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MICROBIOLOGICAL STUDY OF DRINKING WATER: QUALITATIVE AND QUANTITATIVE APPROACH

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Abstract

Consumption of drinking water contaminated with fecally originated pathogenic bacteria is mostly responsible for the onset of water borne disease outbreaks especially in developing countries. Current study attempted to analyze 25 treated drinking water samples both qualitative- and quantitatively from different areas of Alsisar block, jhunjhunu where 90% cases of diseases (dysentery, typhoid, cholera and diarrhea) have long been reported due to the water borne microorganisms diseases. Through the most probable number (MPN) method, 5 samples out of 25 were found to be non-potable as they had been contaminated with Escherichia coli indicating the risk for fecal contamination responsible for disease outbreaks. Other contaminating Gram negative bacteria were characterized as Klebsiella spp., Alcaligenes faecalis, Pseudomonas spp. and Aeromonas spp. Interestingly the presence of E. coli was detected in the same 5 samples within a range of 3.0×102 to 1.7×105 cfu/ml by the conventional cultural and biochemical methods. Moreover, a huge array of other pathogenic bacteria was also detected (5.0×102 - 3.5×108 cfu/ml) through this method. Further detection of drug resistance traits among the bacterial isolates would be of public health significance.

Key words: MPN (Most Probable Number), Fecal contamination, Indicator bacteria, Pathogens, Drinking water

INTRODUCTION

Contamination of drinking water sources both by biological or chemical contaminants has been a quite common scenario in an over-crowded country. Both ground water and surface water may get microbiologically contaminated by a variety of means including agricultural runoff, sanitation difficulties, accumulation of minerals, etc. (Geldreich, 1990; Grabow et al., 1996; WHO, 2008). Leakage of sewage pipelines or improper management of waste disposal may account for the ground water contamination (AWWA, 1971; Acharjee et al., 2013). Inadequate processing of the water may result in gastroenteritis (i.e., cholera, diarrhea, dysentery, etc.) in the consumers caused by the enteric pathogens including Escherichia coli, Salmonella spp., Shigella spp., Vibrio spp. and others (Cray and Moon, 1985; Cunningham, 2005; Acharjee et al., 2011; Nahar et al., 2011; Munshi et al., 2012; Acharjee et al., 2013). Presence and transfer of drug resistance traits among the pathogens present in drinking water may hinder medication and thus may impart a deep impact on the overall public health situation

(Schwartz, 2003; Canton, 2009; McAurthur et al., 2011). Presence of coliforms and other pathogens in treated drinking water may be due to ineffective or poor application of water treatment techniques (McFeters et al., 1986; Kamal et al., 1999; Mead et al., 1999; Subramania, 2004; Nahar et al., 2011; Acharjee et al., 2013). As coliforms are the indicators of bacterial contamination, their presence indicates fecal contamination of water which may account for severe water borne fatal diseases. Rapid detection of coliforms in drinking water is therefore necessary which can easily be carried out by MPN (Most Probable Number) method, a qualitative test to detect coliform and thereby to determine simply the potability or safety of water (Goel et al., 2007; Abera et al., 2011). This test is incorporated with three sequential steps: presumptive test, confirmed test and completed test (Cuppuccino and Sherman, 1996). The detectable gas production in the presumptive test is recognized as coliform positive from which the presence of E. coli can be confirmed by observing green metallic sheen on eosin methylene blue (EMB) agar plate followed by Gram staining procedure as the completed test. However, MPN method can only determine the presence of coliform bacteria, but cannot estimate the actual load of coliform and other pathogenic bacteria in drinking water (Rompre et al., 2002). The enumeration and identification of the pathogens are usually carried out by means of cultural and biochemical techniques, often followed by molecular studies or specific antigen detection (Acharjee et al., 2013; Ngwa et al., 2013). To confer safety of water, the World Health Organization (WHO) has already took initiatives to promote preventive approaches for water quality management using Water Safety Plans (WSP) and support the development of appropriate sanitation technologies for flood and other disaster prone areas, including ecological sanitation (WHO, 2008). Prompt and proper determination of microbiological quality of water can further aid in such approaches and in taking appropriate action by the government and other organizations to ensure safety of drinking water across the country. Along these lines, present study attempted to project a model on the overall microbiological quality of drinking water randomly collected from different areas of Dhaka city in both qualitative and quantitative means. We measured the quality of drinking water samples as potable or not by detecting the indicator bacteria through MPN technique as well as quantified the load of pathogenic bacteria present in the samples by conventional culture and biochemical tests to excel the accuracy of our study findings.

