Prevalence of Antibiotic Resistance in Enteric Pathogens in Drinking Water Supplies

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SYNOPSIS

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Introduction

Water is essential for life and the most important natural resource. Fresh water is a finite resource essential to agriculture, industry and even human existence, without fresh water of adequate quantity and quality, sustainable development will not be possible. Therefore, much of the current concern with regards to environmental quality is focused on water because of its importance in maintaining the human health and health of the ecosystem (Kumar, 1997).

Safe drinking water is essential to humans and other life forms. In both developed and developing countries water quality continues to be a major health concern. Access to safe drinking water has improved over the last decades in almost every part of the world, but approximately one billion people still lack access to safe water and over 2.5 billion lack accesses to adequate sanitation (MDG Report, 2008). Environmental waters that are used as sources of drinking water may be contaminated with a wide range of pathogenic microorganisms including enteric pathogens intermixed with a dominant background of naturally occurring non-pathogenic microbial populations. Enteric pathogen are gastrointestinal organisms which may be present naturally in aquatic environments or, more commonly, are introduced through human activities such as leaking sewage and septic systems, urban runoff, and in the case of estuarine and marine waters, sewage outfall and wastewater discharge, insufficiently treated water, drinking water and private wells that receive treated or untreated wastewater either directly or indirectly (Fong and Lipp, 2005). Also, the occurrence and spread of Antibiotic Resistant Bacteria (ARB) are pressing public health problems worldwide, and is an emerging issue for the general public and drinking water industry (Armstrong *et.al.*, 1982; Schwartz *et.al.*, 2003). However little is known about the fate of Antibiotic Resistance Genes (ARGs) in drinking water system. It had been proposed that ARGs are emerging contaminants (Pruden *et.al.*, 2006).

Many studies have been done to examine the quality of water to determine the prevalence of waterborne disorders of bacterial origin, especially in developing countries like India (Saxena and Chhabra, 2004; Rajendran *et.al*., 2006; Pant and Mittal, 2007). Knowing the importance of water for sustenance of life, the need for conservation of water bodies especially the fresh water bodies had been realized everywhere in the world. This need has added significance to water stressed region such as Rajasthan (Gupta *et.al.*, 2011).

Rajasthan is the driest state of the country with a current population of 5.6 crores. Jaipur city which is a metropolis with a population of 23.24 lakhs has the highest growth rates amongst the metropolitan cities in India. All the 183 municipal towns have piped water supply system out of which only 23 towns get more than 100 liters per capita of daily water supply against the desired standard of 135. The current high population growth rate, urban sprawl, increase in irrigation demand and with the growth of industrialization, causes increase in the water pollution and water demand that at present exceeds the available water resources (RWSRP, 2009).

Therefore, it is important to investigate the prevalence of microbial pathogens and antibiotic resistant genes in drinking water to protect public from outbreaks of waterborne disease and for preservation and promotion of public health.

REVIEW OF LITERATURE

Water borne Diseases- A Threat to the Health

Water might be the source of life but in many parts of the world, it can also be the cause of death. The developing countries with poor sanitation are fertile grounds for the spread of diseases affecting health of millions of people around the world (Malhotra, 2009).

Waterborne diseases are caused by pathogenic microorganisms which are directly transmitted when contaminated fresh water is consumed. Waterborne diseases of major public health concern include typhoid caused by *Salmonella typhi*, Cholera by *Vibrio cholerae,* Dysentery by *Shigella*, Diarrhea by Protozoan *Giardia*, Acute infantile Diarrhea by rotavirus and jaundice by hepatitis A and E viruses (Singal and Sood, 1997). In India, 1 million people loose their lives to Diarrhea every year. The World Bank estimates 21% of communicable diseases in India are water related, of these diseases, Diarrhea alone killed over 70,000 Indians in 1999(estimated) over 1,600 deaths each day (WHO, 2002). There are over 100 different types of bacteria and viruses that can be found in contaminated water (groundwater and surface waters). Contaminated drinking water due to inadequate and unsanitary disposal of sewage and excreta continue to pose a threat to the health in many communities all around the globe. While correctly operating water treatment facilities will remove the vast majority of microbial contaminants, one of the best protective measures against waterborne disease outbreaks is to protect watersheds from contamination in the first place. An important aspect of such protection is the ability to detect pathogens with specific and a sensitive assay. Therefore, it is important to critically examine the occurrence of these waterborne pathogens.

