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***Escherichia coli* as Microbiological Quality Water Indicator: A High Importance for Human and Animal Health**

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ABSTRACT: The purpose of this review is to demonstrate the importance of monitoring microbiological quality in water for animals and humans, which relies primarily on coliforms, mostly *Escherichia coli*. Fecal coliforms, such as *E. coli*, are more specific indicators of fecal pollution. A fecal contamination detection can be performed not just in drinking water, but also in the environment. As a result of improved detection methods for *E. coli*, the drinking water is becoming more reliable as a result of the use of *E. coli* as an indicator of fecal pollution. The drinking water contamination by feces is currently best detected by *E. coli*. Accordingly, temperate environments are more likely to have fecal coliforms than the tropical environments, the human and animal feces have high levels of *E. coli* in comparison to other fecal coliforms, and *E. coli* detection methods are more affordable, fast, sensitive, specific, and easy to use than the other fecal coliforms.

Keywords: *Escherichia coli*; water quality; microbiology; safety; animals

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INTRODUCTION

To sustain life, the water is an essential natural resource (George et al., 2018; Ochilova et al., 2021; Zafar et al., 2019). Providing fresh, clean water plays a key role not only in the economic development and welfare, but also in the production of food, reducing poverty, and improving health (Cosgrove & Loucks, 2015; Tallis et al., 2008). Nevertheless, 1.1 billion people worldwide lack access to safe drinking water, and approximately 400 children under the age of five die every hour as a result of biological contamination of the drinking water (Lea, 2014). The fluids in the body, such as blood and lymph, are maintained at normal volume and consistency by water. Water also regulates body temperature, removes poisons through urine, sweat, and breathing, and regulates the structure and functions of the skin (Odonkor & Ampofo, 2013). Each day, the body of an animal or human loses several litres of water. Therefore, both animals and humans must drink at least the equivalent amount of quality water every day to replenish this volume (Asano & Cotruvo, 2004). Recent years have seen a rapid increase in the demand for clean drinking water in the developing countries with deteriorating environments (Luo et al., 2022). Most communities in some countries do not have access to piped water, and both the quantity and quality of public water supply are inadequate (Hunter et al., 2010). There are only 40% of urban residents with direct access to piped water. Overall, only 10.3 million people have access to improved water supplies (Shamsudduha et al., 2020). It is common for people without access to safe water, or those who have access but cannot afford it, to use other sources of water with questionable qualities such as wastewater (de Llanos et al., 2022; Puvača et al., 2021).

A growing concern among consumers, water suppliers, regulatory agencies, and public health authorities is the microbiological quality of drinking water (Olaoye & Onilude, 2009). Various levels of economic development have documented the risk of drinking water carrying microbial pathogens, causing subsequent illnesses in animals and humans. A majority of sporadic cases of waterborne intestinal illness will not show any signs of detection, or if they do, they may not be identified as such. Waterborne diseases have been estimated by several researchers worldwide (Almeria et al., 2021; Cann et al., 2013; Hennebique et al., 2019; Hunter et al., 2001; Leclerc et al., 2002; Semenza, 2020). Intestinal infections caused by waterborne diseases account for about one-third of all in-

testinal infections worldwide, whilewater, sanitation, and hygiene are responsible for 40% of all deaths and 5.7% of all diseases (Malhotra et al., 2015). There is a risk of waterborne disease associated with fecal contamination from humans, food animals, and wild animals (Sinton et al., 1998). Human fecal waste is generally associated with the greatest risk. The presence of pathogenic agents in water cannot be routinely monitored due to the wide spectrum of the present pathogenic agents. The microorganisms of fecal origin have traditionally been checked for in drinking water to confirm the microbial safety (Leclerc & Moreau, 2002). Several recorded outbreaks of the piped drinking water-related diseases are connected to the quality changes in distribution, based on the evidence relating to the frequency and extent of the known quality changes (Ahmed et al., 2016; Cann et al., 2013). The water distribution systems play a crucial role in the quality and safety of the drinking water (Horvat et al., 2021). There must be a high level of microbiological safety in the water entering the distribution system, as well as a high level of biological stability. When water is transported from the treatment plant to the user, it must be protected from contamination after the treatment.

Based on all mentioned above the aim of this review is to demonstrate the importance of monitoring the microbiological quality in water for animals and humans, which relies primarily on coliforms, *E. coli*.

