water. The tubes which may showing the acid and gas production are further inoculated on EMB agar plates and incubated at 37 °C for 24 hours.

Purification of colonies

Characteristic appearance of green metallic colonies on EMB plate were considered to be presumptive coliforms. The pure cultures were streaked on Nutrient agar and incubated for 24 hours at 37 °C and were further characterized by biochemical tests.

Biochemical examination

Biochemical tests were performed to confirm *E. coli as* well as thermotolerent fecal *E. coli* using Gram's stain, IMViC (Indole, Methyl red, Voges Proskauer and Citrate) test at 37 °C for *E. coli* and for confirmation of thermotolerent fecal *E. coli* indole and Mac Conkey Broth test at 44°C incubation were carried out.

Antibiotic susceptibility assay

All the isolates were subjected to antibiotic sensitivity testing by standard disc diffusion method on Muller-Hinton agar (Himedia) according to the National Committee of Clinical Laboratory Standards (NCCLS) recommendations (NCCLS, 2014).The following antibiotic discs (HiMedia) at the final concentrations that are indicated were used: Cephalothine (CEP) 30µg, Amoxycilline (AMX) 30µg, Tetracycline (TE) 30µg, Chloramphenicol (C) 30µg, Trimethoprime (TR) 30µg and Gatifloxacin (GAT) 10 µg. Colonies were picked from each sample and each colony was transferred into Mueller-Hinton broth to prepare bacterial suspension. Aliquots of 100 μL from each suspension were spread-plated on Mueller-Hinton agar plates. Antibiotic discs were applied on to the plates using sterile forceps and the plates were incubated at 37°C

for 24 hours. After incubation, the antibiotic inhibition zone diameters (IZD) were measured. Results obtained were used to classify isolates as being resistant, intermediate resistant, or susceptible to a particular antibiotic using standard reference values according to National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 2014).

MAR index

The multiple antibiotic resistance MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (Krumpernam, 1983).

MAR index = Number of antibiotics to which isolate is resistant / Total number of antibiotics tested.

RESULTS AND DISCUSSION

Out of 73 water samples processed, 11 (15.06 %) samples yielded the growth of metallic seen on EMB agar plate were identified as *E. coli* and further identification of bacterium done by morphological, cultural and biochemical characteristics. The biochemical test result revealed that all the 11 isolates were show positive reaction for IMViC test at 37 °C. All the isolates were further determined as thermotolerant fecal *E. coli* by Indole and Mac Conkey broth test (Acid & Gas) at 44°C incubation and all isolates were show positive reaction towards both biochemical tests. All 11 samples from which thermotolerant fecal *E. coli* was recovered having presumptive number of coliform (MPN/100 ml) ranging from 13-242, which were higher than the standard number according to BIS. Result of antibiotic sensitivity revealed that all the 11 thermotolerent fecal *E. coli* isolates were resistance to Amoxycilline (100%), were only determined. Out of 11 isolates 5 were susceptible to Cephalothine (45.45%), 2 were resistant to Cephalothine (18.18%), and 4 isolates were Intermediate resistance to Cephalothine (36.36%), Out of 11 positive isolates 10 were susceptible to Tetracycline (90.90%), and only 1 isolates were resistant to Tetracycline (9.09%), Out of 11 isolates 10 were susceptible to Chloramphenicol (90.90%), and only 1 were resistance to Chloramphenicol (9.09%), Out of 11 isolates 9 were susceptible to Trimethoprime (81.81%), and 2 were resistant to Trimethoprime (18.18%), Out of 11 isolates 5 were susceptible to Gatifloxacin (45.45%), and 2 were resistance to Gatifloxacin (18.18%), and only 4 were Intermediate resistance to Gatifloxacin (36.36%). Antimicrobial Sensitivity pattern of all the Thermotolerant fecal *E. coli* isolates were given in Table.1 and Figure.1. The multiple antibiotic resistance MAR index was determined for each isolate which may raging from 0.16-0.83. The MAR index of all the Thermotolerant fecal *E. coli* isolates were given in table:2.

