Available online at www.ilns.pl

International Letters of Natural Sciences

12(2) (2014) 122-131 ISSN 2300-9675

Detection of coliforms in drinking water and its effect on human health - A Review

Partha Pal

Department of Zoology, Scottish Church College, 1 & 3 Urquhart Square, Kolkata - 700006, India
Phone: 91-33-2350-3862

E-mail address: parthapal_iicb@yahoo.co.in

ABSTRACT

The coliform group has been used extensively as an indicator of water quality and has historically led to the public health protection concept. Total coliforms are a group of bacteria commonly found in the environment, for example in soil or vegetation, as well as the intestines of mammals, including humans. Total coliform bacteria are not likely to cause illness, but their presence indicates that the water supply may be vulnerable to contamination by more harmful microorganisms. *Escherichia coli* (*E. coli*) is the only member of the total coliform group of bacteria that is found only in the intestines of mammals, including humans. The presence of *E. coli* in water indicates recent fecal contamination and may indicate the possible presence of disease-causing pathogens, such as bacteria, viruses, and parasites. Although most strains of *E. coli* bacteria are harmless, certain strains, such as *E.coli* 0157:H7, may cause illness. About 80 % of communicable diseases in the world are waterborne. According to WHO estimate about 80 % of water pollution in developing country, like India is carried by domestic waste. In India 70 % of the water is seriously polluted and 75 % of illness and 80 % of the child mortality is attributed to water pollution. The improper management of water systems may cause serious problems in availability and quality of water. The major pathogenic bacteria responsible for water borne disease are spread by the faeco-oral route, in which water may play an intermediate role. The aim of this review is to examine methods currently in use for the detection of coliforms in drinking water and also to evaluate the possible health hazards associated with drinking water contaminated with coliforms.

***Keywords*:** Coliforms; Faeco-oral route; *E. coli*; Waterborne; Contamination

1. INTRODUCTION

In developing countries, 2.6 billion people lack access to basic sanitation and 1.1 billion do not have access to improved water sources. This combination leads to 1.6 million deaths each year from preventable diarrheal diseases, 90 % of which are among children less than 5 years of age. (WHO, 2004). Public and environmental health protection requires safe drinking water, which means that it must be free of pathogenic bacteria. Among the pathogens disseminated in water sources, enteric pathogens are the ones most frequently

encountered. As a consequence, sources of fecal pollution in waters devoted to human activity must be strictly controlled. Entero-pathogens, such as Escherischia coli O157:H7, are generally present at very low concentrations in environmental waters within a diversified microflora. Complex methods are required to detect them, and these are extremely time-consuming. Most coliforms are present in large numbers among the intestinal flora of humans and other warm-blooded animals, and are thus found in fecal wastes. As a consequence, coliforms, detected in higher concentrations than pathogenic bacteria, are used as an index of the potential presence of entero-pathogens in water environments. The use of the coliform group, and more specifically E. coli, as an indicator of microbiological water quality dates from their first isolation from feces at the end of the 19th century. Coliforms are also routinely found in diversified natural environments, as some of them are of telluric origin, but drinking water is not a natural environment for them.

Their presence in drinking water must at least be considered as a possible threat or indicative of microbiological water quality deterioration. Positive total coliform samples in a treated water which is usually coliform-free may indicate treatment ineffectiveness, loss of disinfectant, breakthrough (McFeters et al., 1986), intrusion of contaminated water into the potable water supply (Geldreich etal., 1992; Clark et al., 1996) or regrowth problems (LeChevallier, 1990) in the distribution system, and, as a consequence, should not be tolerated. The use of the coliform group as an indicator of the possible presence of enteric pathogens in aquatic systems has been a subject of debate for many years. Many authors have reported waterborne disease outbreaks in water meeting the coliform regulations (Payment et al., 1991; Moore et al., 1994; MacKenzie et al., 1994; Gofti et al., 1999).

