Microbiological Indicators: A Veritable Tool for Monitoring Public and Private Drinking Water Sources and Distribution Systems

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Abstract: Water is essential for life. It covers about 70% of Earth's surface and makes up about 70% of body mass. Diseases caused by unsafe or drinking microbial-polluted water and inadequate sanitation are a serious public health concern, causing 80% of illnesses in developing countries and killing 2 to 5 million people, mainly young children, every year. This review, therefore, is on microbiological indicators as a veritable tool for monitoring public and private drinking water sources and distribution systems. Its ultimate goal is to assure the water is free from pathogenic microorganisms. Its absence is a good bacteriological indicator of safe water and it grows readily on simple media. Most coliform bacteria do not cause disease. Recent outbreaks of disease caused by *E. coli* 0157: H7 have generated much public concern. This pathogenic strain has been found in cattle, chickens, pigs and sheep. Cases of diarrhea caused by contaminated drinking water supplies are frequent. These could be due to fecal pollution. Fecal coliform may be introduced into water by both point and non-point sources. Streptococci are present in the stomach of human and animal intestines. Many species of strepotcoccus are pathogenic, causing diseases such as bacterial pneumonia, ear infection and bacterial meningitis. The presence of these microbiological indicator organisms would only indicate that pollution has occurred.

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Introduction

Water is the only substance that exists naturally on Earth in all three physical states of matter (e.g., gas, liquid and solid) and it is always on the move among them (CDC, 1996; WHO, 2002). Water pollution is gaining global attention.

Pollution could be defined as the introduction by man into the environment of any material that has detrimental effects on the vital components of the ecosystem.

Water pollution could trigger sustainability issues which arise wherever there is a risk of difficult or irreversible loss of the things or qualities of the environment that people value.

Threats to the environment mean that there is a risk that it will not be sustained and whenever there are such risks, there is a degree of urgency to take action and keep the ecosystem safe (Solomon *et al*., 2016).

Microorganisms are ideal indicator organisms in the evaluation and monitoring of public and private drinking water sources and distribution systems. Indicator organisms or bioindicators are plant or animal species (living organisms) known to be either particularly tolerant or particularly sensitive to environmental pollution and/or changes (Ackman *et al*., 1997).

The health of an organism can often be associated with a specific type or intensity of pollution and its presence can then be used to indicate polluted conditions relative to unexpected conditions (Wosu Kinika *et al*., 2016).

Microbial indicator organisms are a basic monitoring tool used to measure both changes in environmental water quality or conditions and the potential presence of hard-to-detect pathogenic organisms.

These organisms provide evidence of the presence or absence of a pathogenic organism that survives under similar physical, chemical and nutrient conditions. It is important to note that an indicator organism is not necessarily a pathogen (CDC, 1993).

Although some strains of *Escherichia coli* are pathogenic, the reasons *E. coli* and *Enterococci* are used are, because they have been shown to be indicative of recent fecal contamination (Boehm, 2007; Davies *et al*., 1995).

In addition, their behaviours, such as, viability, longevity and movement in the environment are assumed to be similar to actual pathogens of concern, and there is a relatively fast method of analysis available. Index organisms are organism or group of organisms that is indicative of a specific pathogen (U.S EPA, 2004; WHO, 2003).

Bioindicators

Since most disease outbreaks associated with consumption of shellfish originated with fecal contamination, a logical approach is to seek a microbial indicator group commonly found in the feces of all warm-blooded animals (Camper *et al.,* 1991). Ideally an indicator organism should satisfy the following criteria:

1. It should always be present in waters whenever pathogens are present.
2. It should occur in much greater numbers than the pathogens.
3. It should be absent, or at least very few numbers in clean waters.
4. It should not be able to proliferate to any greater extent than pathogens in aquatic environment.
5. It should respond to natural environmental stress and wastewater treatment processes and disinfectants in a manner similar to the pathogens of interests.
6. Indicator density should bear some relation to the degree or extent of pollution.
7. It should be easy to isolate, identify, and enumerate by routine laboratory procedures.

Unfortunately, no organism meets all these criteria. It is doubtful if an ideal indicator exists or will ever be found for bacterial, protozoa and viral pathogens. So we must, therefore, deal in terms of the best indicator available.

The best indicator would obviously be the one whose density correlates best with the health hazards associated with fecal contamination (Ferlay *et al*., 1989; Boehm, 2007). A variety of groups of bacteria and viruses have been used or recommended to measure the sanitary quality of recreational and shellfish growing waters (WHO, 2003).

