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Review article

Isolation and Identification of Pathogenic Bacteria from Drinking Water

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Abstract

Drinking water microbiological quality was primarily determined by enumeration of "indicator organisms", whose presence indicates faecal contamination. The presence of the indicators is often a key in assessing potential public health risks due to bacterial pathogens and is used in drinking water quality regulations and guidelines in many countries. The enumeration of fecal coliform specifically of Escherichia coli (indicator of fecal contamination) from human as well as animal sources per 100 ml of municipal water used for drinking purposes has been recommended by World Health Organization (WHO) worldwide to monitor the quality of drinking water. According to World Health Organization (WHO) guideline standards for drinking water total and fecal coliform the indicator of fecal contamination must not be detectable in any 100 ml samples. The disinfection treatment of drinking water has important role in reducing the waterborne epidemics. The inadequate disinfection treatment of municipal water results in the provision of unsafe drinking water to the people which can pose a great threat and risk of waterborne epidemics by bacterial pathogens to the population consuming it. Such negligence may result in a catastrophic disaster in the area. Water supplying authorities should take account of this situation and take measures for the provision of contamination free drinking water to prevent waterborne disease outbreaks by bacterial pathogens.

Keywords: Drinking water, waste water treatment, waste water, indiccator organism, pathogens , TC, TTC and water quality

1. Introduction

As water is a universal solvent it dissolves salts, inorganic and organic compounds and gases that take part in metabolic reactions, maintain the macromolecular framework, stabilize plasma membrane, thermoregulation, transport nutrients, and maintain hemostasis and body volume/weight (Armstrong *et al*.,2007).Water is an important component of all cells and is prerequisite of life on earth. The accessibility of potable water remains a key issue of development. Enough water is necessary for development. The demand and supply of water use-cycle puts pressure on human needs for fresh water. Displacements of people, loss of wild life and continuous alteration in river ecology and hydrology is the another effect (Botkin and Keller,2005). Direct discharge of domestic wastes leaching from poorly maintained septic tanks and improper management of farm wastes are suspected as the major sours of waterborne diseases. Water contamination refers to degradation of water quality from a public health or ecological view point. A pollutant is any biological physical and chemical substance that is present in an identifiable surplus and is known to be injurious to other desirable living organisms. Water pollutants include heavy metals, sediments, certain radioactive isotopes, phosphorus, nitrogen, sodium, arsenic, heat, fecal coliforms bacteria, other pathogenic bacteria, virus and protozoan pathogens. The pollution of municipal water by human and animal sources is the major threat to the public health in poor countries. Water contaminated with excreta from animal or anthropogenic sources, which may be the carrier or active cases of infectious diseases serve as the vector of disease.

2. Indicator organisms

Since it would be practically impossible to test water for each of the wide variety of pathogens that may be present, microbiological water quality monitoring is primarily based on the tests for indicator organisms' http:// www.who.Int/water_ sanitation_ health. There is no single indicator organism that can universally be used for all purposes of water quality surveillance. Each of the wide variety of indicator available for this purpose has its own advantages and disadvantages, and the challenge is to select the appropriate indicator, or combination of indicators, for each particular purpose of water quality assessment. Indicators most commonly used are of faecal or sewage origin and the following are some of the most important requirements of such indicators:(1) presentwhenever pathogens are present, (2) Present in same or higher numbers than pathogens, (3) Specific for faecal or sewage pollution,(4)At least as resistant as pathogens to conditions in natural water environment, and water purification and disinfection processes,(5) Non- pathogenic,(6) Detectable by simple, rapid and inexpensive methods. Ideally, various other properties are desirable, such as counts which are directly related to those of pathogens. However, the fundamental and most important requirement is that pathogensshould be absent or inactivated whenever indicators are absent or inactivated http:// www.who.Int/water_ sanitation_ health. Many indicators have been studied and recommended for water quality assessment (ISO, 1990). Evaluation of the reliability of indicators is carried out by comparison of their incidence and survival in the water and treatment processes with that of selected pathogens, by epidemiological studies, on the consumers of the water supplies, by thecalculations based on the minimal infectious dose of pathogens, and by experiments with human volunteers (Regli *et al*.,1991). The following is a summary of the most important features of commonly used indictors: *Escherichia coli*, this species are a member of the group of

faecal coliform bacteria. Escherichia coli have the important features of being highly specific for the faeces of man and warm-blooded animals. For all practical purposes these bacteria cannot multiply in natural water environment and they are therefore used as specific indicator for fecal pollution. They generallydistinguished from other thermo-tolerant coliform by the ability to yield a positive indole test within 24 hours at 44^0C http:// www.who.Int/water_ sanitation_ health.