The need for more rapid and sensitive tests is constant in the water industry, with the ultimate goal being the continuous on-line monitoring of water leaving treatment plants. Recent study indicates that drinking water contaminated with antibiotic-resistant enterotoxigenic fecal bacteria may be responsible for presence of waterborne diarrheal diseases attributed to therapeutic agents used by urban populations in the tropics (Pathak and Gopal, 2008). However, the purpose of this review is not to discuss the indicator concept, but rather to identify methods currently in use for the detection of coliforms in drinking water and the potential health hazards associated with drinking coliform contaminated water.

2. COLIFORMS

The coliform group includes a broad diversity in terms of genus and species, whether or not they belong to the Enterobacteriaceae family. Most definitions of coliforms are essentially based on common biochemical characteristics.

The definition of coliform bacteria differs slightly depending on the country or on the organization in charge of the microbiological monitoring regulations. In Canada, the definition is the same as in the US, and differs in some European countries. For example, the French Standardization Association (NFT90-413 and NFT90-414; AFNOR, 1990), which can be considered as a representative model for European legislation, defines total coliforms (TC) as:

Rod-shaped, non-spore-forming, Gram-negative, oxidase-negative, aerobic or facultative anaerobic bacteria that are able to grow in the presence of bile salts or other replacement surface active agents having an analogous growth inhibitory effect and that ferment lactose with gas and acid (or aldehyde) production within 48 h at 37 ±1 °C. AFNOR

(1990) goes further by defining other coliform groups, including the thermotolerant coliforms (also called fecal coliforms, FC) and, more specifically, *E. coli*:

Thermotolerant coliforms have the same fermentation properties as total coliforms (TC) but at a temperature of 44 ±0.5 °C.

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. The use of the coliform group as an indicator of fecal contamination is subject to strict governmental regulations. *E. coli* is the most common coliform among the intestinal flora of warm-blooded animals and its presence might be principally associated with fecal contamination. No *E. coli* are therefore allowed in drinking water (Rompre et al., 2002).

The four genera *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter* are generally accepted as comprising the total coliform population (Clark and Pagel, 1977). Enumeration of this component of the microbial aquatic ecosystem has been universally applied to document the sanitary quality of water. The usefulness of the total coliform count as an indicator of bacterial water pollution has been questioned, partly because coliform detection methods are potentially subject to interferences (Geldreich et al., 1978: Hutchison et al., 1943).

Interference with coliform detection or coliform suppression in presumptive media (Evans et al., 1981) has been thought to result from competition by noncoliform bacteria for nutrients (Reitler and Seligmann 1957). Other proposed causes of coliform suppression are the production of inhibitory products by noncoliform bacteria (Hutchison et al., 1943) and then failure of brilliant green lactose bile broth to recover coliforms from gas-positive presumptive tubes (Chambers, 1950; Ruchhoft, 1935).

Recently, a modified most-probable-number (M-MPN) procedure was developed to document the magnitude of interference with total coliform detection in the standard MPN (SMPN) technique (Evans et al., 1981). Coliform suppression in the presumptive and confirmed tests was found to contribute significantly to the underestimation of coliform numbers in the S-MPN technique. In addition to the quantitative impact of suppression on coliform enumeration, other factors influence the qualitative recovery of the component coliform genera. It has been illustrated with polluted specimens that the kind of water examined (sewage, unchlorinated sewage effluent, surface water), as well as media and techniques, will affect the isolation frequency of the four coliform genera (Clark and Pagel, 1977; Dutka and Tobin, 1976). Treatment of raw water may also influence the percentage distribution of component coliform genera found. Clark and Pagel reported that the percentage of *Escherichia* found in the component genera of contaminated drinking water samples was reduced compared to the untreated surface water source (Clark and Pagel, 1977). Chlorination has been reported by others to increase the percentage of *Klebsiella* in the component coliform genera isolated from drinking water samples (Ptak et al., 1973).