These range from a broad spectrum group, such as the total plate counts, to a narrow spectrum group such as *Escherichia coli* and specific pathogens. Of these various groups, the most commonly used are the total coliform, fecal coliform and fecal streptococcus groups (APHA *et al*., 1998).

Requirements for bioindicator

Traditional requirements for bioindicator of food/ water safety include:

1. Easily and rapidly detectable.
2. Easily distinguishable from food flora.
3. History of association with pathogen.
4. Present with pathogen.
5. Numbers correlate with pathogen.
6. Growth requirements/rate equal to pathogen.
7. Die off rate parallels pathogen.
8. Absent from food free of pathogen.

Limitations of pathogens as biondicators

1. Concentrations may be very low and difficult to relate.
2. May not compete well with food flora.
3. Presence may not relate to another pathogen.
4. Presence may initiate regulatory action – may be considered adulteration.
5. Pathogens require special laboratory skills.

Proposed/adopted indicator organisms

Water pollution caused by fecal contamination is a serious problem due to the potential for contracting diseases from pathogens (Craun *et al*., 1997). Frequently, concentrations of disease-causing organisms from fecal contamination are small and the number of different possible pathogens is large (Doyle and Erickson, 2006).

As a result, it is not practical to test for pathogens in every water sample collected. Instead, the presence of pathogens is determined with indirect evidence by testing for an "indicator" organism such as coliform bacteria.

Coliform bacteria are a commonly used indicator of sanitary quality of foods and water. Proposed or adopted indicator organisms include members of the family Enterobacteriaceae (these includes collectively to coliform, fecal coliform and *Escherichia coli*).

Test for microbial indicators

The most basic test for bacterial contamination of a water supply is the test for total coliform bacteria. Coliforms come from the same sources as pathogenic organisms. Coliforms are relatively easy to identify, are usually present in larger numbers than more dangerous pathogens, and respond to the environment, wastewater treatment, and water treatment similarly to many pathogens (Boehm, 2007).

As a result, testing for coliform bacteria can be a reasonable indication of whether other pathogenic bacteria are present. Test for microbial indicators include testing for the following group of indicators organisms: coliform, fecal coliform, enterococci, anaerobic bacteria and bacteriophage respectively (CDC, 1996).

Group 1: Total Coliforms

Total coliform counts give a general indication of the sanitary condition of a water supply. Total coliforms are defined as rod-shaped Gram-negative non-spore forming and motile or non-motile bacteria which can ferment lactose with the production of acid and gas when incubated at 35–37°C (Erickson and Dufour, 1986).

They include bacteria that are found in the soil, in water that has been influenced by surface water, and

in human or animal waste. Total coliforms include *Klebsiella* sp., *Citrobacter* sp. and *Enterobacter* sp.

Drawbacks

- Coliforms may grow in aquatic environments, particularly if organic matter levels and temperatures are elevated.
- Coliforms may form biofilms in drinking water distribution systems – this is a problem because, for example, *E. coli* is 2400 times more resistant to chlorine in a biofilm than when planktonic.
- Coliforms may recover from disinfectant injury.
- Growth of heterotrophic bacteria on media selective for coliforms can mask coliform population in water (occurs when heterotrophic counts exceed 500/mL).
- More vulnerable to disinfection and environmental trauma than enteric viruses or parasites.
- Do not necessarily indicate fecal contamination.

Group 2: Fecal Coliforms

Fecal coliform is a type of bacteria found in the feces of humans and other warm-blooded animals (Byappanahalli *et al*., 2012). The bacteria naturally occur in the human digestive tract and aid in the digestion of food (Ferguson *et al*., 1996).

Fecal coliform is usually not pathogenic by itself, but when found in a water system, it is generally indicative of the existence of other pathogenic organisms. When calculating the Overall Water Quality Index for any water system, fecal coliform is the second most important factor in the calculation (Byappanahalli *et al*., 2012).

Fecal coliform is a good indicator because it can be tested for at low cost, it is measurable, credible, significant, and easily interpreted. The laboratory test takes diluted samples of water from the stream and filters it with the use of a vacuum.

Any bacteria are thus collected on the filter papers, which are placed in Petri dishes containing an ampoule broth. The samples are then incubated to allow bacteria colonies to form. The total colonies are easily counted and the average fecal coliform colonies calculated. A fecal coliform is a facultatively anaerobic, rod-shaped, gram-negative, non-sporulating bacterium (Doyle and Ericksen, 2006).

They are capable of growth in the presence of bile salts, are oxidase negative and produce acid and gas from lactose within 48 hours at 44 ± 0.5°C (Doyle and Erickson, 2006). The term "thermotolerant coliform" is more correct and is gaining acceptance over "faecal coliform".

