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PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIAL ISOLATES AND CHARACTERIZATION OF PLASMID DNA CONFERRING MDR VIA CONJUGATION FROM POTABLE WATER SAMPLES

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ABSTRACT
RO and RO + UV treated water quality of various water coolers and purifiers of Indore region were analyzed for the detection of total coliforms and multiple drug resistant (MDR) bacteria. Most of the samples from only RO water purifier have shown >240 coliforms/100ml while some have 21, 12, 2.2 coliforms respectively. Lactose fermenting and non-lactose fermenting organisms were identified on the basis of biochemical reactions. Mostly *E. coli, Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa and Proteus* sp. organisms were observed to contaminate water. Mueller Hinton Agar Medium was used to check the susceptibility of isolates towards various antibiotics. All the isolates were found to be resistant to Ampicilin. Some isolates were resistant to Streptomycin, Gentamycin, Amikacin, Chloramphenicol, Ampicilin, Ofloxacin and Ciprofloxacin. The percentages of antibiotic resistance of isolates are *Escherichia coli* (57%), *Klebsiella pneumoniae* (33%), *Enterobacter aerogenes* (38%), *Pseudomonas aeruginosa* (19%), *and Proteus* sp. (14%). A large number of *E. coli* isolates (57%) exhibited resistance to Ampicilin (100%), Streptomycin (25%), Gentamycin (25%), Amikacin (16.66%), Chloramphenicol (8.33%), and Ofloxacin (8.33%) antibiotics. Among all the isolates, *E. coli* (14.25%) was found to be multiple drug resistant bacteria. The extracted plasmid DNA of ˜ 1.8kb was isolated from this amp resistant *E. coli.* The plasmid DNA of amp resistant *E. coli* bacteria was successfully transferred to amp sensitive bacteria via conjugation process. All these drug resistant bacteria were isolated from RO water purifier which concludes that RO water samples was of poor microbial quality while RO+UV treated water was found to be safest for drinking purpose.

KEYWORDS: Water Purifier, Coliforms, Antibiogram, MDR, Plasmid DNA, Conjugation.

INTRODUCTION

The bacteriological quality of drinking water is of paramount importance and monitoring must be given highest priority (Bharti *et al*., 2011). The most dangerous form of water pollution occurs when fecal contaminant like *Escherichia coli* enter the water supply. Contaminants ingested cause many diseases. Examples of such pathogens are *Salmonella* sp., *Shigella* sp., *Vibrio cholerae* and *E. coli* (Tortora *et al*., 2002). The quality of potable water and treatment of water borne diseases are critical public health issues. Bacterial contamination of drinking water sources is the most common health risk. Majority of the water sources were not safe for drinking (Solomon *et al*., 2011). Thereby most of the people rely on RO and RO+UV systems for the purification of water. In RO systems, reverse osmosis treatment is an effective method of reducing the concentration of total dissolved solids (TDS) and many impurities found in water whereas RO membrane can remove all microorganisms while UV kills all remaining bacteria and viruses (Kneen *et al*., 2005). The Microbiological quality of drinking water has attracted great attention worldwide because of implied public health impacts (Amira *et al.*, 2011). Total and fecal coliforms have been used extensively from many years as indicator for determining the sanitary quality of water sources (Solomon *et al*., 2011).

Fecal coliforms are a group of bacteria, which are natural inhabitants of the gut of humans and other warm-blooded animals. *Escherichia coli* (*E. coli*) is a member of fecal coliforms that contaminate the drinking water from human and animal fecal waste (Ahmed *et al*., 1996). *E. coli* is an opportunistic pathogen in neonatal and immuno-compromised patients (Annette *et al*., 1998). Bacteremia, wound infections, urinary tract infection, and gastrointestinal infections are the diseases associated with *E. coli* and are often fatal in newborns (Raina *et al*., 1999). Food and water borne out breaks of *E. coli* have been documented from a number of countries (Ogden *et al*., 2001; Bartlett *et al*., 1996). Maintenance of the microbiological quality of water has been used as an important means of preventing waterborne disease (WHO Report, 2008). Antibiotic resistance is becoming a very large problem throughout the world (Bergeron *et al*., 2015). The occurrence and spread of antibiotic resistant bacteria (ARB) are pressing public health issues worldwide, and aquatic ecosystems are a recognized reservoir for ARB and antibiotic resistance genes (ARGs) (Baquero *et al.*, 2008; Cook *et al.*, 1975; Gonzal *et al.*, 1979; Klare *et al.*, 1995; Kummerer *et al.*, 2004; Martinez *et al.*, 2008; Zhang *et al.*, 2009). Antibiotic resistance in bacteria has been arise from the antibiotic residues which is discharged into the environment when it is not fully metabolized by the body and excreted in its original form