2.1. Thermo-tolerant coliform bacteria

This term refers to certain members of the group of total coliform bacteria which are moreclosely related to fecal or sewage pollution which generally do not redially replicate in water environment. This group of bacteria is also known as fecal coliform, presumptive E. coli fecal *E. coli*, etc http:// www.who.Int/water_ sanitation_ health. Thermo-tolerant coliforms are primarily used for the assessment of fecal pollution in waste water and raw water sources. They are detectable by simple and inexpensive tests and widely used in water quality monitoring. The test methods used are the multiple tube and membrane filtration using membrane Faecal Coliform (mFC) medium and incubation for 24 hours at 44^0C. In the membrane filtration the individual colonies can be identified and the presence of E. coli provides strong evidences of fecal pollution
http:// www.who.Int/water_ sanitation_ health

2.2. Coliform bacteria (total coliform)

The term total coliform refers to a vaguely defined group of gram negative bacteria which have long history in water quality assessment. In outdated literature these bacteria go by all sorts of names including coliform, colis etc. some of the bacteria included in this group are almost conclusively of fecal origin while other members may also replicate in suitable water environment. These bacteria which can be determined by simple and inexpensive test are primarily used for assessment of general sanitary quality of finally treated and disinfected drinking water.

2.2.1. Fecal Indicator Organisms of Water Quality

Historically, improvement of principles, rules and regulations associated to drinking water quality targeted water distribution systems. In the nineteenth century in Europe, this was to find out the solution to problems of waterborne epidemics of infectious disease (Tollon, 2005). For the provision of safe drinking water, the guideline standards for drinking water were established. The filtration and, later, disinfection techniques were adopted to control the pollution of drinking water. The term indicator was emerged for indication of the occurrence of fecal coliforms as well as pathogenic bacteria in drinking water. This technique was adopted for analysis of possiblepresence of bacterial pathogens (Tollon, 2005).Historically, fecal indicator bacteria (FIB) including total and fecal coliforms and enterococci have been used in many countries as a monitoring tool for microbiological impairment of water and for prediction of presence of bacterial, viral and protozoan pathogens. These microorganisms are of fecal origin from higher mammals and birds, and their presence in water may indicate fecal pollution and possible association with enteric pathogens. Currently, there are very few review papers critically evaluating the relationships between

conventional and alternative fecal indicators and the presence of bacterial, viral and protozoan pathogens (Horman *et al*., 2004).

2.3. Alternative indicators of fecal pollution

Alternative fecal indicators such as fecal anaerobes (genera Bacteroides and Bifidobacterium, spore-forming *Clostridium perfringens*), viruses (B. fragilis phage, coliphages (FRNA phage)), and fecal organic compounds (coprostanol) have been increasingly applied. It seems that the use of alternative indicators together with conventional fecal markers is very promising to identifythe source of fecal pollution and associated pathogens. However, replacement or combination of any indicators requires adequate epidemiological studies to support their use.

2.3.1. Fecal anaerobes

Fecal anaerobes account for a significant portion of fecal bacteria (Matsuki *et al*., 2002) and are limited to warm-blooded animals. The primary weakness of fecal anaerobes as indicator is short survival in non-host environments due to their low oxygen tolerance.

2.3.2. Bacteroides spp.

Until now, the need to maintain anoxic conditions for cultivation, isolation and biochemical identification limited the usage of anaerobic Bacteroides species as fecal indicator. Importantly, since certain Bacteroides species are highly host-specific, it is possible to identify the source of fecal contamination by tracking host-specific Bacteroides species (Simpson *et al*., 2004). Only few studies using this indicator have been conducted, indicating the need for future studies at larger scales including studies on geographical distribution of host-specific Bacteroides genetic markers (Bonjoch *et al*., 2004).

