Lec.3 *Indicators of Microbial Contamination of Drinking Water*

The presence of enteric pathogens in drinking water is of great concern. Hence, the most useful tool to detect pathogens in the water environment is the simultaneous analysis of several microorganisms classed as 'Indicator' microorganisms (IMs). To avoid ambiguity in the term 'Microbial Indicator' .The most widely used IMs are total coliforms (TCs), fecal or thermotolerant coliforms, *Escherichia coli*, *Enterococci* (fecal *Streptococci* or intestinal *Enterococci*) and bacteriophages.

The potential application of an IM should be to indicate:

a- The fecal pollution.

b- The presence of domestic sewage.

c- The presence of pathogenic microbes.

d- The efficiency of a particular water or wastewater treatment process.

e- The environmental fate of a target pathogen.

f- The movement of particles suspended in water during subsurface transport.

Traditional indicators:

Coliforms or total coliforms:

The coliforms belong to the family Enterobacteriaceae, which includes harmless *E. coli* and *Enterobacter*, the common intestinal organisms and occasional pathogens like the genus of *Klebsiella, Citrobacter, Kluyvera, Leclercia and Serratia*. These bacteria are classically used as an indicator of fecal contamination in water because they are considered as the residents of intestinal tracts of homoeothermic animals and, thus, are of sanitary significance. Their presence indicates the deterioration in water quality, possibly via bacterial re-growth problems or post-treatment contamination in drinking water.

There are some limitations in the general use of coliforms as IMs, which include:

(a) Their ability to grow in natural water.

(b) Lack of correlation between the number of coliforms and those of microbial pathogens. Therefore, these can serve as an indicator of treatment efficiency of wastewater treatment plants because of their sensitivity to chlorine.

Fecal coliforms or 'thermotolerant coliforms'

Coliforms that are able to grow and ferment lactose with the production of acid and gas in the presence of bile salts are grouped as fecal coliforms (FCs). For this reason, 'thermotolerant coliforms' would be the scientifically more accurate term for this group. Thus, the bacteria of this coliform subgroup have been found to have a positive correlation with fecal contamination of warm-blooded animals. The physiological basis of the elevated temperature phenotype in FCs has been described as 'thermotolerant adaptation of proteins' Therefore, their stability at temperatures found in the enteric tracts of animals is both constant and higher than the temperature in most aquatic and terrestrial environments. *Escherichia coli* are the most reliable indicator of enteric pathogens; it is therefore the indicator of choice to indicate the occurrence of recent fecal contamination in drinking

water. However, *E. coli* appears to provide the best bacterial indication of fecal contamination in drinking water. This is based on the following:

(a) The prevalence of thermotolerant (fecal) coliforms in temperate environments as compared to the rare incidence of *E. coli*.

(b) The prevalence of *E. coli* in human and animal feces.

(c) The availability of fast, sensitive, specific and easier test methods to detect *E. coli*.

Therefore, *E. coli* is the best and commonest microbial indicator available to date to inform public health risks associated with the consumption of contaminated drinking water.

Fecal Streptococci, Enterococci or intestinal Enterococci

Fecal Streptococci (Enterococci)

The fecal *Streptococci* are a group of gram-positive and belong to the genera *Enterococcus* and *Streptococcus* .The genus *Enterococcus*   includes all streptococci that share certain biochemical properties, and have a wide range of tolerance of adverse growth conditions. The enterococci can be found in soil, water, dairy products and food. They are differentiated from other streptococci by their ability to grow in 6.5% NaCl and pH 9.6 such as *E. faecalis*. Of the genus *Streptococcus*, the *S. bovis* is considered to be true fecal streptococci. It has been suggested that a fecal coliform/fecal streptococci ratio of 4 or more indicates a contamination of human origin, whereas a ratio below 0.7 is indicative of animal pollution.

It is advantageous to use fecal Streptococci, Enterococci and intestinal Enterococci as a useful indicator of the microbiological quality of drinking water because:

(a) They show a close relationship with the health risks due to the consumption of contaminated drinking water, mainly for gastrointestinal symptoms.

(b) They are always present in the feces of warm-blooded animals.

(c) Their inability to multiply in sewage-contaminated water resources.

(d) They are not ubiquitous as coliforms.

(e) Their die-off rate is slower than that of coliforms in water as well as their persistence pattern being similar to that of potential waterborne bacterial pathogens.

Alternative IMs:

The indicators named 'alternative' used to determine the possible threats to the public health.

Sulfite-Reducing Clostridia:

The genus *Clostridium perfringens* as an indicator of fecal contamination and sanitary quality of water is based on the following assumptions:

(a) The presence of these microorganisms in the feces of all warm-blooded animals as well as in sewage.