which results in antibiotic resistant bacteria . Bacteria can easily acquire resistance against those antibiotics and release their antibiotic resistance genes (ARGs) into the environment (Auerbach *et al*., 2007; Zhang *et al*., 2009). These released ARGs through genetic transformation can get easily be transferred to the environmental bacteria and pathogens, increasing risks and dangers to environment and human (Liu *et al*., 2012).

This study was aimed to check the prevalence of antibiotic resistant bacterial isolates and characterization of plasmid DNA conferring MDR *via* conjugation from potable water (RO and RO+UV) samples.

MATERIALS & METHODS

Sample collection

From different locations of Indore city, 30 water samples were collected from various water coolers and purifiers (RO and RO+UV) in non-reactive borosilicate glass/ plastic bottles sterilized by autoclaving at 121^{o}C, 30 minutes.

Microbiological analysis of water

Samples were assessed within 24 hrs for the presence of coliforms (Total coliforms and fecal coliforms) by using standard protocol of American Public Health Association (APHA 2005). All samples were analyzed for total coliform count by Most Probable Number (MPN) method. Positive tubes were streaked on MacConkey agar plate to differentiate between lactose fermenting and non-lactose fermenting organisms which were identified on the basis of biochemical reactions.

Antibiotic Resistance Profile

Antibiotic susceptibility test was performed using standardized Kirby Bauer Disc Diffusion Test (Bauer *et al*., 1966). Mueller Hinton Agar Medium was used to check the susceptibility of isolates towards various antibiotics. The isolated bacterial colonies which were identified by standard biochemical characters were tested against seven commonly used antibiotics, *i.e*. Streptomycin (10µg), Gentamycin (10µg), Amikacin (30µg), Chloramphenicol (30µg), Ampicilin (10µg), Ofloxacin (5µg) and Ciprofloxacin (5µg). Bacteriological culture media and antibiotic discs of Hi-Media Pvt. Ltd. Mumbai were used.

Isolation of plasmid DNA

Alkaline lysis method was used for purification of plasmid DNA from isolated MDR *E. coli* bacteria. Agarose gel electrophoresis was performed for the separation of plasmid DNA on the basis of its size and shape

Conjugation

Lactose fermenting and ampicillin resistant donor *E. coli* bacterial culture was used for the transfer of plasmid DNA into non lactose fermenting ampicillin sensitive *Salmonella typhi* bacteria.

RESULTS & DISCUSSION

It was analyzed from 30 investigated samples depicted in Table 1 shows >240 coliforms/100 ml contamination in 40% of RO treated water samples which was highly contaminated and not fit for drinking purpose. In 7% and 3% of RO treated water samples, 21 coliforms /100 ml and 12 coliforms /100 ml were observed respectively which shows that the quality of water sample is unsatisfactory. 2.2 coliforms /100 ml were observed in 10% of water samples, which indicates that water sample is unsatisfactory. Zero coliforms were observed in 40% of RO+UV treated water sample. Kumar *et al.,* 2013 also observed that out of 26 water samples of water cooler, 18 samples were found to be unsatisfactory. He reported 90 coliforms /100 ml from water cooler which was non potable. Coliforms identified as *Escherichia coli* (Figure - 1 & 2), *Klebsiella pneumoniae, Enterobacter aerogenes* were present in 40%, 23%, 27% of samples, respectively while non lactose fermenting organisms identified as *Pseudomonas aeruginosa* and *Proteus* sp. were found in 13%, 10% of samples, respectively. Similar coliforms *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* were identified by Pathak *et al*., 2008, Sapkota *et al*., 2012 & Bergeron *et al*., 2015. *Escherichia coli*, *Pseudomonas aeruginosa* were detected from potable water in public water supply within Lagos University by Ojo *et al.,* 2008.