3. DETECTION OF COLIFORMS

Since drinking waters constitute oligotrophic systems, the lack of sensitivity of cultivation methods in the detection of stressed and starved bacterial cells can generate serious limitations due to contamination-level underestimation. There exist other methods which may be used for coliform detection, and these are in various states of development and application. This review describes the principles and the usual protocols of the classical methods, as well as some innovative methods and emerging approaches.

3. 1. Multiple-tube fermentation technique

The technique of enumerating coliforms by means of multiple-tube fermentation (MTF) has been used for over 80 years as a water quality monitoring method. The method consists of inoculating a series of tubes with appropriate decimal dilutions of the water sample. Production of gas, acid formation or abundant growth in the test tubes after 48 h of incubation at 35 °C constitutes a positive presumptive reaction. Both lactose and lauryl tryptose broths can be used as presumptive media. All tubes with a positive presumptive reaction are subsequently subjected to a confirmation test. The formation of gas in a brilliant green lactose bile broth fermentation tube at any time within 48 h at 35 °C constitutes a positive confirmation test. The fecal coliform test (using an EC medium) can be applied to determine TC that are FC (APHA et al., 1998).

The results of the MTF technique are expressed in terms of the most probable number (MPN) of microorganisms present. This number is a statistical estimate of the mean number of coliforms in the sample. As a consequence, this technique offers a semi-quantitative enumeration of coliforms. Nevertheless, the precision of the estimation is low and depends on the number of tubes used for the analysis. But, if five tubes, each with 1 ml sample, are used, a negative result may be expected less than 1% of the time (APHA et al., 1998).

3. 2. Membrane filter technique

The membrane filter (MF) technique is fully accepted and approved as a procedure for monitoring drinking water microbial quality in many countries. This method consists of filtering a water sample on a sterile filter with a 0.45-mm pore size which retains bacteria, incubating this filter on a selective medium and enumerating typical colonies on the filter. Many media and incubation conditions for the MF method have been tested for optimal recovery of coliforms from water samples (Grabow and du Preez, 1979; Rice et al., 1987). Among these, the most widely used for drinking water analysis are the m-Endo-type media in North America (APHA et al., 1998) and the Tergitol-TTC medium in Europe (AFNOR, 1990). Other media, such as MacConkey agar and the Teepol medium have been used in South Africa and Britain. However, comparisons among the media have shown that m-Endo agar yielded higher counts than MacConkey or Teepol agar (Grabow and du Preez, 1979). To enumerate FC, the APHA et al. (1998) proposes that filters be incubated on an enriched lactose medium (m-FC) at a temperature of 44.5 °C for 24 h.

3. 3. Enzymatic methods

Chromogenic and fluorogenic substrates produce color and fluorescence, respectively upon cleavage by a specific enzyme. These substrates have been used to detect the presence or the activity of specific enzymes in aquatic systems (Chröst, 1991). To detect the presence of b-D-glucuronidase in E. coli, the following chromogenic substrates were used: indoxyl-b-D-glucuronide (IBDG) (Brenner et al., 1993).

Several commercial tests were then developed based on the defined substrate technology: Colilert (IDEXX Laboratories, Portland, ME, USA), Colisure (Millipore corporation, Bedford, MA, USA), Coli-Quick (Hach, Loveland, CO, USA). Most of these are available for a presence/absence response and for enumeration by the multi-tube technique. The most widely used among them is the Colilert test, which utilizes the defined-substrate technique with ONPG and MUGlu. Numerous and very extensive comparisons between these commercial tests and the classical MTF and MF approaches have been performed to enumerate TC and *E. coli* in various types of waters (Edberg et al., 1988, 1989, 1990; Clark

et al., 1991; Olson et al., 1991; McCarty et al., 1992; McFeters et al., 1993; Palmer et al., 1993; Clark and El-Shaarawi, 1993; Colquhuon et al., 1995; Fricker and Fricker, 1996).