Over the past 15 years, the polymerase chain reaction (PCR) and other molecular biology based techniques have begun to revolutionize the detection of pathogenic bacteria, viruses and protozoa in clinical and environmental samples.

Global Burden of Waterborne Diseases

The disease burden from water, sanitation, and hygiene at the global level has taken into account various disease outcomes, principally diarrheal diseases. Pruss *et.al.,* (2002) estimated that water, sanitation, and hygiene was responsible for 4.0% of all deaths and 5.7% of the total disease burden occurring worldwide (accounting for diarrheal diseases, schistosomiasis, trachoma, ascariasis, trichuriasis, and hookworm disease).
According to World Development Report (1992), Diarrheal diseases traceable to contaminated water world wide has been estimated to cause 900,000,000 episode of illness per year and approximately 2,000,000 deaths per year in children. Among the diseases caused by waterborne pathogens , such as *Vibrio cholera*, Hepatitis E virus and *Escherichia coli O 157:H7*, are known to have high mortality rates (Hunter, 1997).On global basis, morbidity and mortality from *Escherichia coli* are thought to exceed those of cholera and others identified waterborne disease. *Enterotoxigenic E.coli* (ETEC) is estimated to cause approximately 400

million diarrheal episodes, with 700,000 deaths each year among children younger than five (Chakraborty, 2001).

Since (2002), World Health Organization (WHO) has identified risk factor for the global burden of disease (Lopez *et.al.,* 2006). World Health Organization (WHO, 2008), estimates that diarrheal disease accounts to an estimated 4.1 percent of total DALYs (Disability-adjusted life years) and is responsible for the deaths of 1.8 million people every year. World Health Organization recently stated (July 2011) that despite significant progress made in recent years and the availability of many technically feasible and low-cost solutions, almost one in five people in South Asia still lack improved water resources and over two million people die due to wate-borne diseases like typhoid and cholera annually. According to the report 90 percent of wastewater discharged daily in developing countries is untreated, contributing to the deaths of some 2.2 million people a year from diarrheal diseases caused by unsafe drinking water and poor hygiene.

It can therefore be understood that these water-borne diseases are a great burden to the health system.

Common Water borne Diseases Outbreaks

In recent years, investigators have identified a large number of pathogens responsible for outbreaks and research has focused on their sources, resistance to water disinfection and removal from drinking water.

Water-related outbreaks of diseases are frequently caused by the consumption of water that is contaminated with human or animal fecal material and have been reported from various parts of world like Nakuru, (Chabalala and Mamo, 2001), Nigeria (Oguntoke *et.al.,* 2009), United States (Craun *et.al*., 2006; Craun and Wade, 2008) and New Zealand (Till *et.al.*, 2008) . In India, various water-borne outbreaks have also been reported from Rajasthan (Saxena and Chhabra, 2004), Pune (Sahasrabuddhe *et.al.,* 2003), Tamil Nadu (Rajendran *et.al.,* 2006), and Delhi (Pant and Mittal, 2007). Researchers have investigated the presence of more than 140 microorganisms, that are known to be associated with waterborne diseases including bacteria (*Campylobacter, Escherichia coli* and *Vibrio cholera*), protozoa (*Cryptosporidium and Giardia*) and Viruses (Norovirus [NoV], Adenovirus [ADV], Hepatitis A virus, and Enterovirus [EV]) (Reynolds *et.al.,* 2008). Even with the advancement of drinking water (DW) treatment processes (e.g., membrane filtration and alternative disinfectants, such as