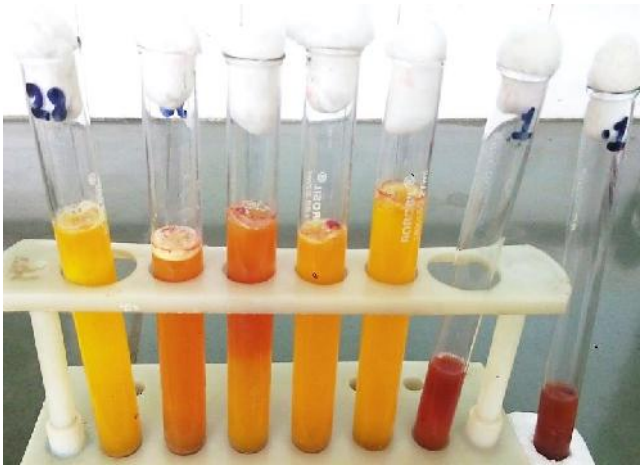

FIGURE 1: MPN analysis for coliforms (sample no. 28)

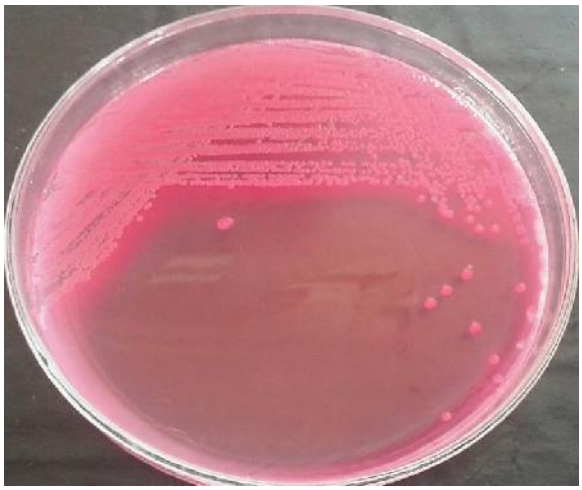

FIGURE 2: Confirmatory test for *E. coli* on MacConkey agar medium plate (sample no. 28)

Another important work of the present study was to check the prevalence of multiple drug resistance (MDR) in the bacteria isolated from water samples and transferability of the antibiotic resistance markers was also investigated in *Escherichia coli* (MDR) (Figure–3) isolate via conjugation process. The results of the antibiotic susceptibility of the bacteria showed that all the isolates were found to be resistant to Ampicilin while some isolates were resistant to Streptomycin, Gentamycin, Amikacin, Chloramphenicol, Ampicilin, Ofloxacin and Ciprofloxacin (Table 1). Figure -4 shows the percentage of antibiotic resistance of *Escherichia coli* (57%), *Klebsiella pneumoniae* (33%), *Enterobacter aerogenes* (38%), *Pseudomonas aeruginosa* (19%), and *Proteus* sp. (14%). Similar Antibiotic resistant bacteria were observed in treated drinking water by Bergeron *et al*., 2015. A large number of *E. coli* isolates (57%) exhibited resistance to Ampicilin (100%), Streptomycin (25%), Gentamycin (25%), Amikacin (16.66%), and Chloramphenicol (8.33%), Ofloxacin (8.33%) antibiotics (Figure– 5). Among all the isolates, *E. coli* (14.25%) was found to be multiple drug resistant bacteria. Walia *et al*., 2012 also isolated *E. coli* which was found to be resistant to Amikacin (8%), Streptomycin (49%) and Chloramphenicol (9%). Antibiotic resistant coliform bacteria are common in the intestine of man and as a result of sewage pollution they may become widely disseminated into the environment and transfer antibiotic resistance to other sensitive coliforms or enteric pathogens (Adesoji *et al*., 2013, Osterblad *et al*., 2000). Presence and survival of cultivable bacteria in drinking water can act as a vehicle to disseminate virulence genes to other bacteria. This can result in high morbidity and mortality, and the failure of the treatment of life threatening bacterial infections in humans and animals (Walia *et al*., 2012).