PAST AND BASIS OF COLIFORMS AS INDICATORS OF WATER CONTAMINATIONS

Klebsiella pneumonia and *K. rhinoscleromatis*, microorganisms characteristic of human feces, were described by Von Fritsch in 1880 as the indicators of the water quality. Later, Robert Koch's solid gelatin media were used by Percy and Grace Frankland to count bacteria in water for the first time in 1885 in London (Cabral, 2010). *Bacillus coli* was also described and renamed *Escherichia coli* by Escherich in the same year (Friedmann, 2006). To provide the evidence of the potentially dangerous pollution, the Franklands proposed that organisms characteristic of the sewage must be identified. Using the concept of acid from lactose as a diagnostic feature, the Wurtz method of enumerating *E. coli* in water samples using litmus lactose agar was in use by 1893 (Mishra et al., 2018). The Durham tube was introduced later, followed by gas production. In 1901, Great Britain was using the concept of coliform bacteria, which are the bacteria similar to *E. coli* (Foppen et al., 2005).

In the first report, however, the colony count for bacteria in water was formally introduced. At the start of the twentieth century, the bacteriologists recognized the importance of finding various coliforms, streptococci, and *C. perfringens* (Cabral, 2010). The famous MacConkey's broth, which was diagnostic for lactose-fermenting bacteria tolerating bile salts, was not described until 1905 by the author itself. Although coliforms included many organisms outside the fecal origin, they were still considered heterogeneous groups of organisms (Scott et al., 2002). It could be argued that *E. coli* originated largely from feces, while other Coliforms did not (Lalancette et al., 2014).

***E. coli* AS BIOLOGICAL INDICATOR OF WATER CONTAMINATION**

Human and animal colonic normal bacteria are dominated by *E. coli*, which is a facultative anaerobic bacterium (Puvača & de Llanos Frutos, 2021). As *E. coli* can only survive in the large intestine of warm-blooded animals (Savageau, 1983), its presence in the environmental samples such as water usually indicates recent fecal contamination or poor sanitation practices at the water treatment facilities (Jang et al., 2017). The fecal pollution, improper storage conditions, and lack of hygienic practices all influence *E. coli* populations in water samples (Odonkor & Mahami, 2020). If there are *E. coli* organisms present in food or water, it does not necessarily mean that pathogenic bacteria are present, but rather that there is a higher risk of the presence of other fecal-borne bacteria and viruses, many of which are pathogenic. It

is for this reason that *E. coli* is widely used as an indicator organism for the fecal contamination in water samples (Table 1). Because fecal coliforms also detect thermotolerant non-fecal coliform bacteria, *E. coli* is considered a more specific indicator of fecal contamination than fecal coliforms (Ferguson & Signoretto, 2011). Testing for the absence of enzymes that are selective for the *E. coli* organism is required by the Environmental Protection Agency (EPA) to confirm presumptive fecal coliforms (Schraft & Watterworth, 2005). Using this test, *E. coli* is distinguished from coliforms that are not thermotolerant in the feces.

BACTERIOLOGICAL SOURCES OF WATER-BORNE ILLNESSES IN HUMANS

The majority of *E. coli* strains are harmless commensal bacteria, but some strains can cause disease in animals and humans (Puvača & de Llanos Frutos, 2021). Bloody diarrhea, hemolytic uremic syndrome, hemorrhagic colitis, and other potentially fatal human diseases have been associated with Shiga toxin-producing *E. coli* (STEC), including enterohemorrhagic *E. coli* (EHEC) (Hughes et al., 2006; Lika et al., 2021; Paton et al., 1996; Tarr et al., 2005). The waterborne illnesses have been caused by *E. coli* O157:H7, one of the most widely recognized serotypes of EHEC (Lim et al., 2010). There are at least five other *E. coli* pathogroups (Brundre et al., 1999) besides STEC and EHEC (Table 2). Most infants in the developing countries suffer from watery diarrhea caused by enteropathogenic *E. coli* (EPEC) (Clarke et al., 2002).

Table 1. Public health indicator and index microorganism definitions.

Group	Description
Process indicator	Total heterotrophic bacteria or total coliforms demonstrate the efficacy of chlorine disinfection.
Fecal indicator	Usually, thermotolerant coliforms or <i>E. coli</i> indicate the presence of fecal contamination. As a result, pathogens may only be present if they have been detected.
Index and model organisms	Groups or species of pathogens indicating their presence or behaviour, such as <i>Salmonella</i> , and F-RNA coliphage models indicate human enteric viruses.

Table 2. Different types of *E. coli*.

