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E-Coli As A Gauge of Bacteriological Quality of Water: an Overview

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ABSTRACT

Monitoring the microbiological quality of drinking water relies largely on examination of indicator bacteria such as coliforms, Escherichia coli, and Pseudomonas aeruginosa. E. coli is a member of the faecal coliform group and is a more specific indicator of faecal pollution than other faecal coliforms. Two key factors have led to the trend toward the use of E. coli as the preferred indicator for the detection of faecal contamination, not only in drinking water, but also in other matrices as well: first, the finding that some faecal coliforms were non faecal in origin, and second, the development of improved testing methods for E. coli. The faecal coliform definition has also been revised to coincide better with the genetic make-up of its members and now includes newly identified environmental species. As a result, faecal coliforms are increasingly being referred to as thermotolerant coliforms. This, combined with improved detection methods for E. coli, has started a trend toward the use of E. coli in place of thermotolerant coliforms as a more reliable indicator of faecal pollution in drinking water. At present, E. coli appears to provide the best bacterial indication of faecal contamination in drinking water. This is based on the prevalence of thermotolerant (faecal) coliforms in temperate environments as compared to the rare incidence of E. coli, the prevalence of E. coli in human and animal faeces as compared to other thermotolerant coliforms, and the availability of affordable, fast, sensitive, specific and easier to perform detection methods for E. coli.

KEYWORDS :

Introduction

Water is a natural resource and is essential to sustain life. Accessibility and availability of fresh clean water does not only play a crucial role in economic development and social welfare, but also it is an essential element in health, food production and poverty reduction.1 However, safe drinking water remains inaccessible for about 1.1 billion people in the world and the hourly toll from biological contamination of drinking water is 400 deaths of children below the age five.2 Water helps maintain the moisture of internal organs of the body;3 maintains normal volume and consistency of fluids such as blood and lymph;4 regulates body temperature; removes poisons or toxins from the body through urine, sweat and breathing;5 and is essential for regulating the normal structure and functions of the skin.6 The body loses about four liters of water every day.3 It is therefore necessary to replenish this volume by drinking at least the equivalent amount of quality water every day. In developing countries with deteriorating environments, the demand for clean drinking water supply is growing rapidly in recent times.7 In Ghana, the supply of piped water is inadequate in most communities. This inadequacy is both in quantity and quality of public water supply. Only 40% of the total urban population has direct access to piped water. On the whole, only about 10.3 million people (approx. 51% of the population) are reported to have improved water supplies.8 Those who do not have access to safe water, as well as those who have access but cannot afford, rely on other sources of water with questionable quality.9

The microbiological quality of drinking water is a concern to consumers, water suppliers, regulators and public health authority alike. The potential of drinking water to transport microbial pathogens to great number of people, causing subsequent illness is well documented in countries at all levels of economic development.10,11 It is stated that, most sporadic cases of waterborne intestinal illness will not be detected or if detected, may not be recognized as water related.12 Several researchers have attempted to estimate the total burden of waterborne diseases world-wide. Waterborne disease might account for one-third of the intestinal infections world-wide,13 while it is estimated that water, sanitation and hygiene were responsible for 40% of all deaths and 5.7% of the total disease burden occurring worldwide.14 Human, livestock and wild animals are all sources of faecal contamination; in general, human faecal waste gives rise to the highest risk of waterborne disease.15 A wide spectrum of pathogenic agents can be found in water and monitoring for their presence on a routine basis is impractical. Traditionally, microbial safety of drinking water has been confirmed by monitoring for absence of microorganisms of faeces origin.16 The importance of quality changes in distribution is based upon evidence concerning the frequency and extends of known quality changes and their impact upon human health, a significant proportion of recognized piped drinking water-related disease outbreaks are related to quality deterioration in distribution.17 Piped distribution systems for drinking water are as important to the quality and safety of drinking water as the treatment itself. Water entering the distribution system must be microbiologically safe and ideally should be biologically stable. The distribution system itself must provide a secure barrier to posttreatment contamination as the water is transported to the user.18 Potentially pathogenic bacteria from shower water and air of stem cell transport unit was isolated,19 while Enterio - coccus faecalis, Clostridium perferns spore and Cryptosporidium parvum oocyst was recovered from water by using MS2 bacteriophage.20

