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RESEARCH ARTICLE

PREVALENCE OF ANTIBIOTIC RESISTANT *E. COLI* FROM DRINKING WATER SAMPLES OF MYSORE DISTRICT

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ABSTRACT

Water-borne diseases are the leading cause of deaths in developing countries. Among the coliform *Escherichia coli* is used as the principle indicator for drinking water pollution monitoring and plays an important role in the sanitary analysis of water. Indiscriminate use of antibiotics leads to antibiotic resistant *E. coli* in drinking water. In this study microbial quality of drinking water at the point of use has been evaluated in different taluks of Mysore district and it was found that the household stored water was more contaminated than the tap water. The *E. coli* isolated from 83% of the household stored water and 13% tap water samples showed resistance to different antibiotics. Nearly 54 % of *E. coli* strains were resistant to various antibiotics. 30% of *E. coli* strains isolated had intermediate, and 16% were susceptible to antibiotics. The high frequency of antibiotic resistance (100%) was observed against Nystatin, Cephalothin, Penicillin, Vancomycin, and Aztreonam whereas were sensitive to Chloramphenicol (100%) and intermediate to Nitrofurantoin (90%), Trimethoprim (82%), and Amikacin (63%). The study indicates the poor sanitary conditions of the sampling sites and unhygienic practices of people leading to the prevalence of the Antibiotic Resistant *E. coli (ABR E.coli)* drinking water.

INTRODUCTION

Water is the most innocuous health drink int the world. Water, sanitation, and hygiene-related diseases are the most common cause of death in the developing countries and the most common cause of diseases such as cholera, gastroenteritis, and diarrhea. It was estimated that 88% of global burden of disease is attributed to unsafe water supply, sanitation, and hygiene. Conventional drinking water treatment consists of a series of barriers to remove contaminants from water. But the waterborne pathogens continued to emerge for a number reasons. Even when water sources are improved, water-quality risks may still exist at the point of consumption (Shaheed *et al.*, 2014). Due to inadequate, unsanitary storage conditions and contaminated hands allow the introduction of disease-causing microbes. It has frequently been observed that the microbiological quality of water in vessels in the home is lower than that at the source, suggesting that contamination is widespread during collection, transport, storage and drawing of water (Van Zijl, 1966; Lindskog and Lindskog, 1988). Microbial pollutants often result in infectious diseases through drinking water. Among the coli form *Escherichia coli* is used as the principle indicator for drinking water pollution monitoring and plays a major role in the sanitary analysis of water. *E. coli* is an opportunistic pathogen in neonatal and immune compromised patients. The native habitat of *E. coli* is the enteric tract of humans and other warm-blooded animals. Most of *E. coli* strains are normal flora of the small intestine and colon and do not cause disease in the gut and these non-pathogenic *E. coli* can cause disease if they spread outside of the intestines. Human feces potentially carry all enteric diseases to which humans are susceptible (Paul, 1983). Its presence in water indicates the direct or indirect recent fecal contamination and the possible presence of enteric pathogens.

Widespread use of antibiotics in medicine and animals for various purposes are released unchanged into the environment after use which causes the probability of the spread of resistant strains in animals and humans. Thus, healthy individuals can

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become carrier hosts for multiple antibiotic-resistant bacteria (Reinthaler *et al.,* 2003) which can enter the household drinking water through unhygienic human activities leading to increase in antimicrobial-resistant bacteria. The use of antibiotics also may accelerate the development of antibiotic resistance genes (ARGs) and bacteria, which shade health risks to humans and animals (Kemper, 2008). Antibiotic-resistant bacteria were detected in drinking water as early as the 1980s (Armstrong, *et. al.*, 1981, Kolwzan *et al*., 1991). Several studies have reported that ABR is common in drinking water (Armstrong, *et al*., 1982; Armstrong, *et al*., 1981; Pathak and Gopal, 2008; Ramteke, *et al*., 1990; Shrivastava, *et al*., 2004). Interestingly, in the study carried out by Walia *et al*., 2004, a higher number of multiple antibiotics resistant (MABR) *E. coli* were found in drinking water as compared to the human urine samples. There are some reports that the, *E. coli* that make it resistant to antibiotics can efficiently exchange genetic material with pathogens such as *Salmonella, Shigella, Yersinia,* and *Vibrio* species, as well as pathogenic *E. coli*. (Levy, 1997; Tauxe *et. al.,* 1989). The purpose of the study was to assess the antibiotic susceptibility of *Escherichia coli* isolated from household drinking water and to evaluate the hygiene practices. Water samples were collected from urban areas of different taluks of Mysore district. The Mysore district falls in the survey of India degree sheet Nos. 48P, 57D, 57H and 58A. The district is bounded by latitudes 11045' N - 12040' N and longitudes 75^{0}59' E-77005' E covering an area of 6269 Sq. km. Mysore district divided into seven Taluks viz, H.D Kote, Hunsur, K.R.Nagar, Mysore city, Nanjangud, Piriyapatna, and T. Narasipura. The main source of water to these taluks are rivers Cauvery, Kabini, Nugu, Gundal, Lakshmanathirtha and ground water. River water is treated at the treatment plant and then distributed to the consumer points whereas ground water is supplied untreated. The antibiotics used in the study are commonly used in human and veterinary medicine.