Different commercial agar media are now available for the detection of TC and E. coli. They include classical agar media used for *E. coli* and coliform enumeration modified with specific chromogenic and/ or fluorogenic substrates for the detection of b-Dglucuronidase and/or b-D-galactosidase: Chromocult Coliform Agar (Merck, Darmstadt, Germany), Fluorocult *E. coli* Direct Agar (Merck) and m-ColiBlue24 broth (Hach) (Grant, 1997).

More recently, the b-D-galactosidase and b-D-glucuronidase properties of TC and *E. coli* have been exploited on freshwater (Berg and Fiksdal, 1988; George et al., 2000) and seawater (Davies et al., 1995) samples in rapid assays without any cultivation steps.
George et al. (2000) finalized a protocol based on the fluorogenic substrates MUGal and MUGlu for a direct enzymatic detection of FC in freshwaters in 30 min. These methods allow a rapid and direct estimate of the level of microbiological contamination of surface water, but their detection limits (20 CFU/100 ml for FC to about 340 CFU/100 ml for TC) preclude their use for the monitoring of drinking water.

Chromogenic membrane filtration culture-based methods have been recently used to assess microbiological water quality. This assay has the ability to detect *E. coli* colonies on confluent growth plates (Maheux et al., 2014a)

3. 4. Molecular methods

Most of the nucleic acid methods use molecular hybridization properties, which involve the complementary sequence recognition between a nucleic probe and a nucleic target. A hybridization reaction can be realized between a nucleic DNA probe and a chromosomic DNA sequence (DNA–DNA hybridization) or an rRNA or tRNA sequence (DNA–RNA hybridization). The more frequently used nucleic-acid-based methods for coliform detection in drinking water are the polymerase chain reaction (PCR) and the in situ hybridization (ISH) methods.

Primers based on the lacZ gene have been used for the detection of coliforms because conventional coliform monitoring methods are based on the expression product (b-galactosidase) of this gene (Bej et al., 1990, 1991a, b; Fricker and Fricker, 1994). Other primer sets designed for two different regions have been proposed for the detection of E. coli, one of them coding for an outer-membrane protein (phoE gene) (Spierings et al., 1993) and the other coding DNA sequences for the V3 and V6 regions of the 16S rRNA genes of pathogenicand non-pathogenic strains of E. coli (Tsen et al., 1998). These primer sets permit the specific detection of *E. coli*, but also of *Shigella* species when the suggested sequences are amplified.

Multiplex-PCR, which consists of simultaneously amplifying different DNA fragments. Bej et al. (1991b) modified this approach to detect gene sequences related to the TC group and those associated with enteric pathogens including *E. coli*, *Salmonella spp*. and *Shigella spp*. Non-radioactive detection systems, like biotinylated primers, may be a useful substitute for more rapid detection of target DNA-probe hybrids. Juck et al., (1996) developed a protocol for the rapid detection of low concentrations of E. coli in water samples based on the nested PCR protocol and a modification of the filtration protocol proposed by Bej et al. (1991a).

Nested PCR protocols were used for the detection of E. coli (Juck et al., 1996) and some pathogens (Delabre et al., 1997) in drinking water, whereas Waage et al. (1999a, b) applied them to the detection of low numbers of *Salmonella spp*. and *Y. enterocolitica* cells in waters. This technique permits a more rapid detection (6 to 8 h) than the usual PCR (few

days), since confirmation of the correct sequence amplification by probe hybridization is no longer necessary.

Another promising approach is real-time quantitative PCR, which consists in monitoring the fluorescently PCR products as they are amplified (Heid et al., 1996). This approach has recently been applied to the detection of enterohemorhagic and enterotoxigenic E. coli strains in clinical microbiology (Bellin et al., 2001; Carroll et al., 2001). When amplified PCR products are stained with SYBR Green dye, using a LightCycler (Roche, Mannheim, Germany) or a GeneAmp (Applied Biosystems, Foster City, USA), the real-time PCR method showed greater rapidity and higher sensitivity and specificity in comparison to the duplex PCR assay with traditional gel analysis (Carroll et al., 2001).