ozone or UV radiation) and more stringent regulatory requirements , waterborne disease outbreaks (WBDOs) associated with drinking water still occur in the United States (Liang *et.al*., 2006;Yoder *et.al*., 2008). Among the diseases, diarrhea, dysentery, jaundice and typhoid are some of the most common water borne diseases that have been reported (Chabalala and Mamo, 2001; Rajendran *et.al.,* 2006; Craun and Wade, 2008). These outbreaks were observed to be seasonal, with highest incidence during the summer, followed by winter and monsoon (Saxena and Chhabra, 2004). A most extensive study has been done on Recreational waterborne outbreaks between 1995 and 2004 in United States. It was noted that the most frequently associated illness type was the gastroenteritis and of these 71% were attributed to a bacterial or protozoan source and 8% of the illnesses were attributed to viral exposure (Craun and Wade, 2008). Above studies suggests that Pathogenic bacteria and enteric viruses can be introduced into the environment via human waste discharge. Thus methods for rapid detection and quantification of human viruses and fecal indicator bacteria in water are urgently needed to prevent human exposure to pathogens through drinking and recreational waters.

Detection of Enteric Bacteria in Water Resources

Indicator bacteria such as *E.coli* are commonly used to determine the relative risk of fecal contamination and also used to assess the potential public health risk of drinking water and their presence or absence, are key elements of most drinking water quality guidelines (WHO, 1997). Indicator organisms, however, have several disadvantages that make them less ideal for indicating the possible presence of microbial pathogens. Consequently methods to directly detect microbial pathogens in water and wastewaters are being investigated. Polymerase chain reaction (PCR) is one of the main alternative detection methods being trialed (Toze, 1999). This technique has rapidly evolved over the last few years and the growing interest in quantitative applications of the PCR has favored the development of real-time quantitative PCR which have been used by researchers to identify the bacterial pathogens. Many studies have been done to examine the quality of water to determine the prevalence of waterborne disorders of bacterial origin, especially in developing countries like India. Some of the common water borne bacteria comprises *Escherichia coli, Salmonella sp., Vibrio cholerae, Clostridium sp., Pseudomonas sp., Campylobacter sp., Shigella sp., Arcobacter sp.* and *Aeromonas hydrophila*. Researchers around the globe are working to make the detection of these bacterial pathogens sensitive, specific and rapid.

Escherichia coli are used as indicator of water safety regarding fecal contamination in almost all water quality legislation in the world. Culture techniques have been routinely used for the examination of the presence of *E.coli*. These methods were robust and sensitive, but the lack of speed is an important drawback. Therefore different PCR methods have been developed to directly detect *E.coli* from water samples (Bej *et.al*., 1991; Frahm and Obst, 2003; Heijnem and Medema, 2006.) *Escherichia coli* have been detected in water samples using primers specific for *lacZ* gene which produces an amplified product of size 180 bp (Shaban and Malkawi, 2007) using Conventional PCR. Also *uidA* gene of 147 bp which encodes for the β-D-glucuronidase enzyme has been reported to be specific for respective bacteria (Bej *et.al*., 1991; Tsai *et.al.,* 1993; Heijnen and Medema, 2006; Momba *et.al.,* 2006). Ram and Shanker (2005), suggested the rapid assessment of virulence pattern of *E.coli* isolates by Multiplex Real –Time PCR probes like TaqMan. *E.coli* virulent genes like Shiga like toxin type 1 (*stx1*) gene of 102 bp, Shiga like toxin Type2 (*stx2*) gene, Enterohemolysin (*hlyA*)gene of 141bp , Heam iron transport (*chuA*) gene of 147 bp, Attaching and effacing proteins (*eae*)gene of 200bp and House keeping Genes like β- galactosidase (*lacZ*)gene of 228 bp , Maltose Transport Protein (l*amB*)gene of 86bp and Fimbrial Major Subunit type1 (*fimA*)gene of 79 bp were chosen based on proven role in *E.coli* Pathogenicity using Taq Man probes for detection of Enterohemorrhagic *E.coli* (EHEC). The study concluded that multiplex Real-Time PCR oligomers and TaqMan probes designed and validated *in silico* will be helpful in management of water quality and outbreaks, by improving specificity and minimizing time needed for *in vitro* verification work.