METHODS AND MATERIALS

Study area and sampling Drinking water consumed by the habitants of different areas of the alsisar block, jhunjhunu after some home-based easy treatments (boiling, sieving and filtration) were tested in this experiment. Twenty-five processed drinking water samples were collected aseptically in sterile plastic bottles kept at 25 o C to maintain homeostasis (Acharjee et al., 2013) in a thermal stabilizer boxand carried to the laboratoryas soon as possible to start themicrobiological assays. Qualitative analysis through MPN method Presumptive Test Presumptive test is the very first portion of the MPN test method. This is primarily done for detection of the Gram negative coliform bacteria in the water samples. For this task, 15 series of test tubes containing 10 mL of lactose fermentation broth were needed for each sample. Ten mL, 1 mL and 0.1 mL samples were added sequentially in 5 test tubes containing 10 mL lactose fermentation broth 2X, 5 tubes containing 10 ml lactose fermentation broth 1X and 5 test tubes containing 10 ml 1X lactose fermentation broth. Each tube was incorporated with a Derhum tube indicating gas formation after lactose fermentation by coliform bacteria (Cappuccino and Sherman, 1996). Confirmed test The test tubes showing positive results by the accumulation of gas in the Derhum tubes were selected for the confirmed test to determine the presence of E. coli in the respective water samples. The loopful sample from the broths which gave positive result in the presumptive test, were inoculated on EMB agar to detect as well as differentiate E. coli and other Gram negative coliform bacteria. The plates were incubated at 37o C for 24 hours (Cappuccino and Sherman, 1996). Completed test This is the final part of the MPN test procedure which was completed after the confirmation of the indicator bacteria Escherichia coli found in the EMB agar medium. The suspected E. coli from a single colony of green metallic sheen was introduced into a lactose fermentation broth 1X again for the assurance of the gas production after fermentation of lactose. Gram staining was also performed for the confirmation of E. coli isolates (Cappuccino and Sherman, 1996). Biochemical identification of other gram negative Coliform bacteria Besides the presence of E. coli colony with green metallic sheen, there were also other colonies in the EMB medium which were identified by using standard biochemical methods to