In this study we describe the isolation, identification and antibiotic susceptibility characterization of thermotolerant fecal *E. coli* from ready to use water obtained from different places of Navsari city. The results of present prevalence study and other author's results from various parts of the world are differing vastly. However, results of study results at Navsari district region showed prevalence of 15.06 percent. The comparison of the percentage of resistant strains with published work from other times and places were complicated because researchers have used different numbers and kinds of antibiotics in their studies.

Avşar and Berber. (2014) reported that all the isolates were resistant to bacitracin (100%), novobiocin (100%), ampicillin (12.5%), tetracycline (7.5%), ceftazidime (5%), and imipenem (2.5%) respectively, whereas the strains were susceptible to polymyxin B (100%). The multiple antibiotic resistance values for the strains were found in range from 0.28 to 0.57. Watkinson *et al.* (2007) determined the antibiotic resistance (AR) patterns of 462 *Escherichia coli* isolates from wastewater, surface waters, and oysters. Rates of AR and multiple-AR among isolates from

surface water sites adjacent to wastewater treatment plant (WWTP) discharge sites were significantly higher (P < 0.05) than those among other isolates, whereas the rate of AR among isolates from oysters exposed to WWTP discharges was low (<10%). Chandran *et al.* (2008) observed the antibiotic high resistant against vancomycin (93%), followed by novobiocin (91%) kanamycin (85%) and oxytetracycline (84%). The MAR indexing of the isolates in this study ranged from 0.33 to 1 and it is greater than 0.25 and probability originated from high risk source of contamination. McKeon *et al*. (1995) observed that the resistance against novobiocin, ampicillin and tetracycline are most common among gram negative bacteria in water. The frequency of multiple antibiotic resistance (MAR) within each species was also determined. Approximately 60% of the coliform were MAR, including 14, 64, and 94% of *E. coli*, *Citrobacter freundii*, and *Enterobacter cloacae* isolates respectively. A high ampicillin resistance was noted from rural and urban waters and much lower resistance to ampicillin were reported by Parveen *et al.* (1997). Amundsen *et al*.(1988) observed the most common resistace directed towards ampicillin , cephalothin, nitrofurantoin, and tetracycline. Gomathinayagam *et al*. (1994) reported that *E. coli* showed predominant resistance to penicillin G, novobiocin and neomycin. Lin *et al*. (2005) observed a high resistance of enteric bacteria against novobiocin and ampicillin. Gaur *et al*. (1992) isolates a total of 231 thermotolerant coliforms from rural drinking water from four states of India. Of these, 220 isolates were resistant to ampicillin, chloramphemicol, streptomycin and tetracycline. Multiple (MAR), double and single antibiotic resistances were observed in 31-4, 48-6 and 13-7 % of the isolates, respectively.

The pollution of water by human and animal sources is the major threat to the public health in major countries including India. Water contaminated with excreta from animal or anthropogenic sources, which may be the carrier or active cases of infectious diseases serve as the vector of disease.

Table 1. Antibiogram of Thermotolerant fecal *E. coli* isolated from ready to use water samples

Antibiotics used	n=11					
	Resistant		Intermediate resistant		Sensitive	
	no. of isolates	%	no. of isolates	%	no. of isolates	%
Cephalothine (CEP)	2	18.18	4	36.36	5	45.45
Amoxycilline (Amx)	11	100	-	-	-	-
Tetracycline (TE)	1	9.09	-	-	10	90.90
Chloramphenicol (C)	1	9.09	-	-	10	90.90
Trimethoprime (TR)	2	18.18	-	-	9	81.81
Gatifloxacin (GAT)	2	18.18	4	36.36	5	45.45

Table 2. Multiple antibiotic resistance (MAR) index of thermotolerent fecal *E. coli* isolates.