Escherichia coli

Escherichia coli is the major species in the fecal coliform group. Of the five general groups of bacteria that comprise the total coliforms, only *E. coli* is generally not found growing and reproducing in the environment. Consequently, *E. coli* is considered to be the species of coliform bacteria that is the best indicator of fecal pollution and the possible presence of pathogens (Camper *et al.,* 1991).

Escherichia coli, a rod-shaped member of the coliform group, can be distinguished from most other coliforms by its ability to ferment lactose at 44°C in the fecal coliform test, and by its growth and color reaction on certain types of culture media. When cultured on an eosin methylene blue (EMB) plate, a positive result for *E. coli* is metallic green colonies on a dark purple media (Davies *et al*., 1995).

Escherichia coli have an incubation period of 12–72 hours with the optimal growth temperature being 30–37°C. Unlike the general coliform group, *E. coli* are almost exclusively of fecal origin and their presence is thus an effective confirmation of fecal contamination (Davies *et al*., 1995).

Drawbacks

- Same drawbacks as for total coliforms.
- Indicates fecal contamination for sure, but can't distinguish between animal and human feces.
- Can survive and grow for extended periods of time in tropical waters.
- May be natural inhabitants of these waters!

Group 3: Fecal streptococcus

The third group of bacteria, the fecal streptococcus, has been suggested as a useful indicator of fecal contamination because they are present in large numbers in feces. They do not multiply in surface waters and are more resistance to adverse environmental conditions (Boehm, 2007).

Unfortunately, the fecal streptococcus, like the coliform group, also includes several biotypes which are widely distributed in nature and are of limited sanitary significance. Many species of strepotcoccus (enterococcus) are pathogenic.

They cause diseases such as bacterial pneumonia, ear infection and bacterial meningitis. Faecal strepotococci are a subgroup of the genus streptococcus. They do not multiply in water. Others are:

- They are more resistant to stress/disinfection
- Last longer in the environment.
- Used as indicators of enteric viruses, and gastroenteritis for swimmers.
- Members of the lactic acid bacteria.
- Gram positive, non-motile, non-spore-forming, aerotolerant anaerobic bacteria that ferment sugars to lactic acid.
- FC/FS ratio - ratio of fecal coliform counts to fecal strep counts.
- FC/FS >4: fecal contamination of human origin.

• FC/FS < 0.7: fecal contamination of animal origin.

• This relationship is only valid for recent fecal contamination (within the last 24 hours).

Group 4: Anaerobic bacteria

Members of the group have the following characteristics:

• Gram positive, anaerobic spore-forming rod-shaped bacterium.

• Spores are heat resistant (can survive 75°C for 15 min), resist disinfection; can remain viable in the environment for a long time.

• May be used as indicator of resistant pathogens (viruses, parasites), past fecal contamination, or tracing fecal contamination in a marine environment.

• *Clostridium perfringens* is one example.

Drawbacks

• A common soil bacterium; may not necessarily indicate fecal contamination.

• Pathogenic (causes gas gangrene if it infects wounds, produces enterotoxin in small intestine causing gastroenteritis).

• Anaerobic culture is difficult.

Bacteriophage

Coliphage is one example.

• bacteriophage that infect coliforms, particularly *E. coli.*

• similar to enteric viruses in size, morphology, and performance in environment.

• found in higher numbers than enteric viruses in wastewater and other waters.

• rapid and easy detection methods available.

• survive for 7 days in shellfish without increasing in numbers.

• routinely used as indicator microorganisms to determine the effectiveness of wastewater treatment processes.

• resistant to disinfection.

Most Probable Number (MPN)

The MPN method is used to detect coliforms and consists of three steps: presumptive, confirming and completed test.

• Presumptive test: dilute water sample.

• Inoculate 3 or 5 tubes of lauryl sulfate-tryptose-lactose broth containing upside-down Durham tubes with water dilutions.

• Incubate at 35°C for 48 hours.

• Determine number of tubes at each dilution that are positive for gas production (contain bubble in Durham tube).

Membrane Filter Test

• Used to detect coliforms.

• Filter 100 mL water through a 0.45 μm filter.

• Incubate filter on pad soaked with a differential medium (Endo medium; contains lactose and Basic Fuchsin dye) at 35°C for 18-24 hours.

• Count colonies that grow on filter.

coliforms will be dark red with metallic gold sheen.