MATERIALS AND METHODS

Tap water from consumer point and stored household water drinking samples were collected in replicates. The water samples were collected in a sterile container containing 4-5 drops of 1% Sodium Thiosulphate to neutralize any residual chlorine and were placed in 4^0 C cooling boxes for transportation to the laboratory and processed within six hours of the collection. Bacterial identification was performed by, conventional microbial culture method Multiple-Tube (MPN) Fermentation Technique; positive cultures were grown on EMB agar (APHA 1998), and the organisms were identified up to the species level using biochemical tests using biochemical identification kit (Himedia, Mumbai, India).

Antibiotic susceptibility testing

Antimicrobial Susceptibility Testing was performed according to the Clinical Laboratory and Standards Institute (CLSI) guidelines for antimicrobial susceptibility testing by standard Kirby-Bauer disc diffusion technique. Suspensions of *E. coli* strains grown in Tryptone Soya Broth were made in sterile 1x phosphate buffered saline (PBS) pH 7.2 to match a 0.5 McFarland Standard to achieve an inoculum density of approximately 1 x 10^8 cfu/ml. *E.coli* from the standardized suspensions were inoculated on the Muller Hinton agar using sterile, nontoxic cotton swabs. Antimicrobial disks were placed on the surface of the inoculated plates. Plates were incubated at 35^{0}C for 18-24 hours and zones of inhibition were measured in millimeters. The commercially available antibiotic discs (Hi-Media, Mumbai, India) used in the study are (potency in µg/disc): Amikacin (AK10), Ampicillin (AMP 25), Ampicillin (AMP 10), Aztreonam (AT 30), Cefotaxime (CTX 30), Cephalothin (CEP 30), Ciprofloxacin (CIP 5), Chloramphenicol (C 30), Erythromycin (E15), Gentamycin (GEN 10), Kanamycin (K30), Nalidixic Acid (NA 30), Nitrofurantoin (NIT 300), Nystatin (NS 50), Oxytetracycline (O30), Penicillin (P 2), Streptomycin (S 10), Tetracycline (TE30), Tetracycline (TE30), Trimethoprim (TR 5), Vancomycin (VA 30). Minimum inhibitory concentrations (MIC) of antibiotics were determined by Antibiotic Zone Scale-(Hi-Media, Mumbai, India). An isolate was designated as antibiotic resistant (AR) if it was resistant to at least one of the antibiotics tested and multiple antibiotic resistant (MAR) if it was resistant to at least two antibiotics tested (Robert E. Brennan and Steven Everman, 2012).

RESULTS AND DISCUSSION

Nearly 90 water samples were collected from the hand pumps, taps and stored household water. Out of which 72% of the samples showed coliform contamination. 90% of the tap water samples were detected with Coliforms, and 13% were positive for the presence of *E. coli*. In the other hand, all the stored household water samples (100%) were contaminated with coliform compared to tap water out of which 83% were positive for *E. coli*. No had pump water samples were found to be positive for *E. coli*. The *E.coli* strains isolated from both tap and stored household water have shown resistant to different antibiotics. Disk diffusion zone sizes were examined using antibiotic zone scale. About 54 % of *E.coli* were resistant, 16% of *E. coli* had intermediate and 30% susceptibility to the antibiotics used (fig. 1). The highest level of resistant shown by *E. coli* isolates was to Nystatin, Cephalothin, Cefotaxime, Penicillin, Ampicillin 10, (100%) followed by Vancomycin (93%), Aztreonam (93%) and Streptomycin (87%). There was a significant difference in the diffusion zone among the isolates (fig. 2). Chloramphenicol (C30) (100%) and Tetracycline (TE30) (53%) were active against most of the *E. coli* isolates with a large zone of inhibition. The isolates HS1, HS12 and NS1 from the household stored water showed 76%, 71%, and 71% resistance to the different antibiotics respectively. 100% resistant to more than nine antibiotics namely AMP10, CTX30, CEP30, NS50, E15, NA30, O30, AT30, CIP5, K30, P, S10, TE30, VA30 and AMP25. However, there were wide variations in the susceptibility between *E. coli* species of this study. *E. coli* is one of the most common pathogens of *Enterobacteriaceae* family responsible for nosocomial infections (Atul Khajuria, 2014). Several groups have reported the presence of antibiotic resistant *E. coli* isolated from environmental samples like sewage, waste water, urine samples, nursing samples (Urine and Feces) from human and animals, under grown water, surface water, drinking water at distribution system and point of use. Although several studies have detected ABR in drinking water systems (Armstrong *et al*., 1981; Armstrong *et al*., 1982; Schwartz *et al.,* 2003; Zhang *et al*., 2009),