2.3.3. Bifidobacterium spp.

Ecological distribution of Bifidobacterium spp. is highly variable in animals (Bonjoch *et al*., 2004). Although feces from humans, chickens, cows, dogs, pigs, horses, cats, sheep, beavers, goats, and turkeys were investigated, Bifidobacterium spp. was isolated from only feces of humans and swine. They were also frequently detected mainly from raw sewage and septic tanks (human feces), even though there was no significant difference in the concentrations of fecal coliforms, enterococci, or clostridia between human and animal fecal samples (Bonjoch *et al*., 2004).

2.3.4. *Clostridium perfringens*

C. perfringens has been successfully used as fecal indicator for sewage-contaminated streams, ocean environments (Hurst *et al*., 2002) and sea water. As the majorityof clostridia population forms spore, they are extremely resistant to the environmental stress and persist for longer time than other indicator bacteria (e.g., fecal coliforms and fecal streptococci) and most of pathogens do (Horman *et al*., 2004). Spore-forming bacteria are especially useful to

determine the ultimate fate of sewage or storm water released into water body. C. perfringens may be ideal microorganisms to evaluate the completeness of disinfection in drinking water treatment processes (Payment *et al*., 2000).

2.4. Bacterial Pathogens in Drinking Water

Some waterborne bacterial pathogens, such as *Legionella* and atypical *Mycobacteria* that can grow in water and soil reach the host by inhalation or direct contact where they can cause infections of respiratory tract, skin or brain tissues (WHO, 2006).

2.4.1.*Salmonella*

Salmonella, a very large group of rod shaped Gram negative bacteria comprising more than 2000 known serotypes that are members of family *Enterobacteriaceae.*These serotypes are virulent to humans and can cause a variety of symptoms from mild gastroenteritis to severe disease or death (Maier *et al*., 2000). The majority of *Salmonella* are of enteric origin of animals particularly of pigs, cows, goats, sheep, rodents, hens, ducks and other poultry. Although *S typhi* and *S. paratyphi* are usually restricted to humans.The pathogenicity of Salmonella species varies in terms of serotype, number of organism and on host status. *S. typhi* is a typical human pathogen and infective dose for Salmonella in humans is 1×10^5 organisms (Levinson& Jaw, 2000). *S. typhi*, *S. paratyphi A*, and *S. paratyphi B*, cause septicemia with high temperature without diarrhea, a condition known as enteric fever. Many serogroups can cause a transitory enteric infection like gastroenteritis with diarrhea in susceptible individuals. However certain serotypes can cause asymptomatic infection (Maier *et al*., 2000).

2.4.2. Escherichia coli

The human and warm-blooded animals contain E. coli in their excreta. This organism is a Gram negative rod, aerobic or facultative anaerobic, cannot produce spores, and is motile by peritrichousflagella and ferments lactose and glucose with production of acid and gas. All strains are catalasepositive; nitrate positive and oxidase negative (Prescott *et al*., 1999).

2.4.3. Vibrio cholerae

Vibrio species are motile, possessing monotrichous flagellum, non-sporing, Gram negative,comma shaped morphology and are the members of family Vibrionaceae. The members of this family are aerobes and facultative anaerobes. They are catalase and oxidase positive and nitrates positive.

2.4.4. Shigella spp.

The members of genus *Shigella* are non-motile, non-spore-forming rods. The Gram reaction is negative and they are capable of growing in aerobic and anaerobic conditions. Biochemically these bacteria ferment sugars with

production of acid but no gas, lactose fermentation is very rare and, excluding *Shigella dysetriae* serotype 1, all members are catalase and nitrate positive, oxidase test is negative in all members except *Shigella dysenteriae*type 1. O antigen is the basis of serogroups among *Shigellae* (WHO, 1996). In certain reports it is mentioned that among *Shigella flexneri* the lysogenic bacteriophage is the source of antigenic specificity determination. Serological typing has a reliable application for all species excluding *Shigella sonnei* (Levinson & Jawetz, 2000).

2.4.5. Pseudomonas aeruginosa

P. aeruginosa belongs to the family Pseudomonadaceae. It is a Gram negative rod, motile with monotrichous flagella. The organism ferments sugar by oxidation. Pseudomonas aeruginosa is oxidase, catalase and citrate positive. It is also capable of growing on higher temperatures rangesfrom 41-42OC. Blue-green fluorescent color (pyocyanin) is the mark of identification for *P. aeuginosa* (Gillespie, 1994).