Escherichia coli serotype O157:H7 have been detected from the Ganges River using *stx1*, *stx2,* and *eae* target gene sequences using conventional PCR (Hamner *et.al*., 2007). Similar study have been conducted by Mull and Hill (2009), in surface water using hollow-fibre Ultrafilteration (UF) and real time PCR using Shiga toxin genes, *stx1* and *stx2* (Bellin *et.al*., 2001; Heijnen and Medema, 2006) and *rfbE* gene Targets (Fortin *et.al.,* 2001; Heijnen and Medema,2006) which encodes for an enzyme involved in biosynthesis of O157 antigen .He also suggested that in conjunction with the UF culture, a suite of real time PCR assays can be effectively used for specific detection of Enterohemorrhagic *E.coli* (EHEC). Sensitive detection and quantification of Enterotoxigenic *Escherichia coli* (ETEC) in different water samples using enterotoxin genes *estA (STh), estB (STp*) and *eltB* have also been reported by Lothigius *et.al.*(2004) and Singh *et.al.* (2010).

Vibrio cholera is the etiological agent of epidemic cholera, which causes watery diarrhea that can result in the rapid dehydration and death of infected persons. Coastal waters are an important reservoir of *V. cholerae*, and cholera is generally transmitted to humans via water or seafood (Colwell *et.al*., 1977; Colwell *et.al*., 1981). Several PCR protocols have been developed for *V.cholerae* (Lyon, 2001; Singh *et.al*., 2001; Lipp *et.al*., 2003; Gubala, 2006; Gubala and Proll, 2006). However these reports mostly describe conventional, time consuming and laborious methods of PCR product characterization. Therefore real time PCR for detection of *V.cholerae* have been developed and most commonly detected in both surface and ground water sources (Momba *et.al.*, 2006). Use of this technique provides a sensitive and cost-effective monitoring of environmental and drinking water samples (Gubala and Proll, 2006). Bacteria have been detected by targeting various virulence and regulatory genes *(ctxA, stn, OmpW and tcpA*). Collectively, the four unique gene targets cover a range of gene sequences essential for the virulence and survival of *V.cholerae* (Gubala and Proll, 2006; Sharma and Chaturvedi, 2006).

Fykse *et.al*.(2007), claimed to be first to described a sensitive multitarget real- time Nucleic Acid Sequence-Based Amplification (NASBA) application for the specific detection of *V.cholerae* cells in water. The genes encoding the 151 bp fragment of cholera toxin *(ctx A) ,* 102 bp fragment of the toxin coregulated pilus (*tcp A;* colonization factor), 151 bp fragment of the *ctx A* toxin regulator *(tox R),* 135bp fragment of Hemolysin (*hy A)*, and 116 bp fragment of the 60 K Da chaperonin product *(groEL)* were selected as target sequences for detection. They reported that general markers *groEL* and *toxR* detected all *V.cholerae* strains. The sensitivity of the *groEL* assay was 10 fold higher than that of *tox R* assay. The study suggests that in order to detect toxigenic strains, *tcpA* and *ctxA* markers should be used and the combination of *groEL* , *toxR*, *tcpA* and *ctxA* markers provide maximum specificity for the detection of all *V.cholerae* strains. The author also reported that the NASBA assay detected *V.cholerae* at 50 CFU/ml by using the genetic marker *groEL* and *tcpA* that specifically indicates toxigenic strains.

Salmonellosis accounts for 60% of all bacterial disease outbreaks in the United States (Feng, 1992). A number of PCR assays specific for *Salmonella* have been developed (Cohen *et.al*., 1992; Jones *et.al*., 1993; Bej *et.al*., 1994). Real time PCR have been conducted using molecular beacon (Tyagi and Kramer, 1996; Chen *et.al*., 2000) as well as Taqman probes (Chen *et.al*., 1997). Salmonella has been detected in various water sources (Abd-El-Halem

et.al., 2003*)* mostly in groundwater samples (Momba *et.al.*, 2006).The genes coding for 16S rRNA , 314 fragment of *ipaB* gene encoding the invasion plasmid antigen B (Kong *et.al*., 2002; Momba *et.al*., 2006), 288 bp fragment of the *invA gene,* and122 bp fragment of *himA gene (Bej et.al., 1994)* have been used for the detection of the strain (Fey *et.al*., 2004; Chen *et.al.,* 2000).