• To enumerate Fecal Streptococci, grow on Streptococcus agar at 37°C for 24 hours. Fecal streptococci reduce 2, 4, 5-triphenyltetrazolium chloride to formazan, which makes colonies appear red.

• Much quicker and easier than MPN method.

Heterotrophic Plate Count (HPC)

• Enumeration of all aerobic and facultative anaerobic chemoheterotrophs in water.

– Includes members of the family Pseudomonas, Aeromonas, Klebsiella, Flavobacterium, Enterobacter, Citrobacter, Serratia, Acinetobacter, Proteus, Alcaligenes and Moraxella.

• Varies from 1 to 10^4 CFU/mL, and depends on temperature, residual chlorine concentration, and availability of organic nutrients.

• Indicates general quality of water (particularly levels of organic matter in water).

• HPC > 500 CFU/mL indicates poor water quality.

Plaque assay

• Used to detect bacteriophage.

• Filter phage from water with charged membrane filter.

• Elute with beef extract, pH 9.0.

• Flocculate solids (including phage) with HCl.

• Centrifuge. Remove supernatant and resuspend pellet in beef extract. Neutralize solution.

• Inoculate 4 mL loose (0.7%) agar with host bacterial culture and 100 μL phage concentrate.

• Pour loose agar onto a solid agar plate. Incubate for 8-18 hours.

• Host bacteria will form lawn on plate. Bacteriophage will lyse small holes in the lawn (plaques).

• Count plaques and compare to the volume of filtered water to determine bacteriophage population in the water sample.

Presence-Absence Tests

• Colilert Test is used. It is qualitative and not quantitative.

• Used to detect total coliforms and *E. coli.*

• Add packet of salts and nutrients to water sample and incubate 24 hours.

• Total coliforms can convert *o*-nitrophenyl-β-D-galactopyranoside (ONPG) to yellow nitrophenol with β-galactosidase.

• *E. coli* can metabolize 4-methylumbelliferone glucuronide (MUG) to a molecule that fluoresces under UV light with glucuronidase.

- May not detect up to 1/3 of *E. coli* strains (including pathogenic ones!).
- Broth and agar plate techniques involving ONPG and MUG also exist.

Conclusion

Indicator organisms are used in safety and quality control procedures whenever a problem organism cannot be detected with ease and reliability. For safety, an indicator organism should be at least as resistant or persistent as the problem organism. Its presence and survival should indicate the potential presence of viable problem organisms. For easy detection, indicator organisms should be more numerous then the problem organisms and they should be easy to grow and to identify.

In sterilization quality control, each sterilization batch includes at least one sample intentional contaminated with an organism that is highly resistant to the treatment. These contaminated samples are incubated after the sterilization process under appropriate condition; lack of growth indicates the success of the sterilization process (Erickson and Dufour, 1986).

An easy way to differentiate between different types of coliform bacteria is by using an eosin methylene blue agar plate. This plate is partially inhibitory to Gram (+) bacteria, and will produce a color change in the Gram (-) bacterial colonies based on lactose fermentation abilities.

Strong lactose fermenters will appear as dark blue/purple/black and *E.coli* (which also ferments lactose) colonies will be dark colored, but will also appear to have a metallic green sheen. Other coliform bacteria will appear as thick, slimy colonies, with non-fermenters being colourless, and weak fermenters being pink.

Recreational water quality standards

Water quality criteria and guidelines comprise recommendations for acceptable levels of indicator microorganisms. Water quality standards are legally enforceable. They are regulated at both the Federal and State levels. Table 1 shows the guidelines for recreational water quality standards.

Table 1: Guidelines for Recreational Water Quality Standards

Country	Criteria or standard
U.S.E.P.A Safe Drinking Water Act Clean Water Act Wastewater discharges Sewage sludge	0 coliforms/100 ml 200 fecal coliforms/4 g <1000 fecal coliforms/4 g <3 Salmonella/4g <1 enteric virus/4 g <1 helmintha ova/4 g
California Wastewater reclamation for Irrigation	<2.2 MPN coliforms
Arezona Wastewater reclamation for Irrigation of golf courses	25 fecal coliforms/100 ml 125 enteric virus/40 L No detectable Giardia/40 L

Source: (WHO, 2003; U.S. EPA, 2004)

Recommendations

The qualitative determination of microbial indicators is never intended to be the sole information to judge the health hazard associated with particular water.

Detail knowledge of the sanitary conditions of the study area is essential to make proper judgment. Bacteriological measurement of shellfish growing water quality must be based on the detection of fecal contamination by all warm-blooded animals. It must be emphasized that the detection and enumeration of indicator organisms should be interpreted only as what they are intended to indicate.

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