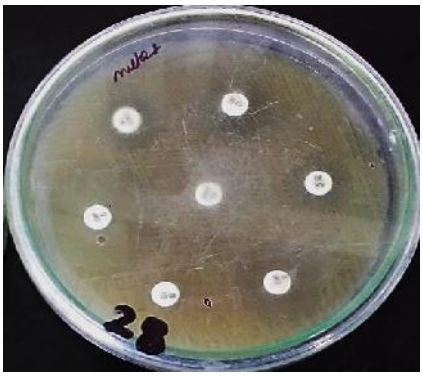

FIGURE 3: Antimicrobial susceptibility test for *E. coli* on Mueller Hinton agar medium plate (sample no. 28)

TABLE 1: Antibiotic resistance pattern of bacterial isolates obtained from water samples

Sample No.	Site of sample collection	Type of water Source	Total Coliform count /100ml (MPN)	Bacterial Isolates	Antibiotic Resistance
1	College Campus Nasia Road (office)	Water cooler With RO	>240	*E. coli* *Enterobacter aerogenes*	Ampicillin Streptomycin, Ampicillin, Ciprofloxacin
2	College Campus Nasia Road (quadrangle)	Water cooler With RO	>240	*E. coli*, *Klebsiellae*	Ampicillin
3	College Campus Nasia Road (comp. dept)	Water Cooler With RO	>240	*Klebsiellae*	Ampicillin
4	Railway Station	Water cooler	>240	*E. coli*	Ampicillin

		With RO			
5	Sarvate Bus Stand	Water cooler With RO	>240	*Pseudomonas* *Enterobacter aerogenes*	Ampicillin -
6	Chawani	Water cooler With RO	>240	*Klebsiellae*	Ampicillin
7	Dawa bazaar	Water cooler With RO	>240	*Enterobacter aerogenes* *Proteus*	Ampicillin Ampicillin, Ofloxacin
8	MR -10	Water Cooler With RO	12	*E.coli*, *Enterobacter aerogenes*	Ampicillin, Ofloxacin, -
9	Bapat Square	RO	21	*Klebsiellae*, *Enterobacter aerogenes* *Pseudomonas*	Streptomycin, Ampicillin Ofloxacin, Ampicillin, Sterptomycin -
10	Sanyogitaganj	Water Cooler With RO	21	*E.coli*, *Enterobacter aerogenes* *Klebsiellae*	Ampicillin Ampicillin -
11	Vijay Nagar	RO+UV	0	MPN –ve	-
12	Clerk Colony	Water cooler With RO	>240	*E.coli*, *Proteus*	Ampicillin Ampicillin
13	Bhandari Hospital (ground floor)	Water Cooler With RO	2.2	*Klebsiellae*	Ampicillin
14	Bhandari Hospital (1st floor)	Water Cooler With RO+UV	0	MPN –ve	-
15	Pardeshipura	Water Cooler With RO+UV	0	MPN –ve	-
16	Subhash Nagar	RO	2.2	*E.coli* *Pseudomonas*	Ampicillin Ampicillin, Chloramphenicol
17	Sudama Nagar	RO+UV	0	MPN -ve	-
18	Palasia	RO+UV	0	MPN -ve	-
19	Khandwa Road	Water Cooler With RO	2.2	*E. coli*	Ampicillin
20	Dewas Road	Water Cooler With RO	0	*Proteus*	Chloramphenicol, Ampicillin, Streptomycin
21	Veena Nagar	RO+UV	0	MPN -ve	-
22	Malwa Mill	RO	>240	*E. coli*	Chloramphenicol, Ampicillin
23	LIG Square	RO+UV	0	MPN -ve	-
24	Narayan Bag	RO	>240	*Enterobacter aerogenes*	Streptomycin, Ampicillin, Chloramphenicol, Amikacin
25	Maha Laxmi Nagar	RO	>240	*Enterobacter aerogenes* *Klebsiellae* *E. coli*	Ofloxacin, Ampicillin Ampicillin Streptomycin,Gentamycin, Ampicillin
26	Abhinandan Nagar	RO+UV	0	MPN -ve	-
27	Sukhliya	RO+UV	0	MPN -ve	-
28	Sudama Nagar	RO	>240	*E. coli*	Streptomycin,Gentamycin, Amikacin, Ampicillin
29	Manglaya	RO+UV	0	MPN -ve	-
30	Chotigwaltoli	RO	2.2	*E.coli* *Pseudomonas*	Streptomycin,Gentamycin, Amikacin, Ampicillin Streptomycin, Chloramphenicol, Ampicillin

In our study the extracted plasmid DNA of ˜ 1.8kb was isolated from this amp resistant *E. coli.* The plasmid DNA of amp resistant *E. coli* bacteria was successfully transferred to amp sensitive bacteria via conjugation process. Similar work was performed by Walia *et al.*, 2012, who observed that a majority of the multiple antibiotic resistant *E. coli* isolates contained one or more plasmids (size ranged ˜1.4 Kb to ˜40 Kb). The ABR (Antibiotic resistance) traits were transferable to other bacteria via conjugation. These data raise an important question about the impact of *E. coli* containing self-transmissible R-plasmids as a potential reservoir of virulence genes in drinking water.