Pseudomonas aeruginosa is a ubiquitous environmental organism as it is capable of growing in a wide variety of niches with a preference for moist environments and a major opportunistic pathogen causing human infections. Schwartz *et.al.* (2006), performed Real-time quantification of *Pseudomonas aeruginosa* in various waste water systems including clinical, municipal wastewaters and inflow from a wastewater treatment plant. For this they investigated various wastewaters by real-time PCR with an optimized primer and probe design specific for the 23S rRNA gene, they detected that clinical wastewaters contains highest concentration of *Pseudomonas aeruginosa* .

Hernandez *et.al*. (1995), examined two extraction procedures, and they discovered that DNA recovered from *Campylobacter jejuni* lysed by the Cetyltrimethylammonium bromide (CTAB) method was more suitable for use as a PCR template than DNA released by the boiling method. RT-PCR amplification was conducted using a 1.73 Kb portion of the *flagellinA* gene of *C. jejuni*. Also a VS gene region comprising an 189bp fragment (Yang *et.al*., 2003) and 72 bp fragment of *mapA* gene (Price *et.al.,* 2006; Ahmed *et.al.,* 2008) were used in real time PCR assay.

Presence of *Shigellae* in surface water has been analyzed using PCR. The gene targets used for the identification were the *Shigella*-specific virulence genes including *ipaBCD, ipaH,* and *st*x1 (Faruque *et.al*., 2002).

Aeromonads are gram- negative water borne bacteria. Altwegg (1996) ; Brandi *et.al*. (1996); Janda & Abbott (1998), reported the presence of the aeromonads in environmental habitats such as soil, fresh and brackish water , sewage and wastewater, untreated and treated drinking. Conventional PCR have been used to investigate the occurrence of potentially pathogenic *Aeromonas hydrophila* strains in seawater (Asmat and Gires, 2002). The gene target used for the amplification of this bacteria includes 683 bp fragment of the *aerA* domain , 130 bp fragment of gyrase B subunit (*gyr B*) gene (Asmat and Gires, 2002; Khan *et.al.,* 2009) and 760bp fragment of *lip* gene (Cascon *et.al*., 1996; Ahmed *et.al*., 2008).

The 16S and 23S rRNA sequence specific primers have been used for the detection of *Arcobacter* and *Campylobacter* strains in river water and wastewater samples. Both of these rRNA-based techniques have been found to be as quick and sensitive methods for detection of *Campylobacters* in environmental samples (Moreno *et.al.,* 2003). Similarly Diergaardt *et.al.* (2004), examined the occurrence of *Campylobacters* and *Arcobacter butzleri* in drinking and environmental water sources by 16S rRNA sequence analysis.

Microbial pathogens are one of the major health risks associated with water and wastewaters, above studies showed that the PCR technique is rapid, specific and quick way to detect pathogens from different sources of water.

Antibiotic Resistance Genes As Emerging Contaminants

The occurrence and spread of Antibiotic Resistant Bacteria (ARB) are pressing public health problems worldwide. Many researchers have highlighted aquatic ecosystems as a recognized reservoir for ARB and Antibiotic Resistance Genes (ARGs) (Cooke, 1975; Gonzal *et.al.*, 1979; Klare *et.al*., 1995; Kummerer, 2004; Martinez, 2008; Baquero *et.al.*, 2008; Zhang *et.al*., 2009). However little is known about the fate of ARG in drinking water systems, and it has been proposed that ARGs are emerging contaminants (Pruden *et.al.*, 2006).

Presence of several antibiotic resistance gene like tetracycline resistance genes, *tetM, tetO, tetQ, tetW, tetC, tetH, and tetZ* , ciprofloxacin resistance genes (*gyrA) ,* vancomycin resistance gene *(vanA)*, ampicillin resistance gene *(ampC),* methicillin resistance gene *(mecA)* of E*nterococci, Enterobacteriaceae,* and *Staphylococci* and Kanamycin (*kan*) and ampicillin (*amp*) resistance genes have been reported in different municipal wastewater and drinking water samples (Volkmann *et.al.,* 2004; Koike *et.al*., 2007 ; Samra *et.al*., 2009).