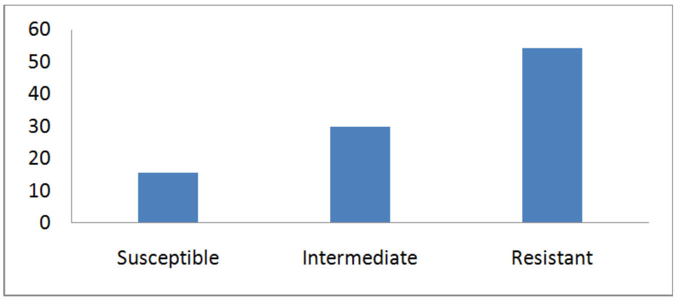

Fig. 1. Resistance pattern of *E. coli* to the antibiotics

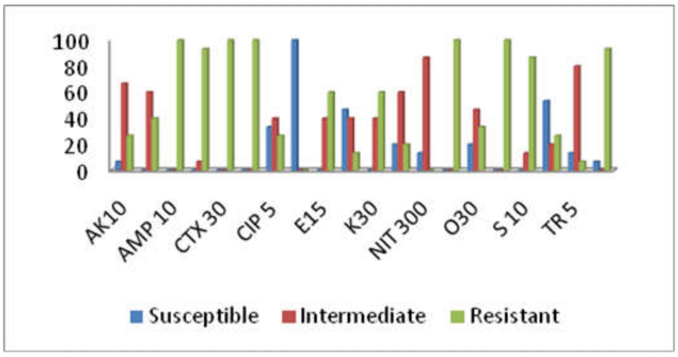

Fig. 2. Percentage response of different *E. coli* isolates for various antibiotics tested

little is known about the fate of ABRs in stored household drinking water. The frequency of *E. coli* resistance to various antibiotics from different environmental samples have been extensively studied such as, Oxytetracycline and Cephalothin resistant (Kerketta *et al*., 2015), Kanamycin and Tetracycline resistant (Satish *et al*., 2004, Cernat, 2007) in drinking water, Trimethoprim resistant from natural water source (Park *et al*., 2003), resistance to tetracycline, Cephalothin and streptomycin from live stacks and wildlife (Sayah *et al.,2005*), Cefotaxime resistance *Enterobacteriaceae* in hospital wastewater and activated sludge (Schwartz, 2003). In another study by Liu, 2013, found that the changes breakpoint in the CLSI guidelines resulted in higher resistance rates to cefotaxime. *E. coli* isolated by Reinthaler and coworkers from sewage plant were resistant to several antibiotics such as ampicillin, piperacillin, cephalothin, cefuroxime,nalidixicacid,tetracycline and sulfamethoxazole. A similar elimination rate was found for ciprofloxacin by Wiethan *et al*., 2001. Walia *et al*., 2004 isolated two strains of *E. coli* resistant to six and one strain to seven antibiotics from drinking water. *E. coli* isolated from tap water by Wose Kinge, *et al.,* 2010, was observed for 50%-90% resistance to chloramphenicol, tetracycline, ampicillin and erythromycin and susceptibility of a few isolates to streptomycin and kanamycin. In the study carried out by Patoli *et al*., 2010, the isolated *E.coli* from drinking water were resistant to nalidixic Acid (92.6%), ampicillin (88.89%), ciprofloxacin (37.04%), cefotaxime (18.52%), gentamicin (18.52%) and susceptibility to amikacin. In the present study, the percentage of resistant by the *E. coli* isolate is more towards more than five antibiotics namely, AMP10, CTX30, CEP30, NS50, P2 with 100%, AT30 (93%), S10 (86%) VA30 (93%) and K30 (60%). The emergence of resistance and decreasing levels of susceptibility of *E. coli* to a wide spectrum of antimicrobials is a matter of concern because it may limit the availability of antimicrobials for clinical management of waterborne outbreaks in the future (Ram *et. al*., 2008). Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control (O'Brien, 1997). The presence of antibiotic resistant *E. coli* in the sewage and its cross contamination with source and the distribution system through the pipeline is common, but contamination of household drinking water are purely on the hygienic practices of the individual when the tap water is without any contamination. Numerous studies have documented inadequate storage conditions and vulnerable water storage containers as factors contributing to increased microbial contamination and decreased microbial quality compared to either source waters or water stored in improved vessels (WHO, 2003).

Conclusion

In many instances, water is collected from contaminated sources. But, in some cases, water is received from the source with safe water quality having an adequate level of residual chlorine. Later becomes contaminated due to unhygienic collection and storage which leads to the introduction of disease-causing microbes. In most of the cases, diarrhoeal diseases are treated at home with self-remedies or by the nonspecific and inadequate quantity of antibiotics giving rise to ABR bacteria. The results of the study indicate the poor sanitary conditions of the sampling sites and unhygienic practices of people leading to the presence of the ABR *E. coli.* The study suggests the additional measures at home are required by the individual to avoid post contamination of drinking water. The health risk can be reduced by employing improved storage conditions and household treatments.

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