2.4.5. Helicobacter pylori

The causative agent of gastritis and peptic ulcer, *H.pylori*exhibits spiral or curved rod-shape morphology. It is a Gram negative, motile bacterium. This human pathogen may be found on the gastric mucosa of man and spread through the gastrointestinal tract (Wisniewska *et al*., 2002). The transmission may be facilitated by saliva or gastric fluids between the members of a population (Mazri-Hiriart *et al*., 2001).

2.4.5. Aeromonas.

Aeromonas are Gram-negative, non-spore-forming, rod-shaped, facultative anaerobic bacilli belonging to the family Aeromonadaceae. Although A. hydrophila is the focus of this section, other aeromonads, such as A. caviae and A. sobria, have also been isolated from human faeces and from water sources (Villari *et al*., 2003). Morphologically, aeromonads are indistinguishable from members of the Enterobacteriaceae family, such as E. coli. They also share many biochemical characteristics, with the differentiation being that aeromonads are oxidase positive and Enterobacteriaceae are oxidase negative.

2.4.6. Yersinia

The genus *Yersinia* is classified in the family Enterobacteriaceae. They are heterotrophic bacteria with 11 recognized species, some of which cause disease in humans, and both pathogenic and non-pathogenic strains of *Yersinia* have been found in surface water and un-chlorinated drinking water. The source of the organism is the environment or non-human hosts, such as wild animals and birds. The species *Y. pestis*, *Y.pseudotuberclosis* and certain serotypes of *Y. enterocolitica* are pathogens for humans. *Yersiniapestis* is the cause of bubonic plague through contact with rodents and their flies. *Yersinia* spp.(WHO, 2000).

2.4.7. Campylobacter.

Campylobacter are mainly spiral-shaped, S-shaped or curved rod-shaped bacteria. There are 16 species and six subspecies assigned to the genus *Campylobacter*, of which the most frequently reported in human disease are *C. jejuni* (subspecies *jejuni*) and *C. coli.C. laridis* and *C. upsaliensis* are also regarded as primary pathogens, but are generally reported far less frequently in cases of human disease. Most species prefer a micro-aerobic (containing 3-10% oxygen) atmosphere for growth. A few species tend to favour an anaerobic environment, although they will grow under micro-aerobic conditions also (WHO, 2000)

2.4.8.Emergenceof New Pathogens

Health authorities, with the passage of time, face the problems of new emerging pathogens. These pathogens overcome the conventional procedures used for water purification and distribution. It has been reported that previously unknown microorganisms in water were found responsible for outbreaks of waterborne diseases (WHO, 1998). Caliciviruses, *E coli* O157:H7, *Helicobacter* sp. *Mycobacterium avium* complex, (MAC), are the candidates for new emerging pathogens (EPA 2002). This problem needs regular attention as to what may pose a "new threat" and also by regular monitoring and development of detection techniques to control the newly emerging pathogens noted by LeChevallier et al. (1999), thereby emphasizes that "knowledge is the first line of defense towards safe drinking water."

Recommendations

Keeping in view the quality of drinking water of area under study following recommendations has been made.

- The drinking water should be boiled before drinking it.
- Reported case of waterborne bacterial pathogens in drinking water is alarming. Government should fulfill its basic complacence of providing safe drinking water to community.
- The regular chlorine disinfection treatment of drinking water may be ensured.
- The quality of drinking water may be checked in light of drinking water guideline established by WHO
- The source of drinking water may be protected from un-necessary human and animal access.
- The general cleanliness and hygiene of water main storage reservoirs may be maintained.
- Sewage water should be treated and disinfected before disposing it.

Future work

Presently little is known about survival /persistence of bacterial, viral and protozoan pathogens in municipal water under different environmental conditions, which is most important in pathogen impact. The better understanding of the source of microbial contaminants (human versus animals), their transport, prevalence and fate in water environment and the resulting public health risk is urgently needed. The distribution of host specific genetic markers including humans has not been extensively investigated yet leaving a large space for additional future researches on identification of sources pathogens in municipal water. In addition to establish the link between waterborne bacterial pathogens and waterborne diseases is an important task for microbiologist to provide more futuristic vision of drinking water quality monitoring.

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