Isolate No.	MAR Index	Antibiotics
1	0.16	Amx
2	0.16	Amx
3	0.33	Amx, GAT
4	0.83	CEP, Amx, TE, C, GAT
5	0.16	Amx
6	0.50	CEP, Amx, TR
7	0.16	Amx
8	0.16	Amx
9	0.16	Amx
10	0.16	Amx
11	0.33	Amx, TR

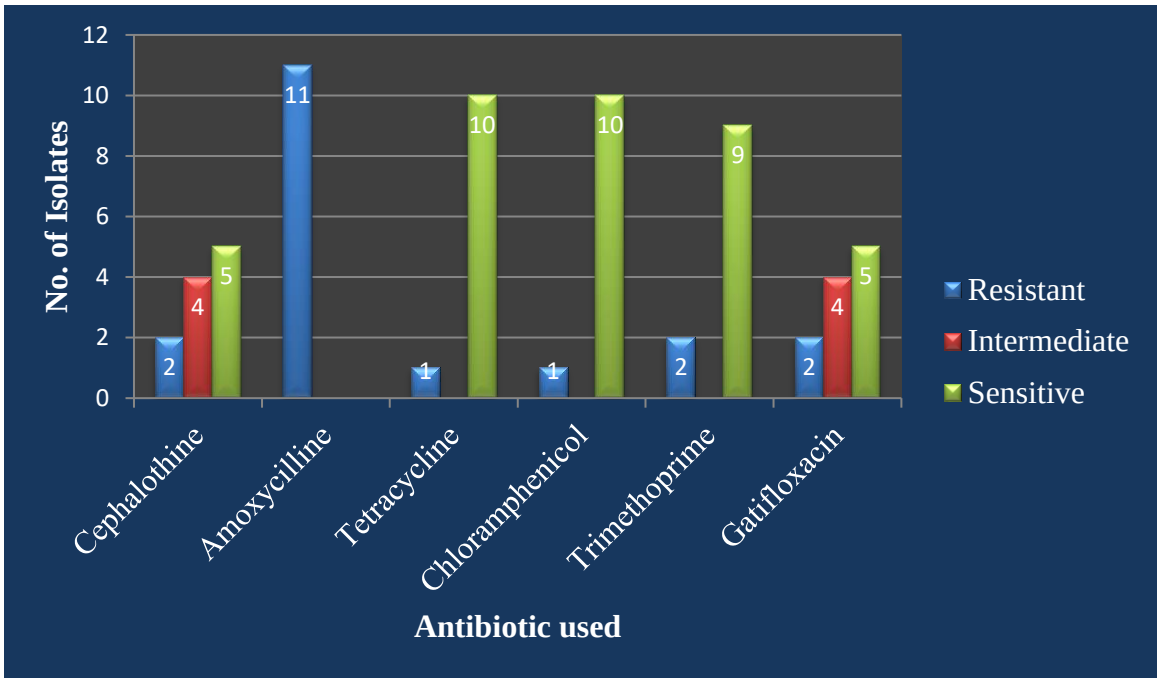

Fig-1: Antimicrobial Sensitivity pattern of Thermotolerant fecal *E. coli* isolated from redy to use water samples.

CONCLUSIONS

Water used for different purposes is contaminated in Navsari city. So water authorities should have to take steps to control coliforms in drinking water in order to prevent population from water borne diseases. An evaluation of the bacteriological quality of ready to use water in the present study confirmed the presence of Thermotolerent fecal *E.coli*. These organisms were resistant to several classes of antibiotics. Undesirable properties of water quality caused by the presence of drug-resistant bacteria can pose a negative impact on human health. The data on multiple antibiotic resistance (MAR) profiles of bacterial isolates from water and the resistance patterns of organisms in ready to use water suggested that there has been an indiscriminate use of the antibiotics tested. The high prevalence of

multiple antibiotic-resistant organisms in the drinking water distribution system could potentially pose a threat to humans consuming this water. Antibiotic resistance surveillance can be used as tool to control the problem of antibiotic resistance and to educate the public on the consequences of the misuse of antibiotics and also to regulate the usage of drugs in both human and veterinary medicine. It is also helpful to formulate guidelines for the optimal use of antibiotics.

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