find a complete microbiological profile of the drinking water samples (Cappuccino and Sherman, 1996; Alfrad, 2007). Quantitative analysis through conventional culture method an aliquot of 0.1 mL of each sample was introduced on to nutrient agar, MacConkey agar, membrane fecal coliform (mFC) agar and cetrimide agar through spread plate technique for the isolation of total viable bacteria (TVB), E. coli, Klebsiella spp., fecal coliform and Pseudomonas spp., consecutively. Plates were incubated at 37 °C for 24 hours except mFC agar plates which were incubated at 44.5 °C. Presence of green metallic sheen on EMB agar media was further specified the presence of E. coli. Considering the presence of viable but nonculturable (VBNC) cells and the stressed cells (Colwell, 2000; Oliver, 2010; Rahman and Noor, 2012; Acharjee et al., 2013; Noor et al., 2013), enrichment was performed for the assessment of Salmonella spp. and Shigella spp., and also for Vibrio spp. in selenite cysteine broth and alkaline peptone water, respectively. The enriched samples were then inoculated onto the Salmonella-Shigella agar and thiosulfate citrate bile salt sucrose agar. For the final identification all isolates were biochemically analyzed by following the standard methods (Cappuccino and Sherman, 1996; Alfrad, 2007). Determination of the antibacterial activity All the isolates were tested against commonly used 13 antibiotics (including first, second and third generation drugs) by disc diffusion assay on Mueller-Hinton Agar (Difco, Detroit, MI) (NeoSensitabs, Rosco, Denmark) according to the modified Kirby-Bauer method (Bauer, 1966; Munshi et al., 2012). Antibiotic discs of appropriate concentrations such as ampicillin (10 µg), amoxicillin (10µg), ciprofloxacin (5 µg), ceftriaxone (30µg), nalidixic acid (30 µg), imipenem (10µg), tetracycline (30µg), erythromycin (15µg), kanamycin (30µg), chloramphenicol (30 µg), azithromycin (15µg), trimethoprimesulfomethoxazole (25µg), gentamycin (10µg), penicillin (10µg), vancomycin (30µg), ofloxacin (5 µg) andpiperacillin (10 µg) were used. RESULTS AND DISCUSSION as stated earlier, in developing countries, bacteriological contamination of drinking water has been one of the most serious problems leading to water borne infectious diseases (WHO, 2003; Haq, 2010; Acharjee et al., 2011; Acharjee et al., 2013). MPN method is very easy and applicable in more efficient way in the indication of the presence of indicator bacteria than the time consuming conventional bacterial culture techniques. Nevertheless, the method imparts only a qualitative result and not the actual microbial load (WHO, 2003). Present experiment was conducted for the determination of the processed drinking water quality to be either potable or non-potable on the basis of the presence of indicator bacteria which indicates the chance of fecal contamination as well as the health associated risks. Moreover, next to the detection of indicator bacteria from the processed drinking water, the load of coliform and other pathogenic isolates present was determined to deliver a complete bacteriological data. Determination of the potability of drinking water samples In the presumptive test, accumulation of gas in the incorporated Derhum tubes was observed in variable number of tubes of all the 25 drinking water samples tested which indicated the possible presence of coliform. The lowest count of MPN/100 ml was 2 which was detected in three samples (sample no. 6, 7 & 15). Most of the samples showed highest number (e”2400) of MPN/100 mL. The presence of the indicator bacteria did not necessarily mean that the fecal contamination was present. It only projected the chance of fecal contamination, although the samples tested in this study were treated by boiling, filtration, or collected directly from deep tube-wells. There might be a possibility that even after processing, the samples were contaminated with bacteria as a result of improper boiling, improper filtration, reuse of the same filter for many times without washing or changing during the filtration, collecting the water in jars or cans which might not have been properly washed or were washed by focally contaminated water (Acharjee et al., 2013). Among the positive samples found in presumptive test, only 5 samples (sample no. 1, 10, 11, 12 &13) showed to be positive for the presence of the indicator E. coli by observing the green metallic sheen on EMB agar. The presence of E. coli isolates was further confirmed by observing the gas formation in lactose fermentation broth 1X and also by visualizing Gram negative, short rod cells under the bright field microscope. Finally, to achieve a complete microbiological profile of the contaminating bacteria in addition of E. coli in the drinking water samples, other bacterial isolates were confirmed after biochemical identification which ensures the presence of Klebsiella spp., Alcaligenesfaecalis, Pseudomonas spp. and Aeromonas spp. Therefore, it was assumed that the overall quality of drinking water sample was not satisfactory to be categorized as potable water heading a major source for public health associated problems experienced frequently. Frequency of pathogenic bacteria The result of conventional culture method was found to be consistent in detecting E. coli when compared to MPN method as the load was found within the range of

3.0×102 to 1.7×105 cfu/mL in samples 1 and 10-13 (Table 4). A large number of other pathogenic bacteria was also quantified. Total viable bacteria were found in a range up to 108 cfu/mL. Klebsiella spp. and Pseudomonas spp. were encountered in most of the tested samples (1.5×104 - 4.0×105 cfu/mL and 1.3×103 - 3.2×107 cfu/mL, respectively). Few samples exhibited the growth of Salmonella spp., Shigella spp. and Vibrio spp. Detection of pathogens in such volume would accelerate the risk as revealed from the MPN result. Prevalence of drug resistant pathogens in drinking water Previous studies and other parts ofthe world portrayed the existence and dissemination of drug resistant pathogens in water samples (Xi et al., 2009; Patoli et al., 2010; Lubick 2011; Munshi et al., 2012; Acharjee et al., 2013). In consistent to the previous findings, most of the pathogenic isolates were found to be resistant against multiple antibiotics in the present study. Higher rates (more than 50%) of resistance were observed against ampicillin (10 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), penicillin (10 µg) and nalidixic acid (30 µg) (Table 5). However, an adequate degree of sensitivity was observed against imipenem (10 µg) and gentamycin (10 µg). Extensive misuse of antibiotics and antibiotic abuse might results in the dissemination of drug resistant pathogens to the water bodies through feces of the diseased individuals which confer the difficulties in eradicating such pathogens (Armstrong, 1981; Schwartz, 2003; Dutta et al., 2013; Khan et al., 2013).

Conclusion

The results of our current investigation projected the microbiological hazards among the drinking water samples studied. Although the sample size was a bit low to present the actual scenario and molecular detection of the virulent genes among the isolated pathogens was not carried out, the present study still enabled to portray the public health significance. Routine estimation of bacterial population as indicted in this study both in qualitative and quantitative methods might nourish the current water quality measurement in a global perspective.

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