ISH uses oligonucleotide probes to detect complementary nucleic acids sequences. This method exploits the ability of nucleic acids to anneal to one another in a very specific complementary way to form hybrids. Current work on rRNA in situ hybridization uses fluorescent-labeled nucleotide probes almost exclusively to detect hybridization (FISH). However, FISH cannot be applied to the detection of nonphylogenetically identified micro-organisms, such as coliforms (Rompre et al., 2002).

Recent work demonstrates the combinations of three different PCR assays targeting LacZ, WecG and 16S rRNA genes respectively have the ability to detect the presence of total coliform (TC) in 100 ml samples of water (Maheux et al., 2014b).

4. EFFECT ON HUMAN HEALTH

Outbreaks of disease attributable to drinking water can lead to serious acute, chronic, or sometimes fatal health consequences, particularly in sensitive and immunocompromised populations. From 1971 to 2002, there were 764 documented waterborne outbreaks associated with drinking water, resulting in 575,457 cases of illness and 79 deaths (Blackburn et al. 2004; Calderon, 2004). Private water supplies are not regulated by the USEPA and are generally not treated or monitored, although very few of the municipal systems involved in documented outbreaks exceeded the USEPA's total coliform standard in the preceding 12 mon (Craun et al., 2002). Studies indicate that 10.7 M infections/yr and 5.4 M illnesses/yr occur in populations served by community groundwater systems; 2.2 M infections/yr and 1.1 M illnesses/yr occur in noncommunity groundwater systems; and 26.0 M infections/yr and 13.0 M illnesses/yr occur in municipal surface water systems (Reynolds et al., 2008) The total estimated number of waterborne illnesses/yr in the U.S. is therefore estimated to be 19.5 M/yr. Others have recently estimated waterborne illness rates of 12M cases/yr (Colford et al., 2006) and 16 M cases/yr (Messner et al., 2006).

Drinking water outbreaks exemplify known breaches in municipal water treatment and distribution processes and the failure of regulatory requirements to ensure water that is free of human pathogens. Water purification technologies applied at the point-of-use (POU) can be effective for limiting the effects of source water contamination, treatment plant inadequacies, minor intrusions in the distribution system, or deliberate post treatment acts (i.e., bioterrorism). One prospective intervention study found that consumers of reverse-osmosis (POU) filtered water had 20-35 % less gastrointestinal illnesses than those consuming regular tap water, with an excess of 14% of illness due to contaminants introduced in the distribution system (Payment 1991, 1997). Two other studies using randomized, blinded, controlled trials determined that the risks were equal among groups supplied with POU-treated water compared to untreated tap water (Hellard et al., 2001; Colford et al., 2005).

For immunocompromised populations, POU water treatment devices are recommended by the CDC and USEPA as one treatment option for reducing risks of Cryptosporidium and other types of infectious agents transmitted by drinking water.

Many classes of pathogens excreted in feces are able to initiate waterborne infections. There are bacterial pathogens, including enteric and aquatic bacteria, enteric viruses, and enteric protozoa, which are strongly resistant in the water environment and to most disinfectants. The early bacterial agents such as *Shigella sonnei* remains prevalent and new pathogens of fecal origin such as zoonotic *Campylobacter jejuni* and *E. coli* O157:H7 are emerging. The emergence in early 1992 of serotype O139 of *V. cholerae* with epidemic potential in Southeast Asia suggests that other serotypes than V. cholerae O1 could also get on epidemic. Some new pathogens include environmental bacteria that are capable of surviving and proliferating in water distribution systems are emerging which may cause serious health hazards (Leclerc et al., 2002).