Historic perspective of indicator organisms

Traditionally, indicator micro-organisms have been used to suggest the presence of pathogens.21 Today, however, we understand a myriad of possible reasons for indicator presence and pathogen absence or vice versa. In short, there is no direct correlation between numbers of any indicator and entire pathogens.22 To eliminate the ambiguity in the term microbial indicator, the following three groups (Table 1) are now recognized: i) general (process) microbial indicators, ii) faecal indicators such as E. coli, iii) index organisms and model organisms. A direct epidemiological approach could be used as an alternative or adjunct to the use of index micro-organisms. Yet epidemiologic methods are generally too insensitive, miss the majority of waterborne disease transmissions and are clearly not preventative.23 Nonetheless, the ideal is to validate appropriate index organisms by way of epidemiological studies. A good example is the emerging use of an enterococci guideline for recreational water quality.24 Often epidemiologic studies fail to show any relationship to microbial indicators, due to poor design and/or due to the widely fluctuating ratio of pathogen(s) to faecal indicators and the varying virulence of the pathogens. 25,26

Development of indicators: the coliforms The use of bacteria as indicators of the sanitary quality of water probably dates back to 1880 when Von Fritsch described Klebsiella pneumonia and K. rhinoscleromatis as microorganisms characteristically found in human faeces.27 In 1885, Percy and Grace Frankland started the first routine bacteriological examination of water in London, using Robert Koch's solid gelatin media to count bacteria.28 Also in 1885, Escherich described Bacillus coli and renamed it Escherichia coli.29 In 1891, the Franklands came up with the concept that organisms characteristic of sewage must be identified to provide evidence of potentially dangerous pollution.28 By 1893, the Wurtz method of enumerating E. coli by direct plating of water samples on litmus lactose agar was being used by sanitary bacteriologists, using the concept of acid from lactose as a diagnostic feature. This was followed by gas production, with the introduction of the Durham tube.30 The concept of coliform bacteria, those bacteria resembling E. coli, was in use in Britain in 1901.31 The

colony count for bacteria in water, however, was not formally introduced until the first report.32 Therefore, the sanitary significance of finding various Coliforms along with streptococci and C. perfringens was recognized by bacteriologists by the start of the twentieth century.28 It was not until 1905, however, that MacConkay described his now famous MacConkay's broth,33 which was diagnostic for lactose-fermenting bacteria tolerant of bile salts. Nonetheless, coli-forms were still considered to be a heterogeneous group of organisms, many of which were not of faecal origin. The origins of the critical observation that E. coli was largely faecal in origin while other Coliforms were not, could be claimed.34

Table 1. Definitions for indicator and index micro-organisms of public health concern.

Group	Definition
Process indicator	A group of organisms that demonstrates the efficacy of a process such as total heterotrophic bacteria or total Coliforms for chlorine disinfection.
Faecal indicator	A group of organisms that indicates the presence of faecal contamination such as the bacterial groups thermotolerant Coliforms or E. coli. Hence, they only infer that pathogens may be present.
Index and model organisms	A group/or species indicative of pathogen presence and behavior respectively such as E. coli as an index for Salmonella and F-RNA coliphages as models of human enteric viruses.