Samra *et.al*.(2009), investigated the prevalence of Kanamycin (*kan*) and ampicillin (*amp*) resistant bacteria in public drinking water of Lahore Metropolitan, Pakistan. Among 625 drinking water samples, 42.5 % and 57.5 % of samples were found positive for *kan* and *amp* resistant bacteria and the results were confirmed by amplification of 810 bp *kan* resistant gene and 850 bp *amp* resistant gene. *Salmonella, Shigella, Staphylococcus, Streptococcus* and *E.coli* accounted for 13%, 30%, 11%, 17% and 29% respectively in *kan* and *amp* resistant bacteria.

The other antibiotic resistance genes investigated includes 573 bp fragment of erythromycin resistance gene (*erm B),* 733 bp fragment of methicillin resistance *(mecA),* 825 bp fragment

of extended β lactam resistance conferring broad resistance to penicillins and cephalosporins *(blaSHV-5),* 822bp fragment of ampicillin resistance *(ampC),* 738 bp fragment of tetracycline resistance *(tetO)* and 572 bp fragment of vancomycin resistance *(vanA)* (Bockelmann *et.al*., 2009). Xi *et.al.* (2009), also detected Antibiotic Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARGs) in source waters, drinking water treatment plants and tap waters from several cities in Michigan and Ohio. The ARGs examined includes beta-lactam resistance genes (bla_{TEM} *and* bla_{SHV}), Chloramphenicol resistance genes (*cat and cmr*), Sulfonamide resistance genes (*suII and suIII*) and tetracycline resistance genes (*tetO and tetW*).They detected that the ARGs and ARB are present in small quantities in finished water and tap water and the quantities of ARGs were found to be greater in tap water than in finished water and source water. Elevated resistance to some antibiotics has been observed during water treatment and in tap water. They suggested that water treatment might increase the antibiotic resistance of surviving bacteria, and water distribution systems may serve as an important reservoir for the spread of antibiotic resistance to opportunistic pathogens.

Tenover (2006), suggested that bacteria may inherit resistance to some antibiotics or can develop resistance via spontaneous mutation or the acquisition of resistance genes. The acquisition of a resistant gene via horizontal gene transfer is the most common and the easiest way for bacteria to develop antibiotic resistance both in the environment and in a host (Rowe-Magnus and Mazel, 1999; Salyers *et.al*., 2004).

Molecular Subtyping of Waterborne Bacteria

The microbiological contamination of water by pathogenic microbes has been and is still, a persistent public safety concern in the countries of the world. As most enteric pathogens are transmitted through the fecal oral route, fecal pollution is generally regarded as the major contributor of pathogens to waterways. Fecal indicator bacteria have been used successfully as the primary tool for microbiologically based risk assessment. However measurement of fecal indicator bacteria does not define what pathogens are present, or define the sources of these bacteria (Yan and Sadowsky, 2007). Therefore to improve public health and to ensure safe life conditions, the development of a method of analysis for tracking sources of fecal contamination in aquatic environments is required.

The process of subtyping was found to be important epidemiologically for recognizing outbreaks of infection, detecting the cross-transmission of nosocomial pathogens,

determining the source of the infection, recognizing particularly virulent strains of organisms, and monitoring vaccination programs (Olive and Bean, 1999). Restriction fragment length polymorphism (RFLP) is one of the most frequently used molecular subtyping tools in epidemiologic investigations. While there are different approaches to RFLP, pulse-field gel electrophoresis (PFGE) has been shown to be a reliable and highly discriminating method for subtyping foodborne pathogens and other bacteria (Georing, 2004).

Numerous studies which define the banding patterns of a variety of bacteria digested with a number of restriction enzymes have been conducted. PFGE is a subtyping method that detects polymorphism in restriction fragments of genomic DNA. Bopp *et.al.* (2003), analyzed the patient and environmental isolated of waterborne *E.coli O157:H7* and *C.jejuni* in United States. Pulsed-field gel electrophoresis (PFGE) was performed using *XbaI* gene. PFGE of *C.jejuni* isolates revealed that 29 of 35 (83%) had indistinguishable PFGE pattern. Similarly Ribot *et.al*. (2006), performed subtyping of *Escherichia coli O157:H7*, *Salmonella* and *Shigella* using *XbaI* gene for detection of foodborne pathogens for PulseNet.