5. DISCUSSION AND CONCLUSION

The emergence of new detection and real-time methods is linked to the need for a better assessment diagnostics for the microbiological quality of water. This objective can be reached through an increase in detection specificity and a reduction in analysis time. Several methods offer direct information at the cellular level through taxonomical and/or physiological investigation. Some of them permit, notably in stressful environments, an increase in detection levels and higher enumerations than standard culturability-based methods. By eliminating the time-consuming confirmation step, they also allow a reduction in analysis time and thus a quicker response regarding health related problems. These methods are still in the development stage, however. Technical complexity and the correspondingly high associated costs limit currently their potential for becoming standardized methods for the detection of coliforms in drinking water samples.

In a recent study conducted in Nepal it was reported that out of the total 506 water samples studied 88.5% samples were positive for total coliform (TC) whereas 56.5% were positive for faecal colifor (FC) particularly *E. coli* bacteria. These findings are of serious public health concern with regard to both endemicity and outbreak of waterborne diseases in the country (Rai et al., 2012).

In the present review, it was concluded that current researches are focusing on the various innovative approaches including molecular technologies for the detection of coliforms in potable water and the elucidation of the various virulent bacterial species that are contaminating the drinking water sources. The presence of coliforms in the water is a major emerging health problem in the current era which may lead to epidemic or pandemic events if left untreated. This present review will provide an insight into the modern aspects of detection of coliforms in drinking water and will open up new vistas for modern research in the field of understanding the evolutionary mechanisms of emergence of new bacterial species in water sources and the potential health hazards associated with them.

References

[1] AFNOR (Association Franc_aise de Normalisation) 4th edn (1990) 735 pp.

[2] APHA, AWWA, AEF, Standard Methods for the Examination of Water and Wastewater. Washington, DC. 20th edn (1998).

[3] Bej A.K., Steffan R.J., DiCesare J., Haff L., Atlas R.M., *Appl Environ Microbiol* 56 (1990) 307-314.

[4] Bej A.K., Mahbubani M.H., DiCesare J.L., Atlas R.M., *Appl Environ Microbiol* 57 (1991a) 3529-3534.

[5] Bej A.K., McCarty S.C., Atlas R.M., *Appl Environ Microbiol* 57 (1991b) 2429-2432.

[6] Bellin T., Pulz M., Matussek A., Hempen H.-G., Gunzer F., *J Clin Microbiol* 39 (2001) 370-374.

[7] Berg J.D., Fiksdal L., *Appl Environ Microbiol* 54 (1988) 2118-2122.

[8] Blackburn B.G., Craun G.F., Yoder J.S., Hill V., Calderon R.L., Chen N., Lee S.H., Levy D.A., Beach M.J., *Morb Mort Weekly Report* 53 (2004) 23-45.

[9] Brenner K.P., Rankin C.C., Roybal Y.R., Stelma G.N., Scarpino P.V., Dufour A., *Appl Environ Microbiol* 59 (1993) 3534-3544.

[10] Calderon R.L., *NEWWA* Water Quality Symposium (2004), Boxborough MA.

[11] Carroll L.E., Adams J.K., Sullivan M., Besser J.M., Bartkus J.M., *ASM General Meeting* Orlando USA (2001).

[12] Chambers C.W., *Public Health Rep* 65 (1950) 619-627.

[13] Chröst, R.J., *Springer-Verlag* New York (1991) 317.

[14] Clark D.L., Milner B.B., Stewart M.H., Wolfe R.L., Olson B.H., *Appl Environ Microbiol* 57 (1991) 1528-1543.

[15] Clark, J.A., Pagel J.E., *Can. J. Microbiol* 23 (1977) 465-470.

[16] Clark J.A., El-Shaarawi A.H., *Appl Environ Microbiol* 59 (1993) 380-388.

[17] Clark, R.M., Geldreich, E.E., Fox, K.R., Rice, E.W., Johnson, C.H., Goodrich, J.A., Barnick, J.A., Abdesaken, F., *J Water SRT-Aqua* 45 (1996) 171-183.