Use of Escherichia coli as indicator organism

Escherichia coli are the predominant member of the facultative anaerobic portion of the human colonic normal flora.35 The bacterium's only natural habitat is the large intestine of warm-blooded animals and since E. coli, with some exceptions, generally does not survive well outside of the intestinal tract, its presence in environmental samples, food, or water usually indicates recent faecal contamination or poor sanitation practices in food-processing facilities.36 The population of E. coli in these samples is influenced by the extent of faecal pollution, lack of hygienic practices, and storage conditions.35 The mere presence of E. coli in food or water does not indicate directly that pathogenic microorganisms are in the sample, but it does indicate that there is a heightened risk of the presence of other faecal-borne bacteria and viruses, many of which, such as Salmonella spp. or hepatitis A virus, are pathogenic. For this reason, E. coli is widely used as an indicator organism to identify food and water samples that may contain unacceptable levels of fecal contamination. E. coli is considered a more specific indicator of fecal contamination than fecal coliforms since the more general test for fecal coliforms also detects thermotolerant non-fecal coliform bacteria. The E. coli test recommended by the United States Environmental Protection Agency (EPA) confirms presumptive fecal coliforms by testing for the lack of an enzyme which is selective for the E. coli organism. This test separates E. coli from non-fecal thermotolerant coliforms.

Scientific classification of Escherichia Coli Kingdom:

bacteria; phylum: proteobacteria; class: Gamma proteobacteria; order: Entero -bacteriales; family: Enterobacteriaceae; genus: Escherichia; species: Escherichia coli. Escherichia coli (commonly abbreviated E. coli; pronounced and named after its discoverer), is a Gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most E. coli strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2,43 or by preventing the establishment of pathogenic bacteria within the intestine. In fact, various classification schemes for coliforms have been emerged. The earliest were those of MacConkay, who recognized 128 different coliform types, while Bergey and Deehan identified 256. By the early 1920s, differentiation of coliforms had come to a series of correlations that suggested indole production, gelatin liquefaction, sucrose fermentation and Voges-Proskauer reaction were among the more important tests for determining faecal contamination. These developments culminated in the IMViC (Indole, Methyl red, Voges-Proskauer and Citrate) tests for the differentiation of so-called faecal coliforms, soil coliforms and intermediates. Water sanitary engineers, however, require simple and rapid methods for the detection of faecal indicator bacteria. Hence, the simpler to identify coliform group, despite being less faecal-specific and broader (for which Escherichia, Klebsiella, Enterobacter and Citrobacter were considered the most common genera) was targeted. One of the first generally an accepted method for coliforms was called the Multiple-Tube Fermentation Test. New strains of E. coli evolve through the natural biological process of mutation, and some strains develop traits that can be harmful to a host animal. These virulent strains typically cause a bout of diarrhea that is unpleasant in healthy adults and is often lethal to children in the developing world. More virulent

strains, such as O157:H7 cause serious illness or death in the elderly, the very young or the immunocompromised. E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped and are about 2 micrometres (μm) long and 0.5 μm in diameter, with a cell volume of 0.6-0.7 μm3. It can live on a wide variety of substrates. E. coli uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when E. coli lives together with hydrogen-consuming organisms such as methanogens or sulfate-reducing bacteria. Optimal growth of E. coli occurs at 37°C but some laboratory strains can multiply at temperatures of up to 49°C. Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide.

Virulence properties of E. Coli

Enteric E. coli (EC) are classified on the basis of serological characteristics and virulence properties (Table 2).

Table 2. Virotypes of E. coli.56

Name	Host	Description
Enterotoxigenic E. coli (ETEC)	Causative agent of diarrhea (without fever) in humans, pigs, sheep, goats, cattle, - The larger of the two proteins, LT enterotoxin, is similar to cholera toxin in structure and function. dogs, and horses	ETEC uses fimbrial adhesins (projections from the bacterial cell surface) to bind enterocyte cells in the small intestine. ETEC can produce two proteinaceous enterotoxins: -- The larger of the two proteins, LT enterotoxin, is similar to cholera toxin in structure and function - The smaller protein, ST enterotoxin causes cGMP accumulation in the target cells and a subsequent secretion of fluid and electrolytes into the intestinal lumen. ETEC strains are non-invasive, and they do not leave the intestinal lumen. ETEC is the leading bacterial cause of diarrhea in children in the developing world, as well as the most common cause of traveler's diarrhea. Each year, ETEC causes more than 200 million cases of diarrhea and 380,000 deaths, mostly in children in developing countries.57
Enteropathogenic E. coli (EPEC)	Causative agent of diarrhea in humans, rabbits, dogs, cats and horses	Like ETEC, EPEC also causes diarrhea, but the molecular mechanisms of colonization and etiologyare different. EPEC lack fimbriae, ST and LT toxins, but they utilize an adhesin known as intimin to bind host intestinal cells. This virotype has an array of virulence factors that are similar to those found in Shigella, and may possess a shiga toxin. Adherence to the intestinal mucosa causes a rearrangement of actin in the host cell, causing significant deformation. EPEC cells are moderately-invasive (i.e. they enter host cells) and elicit an inflammatory response. Changes in intestinal cell ultrastructure due to attachment and effacement are likely the prime cause of diarrhea in those afflicted with EPEC.