Name	Hosts	Consequences on the host
Enterotoxigenic <i>E. coli</i>	Humans; Pets; Food animals	Diarrhea without fever
Enteropathogenic <i>E. coli</i>	Humans; Rabbits; Pets; Horses	Diarrhea
Enteroinvasive <i>E. coli</i>	Humans	Diarrhea and high fever
Enterohemorrhagic <i>E. coli</i>	Humans; Goats; Cattle	Bloody diarrhea without fever; Hemolytic-uremic syndrome;
Enteropathogenic <i>E. coli</i>	Humans	Watery diarrhea without fever

Traveler's diarrhea is most commonly caused by enterotoxigenic *E. coli* (ETEC) (Mendez Arancibia et al., 2009) while persistent diarrhea can be caused by enteroaggregative *E. coli* (EAEC) (Okhuysen & DuPont, 2010). In terms of genetics, biochemistry, and pathogeny, enteroinvasive *E. coli* (EIEC) are closely related to *Shigella* (van den Beld & Reubaet, 2012). It is believed by some researchers that *Shigella* is a subgroup of *E. coli* (Pupo et al., 2000; Sims & Kim, 2011). Unlike uropathogenic and avian pathogenic strains of *E. coli*, extraintestinal pathogenic *E. coli* (ExPEC) are harmless when they are inside the intestinal tract (Johnson & Russo, 2002). However, they can lead to neonatal meningitis and sepsis if they are brought into the body (Smith & Fratamico, 2017). There has been no detailed investigation into the distribution of pathogenic *E. coli* in the environment, however, the presence of EPEC strains in the environment is more frequent than that of STEC strains, according to several studies (Balière et al., 2015, 2016; Ishii et al., 2007). It is thought that EPEC strains are evenly distributed among different human and animal hosts, whereas cattle and other ruminant animals (sheep, goats, and deer) are the most important reservoirs of STEC. The detections of EPEC in the environment may be explained in part by the widespread distribution of EPEC in a wide variety of animal hosts (Jang et al., 2017).

SOURCES OF *E. coli* INFECTION IN ANIMALS

A wide variety of animals, including cattle, sheep, goats, pigs, water buffalo, and wild ruminant species, shed Shiga toxin-producing *E. coli* in their feces. The most important reservoir for zoonotic STEC is ruminants, which may transmit the disease to humans through the ingestion of contaminated food or water or contact with the infected animals (Persad & LeJeune, 2015). Drinking water, feed, and the environment of animals are the main sources of STEC infection (Ahmed et al., 2015). As well as cattle carrying the bacteria, other food animals (poultry, pigs, sheep, goats), companion animals (dogs, cats), wild animals (deer), or insects (flies) may also contribute to the contamination. It is also possible to contract an infection from direct contact with cattle or other animals. In addition to rivers, ponds, lakes, and groundwater supplying wells and springs, water runoff from dairies and pastures where zoonotic STEC-carrying cattle have been grazing can pollute the surface drinking waters. A source of contamination may also be pastures where slurry or manure from cattle (Pedersen &

Clark, 2007) or poultry (Ljubojević et al., 2017) with zoonotic STEC has been spread as fertilizer. Drinking troughs can be contaminated by the water source from fecal contamination, or from cows carrying STEC in their tonsils contaminating the water through oral contact. O157 STEC can survive for several months in water, feces, or sediment from drinking troughs (Avery et al., 2008). As a result of run-off or the spread of manure and slurries as fertilizer, or by wild bird or mammalian feces, feeds such as grain pellets, soybean meal, silage grasses, and grass hay can be contaminated. In addition to contamination during transportation to a feed mill, the feed can also be contaminated during storage. If silage is poorly managed, it may allow STEC to survive on grass that has been contaminated with feces: proper processing of silage normally eliminates the STEC. Animals, wildlife, rodents, birds, and insects, such as flies, may contaminate feed troughs through saliva or defecation. Most of the contamination of cattle environments, including pastures, feed troughs, and pen floors, comes from fecal contamination. One of the most important findings is that some O157 STEC strains may persist for more than two years in a certain farm environment (Ma et al., 2014). Bacteria persistence is strongly influenced by the type of the environment they live in. In contrast, calves raised on pasture on the same farm shed no O157 STEC over a period of six months, possibly because they were exposed to fewer bacteria when kept indoors in pens. The persistence of STEC will also be impacted by poor husbandry (Hellberg & Chu, 2016). O157 STEC was found to be more prevalent in cattle raised in pens with wet, muddy floors than in cattle raised under normal conditions.

It has been shown that age, weaning, movement of the animals, season, feed composition, as well as the ability of the bacteria to persist in the environment, are risk factors for infection with O157 STEC in animals (Hellberg & Chu, 2016).