Kuusi *et.al*. (2004), determined the source and the extent of community wide outbreak of gastroenteritis from a non-chlorinated water supply. Subtyping of *Camphylobacter* strains has been done using Pulsed- field gel electrophoresis. Eight patient isolates and water isolate were genotyped by PFGE using *SmaI, SacII* and *kpnI* enzyme for digestion of DNA. They reported that the water isolate and all but one of the patients isolate were indistinguishable by PFGE. In another study by Borchardt *et.al.* (2003), pulsed- field gel electrophoresis pattern of *Aeromonas* isolates using *SmaI* gene from stool specimens of patients with diarrhea was compared with *Aeromonas* isolates from patients drinking water. They showed that the stool and drinking water isolates were genetically unrelated and suggested that *Aeromonas* gastrointestinal infections were not linked with groundwater exposures. The above studies concluded that the Pulsed-field gel electrophoresis is reproducible and has sufficient discriminatory power to allow detection of minor genetic variations among isolates.

Thus typing of bacterial isolates from different sources is a pre-requisite for intervention and infection control and to contribute to risk assessment studies of sources of water borne diseases.

We hypothesize that drinking water may contains enteric pathogens (bacteria) and also carries genes for antibiotic resistance, therefore detection as well as quantification of enteric pathogens is required. Detection of these pathogens will allow the scientists, industry, and

environment managers to assess the burden of these organisms in water bodies and provide a baseline for developing management plans.

OBJECTIVES OF RESEARCH

- To Determine the Presence and Abundance of Enteric Pathogens to Assess the Quality of Drinking Water.
- Prevalence of Antibiotic Resistance Genes in Enteric Pathogens and their Molecular Subtyping.

Methodology

To determine the presence of enteric pathogens to access the quality of drinking water.

- **Sample Collection:** Water samples will be collected in pre sterilized containers from different areas in Jaipur City. At least 5-10 liters of water will be collected to ensure proper detection. All the water samples will be transferred on ice to the laboratory and will be stored at 4^{o}C till further use.

- **Physico-chemical Characterization of Water:** Physico-chemical characterization will be done for several parameters using standard methods (APHA, 2005).

- pH,
- Electric Conductivity,
- Total dissolved Solids,
- Nitrate,
- Fluoride,
- Chloride.

➢ **Molecular identification of Pathogens**

- **Total Genomic DNA Isolation**: Genomic DNA will be directly isolated from collected water samples.

- **PCR Detection of Bacterial pathogens**: Polymerase chain reaction will be carried out for the bacterial universal 16S rDNA sequence and for eight bacteria chosen for the study using the specific primers.

Table1: Genes Selected For Detection Of Bacteria Using PCR.

Bacteria	Gene Target	Product Size
Universal for Bacteria	*16SrDNA*	1500

Escherichia coli	*LacZ*	180
Salmonella	*Random fragment*	429
Vibrio cholerae	*ctxA*	564
Arcobacter	*16SrRNA*	331
Camphylobacter	*16SrRNA*	439
Pseudomonas	*oprl*	504
Shigella	*ipaH*	300
Aeromonas	*aerolysin*	683

- **Molecular Quantification of the Identified Pathogens**

For Quantification of bacteria, Real-Time PCR will be performed using Primers and Probes specific for Bacteria.

Detection of Antibiotic Resistance Genes in Enteric Bacteria and their Molecular subtyping