Enteroinvasive E. coli (EIEC)	Found in humans,	EIEC infection causes a syndrome that is identical to Shigellosis, with profuse diarrhea and high fever.
Enterohemorrhagic E. coli (EHEC)	Found in humans, cattle, and goats	The most famous member of this virotype is strain O157:H7, which causes bloody diarrhea and no fever. EHEC can cause hemolytic-uremic syndrome and sudden kidney failure. It uses bacterial fimbriae for attachment (E. coli common pilus, ECP), 58 is moderately-invasive and possesses a phage-encoded Shiga toxin that can elicit an intense inflammatory response.
Entero Enteroaggregative E. coli (EAEC)	Found in humans,	So named because they have fimbriae which aggregate tissue culture cells, EAEC bind to the intestinal mucosa to cause watery diarrhea without fever. EAEC are non-invasive They produce a hemolysin and an ST enterotoxin similar to that of ETEC.

Isolation and identification of E. coli

Methods used to isolate E. coli as an indicator organism from food have not proved to be efficient for isolating pathogenic strains of E. coli. This is largely because pathogenic strains often differ considerably from nonpathogenic E. coli in growth patterns.60 Pathogenic strains frequently show delayed growth at 44 and 45.5°C, particularly when initially present in low populations. Some pathogenic strains will not produce acid and gas from lactose in LST, BGLB, or EC broths within 48h. It has also been shown that growth in media containing sodium lauryl sulfate and growth at 44.5°C can cause a loss of plasmids, known to encode many virulence factors associated with pathogenic E. coli strains. One study indicated that up to 95% of E. coli cells lost plasmids during selective enrichment cultures. Therefore, the methods commonly used for detection of E. coli as an indicator organism should not be used to attempt isolation of pathogenic strains from food or water. Isolation of enterohemorrhagic E. coli 0157:H7 must be approached differently than using the methods for isolating otherstrains. E. coli 0157:H7 has some biochemical differences from most of other E. coli strains that can be exploited in isolation and identification methods . E. coli 0157:H7 ferments sorbitol slowly, or not at all and does not produce functional β-glucuronidase, whereas most of the other E. coli strains are positive in both tests. Further, E. coli 0157:H7 strains do not ferment rhamnose on agar plates, whereas 60% of non-sorbitolfermenting E. coli belonging to other serogroups ferments rhamnose on agar plates. Several methods such as DNA probes and polymerase chain reaction (PCR), ELISA procedure utilizing monoclonal antibody (4E8C12) specific for an outer membrane protein of E. coli 0157:H7 and media that can test both sorbitol fermentation and β-glucuronidase activity such as Sorbitol MacConkay agar containing MUG can be used for isolation of this organism. The identification and enumeration of E. coli of sanitary significance relies upon isolate conformance to the coliform and faecal coliform group definitions. E. coli isolates are traditionally identified by their IMViC pattern: + + - - (Type I) and - + - - (Type II). In this scheme I refers to the ability of the organism to produce indole from metabolism of tryptophane; M indicates the ability of the organism to ferment glucose to high acid as detected by E. coli

Methyl Red pH indicator dye in the medium; Vi stands for the production of neutral products 2.3 butanediol and/or acetoin from glucose metabolism, otherwise known as the VoguesProskauer reaction, whereas C represents the ability of the bacterium to use citrateas a sole carbon source. Recent data indicate that defining E. coli by IMViC profile is inadequate for identification of E. coli strains which do not give IMViC reactions corresponding to either Biotype I or Biotype II. The relatively high incidence of Type II E. coli in some specimen is at partly explained by the fact that many isolates require 48 h to produce a detectable amount of indole; hence, additional tests are essential for speciation.