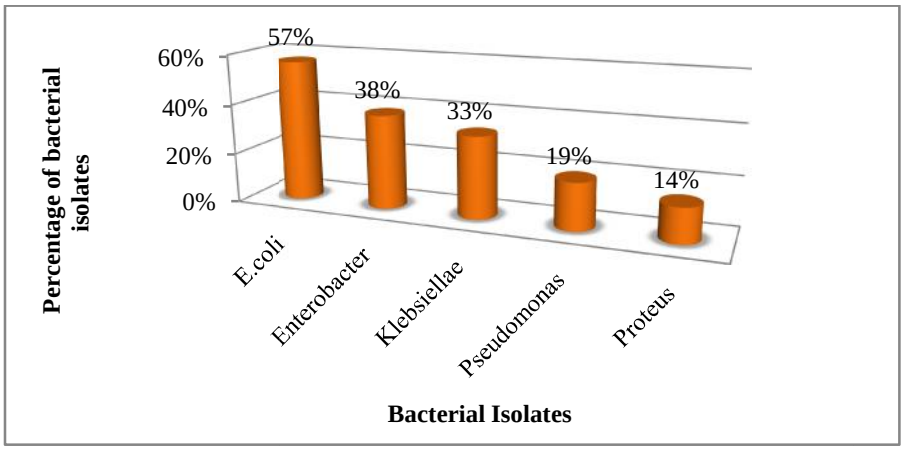

FIGURE 4: Percentage of bacteria isolated from water samples

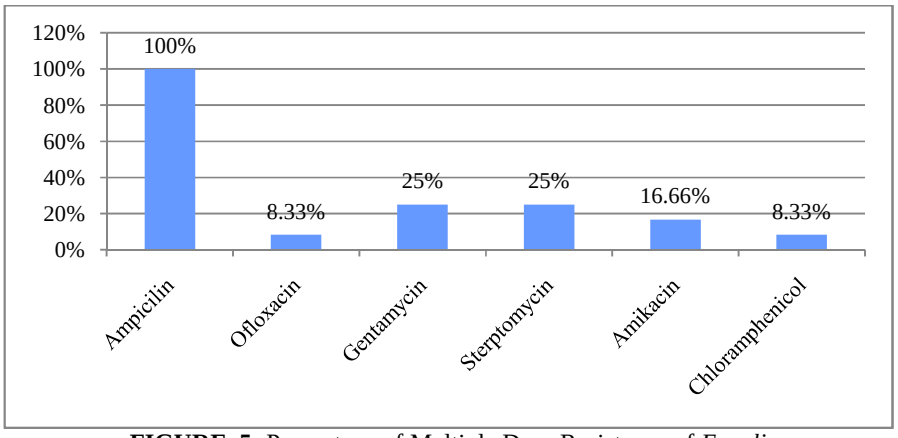

FIGURE 5: Percentage of Multiple Drug Resistance of *E. coli*

CONCLUSION

The results of bacteriological analysis of potable water showed that most of the samples were found contaminated and not fit for drinking purpose. Maintenance of RO system is essential for reliable performance. High level of TDS & microorganisms in the system are commonly the cause of fouled membranes. The treated water should be monitored for TDS and the level of any specific contaminants that may affect the quality of water and family's health. Occurrence and prevalence of these resistant strains in environment is a cause of concern as they can act as a vehicle to disseminate resistance to other bacteria. Much needs to be done to increase awareness of the hazards of drinking contaminated water and of ways to prevent contamination. All these drug resistant bacteria were isolated from RO water purifier which concludes that RO water samples was of poor microbial quality since RO only reduces hardness of water but not concerned with the killing of microorganisms while RO+UV treated water was found to be safest for drinking purpose because it purifies water by ultrafiltration and kills bacteria by UV processes.

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