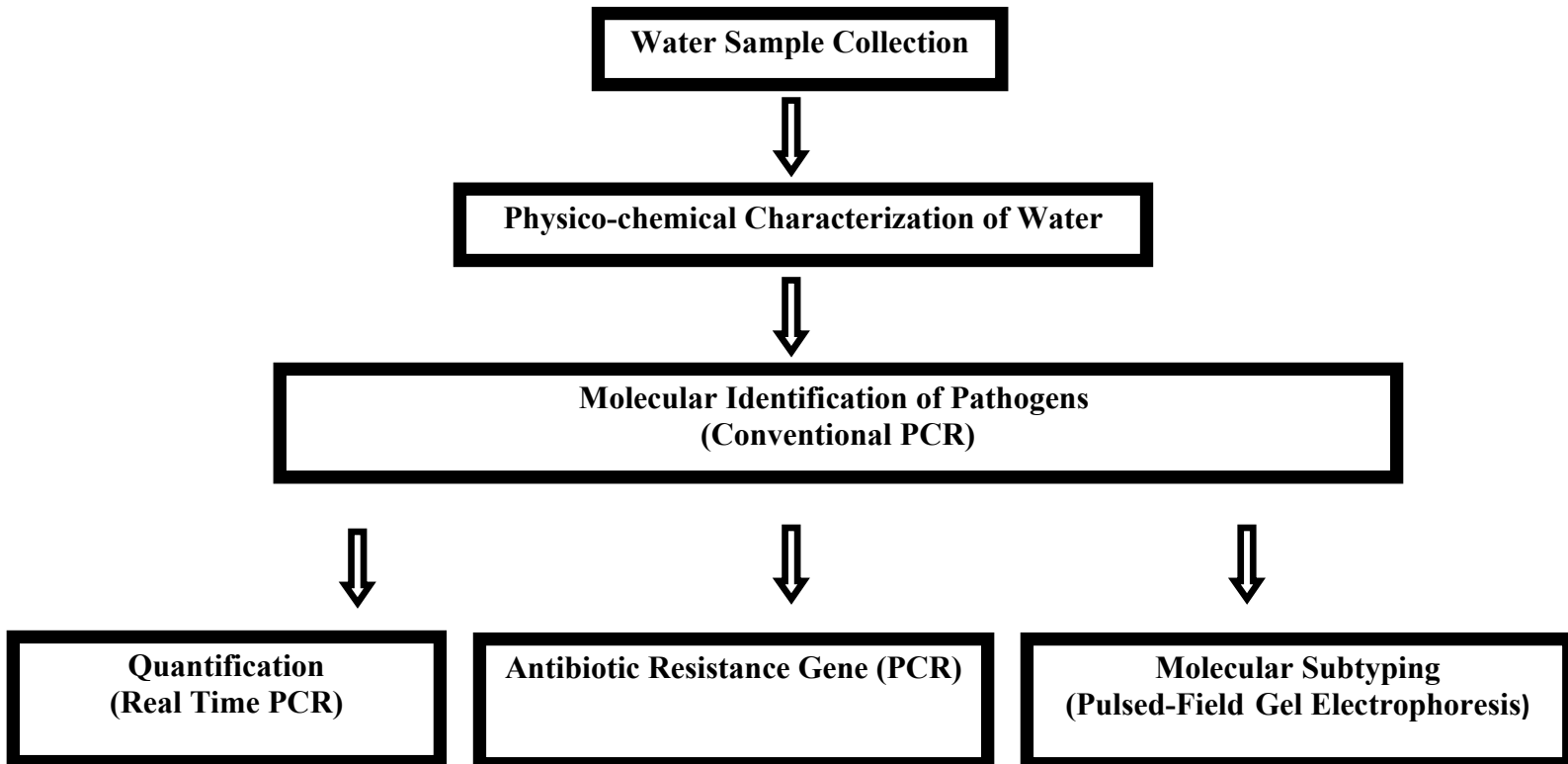
- **Detection of Antibiotic Resistance Genes (ARGs)**

For the detection of Antibiotic Resistance Genes, detected bacteria will be grown on selective media. DNA will be isolated and PCR amplification will be performed using selected ARGs specific primers.

- **Molecular Subtyping**

DNA will be digested with suitable enzymes and then Pulsed-field gel electrophoresis (PFGE) will be performed for molecular subtyping.

PLAN OF WORK


Water Sample Collection
Physico-chemical Characterization of Water
Molecular Identification of Pathogens
(Conventional PCR)
Quantification
(Real Time PCR)
Antibiotic Resistance Gene (PCR)
Molecular Subtyping
(Pulsed-Field Gel Electrophoresis)

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