E. coli METHODS FOR ISOLATION AND IDENTIFICATION

The serological characteristics and virulence characteristics of enteric *E. coli* (EC) determine their classification. Isolating pathogenic strains of *E. coli* from food using *E. coli* as an indicator organism has not been proven effective. In part, this is since pathogenic strains of *E. coli* have very different growth patterns than non-pathogenic strains. Especially when initially present in low populations, pathogenic strains frequently show delayed growth at 44 and 45.5°C (Eblen

et al., 2005).

It is possible for some pathogenic strains to not produce acid and gas from lactose within 48 hours when grown in LST, BGLB, or EC broths. Additionally, plasmids that encode many virulence factors of pathogenic *E. coli* strains are lost in growth media that contain sodium lauryl sulfate at 44.5°C. To isolate the pathogenic strains from food or water, the methods commonly used to detect *E. coli* as an indicator organism should not be used (Tortorella, 2003).

Enterohemorrhagic *E. coli*O157:H7 must be isolated differently from other strains of *E. coli*. The biochemical differences between *E. coli* O157:H7 and most other strains can be exploited for isolation and identification (Table 3). The O157:H7 strain produces no functional beta-glucuronidase and ferments sorbitol slowly, or not at all, whereas most other *E. coli* strains do (Nataro et al., 2011). Further, *E. coli* strains belonging to other serogroups ferment rhamnose on agar plates, whereas *E. coli* strains belonging to O157:H7 do not ferment rhamnose (Nataro et al., 2011).

For sorbitol fermentation and beta-glucuronidase testing, such as Sorbitol MacConkey agar containing MUG, and methods that test both sorbitol fermentation and beta-glucuronidase activity, DNA probes and polymerase chain reactions (PCR) can be used for the isolation of *E. coli* O157:H7. As a result of coliform and fecal coliform group definitions, *E. coli* of sanitary significance are identified and enumerated. Conventionally IMViC pattern: + - - (Type I) and - + - - (Type II) are used to identify *E. coli* isolates (Huang et al., 1997). In this scheme I refers to the

ability of the organism to produce indole from the metabolism of tryptophan; M indicates the ability of the organism to ferment glucose to high acid as detected by Methyl Red pH indicator dye in the medium; V stands for the production of neutral products 2,3 butanediol and acetoin from glucose metabolism, known as the Voges-Proskauer reaction, whereas C represents the ability of the bacterium to use citrate as a sole carbon source (Huang et al., 1997). Furthermore, IMViC profiles do not provide adequate identification of *E. coli* strains which do not give IMViC reactions corresponding to either Biotype I or Biotype II. As many isolates need 48 hours to produce detectable indole, the additional tests are necessary for speciation of Type II *E. coli* in some specimens (de Boer et al., 2010).

RECENT DEVELOPMENTS OF *E. coli* AS CONTAMINATION INDICATOR

Aside from its limitations and problems, the fecal coliform test has many advantages. The most important attribute is that it has proven effective as a regulatory tool for many years. The coliform test has been successfully used for more than 60 years in water quality control (Tiwari et al., 2021). As far as quality water harvesting and contact recreation are concerned, much of the regulatory decision-making will continue to be based on the fecal coliform test. By using PCR, which detects both live and dead bacteria, the primary bias of culturable tests in isolating *E. coli* as an indicator organism has been overcome (Offenbaume et al., 2020). Infectious diseases can be detected molecularly using PCR, which is a rapid and reliable method (Hasseb et al., 2022). *E. coli* has been identified in primary water samples, feces samples,

Table 3. *E. coli* types and their biochemical properties.

Test	Enterotoxigenic	Enteropathogenic	Enteroinvasive
Motility	+	+	++
Voges-Proskauer	++	++	++
Gas from lactose	+	+	++
Lysine decarboxylation	+	+	+++
Ornithine decarboxylation	+++	+++	++
Lactose fermentation	+	+	+++
Beta-Glucuronidase	+	+	+
Indole	+	+	+++
Sorbitol fermentation	+	+	++
Citrate	++	++	++
Methyl red	+	+	+

+, >90% positive; ++, <10% positive; +++, 10-90% positive

and outbreak samples using PCR analysis for screening (Kinnula et al., 2018) .

CONCLUSION

Considering the availability of inexpensive, fast, sensitive, specific, and easier-to-perform detection methods for *E. coli*, it makes sense that *E. coli* appears to be the best indicator of the bacteriological quality in water. However, *E. coli* in water only lives a short time, so it can best determine recent contamination. To

determine the bacteriological quality of water, continuous monitoring for *E. coli* is still required and will be required for long period.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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