Challenges of using E. coli as an indicator organism

As soon as the coliform test came into widespread acceptance, complications with its use and interpretation began to emerge. One concern was the discovery that a variety of microorganisms that read positive in the coliform test were not of fecal origin. As a result, the test method has evolved continually to become more specific. Some of the more significant developments were the so-called fecal coliform test which selects for coliforms of fecal origin by using a higher incubation temperature.

Though, disease-causing strains of E. coli species have been isolated from tap water, drinking water sources and mountain streams, examination of pathogenic E. coli is not easy due to the uncertainty in determining the pathogenic nature of isolated E. coli strains. There is no biochemical marker that can separate pathogenic from non-pathogenic strains and the relationship between serotype and pathogenicity is questionable. The use of E. coli as an indicator organism is somewhat restricted by the fact that E. coli is not a single species; certain genera of the coliform group such as Proteus and Aerobacter are normally found outside the human intestinal tract in soil; other organisms found in water that do not represent fecal pollution possess some of the characteristics attributed to E. coli and E. coli identical to that found in humans is also found in the intestinal tract of other warm-blooded animals. However, primarily, studies have shown that E. coli is a much better indicator of disease risk than is faecal coliform, EPA has therefore, recommended that E. coli be used as a criteria for classifying waters for fresh water contact recreation. Another weakness of the faecal coliform test and perhaps any indicator organism test geared to human waste is that there are some bacterial pathogens which are unrelated to human wastes. To the degree that naturally occurring microbial pathogens become a significant public health concern, completely new test procedures may have to be developed. Furthermore, while E. coli is specific for faecal contamination, there are three inherent problems of using E. coli as a confirmation of faecal contamination: i) it is outnumbered by other types of fecal bacteria making it more difficult to find; ii) it does not survive for long outside of the gut; iii) it can be found in pristine environments in the tropics. Therefore, the absence or presence of E. coli via a culture test does not absolutely confirm the absence or presence of faecal contamination. The E. coli tests used today as an indication of fecal contamination are commonly culture tests although there are PCR tests for the pathogenic strain E.coli O157:H7 and for enterotoxigenic strains. In addition to the inherent differences in the ecology of the above mentioned indicator organism, there is also the problem using culturable tests. All culture tests have an inherent bias in that they always underestimate the number of E. coli present in the sample. This occurrence happens for a number of reasons, but in the instance of recovering faecal indicators, the bias is primarily for two reasons: i) some healthy coliforms are viable but will not grow in the media prescribed for them; and ii) coliforms found in the environment are often stressed thereby making recovery very difficult despite the growth media used.

Current trends of E. coli

as indicator organism While the faecal coliform test has its limitations and problems, it also has many attributes. Perhaps, the most significant attribute is that: as a regulatory tool, it has worked long and well. In the case of water quality regulation, coliform testing has been used successfully for well over fifty years. For the foreseeable future, the faecal coliform test will continue to be the basis for much of the regulatory decision making regarding both quality water harvesting and contact recreation. The primary bias of using culturable tests in isolating E. coli as an indicator organism, has being overcome by using PCR, which detects both live and dead bacteria. The PCR is a rapid and reliable tool for the molecular-based diagnosis of a variety of infectious diseases. PCR analysis for screening drinking water and environmental samples has been reported, and has been utilized to identify E. coli in primary water specimens, stool specimens and outbreaks.

Conclusions

In conclusion its clear that E. coli appears to be the best indicator of bacteriological quality of water, primarily because of the, availability of affordable, fast, sensitive, specific and easier to perform detection methods for E. coli. However the fact remains that the life span of E. coli in water is short, thus it best determines, recent contaminations. It is therefore important that there is continuous monitoring for E. coli to determine the bacteriological quality of water.

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