[18] Colford Jr J.M., Roy S., Beach M.J., Hightower A., Shaw S.E., Wade T.J., *J Water and Health* 4 (Suppl 2) (2006) 71-88.

[19] Colford Jr J.M., Wade T.J., Sandhu S.K., Wright C.C., Lee S., Shaw S., Fox K., Burns S., Benker A., Brookhart M.A., Van Der Laan M.J., Levy D.A., *Am J Epidemiol* 161(5) (2005) 472-482.

[20] Colquhuon K.O., Timms S., Fricker C.R., *J Appl Bacteriol* 79 (1995) 635-639.

[21] Craun G.F., Nwachuku N., Calderon R.L., Craun M.F., *J Environ Health* 65(1) (2002) 16-23.

[22] Delabre K., Mennecart V., Joret J.-C., Cervantes P., *Proceedings of AWWA-Water Quality Technology Conference*, Denver, CO. American Water Works Association (1997).

[23] Dutka B.J., Tobin S.E., *Can. J. Microbiol* 22 (1976) 630-635.

[24] Edberg S.C., Allen M.J., Smith D.B., *Appl Environ Microbiol* 54 (1988) 1595-1601.

[25] Edberg S.C., Allen M.J., Smith D.B., *Appl Environ Microbiol* 55 (1989) 1003-1008.

[26] Edberg S.C., Allen M.J., Smith D.B., Kriz N.J., *Appl Environ Microbiol* 56 (1990) 366-369.

[27] Evans T.M., Waarvick C.E., Seidler R.J., LeChevallier M.W., *Appl Environ Microbiol* 41 (1981) 130-138.

[28] Fricker E.J., Fricker C.R., *Water Res*. 30 (1996) 2226-2228.

[29] Fricker E.J., Fricker C.R., *Lett Appl Microbiol* 19(1994) 44–46.

[30] Geldreich, E.E., Fox, K.R., Goodrich, J.A., Rice, E.W., Clark, R.M., Swerdlow, D.L., *Water Res* 26 (1992) 1127-1137.

[31] Geldreich E.E., Allen M.J., Taylor R.H. *In C W Hendricks* (ed.) (1978) 13-20.

[32] George I., Petit M., Servais P., *J Appl Microbiol* 88 (2000) 404-413.

[33] Gofti, L., Zmirou, D., Murandi, F.S., Hartemann, P., Poleton, J.L., *Rev Epidemiol Sante´ Publi* 47 (1999) 61-75.

[34] Grabow W.O.K., du Preez, M., *Appl Environ. Microbiol* 38 (1979) 351-358.

[35] Grant M.A., *Appl Environ Microbiol* 63 (1997) 3526-3530.

[36] Heid C.A., Stevens J., Livak K.J.,Williams P.M., *Genome Res* 6 (1996) 986.

[37] Hellard M.E., Sinclair M.I., Forbes A.B., Fairley C.K., *Environ Health Perspect* 109(8) (2001) 773-778.

[38] Hutchison D., Weaver R.H., Scherago M., *J. Bacteriol* 45 (1943) 29.

[39] Juck D., Ingram J., Pre´vost M., Coallier J., Greer C., *Can J Microbiol* 42 (1996) 862-866.

[40] LeChevallier, M.W., *J AWWA* 82 (1990) 74-86.

[41] Leclerc H., Schwartzbrod L., Dei-Cas E., *Crit Rev Microbiol* 28(4) (2002) 371-409.

[42] MacKenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Peterson, D.E., Kazmierczak, J.J., Addiss, D.G., Fox, K.R., Rose, J.B.L.,. *N. Engl. J. Med.* 331 (1994) 161-167.

[43] Maheux A.F., Boudreau D.K., Bisson M.A., Dion-Dupont V., Bouchard S., Nkuranga M., Bergeron M.G., Rodriquez M.J., *Appl Environ Microbiol* 25 (2014b).