(b) More stability in environmental water and greater resistance to disinfection processes than most pathogens.

(c) Successful use in the monitoring of sewage contamination in water.

Bifidobacterium:

Bifidobacterium spp. is extremely variable groups of bacteria present in the feces of warm blooded animals, most of which have been detected in the human gastrointestinal tract (GI-tract). In human feces, the species changes with the age of the individual, in the intestines of infants, *B. breve and B. longum* generally predominate. In adults, *B. pseudocatenulatum and B. longum* are the dominant species. In both human and animal feces, *Bifibobacteria* are always much more abundant than coliforms.

Bifidobacteria have been found in sewage and contaminated water but appear to be absent from decontaminated or pristine environments such as springs and decontaminated soils. The presence of *Bifidobacteria* spp. in the water environment is considered as an indicator of fecal contamination of water because some species are specific to humans and animals as well as the alternative water quality indicators in tropical and temperate areas.

Bacteroides:

Bacteroides are among the most oxygen-tolerant microbes of the entire anaerobic microflora found in the human GI-tract. The need to maintain anoxic conditions for cultivation, isolation and biochemical identification has limited the use of anaerobic bacteroides species as a fecal indicator. The survival of bacteroides in water environments is usually much lower than that of coliforms.

Bacteriophages:

Bacteriophages have been proposed as an indicator of fecal and viral contamination and also as models to evaluate the chlorination efficiency of water treatment plants. The proposed groups include:

1- Somatic coliphages (Somatic coliphages are the phages that replicate in *E. coli* after infecting it through the cell wall). Somatic coliphages are specific to *E. coli* and have been commonly used as an indicator of fecal and/or sewage contamination in several water resources.

2- F-specific RNA (F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water). F-specific RNA is used as an indicator of fecal contamination as well as model viruses in water hygiene because of:

(a) Similar size and shape to human enteric viruses.

(b) Direct correlation with degree of sewage contamination.

(c) Inability to replicate in the water environment.

3- Phages of *Bacteroides fragilis*. *Bacteroides fragilis*, is found at high concentrations in the human intestinal tract. The phage of *B. fragilis*, has been reported as a specific indicator of human fecal contamination in the water environment because:

(a) The phages against this bacterial strain are human-specific and are not isolated from the feces of other homoeothermic animals.

(b) *B. fragilis* phages are consistently isolated from the water contaminated with sewage and feces and their sediments.

(c) The number of phages is related to the degree of contamination.

(d) *B. fragilis* phages always outnumber the human enteric viruses.

(e) In model experiments, no replication of these phages has been observed under simulated environmental conditions.

Indicator organisms	Characteristics
Total coliforms	Gram-negative, non-spore-forming, oxidase/indole-negative, rod-shaped facultative anaerobic bacteria that ferment lactose with β-galactosidase to acid and gas in a medium containing bile salts.
Fecal coliforms	Thermotolerant coliforms that produce acid and gas from lactose fermentation.
Escherichia coli	Thermophilic coliforms that produce indole from tryptophan, able to produce β-glucuronidase. Most appropriate group of coliforms to indicate fecal pollution from warm-blooded animals.
Fecal *Streptococci*	Gram-positive, catalase-negative, non-spore-forming cocci, belonging to the genera Enterococcus and Streptococcus. This group had been used in conjunction with fecal coliform to determine the source of recent fecal contamination. Several strains appear to be ubiquitous and cannot be distinguished from the true fecal.
Bacteroides	An anaerobic, non-spore-forming, Gram-negative, pleomorphic bacillus has been proposed as human-specific indicator.
Enterococci	Fecal streptococci that grow at pH 9.6 and in 6.5% NaCl. Enterococci are generally found in lower numbers than other indicator organisms.
Clostridium perfringens	Gram-positive spore-forming, non-motile, strict anaerobe, sulfite-reducing bacilli, ferment lactose and sucrose with the production of gas, produce clot fermentation with milk, reduce nitrate, hydrolyze gelatin and produce lecithinase and acid phosphatase. Spores most often of fecal origin and always present in sewage.
Bifidobacteria	Gram-positive, obligate anaerobic, non-acid-fast, non-spore-forming, non-motile bacilli which are highly pleomorphic and may exhibit branching bulbs. They are all catalase-negative and ferment lactose except *B. indicum* and *B. coryneforme*.
Bacteriophage	Bacterial viruses which are ubiquitous in the environment and resistant to disinfection. For water quality testing and to model human enteric viruses, most interest in somatic coliphages, F-specific RNA coliphages and phages infecting *B. fragilis*