[44] Maheux A.F., Dion-Dupont V., Bisson M.A., Bouchard S., Rodriquez M.J., *J Microbiol Methods* 97 (2014a) 51-55.

[45] McCarty S.C., Standridge J.H., Stasiak M.C., *J AWWA* 84 (1992) 91-97.

[46] McFeters, G.A., Pyle, B.H., Gillis, S.J., Acomb, C.J., Ferraza, D., *Water Sci Technol* 27 (1993) 261– 265.

[47] McFeters, G.A., Kippin, J.S., LeChevallier, M.W., *Appl. Environ. Microbiol* 51 (1986) 1-5.

[48] Messner M., Shaw S., Regli S., Rotert K., Blank V., Soller J., *J Water Health* 4 (suppl 2) (2006) 201-240.

[49] Moore, A.C., Herwaldt, B.L., Craun, G.F., Calderon, R.L., Highsmith, A.K., Juranek, D.D., *J. AWWA* 86 (1994) 87-99.

[50] Olson B.H., Clark D.L., Milner B.B., Stewart M.H., Wolfe R.L., *Appl Environ Microbiol* 57(5) (1991) 1535-1539.

[51] Palmer C.J., Tsai Y.-L., Lee Lang A., Sangermano, L.R., *Appl Environ Microbiol* 59 (1993) 786-790.

[52] Pathak S.P., Gopal K.J., *Toxicol Environ Health A* 71 (2008) 427-433.

[53] Payment, P., Richardson, L., Siemiatycki, J., Dewar, R., Edwardes, M., Franco, E., *Am. J. Public Health* 81(1991) 703-708.

[54] Payment P., Siemiatycki J., Richardson L., Gilles R., Franco E., Prevost M., *Int. J Health Res* 7 (1997) 5-31.

[55] Ptak D.J., Ginsburg W., Willey B.F., *J Am Water Works Assoc* 65 (1973) 604-608.

[56] Rai S.K., Ono K., Yanagida J.I., Ishiyama-Imura S., Kurokawa M., Rai C.K., *Nepal Med Coll J* 14(3) (2012) 234-240.

[57] Reitler R., and Seligmann R., *J Appl Bacteriol* 20 (1957) 145-150.

[58] Reynolds K.A., Mena K.D., Gerba C.P., *Rev Environ Contam Toxicol* 192 (2008) 117-158.

[59] Rice E.W., Fox K.R., Nash H.D., Read E.J., Smith A.P., *Appl. Environ. Microbiol* 53 (1987) 1571-1573.

[60] Rompre A., Servais P., Baudart J., de-Roubin M-R., Laurent P., *J Microbiol Methods* 49 (2002) 31-54.

[61] Ruchhoft C.C., *J Am Water Works Assoc* 27 (1935) 1732-1745.

[62] Spierings G., Ockhuijsen C., Hofstra H., Tommassen J., *Res Microbiol* 144 (1993) 557-564.

[63] Tsen H.Y., Lin C.K., Chi W.R., *J Appl Microbiol* 85 (1998) 554-560.

[64] Waage A.S., Vardund T., Lund V., Kapperud G., *J Appl Microbiol* 87(3) (1999a) 418-428.

[65] Waage A.S., Vardund T., Lund V., Kapperud G., *J Appl Microbiol* 87 (1999b) 814–821.

[66] WHO (2004), http://www.who.int/water_sanitation_health/publications/facts2004/en/.

[67] F. W. Owa, *International Letters of Natural Sciences* 3 (2014) 1-6.

[68] Sajid Qurashi, Omprakash Sahu, *International Letters of Natural Sciences* 5 (2014) 35-44.

[69] Omprakash Sahu, *International Letters of Natural Sciences* 7 (2014) 35-43.

[70] Omprakash Sahu, *International Letters of Natural Sciences* 8(1) (2014) 1-8

(Received 12 June 2014; accepted 22 June 2014)